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Synthesis of peptidomimetic compounds as potential anti HIV and malaria agents

Memory Zimuwandeyi

A thesis submitted to the Faculty of Science

University of the Witwatersrand

Johannesburg

In fulfillment for the requirements of the degree of Master of Science

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Declaration

I declare that the work presented in this thesis was carried out extensively by myself under the supervision of Dr Moira L. Bode and Dr Amanda L. Rousseau. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

_____

Memory Zimuwandeyi

17 March 2015

Abstract

Peptidomimetic compounds have been shown to exhibit both anti-HIV and anti-malarial activity. A multicomponent reaction was used to create a library of peptidomimetic compounds with an α-hydroxy-β-amino acid unit. The Passerini reaction between an aldehyde, carboxylic acid and isocyanide was used to prepare compounds containing both ester and amide functionalities. These compounds were then subjected to a deprotection-acyl migration strategy giving rise to the target compounds. This approach, known as the Passerini Amine Deprotection Acyl Migration (PADAM) sequence was successfully used to create a library of novel peptidomimetic compounds. From this library, 22 compounds were tested for activity against HIV and malaria.

The Passerini reaction gives rise to a product containing a new stereogenic centre, and as the starting aldehyde used (*N*-Boc-phenylalaninal) has a stereogenic centre, the products were isolated as a mixture of diastereomers. Our research was also focused on finding ways of influencing the stereoselectivity of the reaction and the separation of the resulting diastereomers. The diastereomeric ratio of the Passerini products was found to be approximately 2:1 for all the reactions performed. This ratio could be modified slightly when using certain carboxylic acids and isocyanides that were either very bulky or had a stereogenic centre.

Attempts to enzymatically resolve the diastereomeric products were not successful after trials using a library of 25 lipase enzymes. However, use of preparative HPLC enabled the successful separation of most of the diastereomeric mixtures, affording compounds with high purity. X-ray crystallography enabled us to identify the major diastereomers as having the *R*,*S* configuration, whilst the minor diastereomers had the *S*,*S* configuration at the two stereogenic centres.

A possible explanation for the observed stereoselectivity is based on the Felkin-Anh chelation control model. It suggests that mono-protected amino aldehydes follow a chelation controlled mechanism in nucleophilic addition reactions. Chelation occurs, albeit in the form of hydrogen bonding, between the NH and carbonyl oxygen.

Abstract

The library of compounds was tested for activity against both HIV-1 and malaria. Only three compounds showed moderate activity against the malaria parasite, inhibiting parasitic growth by 37-42% at 5 μM respectively. Significantly, all of the active compounds contained an adamantyl moiety. Unfortunately no anti-HIV activity was seen for any of the compounds tested in the HIV-assay.

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Firstly I would like to thank my supervisors Dr M.L. Bode and Dr A.L Rousseau for taking me in as their student. I will never forget the welcoming reception and eagerness to help, that they showed me when I walked into their offices looking for a chance to enroll as a Master's student. I feel very fortunate to have been able to work with you on this project and I will forever be grateful. Without your unending support and guidance, none of this would have been possible. I would also like to thank Prof C. de Koning and Prof J. Michael for the contributions they made for this project to be a success.

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List of abbreviations and formulae

d.r.	diastereomeric ratio
d.e.	diastereomeric excess
e.r.	enantiomeric ratio
e.e.	enantiomeric excess
m.p.	melting point
TosMIC	*p*-toluenesulfonylmethyl isocyanide
DMF	dimethylformamide
EtOAc	ethyl acetate
MeCN	acetonitrile
MeOH	methanol
Et_2O	diethyl ether
Hex	hexane
Ac_2O	acetic anhydride
TFA	trifluoroacetic acid
Et_3N	trimethylamine
DMSO	dimethyl sulfoxide
PADAM	Passerini amine deprotection acyl migration
CDI	carbonyldiimidazole
h	hour (s)
d	day (s)
Boc	*tert*-butyloxycarbonyl
R_f	retardation factor
TLC	thin layer chromatography
NMR	nuclear magnetic resonance
HRMS	high resolution mass spectrometry
IR	infrared
NICD	National Institute of Communicable Diseases

IC_{50}	50% inhibitory concentration
CC_{50}	50% cytotoxic concentration
CC_{20}	20% cytotoxic concentration

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CHAPTER 1: INTRODUCTION

1.1 HIV and AIDS

Acquired Immunodeficiency Syndrome (AIDS) is a condition attributed to the Human Immuno-deficiency Virus (HIV). The virus results in the infected person being susceptible to opportunistic infections and if untreated leads to the total collapse of the immune system and death.

1.1.1 Prevalence

The origin of HIV is still subject to much debate and controversy across the scientific community. The virus is believed to have originated in central Africa during the 1930s, with the first symptomatic outbreak occurring in Kinshasa (Democratic Republic of Congo) during the 1970s before reaching epidemic proportions throughout sub-Saharan Africa during the 1980s.[1] Despite the origin controversy, its devastating effects and fatalities across the world cannot be denied. HIV is transmitted via the exchange of a variety of body fluids from infected individuals, such as blood, breast milk, semen and vaginal secretions.

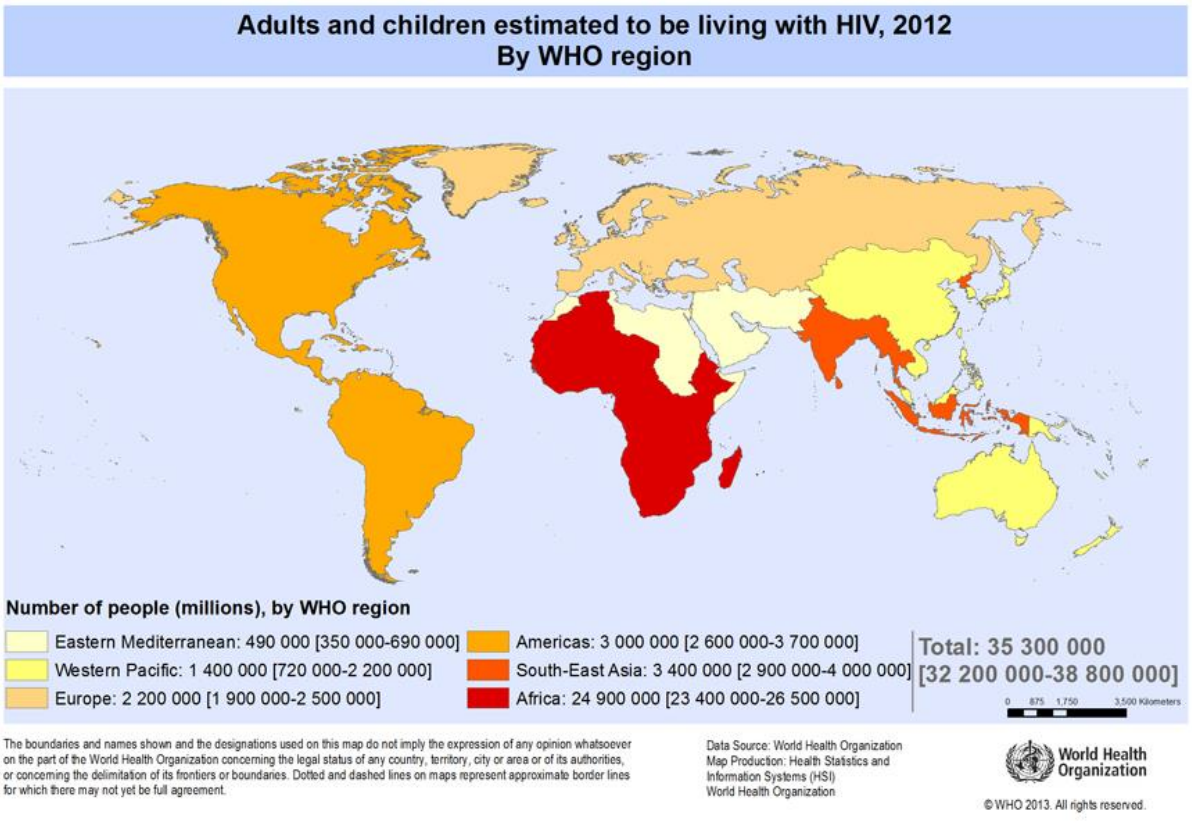


Figure 1: HIV map.[2]

From the onset of the scourge, an estimated 75 million people have been infected with the virus and 39 million of them have died, according to a 2013 WHO report, making it one of the top killer viral infections in recent history.[2] Globally, about 35.3 million people were living with the virus by the end of 2012. Estimates state that about 0.8% of adults of the world population aged between 15 – 49 years are currently infected with the virus. Although the burden of the epidemic continues to vary considerably between countries and regions, sub-Saharan Africa continues to be the most severely affected region, with nearly 1 in every 20 adults living with HIV and accounting for 71% of the people living with HIV worldwide. Furthermore, 70% of the global total cases of new HIV infections emanate from this region.[3] The map depicted (**Figure 1**) shows the spread of HIV infections across the globe, indicating the magnitude to which continents and various regions are affected. Africa is clearly the hardest hit with most of the infected people being the poor, unlikely to be able to afford treatment.

All across the world, women and children continue to be the worst affected and vulnerable groups, owing to the socio –economic, cultural and economic factors of the society that we live in. Stigmatisation of infected people also prevents such people from seeking treatment at an early stage, which increases their risk of contracting opportunistic infections and death. Current mitigation efforts are targeted at these marginalized groups in order to improve their risk profile. Awareness campaigns are also ongoing to ensure that people are conscious of, and knowledgeable about, the disease and the measures which can be taken to control and minimise the effects of HIV. Governments continue to spend millions of dollars towards these efforts; money which could under different circumstances be channelled towards other developmental goals.

1.1.2 HIV Life Cycle

HIV is a lentivirus, which belongs to the genus of retroviruses. The two main viral strains are HIV-1 and HIV-2, with HIV-1 being the most prevalent and fatal viral strain. The name 'lentivirus' literally means 'slow virus' because they take such a long time to produce any adverse effects in the body. The viruses also persist lifelong in the host's body and establish chronic infections marked by continual viral replication. These characteristics emanate from the ability of the viruses to integrate into the host chromosome, whilst evading the host's immunity.

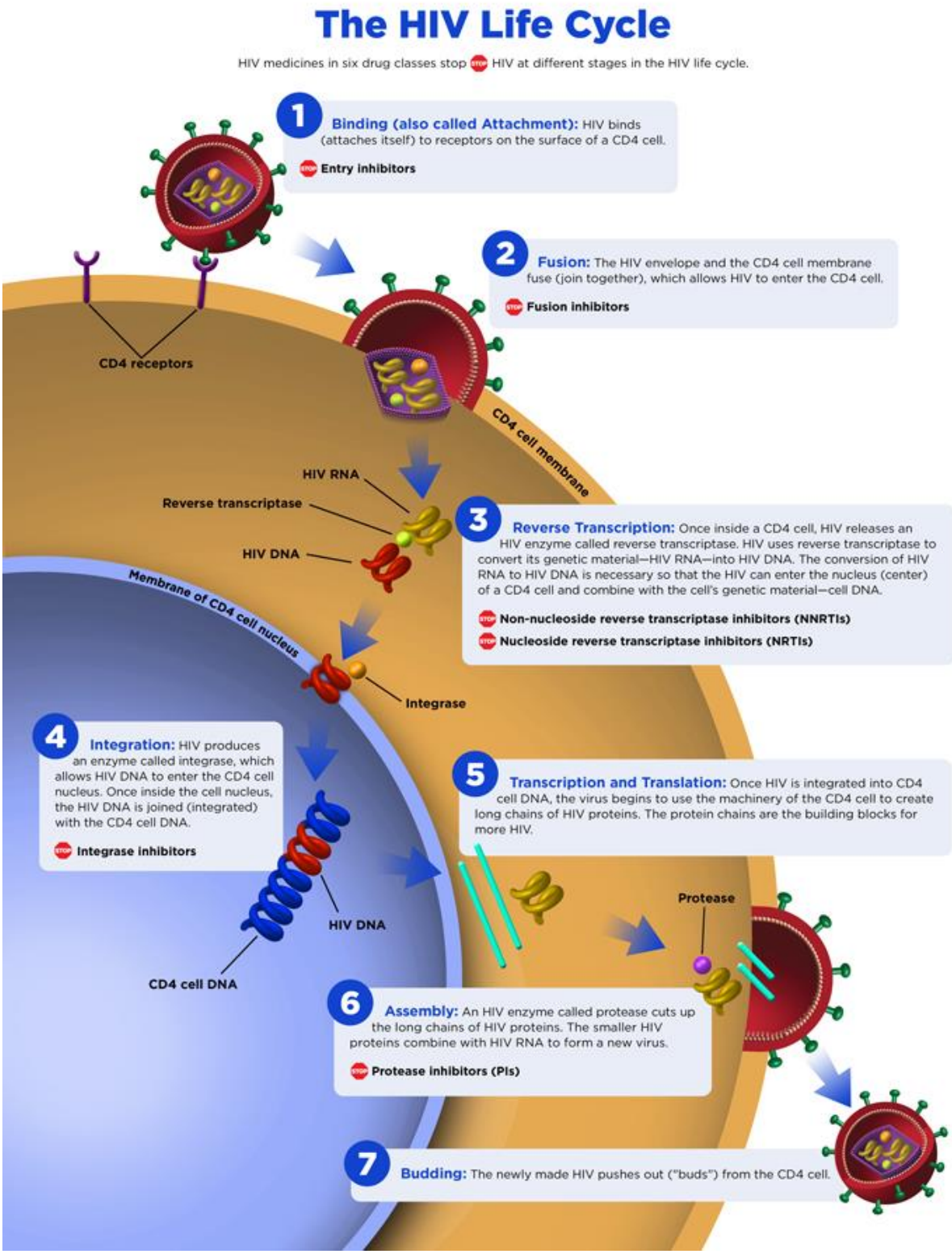


Figure 2: HIV life cycle (http://aidsinfo.nih.gov/images/factsheet/hiv-lifecycle-05.jpg)

As a result of the characteristics of the HI virus, it has a very long incubation period; to the extent that those who are infected by the virus may not be aware that they are HIV-positive until they are clinically tested.[3, 4] This makes the infected person likely to infect other people unknowingly, until the onset of opportunistic diseases triggers testing.

Upon entering the host's body, the HIV attaches itself to the CD4+T lymphocyte receptors and exploits a vast network of human proteins in order to replicate inside human cells.[4] Three constitutive enzymes, namely, reverse transcriptase, integrase and protease, play a pivotal role in this process. The viral replication process goes through several stages as the virus matures and infects new cells, whilst destroying the white blood cells which fight against bacteria and other infections.[5] This causes the infected person to be susceptible to opportunistic infections which leads to death. All the stages of the virus lifecycle are explained and described in **Figure 2**, which also indicates the validated targets for drug intervention at each stage to try and reduce the rate of replication of the virus in the body.

1.1.3 Treatment methods

Since the first confirmed case of HIV was reported in 1981, no cure has been discovered yet and the race is still on for that elusive treatment which will give hope to humankind again.[4] Despite the lack of success in finding a cure, scientists have been persistent in the search for novel drugs to control and minimize the effects of the disease. Research is currently focused on the synthesis of drugs that attack the virus at different stages of its lifecycle, so as to control or eliminate the detrimental effects the virus has on human health, as indicated in **Figure 2**.

During the mid-nineties, scientists discovered antiretroviral drugs (ARVs) that reduced the rate of viral replication in the human body, which in turn reduced the overall rate of collapse of the immune system.[6] To date, 31 ARVs have been approved by the Food and Drug Adminstration (FDA) to treat HIV infection. These treatments do not cure the disease, but rather the drugs reduce the amount of virus in the body after infection or after the onset of AIDS.[7] Initially, AIDS sufferers were not likely to live longer than a few years but now they can live longer than 20 years after being infected. Since 1996, the annual number of AIDS related deaths has steadily declined due to the significant increase in the number of people receiving antiretroviral

therapy. The disease has stopped being the death sentence it previously was. AIDS patients must, however, continue to take the ARVs lifelong in order to maintain their quality of health.

The first regimens of highly active antiretroviral therapy (HAART) were approved in early 1996 by the FDA.[6] Different ARVs are classified according to their target and these classes are: Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Protease Inhibitors (PIs), Fusion Inhibitors (FIs), Entry Inhibitors (EIs) and Integrase Inhibitors (also EIs). These drugs are used in combination from two or more drug classes. Because of the positive outcome of the antiretroviral drugs in managing the epidemic, there is now a drive to get as many infected people on the treatment programme as possible.

According to the WHO website, there is still a wide deficit between those requiring and those receiving treatment, with only 35% of people eligible for antiretroviral therapy in low and middle income countries receiving treatment.[2] Drug cost and availability are the greatest impeding factors preventing people from receiving these lifesaving medications, especially for impoverished countries where infected people rely on donor and government issued drugs. Awareness campaigns have also had a positive impact on reducing the number of new infections. People are now taking preventative measures to protect themselves against being infected or infecting other people. Studies have also shown that risky behaviour of unprotected sex with multiple sexual partners is on the decline, signalling that all efforts targeted at control of the disease are clearly having a positive effect.

There is also a new challenge, which is the development of drug resistance. This mostly occurs when patients fail to take medicines as prescribed, creating an opportunity for HIV to mutate to strains that are resistant to antiretroviral drugs. The mutated virus will then continue to replicate without suppression by the current drugs. It is estimated that 10-25% of newly infected persons harbour at least 1 resistant viral strain.[8] All these challenges provide the need for continual development of drugs to reduce cost, increase availability and combat the drug resistant strains. Although the rate of new drug approval is slow, significant progress is being made. At least several antiretroviral drugs, (darunavir[9], dolutegravir[10], raltegravir[11] and elvitagravir[12]) have been approved in the past 10 years. Raltegravir was the first integrase inhibitor to be FDA approved. Investigation into compounds that act against targets such as

protein cofactors is also underway. This continual search for new methods of treating HIV infections minimises fears of a new scourge emerging, as a result of an increase in drug resistant strains compounded by lack of potent drugs.

1.1.4 Protease inhibitors (PIs)

For this project our interest is the design and synthesis of compounds as possible PIs, thus we will discuss this class in more detail.

The proteins required by HIV for replication are prepared as a polyprotein, with several proteins strung together. In order for the virus to be infectious, this polyprotein must be timeously cleaved to give functional proteins and mature viruses capable of infecting new healthy cells.[13, 14] The protease enzyme plays this vital role of proteolytic processing of the viral polypeptide chain. This action is indispensible and facilitates the replication of the virus in the body. Due to the critical function that protease plays in the viral lifecycle, it was identified as one of the crucial targets for viral control.[15] Inhibition of protease is very important as it disrupts viral replication, producing non-infectious viral particles. The consequence of this is reduction of the viral load in the human host.

1.1.4.1 Structure of Protease enzyme

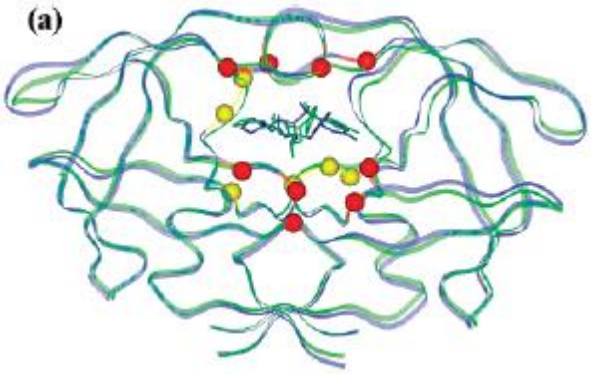


Figure 3: HIV protease enzyme complexed with amprenavir.[5]

HIV-1 protease (PR) is a small enzyme composed of two identical protein chains, each only 99 amino acids long. It functions as a homodimer with only one active site which is C2-symmetric in the free form.[16] More than 140 structures of the HIV-1 PR, its mutants and enzymes

complexed with various inhibitors have been reported so far. **Figure 3** shows one of the reported structures of the enzyme complexed with amprenavir, a protease inhibitor.

The enzyme protein chains assemble to form a long tunnel, covered by two flexible protein "flaps" with a water molecule trapped inside. The flaps open up to allow entry of the viral polypeptide chain. The enzyme then wraps around a viral polypeptide chain, closing and holding it tightly until peptide hydrolysis occurs.[17] There are hydrophobic pockets around the active site where the hydrophobic amino acid side-chains are accommodated, ensuring that the viral protein is perfectly held in the active site. The active site is at the centre of the tunnel, where the water molecule acts as a nucleophile, attacking the carbonyl carbon in the hydrolysis of the proteins. **Figure 4** is a schematic representation of how a peptide is held in the tunnel of the protease enzyme where S_1, S_1', S_2, S_2' etc., indicate hydrophobic pockets filled by amino acid side chains indicated by R_1, R_1', R_2, R_2' etc. Studies have shown that the protease chain is stretched straight through the active site and two aspartic acid residues facilitate cleavage of the peptide bond.

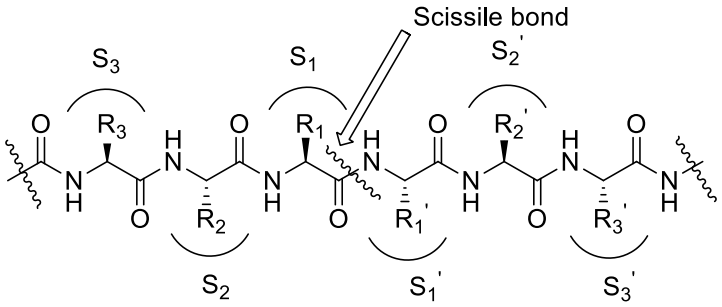


Figure 4: Cleavage site of the viral polyprotein.

Designing of inhibitors to act against HIV protease is now an achievable task because the structure of the protease enzyme has been elucidated and the mechanism of how the viral protein chain binds has been established.[18] From the mechanism (**Figure 5**), the important functionalities were established and compounds mimicking the transition state of peptide cleavage have been synthesised to effectively compete with the natural substrate for this active site. These compounds fit into the active site but will not be hydrolysed because they have a non-scissile bond instead of the amidic group of the natural substrate.

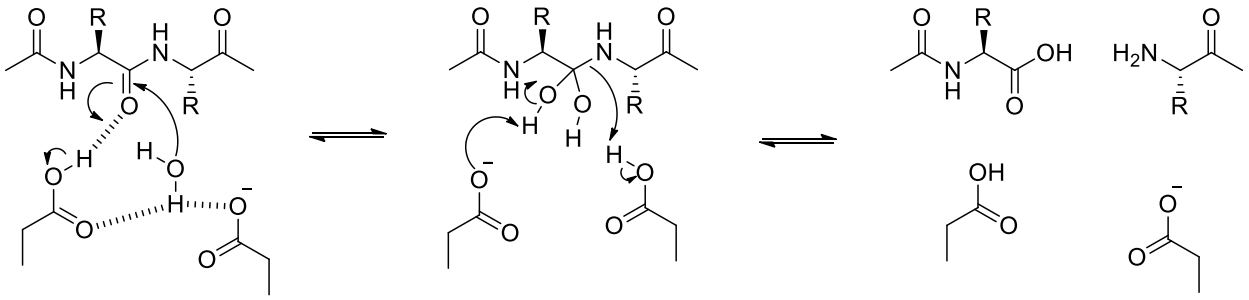


Figure 5: Protease enzyme mechanism.

1.1.4.2 Protease Inhibitor drugs

Due to the vital role of HIV protease, many protease inhibitors have been synthesized to fight HIV as they help to prolong the life of those infected with the virus. To date 10 PIs have been approved by the FDA and these have been shown to reduce the viral load in infected patients.[2] Some of the PI drugs in current use are shown in **Figure 6**.

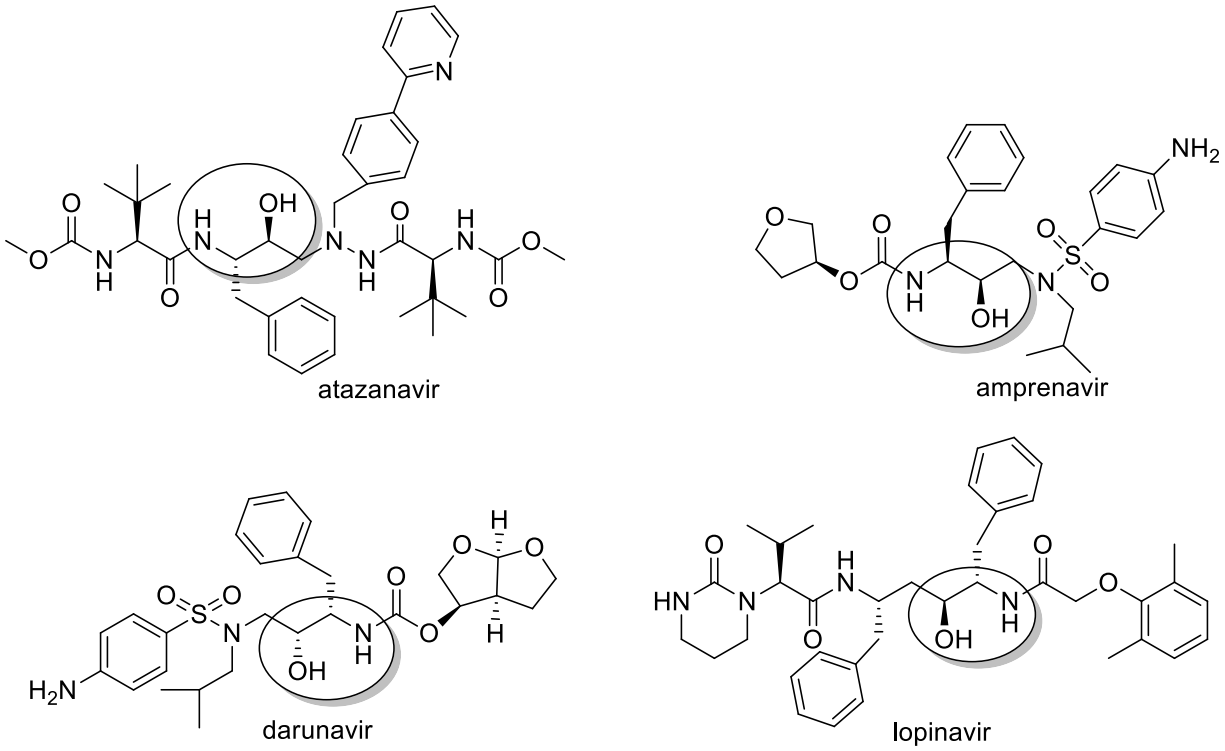


Figure 6: FDA approved PIs.

The inhibitors have at least four hydrophobic groups capable of filling the enzyme's hydrophobic pockets and also have a 1,2-secondary alcohol amino functionality that acts as a transition-state mimic (circled in **Figure 6**). Research has shown that synthesized compounds

with fewer groups show little or no activity against protease because of lack of binding in the active site. The PIs also have two oxygen atoms on either side of the non-scissile bond which interacts with a special water molecule that is trapped under the flaps of the HIV protease. The drugs possess groups capable of participating in hydrogen bonding interactions as well, so as to improve their binding affinity.

PI design is also influenced by the fact that an inhibitor that binds in the enzyme active site via multiple interactions, especially hydrogen bonding with the HIV protease backbone atoms, generally retains these affinities with mutant strains. Ghosh *et al*. showed that minimal conformational changes occur to the backbone structure of mutant enzymes, and hence it can be concluded that similar inhibitor-enzyme interactions should remain, maintaining the inhibitor affinity and potency.[8] Inhibitor-bound HIV protease structures have revealed tight hydrogen bonding between the inhibitor and the protease backbone, hence any new potential inhibitors designed should contain groups capable of forming hydrogen bonds with the enzyme backbone.

Although numerous studies have led to the discovery and development of a number of HIV-1 protease inhibitors, a “perfect” protease drug seems elusive, because therapeutic benefit is short-lived and treatment success is usually limited due to the evolution of drug-resistant variants.[19] Resistance to protease inhibitors is the consequence of amino acid substitutions that emerge either inside the substrate-binding pocket or at distant sites.[20] This search for new PIs provided the basis for this work: synthesis of peptidomimetic compounds as potential HIV protease drugs.

1.2 Malaria

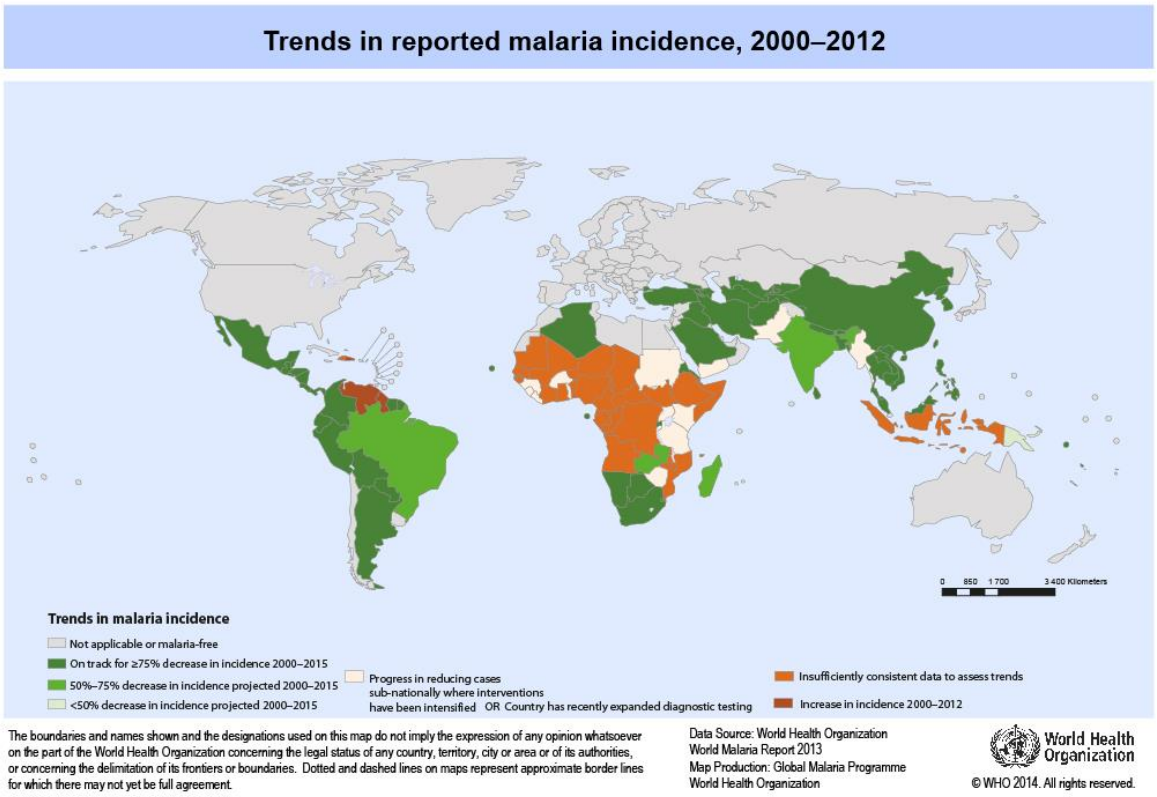


Figure 7: Trends in malaria infections (Source: WHO world malaria report 2013).

Malaria is the most deadly tropical parasitic disease, endemic to about 104 countries, most of which are located within the tropics (**Figure 7**). In 2012 alone, malaria accounted for about 627 000 deaths with 207 million reported cases. Africa accounted for 80% of reported cases and 90% of deaths, the majority (77%) of whom were children below the age of 5.[21]

This tropical disease is caused by five parasitic species that belong to the genus *Plasmodium,* namely *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi.* Of these, *P. falciparum* causes the most deadly form of malaria and it is common in Africa. *P. vivax* is the second most widespread malaria-causing parasite and it is prevalent in countries outside Africa, but the malaria it causes is less severe. This disease is transferred from one person to another by the female *Anopheles* mosquito. This provides a complex life cycle for the disease as it has two hosts, but it also provides a wide scope for drug targets and control.

1.2.1 Current treatment and control methods

Malaria is a preventable and treatable disease provided medication is taken timeously and mosquito breeding grounds are destroyed. Quinine (**Figure 8**) was the first antimalarial drug to be used, but it was largely replaced by its derivative chloroquine due to side effects. Despite this, quinine is still in use in some countries today.[21] Over the years, drug resistance prompted more research into antimalarial drug discovery as more and more people were dying from the disease. As a preventative measure against the disease, anti-malarial drugs such as sulfadoxine-pyrimethamine and amodiaquine were and are offered to the highest risk groups, namely children and pregnant women. Resistance of *Plasmodium falciparum*, the most lethal form of malaria, to these first line drugs, was the biggest culprit in the increasing mortality rate. To combat this problem, artemisinin based therapies used in combination with other drugs, known as artemisinin-based combination therapy (ACT), were introduced to treat severe cases of malaria.[21] Use of bed nets and other social aspects have also been, and are continuously being, promoted.

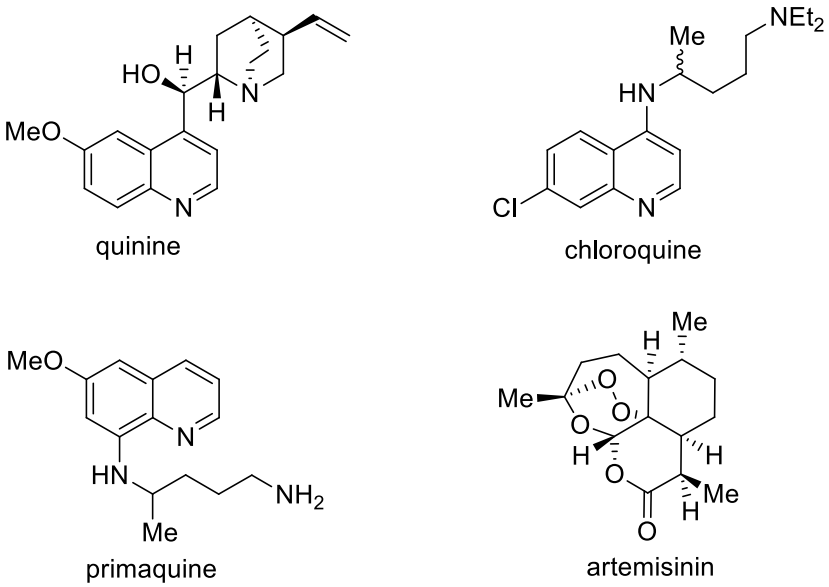


Figure 8: Examples of antimalarial drugs.

The mode of action of quinine, chloroquine and other derivatives is not clearly understood due to the complexity of the process. However, these drugs are believed to act primarily by binding to haem released following the digestion of haemoglobin in the host red blood cells by the parasite.[22] This process prevents detoxification of haem which is normally detoxified by

conversion into insoluble haemozoin (malaria pigment). The binding of the drugs prevents formation of haemozoin and the free drug-haem complex is believed to exert a toxic effect on the parasite. The quinine based drugs are weak bases and also exert their anti-malarial effect by accumulating within the acidic digestive vacuole of the parasite.[23] Consequently the parasite either starves, builds up toxic levels of partially degraded haemoglobin, or both, resulting in parasitic death.

Artemisinin has a totally different mechanism of action from the quinine-based drugs and has therefore been effective against chloroquine-resistant strains of malaria. Its mode of action lies in the endoperoxide group acting as a molecular trigger for the disruption of redox homeostasis within the parasitic cell, eventually resulting in death of the parasite.[24] The difference in the mode of action means that artemisinin has had success in treating chloroquine and quinine resistant malaria. Despite these successes, drug resistance also emerged with artemisinin and continues to be a major problem, which requires continual action through novel drug development.[21] There is a need for the development of new drugs with novel targets so as to circumvent problems associated with drug resistance.

1.2.2 *Plasmodium falciparum* proteases as drug targets

Plasmodium falciparum, the major causative agent of malaria, is found in Africa and encodes over 10 aspartic proteases responsible for the life cycle of the parasite in the human body.[23] These enzymes are known as plasmepsins and appear to be involved in the initial steps of the degradation of haemoglobin. This is one of the critical stages of the life cycle of the parasite during human infection which provides nutrients for parasitic growth and maturation.[25] Because of the importance of the degradation of haemoglobin stage in the parasitic life cycle, plasmepsins make for an attractive antimalarial drug target.[26] Research has identified Plasmepsin I, II, III and IV as attractive drug targets for the treatment of malaria.

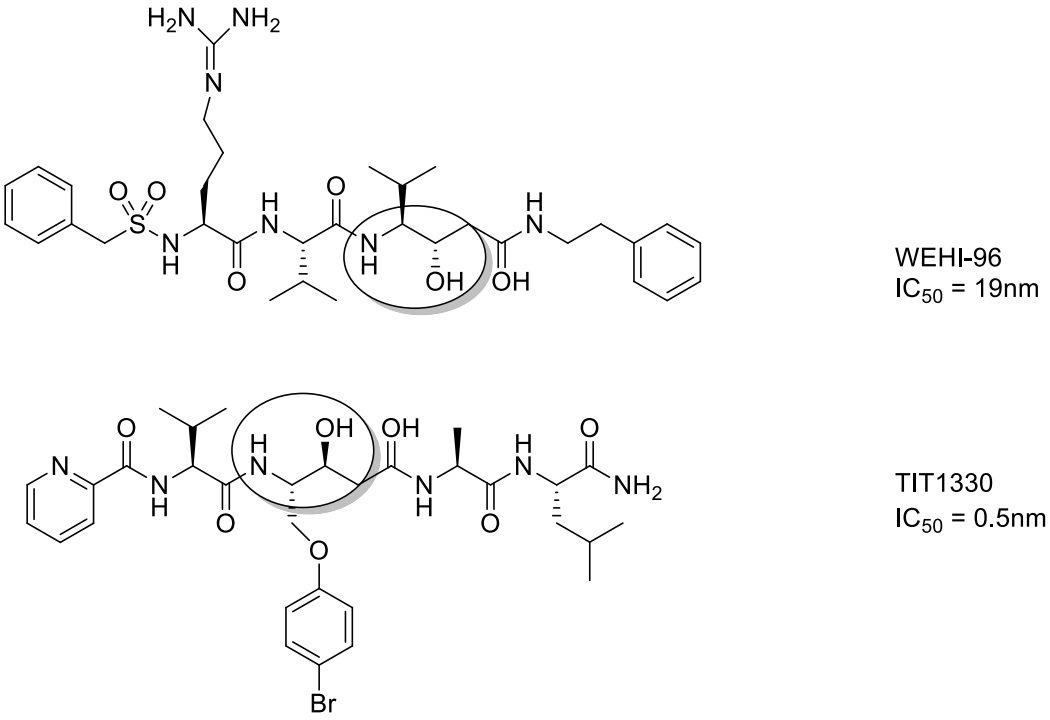


Figure 9: Potential Plasmepsin inhibitors.

Plasmepsin II and IV are two of the better targets as they are responsible for clipping long protein chains to release active protein in the red blood cells, much like HIV protease does. Research has shown that peptidomimetic compounds can be used as inhibitors against Plasmepsin II and IV, which also blocks the degradation of haemoglobin in cell-culture, which kills the parasite by starvation.[27] **Figure 9** shows peptidomimetic compounds which have shown good activity against these enzymes whilst exhibiting low toxicity.[28, 29] These results support plasmepsins as a new promising target for the synthesis of potential new antimalarial drugs.

1.3 Peptidomimetic compounds

Over the last three decades scientists discovered biologically active peptides and characterized them as hormones, vasoactive peptides, enzymes and neuropeptides etc. Their interaction with membrane-bound receptors, influence on cell-cell communication and control of a series of vital functions became of great interest in the biomedical field. This interest prompted investigations into synthesis of peptide mimics which are now known as peptidomimetic compounds. These compounds are described as compounds whose essential elements (pharmacophore) mimic a natural peptide or protein in 3D space and which retain the ability to

interact with the biological target and produce the same biological effect.[30] Their molecular structures are adjusted to enhance stability, biological activity and pharmacokinetic properties.

Historically, peptides did not have much success as drugs because of their unfavourable delivery methods and short plasma half-life. However development of peptidomimetics has provided drugs which are orally available and have a longer half-life which increases efficacy. Much success of oral availability has been seen with HIV antiretroviral drugs. New delivery technologies, such as the use of nano particles, have also stimulated research into finding new peptidomimetics as potential drugs.[30]

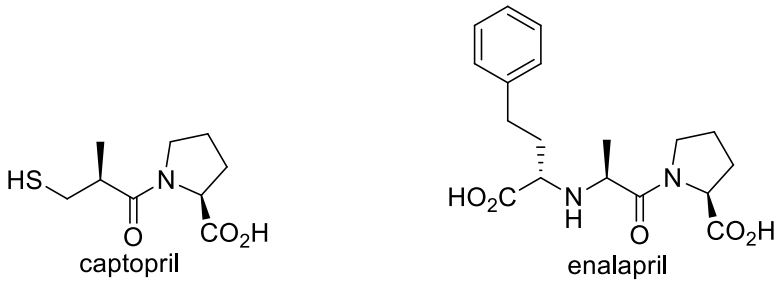


Figure 10: Structures of captopril and enalapril.

These compounds are providing a powerful tool for the development of inhibitors created to mimic the endogenous peptide, which helps in treating various physiological disorders.[31] Current areas of application include endocrinology, urology, obstetrics, oncology and as functional excipients. Captopril and enalapril (**Figure 10)** are examples of peptidomimetic compounds which have been successfully synthesised and are being used for treatment of high blood pressure as angiotensin-converting enzyme (ACE) inhibitors. ACE is a naturally occurring metalloprotease which is responsible for cleaving decapeptide angiotensin to octapeptide angiotensin II by utilising a Zn^{2+} ion trapped in the enzyme.[32] The cleaved angiotensin II has strong hypertensive properties inducing vasoconstriction and augmenting the levels of aldosterone, which in turn promotes the retention of water and sodium ion, consequently increasing blood pressure. Inhibition of this enzyme is paramount in people suffering with high blood pressure, hence the development of captopril and enalapril. Success of these drugs lies in their higher affinity for the enzyme and increased coordination to the Zn^{2+}, which in turn

suppresses the function of ACE. Another advantage of these drugs is that, they are orally bioavailable, have a longer half-life and produce prolonged effects.

Since our interest lies in the inhibition of protease enzymes, we focused our attention on creating a library of peptidomimetic compounds as potential antimalarial and anti HIV drugs. Previous work in our laboratory has shown that such compounds have potential activity against protease enzymes found in both the HIV and malaria parasite. Structural similarities are also present in both FDA approved PI drugs and potential antimalarial agents, as they possess a 1,2-secondary alcohol amino moiety circled in the structures shown in **Figure 6** and **Figure 9**.

The design and development of peptidomimetics initially involves a structure-activity relationship investigation of the natural peptide in order to ascertain and identify major pharmacophore elements that are responsible for the biological effect. In this way, the natural peptide complexity is reduced, making it possible for the active pharmacophore to be synthesised and optimised.[31] In order to find a hit compound, libraries of analogues are then synthesised and screened against the target enzymes. In this process, peptide bonds are replaced with "look alikes" called isosteres which are less likely to be hydrolysed. Rigidification via cyclisation is also utilised to minimise conformational flexibility as this is an important factor to maximise drug-receptor interactions. For our research, the target pharmacophore has already been optimised and what is required is to synthesise a library of analogues in search of hit compounds.[33]

The preparation of peptidomimetics has generally been approached by employing both naturally occurring amino acids and synthetic amino acids (that possess several features which are not present in the naturally occurring amino acids) and combining them in a linear synthesis to produce the desired product. Modification of commercially available enantiopure reagents, such as sugar derivatives, can also be used to make other necessary starting materials for the preparation of the desired isosteres. Synthetic routes used are normally lengthy and complex, and hence there is a need for the development of shorter synthetic routes.

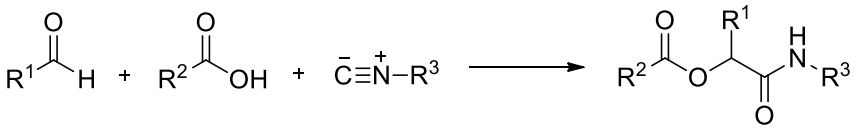
A convenient approach to the synthesis of peptidomimetics is through the use of multi-component coupling reactions (MCRs) in combination with linear synthesis. While each method

has its own set of advantages, MCRs are advantageous in that complex structures are prepared in a single highly atom efficient step.[34] In the majority of commercially available drugs and some of the inhibitors that are currently being tested clinically against HIV protease, a nonhydrolysable hydroxyethylene or hydroxyethylamine moiety is used as the basic core for the development of these inhibitors, a core which is readily achievable by employing the MCR approach.

1.3.1 Multi-component Coupling Reactions (MCRs)

MCRs are reactions where three or more starting materials react with each other in one-pot to produce products such that the majority of the atoms of the reactants are found in the product.[35] They are a versatile means of obtaining complex molecules in one step and have been employed in various applications including the synthesis of polymers and dendrimers.[36, 37] These reactions are often high yielding, simple procedures, which exhibit superior atom economy and are time-saving.[38] A number of such reactions have been developed over the past century and modifications of some of the classical ones have also occurred. The most widely used reactions include, but are not limited to, Passerini 3-component reaction (3CR), Ugi 4-component reaction and Biginelli 3-component reaction.

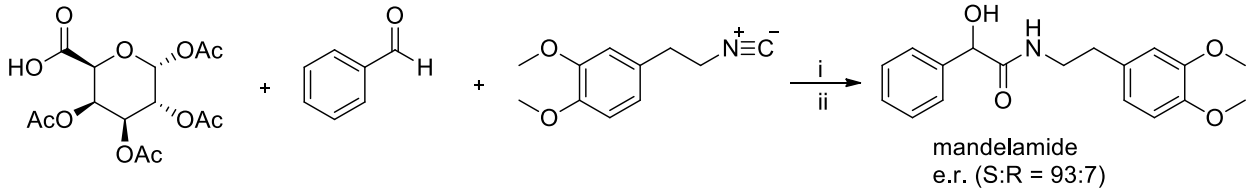
1.3.1.1 Passerini reaction



Scheme 1: Passerini reaction.

The Passerini reaction was discovered in 1923 by Passerini who was a Professor of chemistry in Italy.[38] The Passerini reaction couples an isocyanide, carboxylic acid and aldehyde in a single step in a one pot reaction (**Scheme 1**).[39, 40] This reaction produces a product with one amide and one ester functionality, making it less useful for the synthesis of peptidomimetic compounds. Since not much product diversity was offered by this reaction, it was not popular until recently when researchers started employing amide containing starting materials in the Passerini reaction which offered the diversity they were looking for.

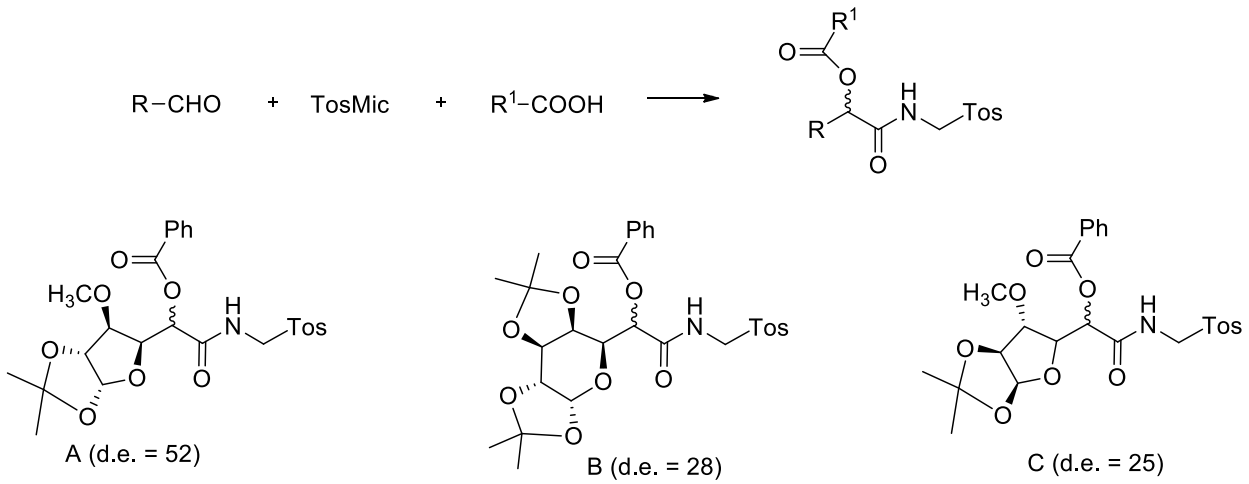
As a result of this advancement, the Passerini reaction continues to be used in organic synthesis. Of significant benefit in employing the Passerini reaction is the high atom economy and the fact that the reaction gives very few or no side products. The reaction is also generally high yielding; with yields ranging between 70% and 100% in most cases.[41, 42] All these advantages make it an attractive tool in drug synthesis. The major drawback of the Passerini reaction is the lack of stereocontrol over the formation of the new stereogenic centre. When achiral starting materials are used, a racemic mixture of products is produced and in general enantiomers are very difficult to separate. This is not ideal, especially when target compounds are potential enzyme inhibitors or drug candidates. Enantiomers have been shown to have different biological effects on different biological targets, hence the need to produce enantiomerically pure compounds.



Scheme 2: Reagents and conditions: i) MeCN, r.t., 16 h, air ii) NaOH, dioxane, r.t., 1 h.

Since the Passerini reaction had already been established as a useful reaction in synthesising complex molecules, researchers embarked on finding ways of making the reaction either enantioselective or diastereoselective. Frey *et al.* developed an asymmetric synthetic route for the synthesis of mandelamides, compounds which have applications in medicine and agriculture.[43] The researchers used 1,2,3,4-tetra-*O*-acetyl-α-D-galacturonic acid as an asymmetric inducing agent to control the stereochemical outcome of the new stereogenic centre (**Scheme 2**) in the Passerini reaction. The acid component was then removed by saponification of the ester bond formed to yield the desired mandelamide with an e.r. of 93:7. The resulting e.r. showed that the Passerini reaction exhibited a high diastereoselectivity, which was the result the research team was after. Configuration assignment of the enantiomers was done by comparison with commercially available D-(-) and L-(+) mandelic acids after mandelamide hydrolysis. However, separation of the enantiomers was not achieved in this study.

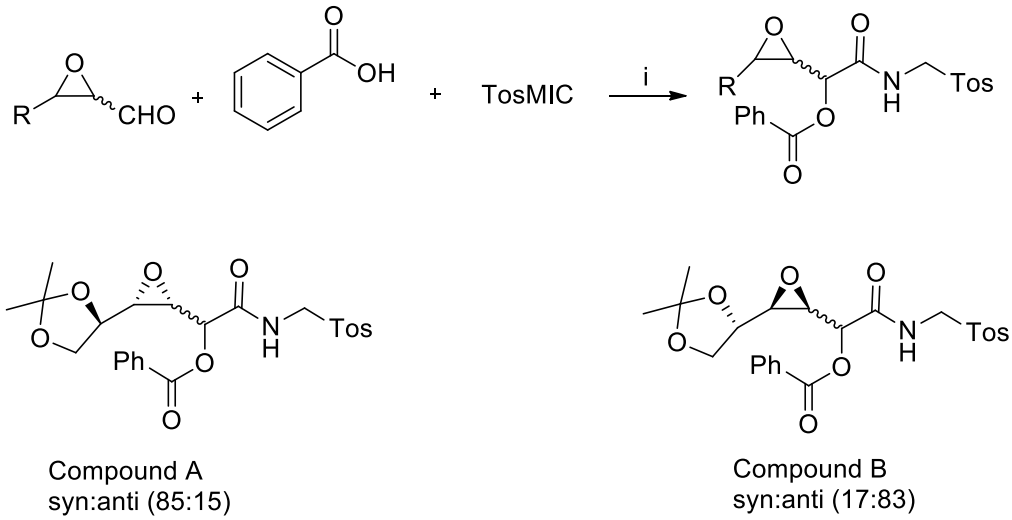
Due to the potential of the Passerini reaction to produce potentially biologically active compounds with ease, a few other researchers have also investigated ways of making the reaction stereoselective.[44, 45]



Scheme 3: Reagents and conditions CH_2Cl_2, r.t., 24-48 h.

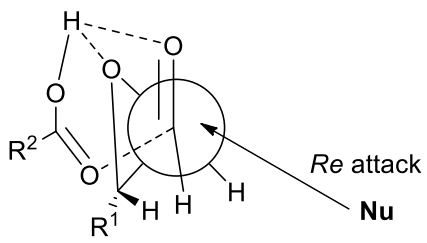
Krishna *et al.* were one of a few groups of researchers to investigate the selectivity of the Passerini MCR.[45] They employed *p*-toluenesulfonylmethyl isocyanide (TosMIC) in the reaction with a variety of chiral aldehydes derived from sugar molecules (**Scheme 3**). A few of the examples of the products and their d.e. are shown in **Scheme 3**. Since the aldehyde already had a fixed stereogenic centre, the reaction would be diastereoselective instead of being enantioselective. For all the combinations tested the de of products ranged from 28% - 90% showing that moderate selectivities could be achieved by varying the reactants. However, it is important to note that the diastereomers produced using this approach were not separable by normal chromatography. They assigned the configurations of the diastereomers based on coupling constants of diastereomeric signals in the ^{1}H NMR spectra.

The success of using TosMIC and chiral aldehydes led the same group of researchers to investigate the selectivity of the Passerini reaction using more sterically hindered aldehydes.[46] They employed chiral epoxy aldehydes to create a library of compounds, with the best results shown in **Scheme 4**.

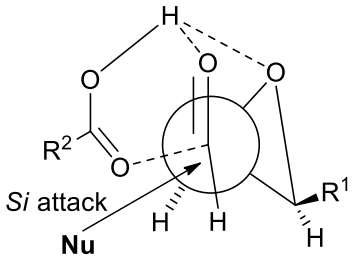


Scheme 4: Reagents and conditions: i) CH_2Cl_2, r.t., 48 h.

From their results, they concluded that steric hindrance of the aldehyde in the Passerini reaction could be used for asymmetric induction as it had, on average, considerably increased the d.r. of the products. Determination of d.r. was made using the ^{1}H NMR spectra by measuring coupling constants of protons adjacent to the epoxide ring, hence the proton at the newly formed stereogenic centre was either *syn* or *anti* to the epoxide ring. The stereochemical result was explained by using the transition state model shown in **Scheme 5**. They proposed that the isocyanide reacts with a loosely bound adduct formed between the carboxylic acid and the aldehyde. The adduct formed results from the protonation of the carbonyl oxygen of the epoxy aldehyde by the carboxylic acid. This provides a stereofacial preference with the isocyanide attacking the carbonyl carbon from the less hindered side, i.e., from the *Re* face to afford a *syn* major product for the *trans*-epoxy aldehyde, and from the *Si* face to afford a major *anti*-product for the *cis*-epoxy aldehyde. The separation of the diastereomers formed was not achieved by column chromatography.



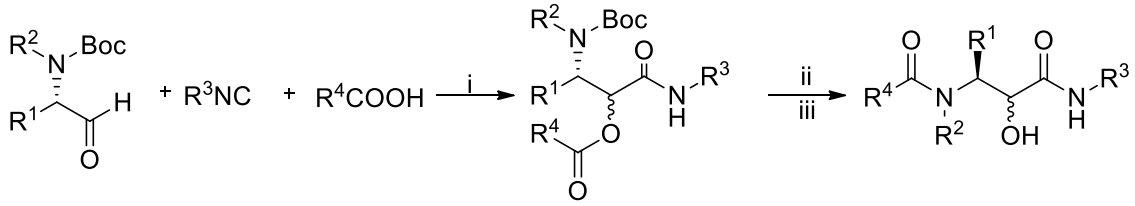
trans-epoxy aldehyde

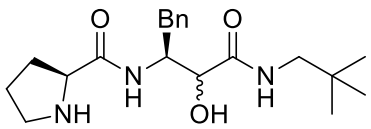


cis-epoxy aldehyde

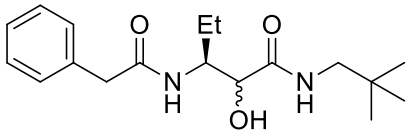
Scheme 5: R^1- alkyl substituent, R^2- Ph.

As the research into improving the selectivity of the MCR continues, there is growing interest in the use of α-amino acid derived aldehydes, carboxylic acids and/or isocyanides in the Passerini reaction.[47] This is done to both investigate selectivity due to the presence of a resolved stereogenic centre and to introduce diversity in the backbone structure of the products.

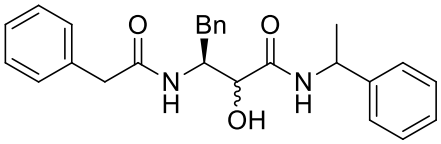




d.r. = 71:29



d.r. = 69:31



d.r. = 67:33

Scheme 6: Reagents and conditions: i) CH_2Cl_2 ii) TFA iii) Et_3N.

Banfi *et al.* investigated the diastereoselectivity of the Passerini reaction by using a variety of amino acids in the preparation of aldehydes which were then employed in the reaction.[41] After the Passerini reaction, the amino group was deprotected and the acyl group migrated to the nitrogen in a reaction sequence known as the "Passerini amine deprotection acyl migration" (PADAM) approach, and it was used to synthesise oligopeptides which were of interest as enzyme inhibitors. Irrespective of the aldehyde used, the d.r. observed was approximately 2:1 for all combinations tested, as shown in **Scheme 6**. However, the identity of the major and

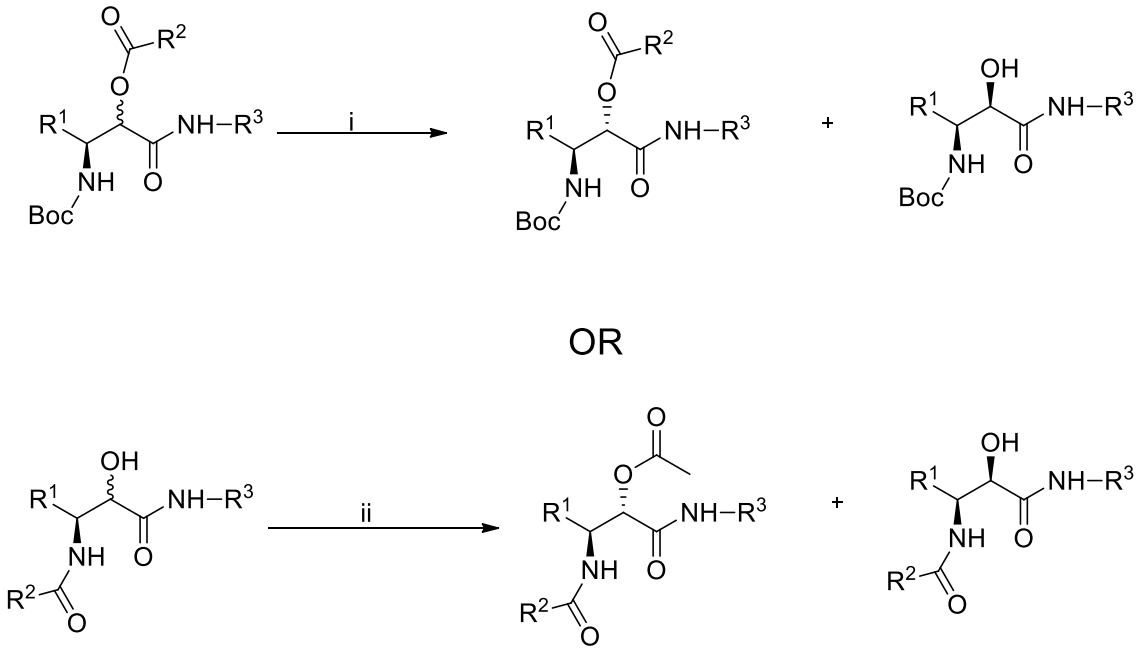
minor diastereomer was not established in this study. When enantiomerically pure carboxylic acids were employed, only two diastereomers were detected by NMR spectroscopy indicating that the starting α-amino acids did not undergo significant racemisation under the reaction conditions. The products of the Passerini reaction can undergo further modification allowing for additional variation of the structure to achieve targeted compounds. It is noteworthy that in all these investigations no one was able to isolate and confirm the absolute configuration of major and minor diastereomers.

1.4 Enzymatic kinetic resolution (Bio-catalysis)

The Passerini reaction using a chiral aldehyde produces diastereomers and therefore it is important to be able to separate the two chiral compounds, as they can have different or opposite biological activities. Separation of the diastereomers by normal column chromatography has not proved to be possible hence other ways of achieving this have to be sought.[41, 42] Biocatalysis is a potential route for the separation of diastereomers. Many different types of enzymes have been used to kinetically resolve potentially bioactive compounds.[48, 49] Among the biocatalysts in organic synthesis, lipases are the most frequently used because this class of enzyme is able to perform both enantioselective hydrolytic reactions as well as the formation of a wide range of ester and amide bonds.[50, 51] Lipase enzymes can be used for both transformations depending on the reaction medium and the presence of an acetate donor. They are most frequently employed in the enantioselective hydrolytic reactions,[52] however esterification is also a selective method which can be used successfully.[53] Lipases are uniquely characterized by their ability to catalyse the hydrolysis of ester bonds at the interface between an organic phase containing the substrate and an aqueous phase in which the enzyme is dissolved. The other advantage of using lipases for the hydrolysis or esterification reaction is that they are very selective and specific with respect to structure and stereochemistry of the substrate and product.

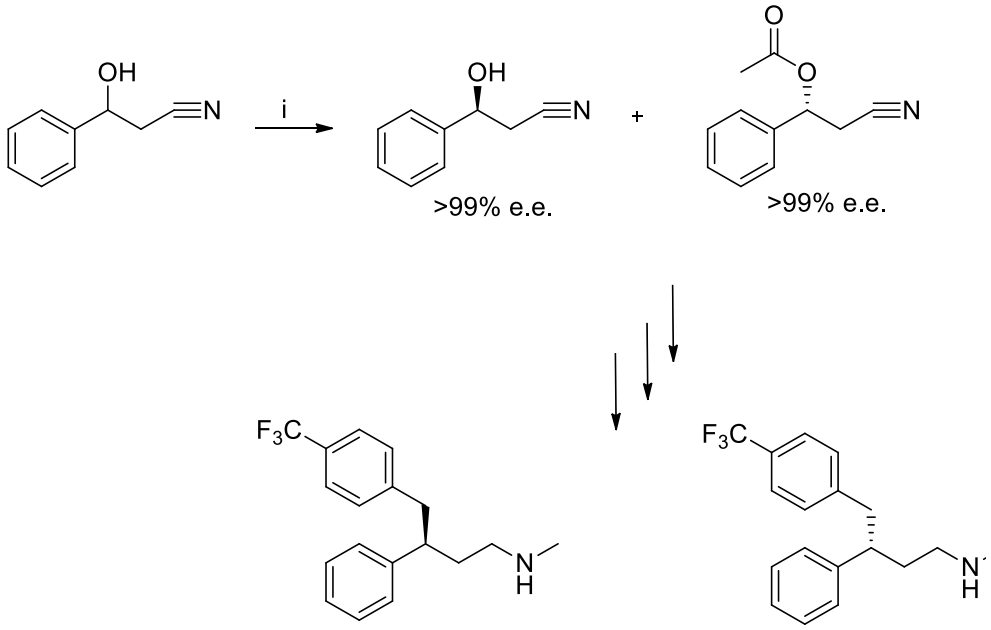
In our research, we anticipated that we could either test the use of lipase enzymes in the selective hydrolysis of the ester functional group in one of the Passerini products, or alternatively, test the selective esterification of the free hydroxyl group of one of the

deprotected and rearranged products (**Scheme 7**). The products formed would be significantly different chemically and separation by either column chromatography or crystallization would thus be possible.



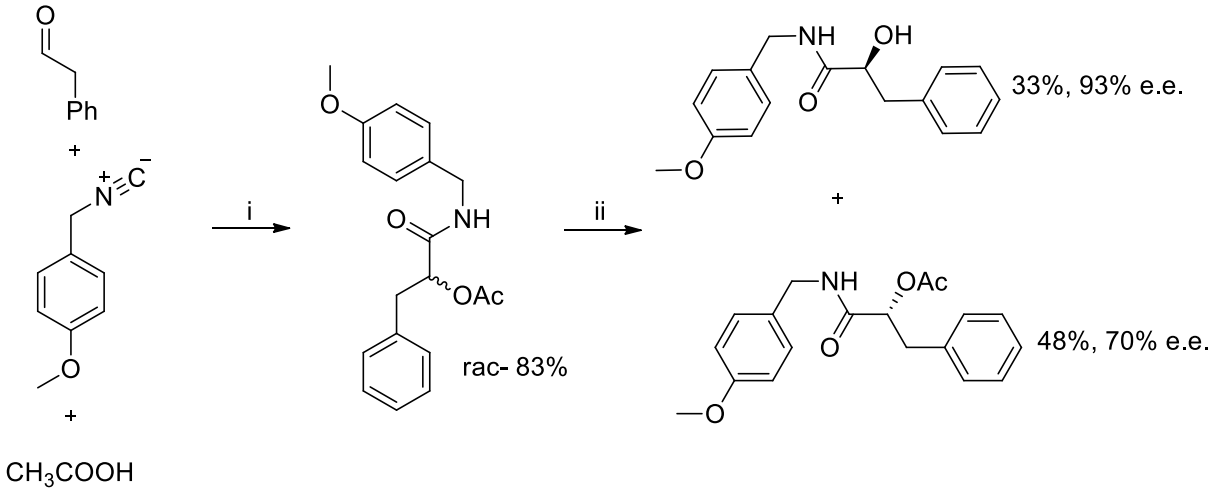
Scheme 7: Reagents and conditions: i) Lipase enzyme, buffer, solvent ii) Lipase enzyme, vinyl acetate, solvent.

A wide variety of organic molecules have been biosynthesized due to the catalytic activities of enzymes. Kamal *et al.* also successfully used lipase enzymes for a critical step in the synthesis of both enantiomers of fluoxetine, the world leading anti-depressant drug (**Scheme 8**).[48] The drug had previously been offered as a racemate, but research had shown that the *R*-enantiomer was more potent, hence the desire to produce enantiomerically pure product.



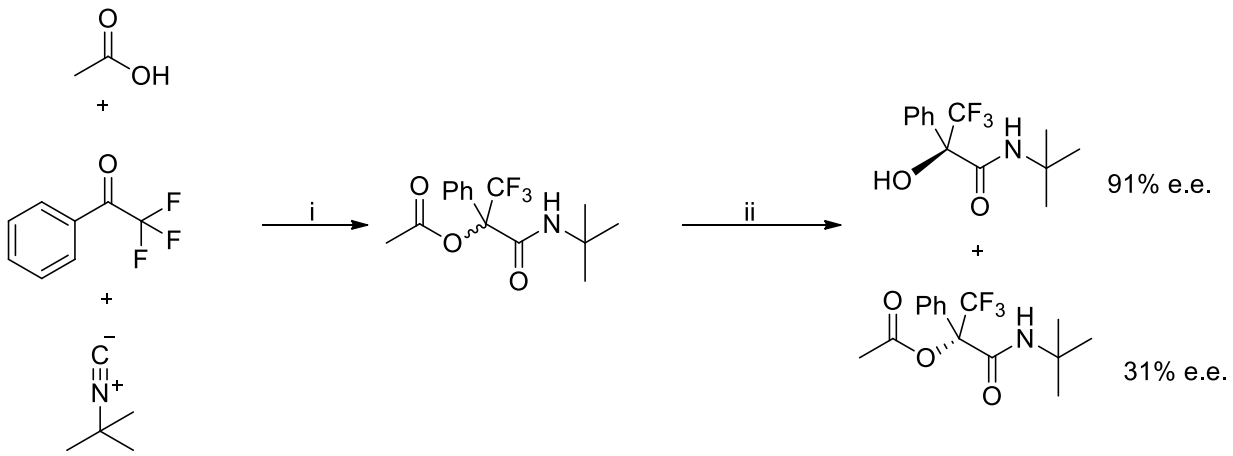
Scheme 8: Reagents and conditions: i) Vinyl acetate, *Pseudomonas cepacia* lipase, Et_2O, 40°C.

MCRs have also already been combined efficiently with biocatalytic methods in, for example, the synthesis of enantiopure precursors or for the enantioselective enzymatic hydrolysis of the products (Passerini reactions).[54] Szymanski *et al*. successfully utilised lipase enzymes to resolve a racemic mixture of Passerini products (**Scheme 9**).[55] This provided enantiopure building blocks for further reactions.



Scheme 9: Reagents and conditions: i) CH_2Cl_2, 20°C, 48 h ii) Lipase from *Wheat Germ*, phosphate buffer 7.0/Et_2O (8:2), 20°C, 48 h.

Although the enantiomeric excess was not as high as expected, the approach offered a new and alternative way to synthesise peptidomimetic compounds which was short, and utilised reagents which were non-toxic, cheap and recyclable, all of which are desirable properties in synthesis. This approach to synthesis was also successfully employed in similar reactions by Kourist *et al.* (**Scheme 10**) with different substrates.[51]

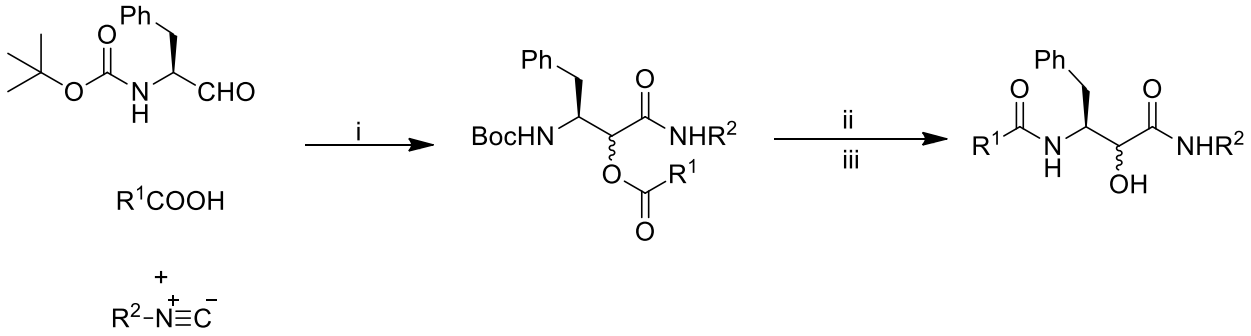


Scheme 10: Reagents and Conditions: i) CH_2Cl_2 ii) Esterase 56, buffer.

The success of combining MCRs with biocatalysis has been reported by other researchers as well, although progress in this field is relatively slow.[56, 57, 58]

1.5 Planned synthetic approach

Previous work done by Gravestock and co-workers involved the use of the PADAM approach for the synthesis of peptidomimetic compounds with the potential to act against HIV-1 protease (**Scheme 11**).[42] In this approach, the Passerini product rearranges via *O*,*N*-migration after *N*-deprotection, to give an *N*-acyl compound with a free hydroxyl group.[42, 47] The free hydroxyl group is a common feature in all FDA-approved HIV protease inhibitors (shown in **Figure 6**). The hydroxyl group is of great importance because PIs have been designed as transition state mimics, where the inhibitors show structural similarity to the tetrahedral intermediate formed during peptide hydrolysis.[59]



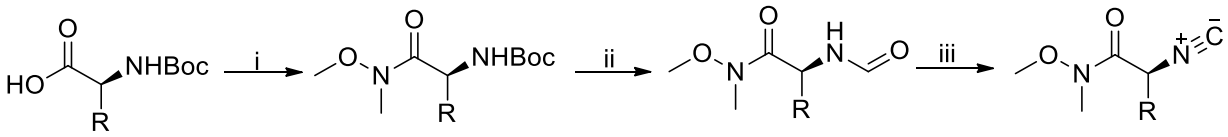
Scheme 11: Reagents and conditions: i) CH_2Cl_2, r.t, ii) TFA 10 equiv iii) Et_3N 10 equiv.

N-Boc protected phenylalanine was used as the aldehyde component of the Passerini reaction because this affords a product bearing a substituent which closely resembles the cleavage site of the natural substrate.[15] A phenylalanine residue of the viral protein has been shown to bind in the hydrophobic pocket of the HIV protease at most of the cleavage sites. In this project we will extend this previous work, using the PADAM approach to prepare a library of compounds for testing against HIV-1 and malaria. Because the aldehyde already has a defined stereogenic center, the product of the Passerini reaction will be a mixture of diastereomers, provided that the stereochemical integrity of the aldehyde is maintained. Fortunately, previous work conducted on the synthesis of the aldehyde has shown that it does not racemise and the integrity of the stereogenic centre is maintained during the PADAM sequence.[42, 47]

N-Boc phenylalaninal can easily be prepared via the Weinreb amide (without racemisation) from the commercially available *N*-Boc-*L*-phenylalanine as reported by Fehrentz *et al*.[60] Use of a chiral aldehyde component will also enable an investigation of the diastereoselectivity of the Passerini reaction. Chiral carboxylic acids and isocyanides will also be introduced to further ascertain the extent to which these components influence the selectivity of the reaction.

Readily available isocyanides will be used initially and then chiral isocyanides (synthesised from amino acids and glucose) will be employed to increase the functionality of the target compounds and add an additional chiral component to the Passerini reaction. Amino acids can be converted into isocyanides (**Scheme 12**) by conversion of the amino group into a formamide, followed by dehydration to the isocyanide. Our plan is to convert the carboxylic acid portion of

the amino acids into the Weinreb amide, which would allow further modification after the PADAM sequence to yield highly functionalised peptidomimetic compounds.



Scheme 12: Reagents and conditions: i) CDI, N,O dimethylhyroxylamine.hydrochloride, CH_2Cl_2 ii) HCOOH, Ac_2O, TFA iii) $POCl_3$, Et_3N, CH_2Cl_2.

1.6 Aims of the project

Since peptidomimetic compounds have been shown to be of medicinal importance as potential drug candidates against HIV-protease and the malaria parasite, this project aims at synthesising a library of such compounds using the PADAM approach. Because the Passerini reaction results in the formation of a new stereogenic centre, it is also of interest to establish whether the diastereoselectivity of the reaction can be controlled by the reagents utilised and if so, to what extent. Once the compounds have been synthesised, we will assess the feasibility of employing biocatalysis to resolve the resulting diastereomers before the compounds are tested for biological activity.

CHAPTER 2: RESULTS AND DISCUSSION

The overall aim of the project was to synthesise a library of peptidomimetic compounds as potential enzyme inhibitors. Thus it was important to include different functionalities that would probe the different possible ligand-enzyme interactions. Different groups may be instrumental in increasing binding interactions between enzyme and substrate via hydrogen bonding, dipole-dipole interactions, ionic interactions and through hydrophobic interactions. Protease inhibitors have been shown to require both hydrophobic groups which fit into various enzyme pockets around the active site and also polar groups which are capable of hydrogen bonding.[18] It is also important to vary the sizes of these groups so as to find the best fit.

With these factors in mind, a total of 7 commercially available carboxylic acids (**1**-**7**, **Figure 11** and 6 isocyanides (**8**-**13**, **Figure 12**) were used in different combinations with (*R*)- or (*S*)-*N*-Boc-phenylalaninal to create a compound library using the Passerini reaction. The diastereoselectivity of the Passerini reaction was also investigated. *N*-Boc-phenylalaninal was always used as the aldehyde component because the HIV protease cleavage site is adjacent to hydrophobic amino acid residues such as phenylalanine in the viral protein.

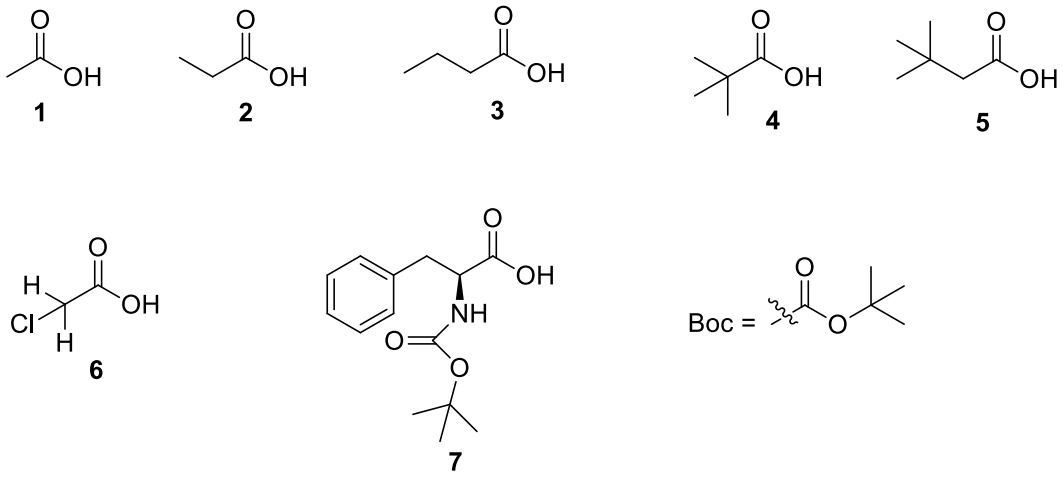


Figure 11: Commercially available carboxylic acids used in Passerini reactions.

Three of the isocyanides (**11**-**13**, **Figure 12**) were synthesised because they were either too expensive to buy or they were not commercially available.

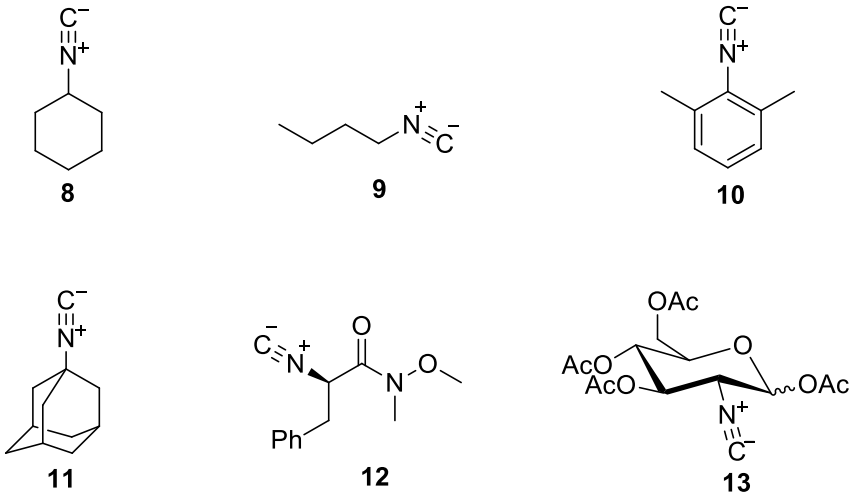
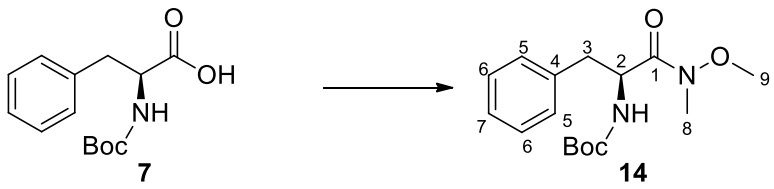


Figure 12: Isocyanides.

2.1 Synthesis of *N*-Boc-L-phenylalaninal

We first prepared the aldehyde component of our Passerini reaction with much success using the method which was developed in 1982 by Fehrentz *et al*.[60]

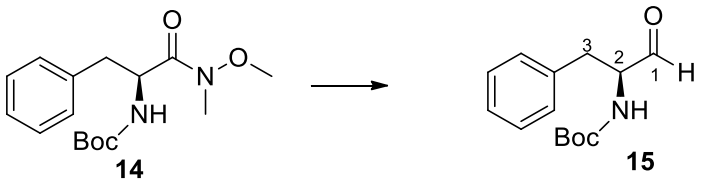


Scheme 13: Reagents and Conditions: (i) 1.2 eq CDI, CH_2Cl_2, rt (ii) 1.2 eq *N,O* dimethylhydroxylamine.HCl, CH_2Cl_2, 82%.

Synthesis began by converting commercially available *N*-Boc-L-phenylalanine **7** into Weinreb amide **14** (**Scheme 13**). Boc protection of the nitrogen was preferred because this protecting group is easy to remove and would allow for the acyl migration to occur in the PADAM sequence. Compound **7** was dissolved in CH_2Cl_2 and reacted with carbonyldiimidazole (CDI), which was added in small portions and effervescence was observed. Gas evolution is a clear indication that the carboxylic acid has been activated for nucleophilic substitution, and this activation step is vital in the synthesis of the Weinreb amide, since addition of the imidazole activates the carbonyl group for nucleophilic substitution.[61] After stirring at room temperature for 1 hour, the reaction mixture was treated with *N,O*-dimethylhydroxylamine hydrochloride and the reaction was left to stir overnight. After this time, thin layer chromatography (TLC)

showed the presence of a new product and no starting material. The desired product was isolated and purified by silica gel column chromatography as a clear viscous oil in a yield of 82%.

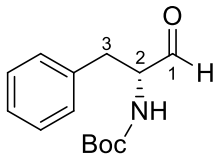
The formation of **14** was confirmed by ^{1}H NMR spectroscopy by the presence of two singlets: one at δ 3.16 for the N-CH_3 protons and one at δ 3.65 for the O-CH_3 protons, with each signal integrating for three protons. This was a clear indication that the Weinreb amide had been formed. The characteristic Boc group protons were observed at δ 1.39 as a singlet integrating for 9 protons. The proton at the stereogenic centre (H-2) appeared at δ 5.04 – 4.86 as a multiplet, whilst the NH was visible at δ 5.27 – 5.09 as a multiplet integrating for one proton. The benzylic protons (H-3) gave rise to 2 signals in the ^{1}H NMR spectrum since they are diastereotopic. One appeared as a doublet of doublets and the other as a multiplet. The multiplet appeared at δ 2.95 – 2.80 integrating for one proton and the doublet of doublets was visible at δ 3.05 with *J* = 13.5 and 6.1Hz. The phenyl protons appeared as a multiplet at δ 7.37 – 7.12 and integrated for 5 protons. The ^{13}C NMR spectrum further confirmed the formation of this compound by the presence of signals in the carbonyl region at δ 172.32 for C-1 and at δ 155.16 for the Boc C=O. The two methyl carbon atoms of the Weinreb amide functional group appeared at δ 61.50 for C-9 and at δ 32.03 for C-8. The quaternary carbon atom of the Boc group gave rise to a signal at δ 79.54, while the Boc methyl groups appeared at δ 28.31 in the ^{13}C NMR spectrum. The IR spectrum showed a strong signal at 1666 cm^{-1} which is characteristic for the carbonyl bond of an amide functional group. The absence of an OH stretch peak was further evidence that the Weinreb amide had been formed.



Scheme 14: Reagents and Conditions: 1.5 eq $LiAlH_4$, dry THF, 0°C, 2 h, 88%.

Having successfully prepared the Weinreb amide **14**, we then reduced it to the desired aldehyde by treatment with $LiAlH_4$ in THF. The desired product **15** was achieved in a yield of 88% (**Scheme 14**). The product was not purified by silica gel column chromatography in order to minimise the chance of racemisation. No primary alcohol, which would have indicated over-

reduction, was observed. This confirms the findings of Fehrentz *et al*. who synthesized a number of aldehydes from naturally occurring amino acids.[60] It is believed that the Weinreb conversion proceeds through a very stable metal chelation intermediate between the carbonyl oxygen and Weinreb nitrogen which collapses during the acid work up. The formation of the product was confirmed by ^{1}H NMR spectroscopy by the disappearance of the two methyl singlet peaks at δ 3.65 and δ 3.16 and the appearance of a new characteristic aldehyde singlet at δ 9.63. ^{13}C NMR spectroscopy also indicated the absence of signals at δ 61.50 and δ 32.03 owing to the methyl groups of the Weinreb amide, and the presence of a new characteristic carbonyl carbon peak at δ 199.44. The IR spectrum showed a strong sharp signal at 3365 cm^{-1} due to the N-H stretch. The melting point obtained (83-84 °C) was comparable with the reported literature value of 86 °C.[60] To ensure that the stereochemical integrity had been retained, optical rotation was performed and the results obtained ($[\alpha_D]^{20}_{589}$ +44.0 (c 2.0, CH_2Cl_2), -44.5 (c 2.0, MeOH)) were comparable to those of previously reported findings ($[\alpha_D]^{20}_{589}$ +40.4 (c 2.0, CH_2Cl_2), -44.4 (c 2.0, MeOH)).[60]

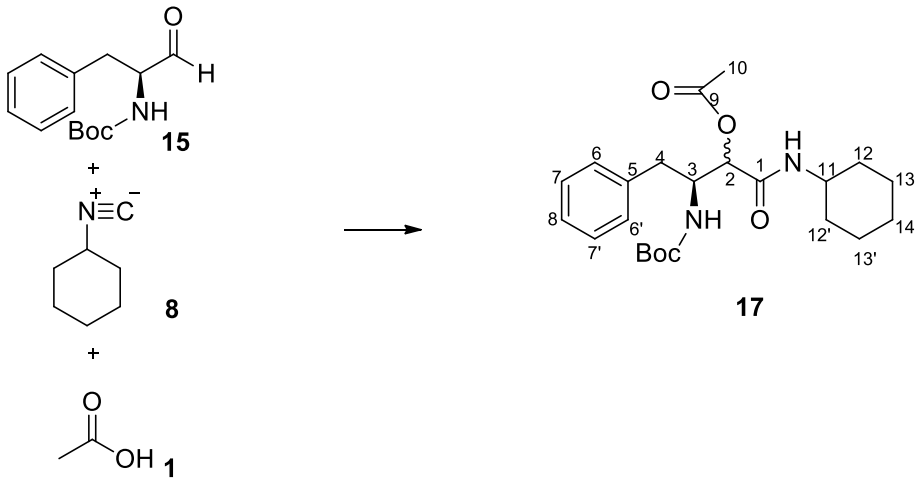


16

The aldehyde derived from the unnatural amino acid, compound **16** (*N*-Boc-D-phenylalaninal), was synthesised using the same method as described for the synthesis of compound **15**. Product formation was confirmed by ^{1}H NMR spectroscopy by the presence of the aldehyde singlet (H-1) at δ 9.61 and the characteristic carbonyl signal in the ^{13}C NMR spectrum at δ 199.44. All the other signals were as expected for the desired product. The melting point obtained was the same as for compound **15** at 83-84 °C whilst the specific optical rotation was ($[\alpha_D]^{20}_{589}$ -36.2 (c 2.0, CH_2Cl_2)) which is comparable to literature values.[62] The optical rotation is roughly equal in magnitude but opposite in sign, which is what is expected for enantiomers.

2.2 Passerini reaction to prepare (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate 17 and subsequent deprotection-acyl migration

After the successful synthesis of *N*-Boc-L-phenylalaninal, the synthesis of a library of peptidomimetic compounds using the Passerini reaction commenced.



Scheme 15: Reagents and conditions: CH_2Cl_2, r.t., 3 d, 93%.

The first compound synthesised in this series was obtained by reacting *N*-Boc-L-phenylalaninal **15**, commercially available cyclohexyl isocyanide **8** and acetic acid **1** in a one pot reaction for 3 days (**Scheme 15**). Equimolar quantities were used for each reactant and the reaction was carried out at room temperature in air. Care was taken with handling the isocyanide as it has a strong pungent smell. After 3 days the product was purified using silica gel column chromatography, with a mixture of both diastereomers collected. Partial diastereomeric separation of the product did not occur using this method. The product was recovered in a good yield of 93%.

The ^{1}H NMR spectrum confirmed the presence of the desired product. A multiplet was observed at δ 7.31 – 7.14 integrating for 5 protons resulting from the aromatic protons. One of the signals from a proton attached to N was split into two: one at δ 6.01 - 5.90 and the other at δ 5.83 - 5.75 with both signals being multiplets and integrating collectively for 1H. This might be due to either the presence of rotamers caused by restricted rotation around the amide or

carbamate C-N bonds, or simply due to the presence of the diastereomers. Variable temperature ^{1}H NMR spectroscopy could have been performed to establish which factor was predominant, but further analysis will be done as future work. However, the ratio of these signals of 2:1 suggests that the observation is due to the diastereomers, as it corresponds to the ratio calculated by HPLC analysis (2.14:1.00 **Figure 13**). The proton at H-2, the newly formed unresolved stereogenic centre, appeared as an overlapping multiplet signal with one of the N-H protons at δ 5.28 – 4.90 (2H). The proton at H-3 also appeared as a multiplet at δ 4.35 – 4.22 integrating for 1 proton. This complex splitting pattern observed is due to coupling with the neighbouring stereogenic centre proton, benzylic protons and the NH combined with the presence of diastereomers. For each of the two diastereomers, the benzylic protons (H-4) appear as a doublet, indicating that no geminal coupling is occurring. The first signal was observed at δ 2.94 with *J* = 7.5 Hz while the second signal was found at δ 2.83 with *J* = 7.2 Hz. The methyl protons of the ester group H-10 appear as two singlet signals, one at δ 2.13 whilst the other signal was at δ 2.09 in a ratio of 2:1.

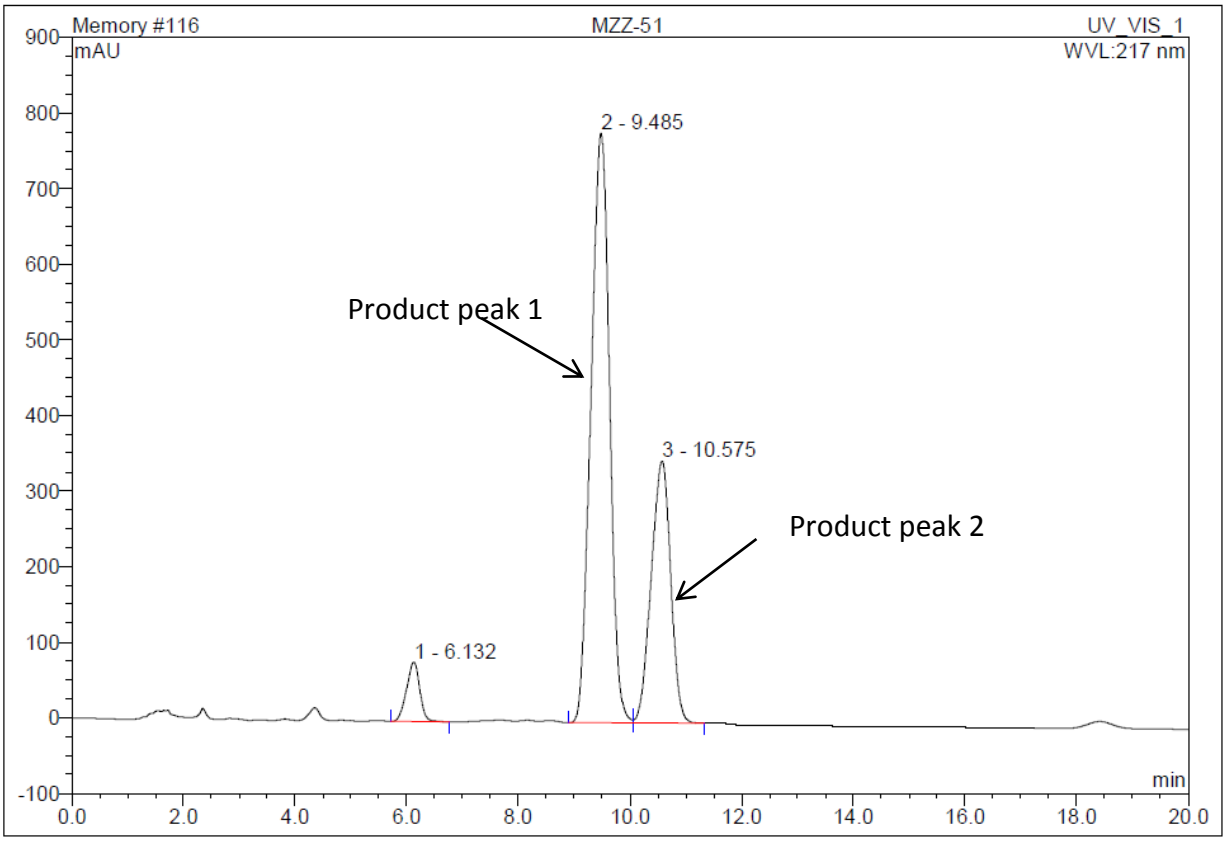
The remaining signal from the Boc methyl protons appeared at δ 1.43 – 1.29 as a multiplet integrating for nine protons. Signals from the protons of the cyclohexyl ring were not clearly resolved but they integrated for 10 protons as expected.

The ^{13}C NMR spectrum further confirmed the presence of diastereomers as doubling of some peaks was observed. Four carbonyl ester signals were observed at δ 169.80, δ 169.09, δ 166.86 and δ 166.59 instead of only 2 signals. The carbonyl carbon characteristic of the Boc group also gave rise to two signals at δ 155.50 and δ 154.94. The newly formed stereogenic centre C-2 appeared as 2 peaks, one at δ 74.32 and the second one at δ 73.73. Doubling of C-3 was also observed with signals appearing at δ 53.27 and δ 52.79. The cyclohexyl C-H, (C-11) appeared at δ 48.29 and δ 48.18 for the two diastereomers and the benzylic carbon C-4 at δ 38.05 and δ 37.27.

In order to determine the diastereomeric ratio of the compound accurately, High Performance Liquid Chromatography was performed. Since this was a new compound, there was no published method in literature for the separation of these compounds and various solvent systems and flow rates were tested to find one which gave maximum baseline peak separation

with an elution time of less than 20 minutes. For the separation of organic molecules, a C-18 column is normally used and it was the starting point for our experimental work. Product (1 mg) was dissolved in 1 ml of solvent and 25 µl of the solution was injected onto the column for separation.

The first mobile phase to be tested was a mixture of *iso*-propanol/hexane using isocratic elution at different ratios, starting with a ratio of 1:9. No separation was observed. HPLC was then performed using gradient elution but still no separation was observed. The mobile phase was then changed to acetonitrile/water (MeCN/H_2O) starting with a 1:1 mixture. Partial separation was observed, but the elution time was too long at 30 minutes. The solvent ratio was then changed to 3:2 and baseline separation was observed, although the elution time was still at 25 minutes. To reduce the elution time whilst maintaining baseline separation of the separated diastereomeric peaks, gradient elution was then used. This was done by gradually increasing the ratio of MeCN/H_2O from 1:1 to 4:1. This method afforded the best chromatogram which had a reduced elution time and baseline separation of the diastereomers (**Figure 13**). The d.r. was then calculated by comparing the area underneath the two product peaks 1 and 2 giving a ratio of 2.14:1.

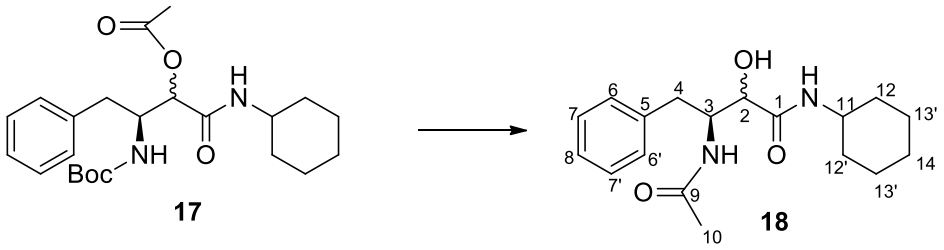


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	6.13	n.a.	78.197	21.530	4.66	n.a.	BMB
2	9.49	n.a.	780.654	300.352	64.97	n.a.	BM
3	10.58	n.a.	346.509	140.432	30.38	n.a.	MB
Total:			1205.360	462.315	100.00	0.000	

Figure 13: HPLC chromatogram of compound 17.

It was clearly evident that formation of one diastereomer was favoured over the other. Any fears of racemisation of the resolved stereogenic centre were allayed by analysing the compound using a Phenomenex 5µ Lux cellulose chiral column. The same method used for separation of the diastereomers on the reverse phase C-18 column was used with the chiral column. Fortunately, the method was transferable, although baseline peak separation was not achieved. Variation of solvent ratios did not provide complete baseline separation, but fortunately only 2 peaks corresponding to the expected diastereomers were observed.

The diastereomeric mixture was then subjected to Boc deprotection, followed by acyl migration for the completion of the PADAM sequence (**Scheme 16**). An excess of trifluoro-acetic acid was added to a solution of compound **17** dissolved in CH_2Cl_2 and the reaction mixture was left stirring for 2 hours. Excess solvent and TFA were removed *in vacuo* and the product was redissolved in CH_2Cl_2 followed by the addition of triethylamine. The PADAM product **18** was then isolated and purified by silica gel column chromatography and HPLC analysis gave a d.r. of 2.08:1. The d.r. shows that the stereogenic centre at C-3 did not racemise during the deprotection and acyl migration steps.



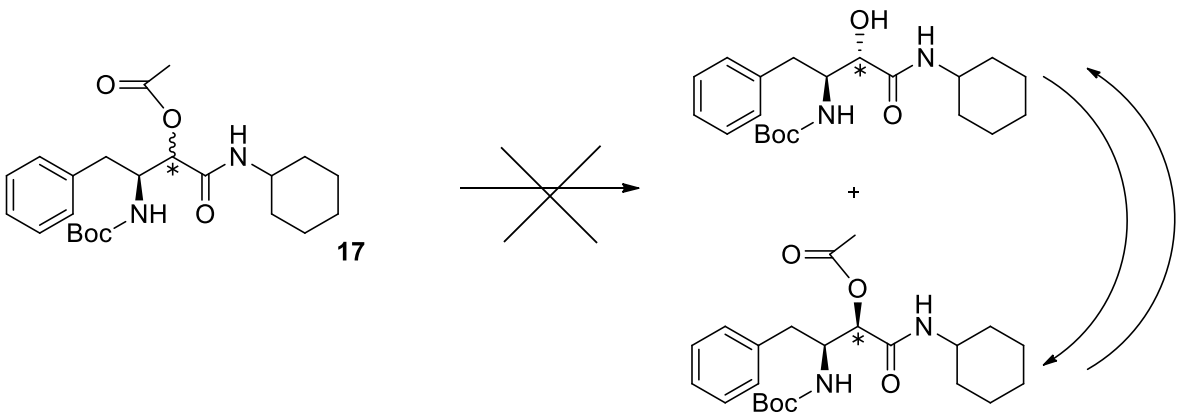
Scheme 16: Reagents and Conditions: (i) 10 eq TFA, CH_2Cl_2; (ii) 10 eq Et_3N, CH_2Cl_2, 94%.

Synthesis of compound **18** was confirmed by the disappearance of the characteristic Boc signals at δ 1.43 – 1.29 in the ^{1}H NMR spectrum and δ 155.50 and δ 154.94 in the ^{13}C NMR spectrum. Further discussion of the spectra will be done at a later stage due to complexity of the spectra of unseparated diastereomers. The diastereomers were ultimately successfully separated using preparative HPLC. Once the PADAM sequence had been shown to afford the desired product, the diastereomeric mixtures of both the Passerini product **17** and PADAM product **18** were individually subjected to enzymatic kinetic resolution.

2.3 Attempted enzymatic kinetic resolution

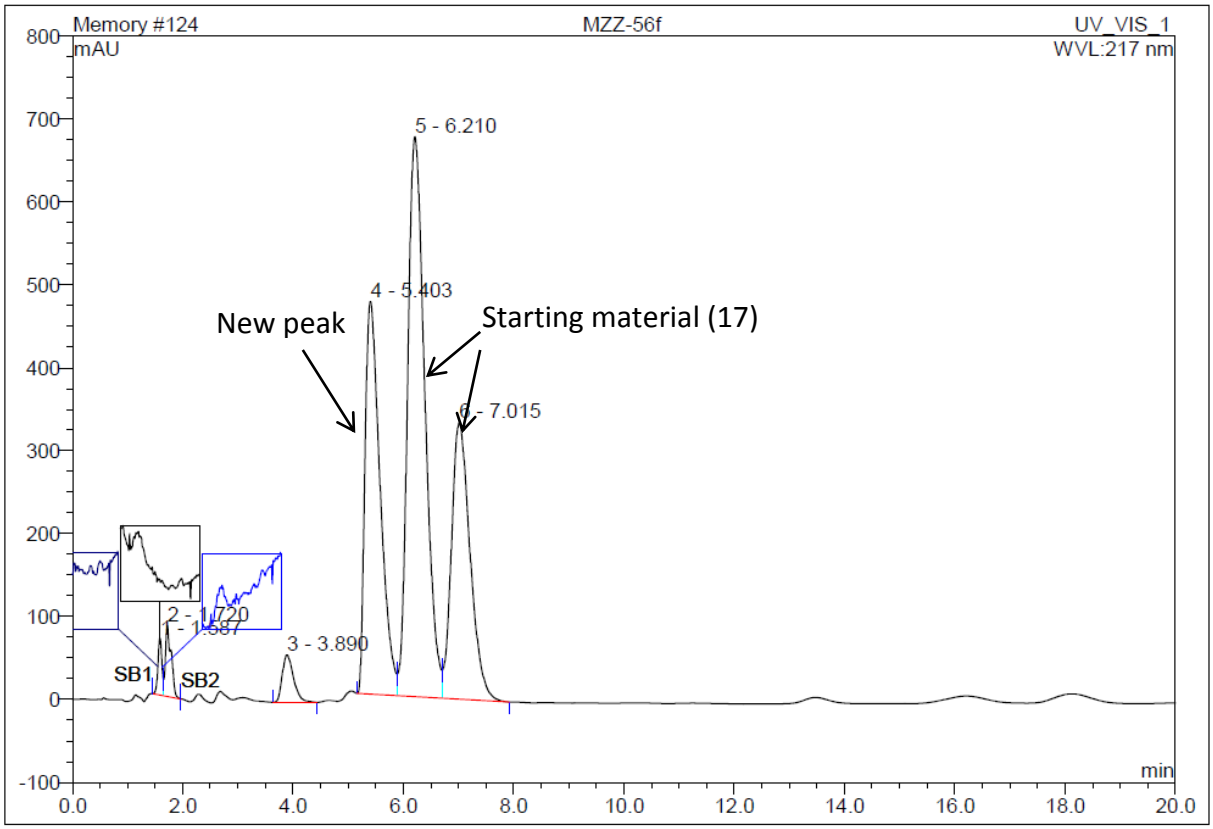
Lipase enzymes have been established to be the most versatile and flexible biocatalysts for organic synthesis and, despite their specific nature, they have been found to be quite compatible with a wide variety of synthetic organic molecules.[51] This provided a basis for our investigation into enzymatic kinetic resolution of diastereomers produced in the Passerini reaction.

All the enzymes used in these experiments were first tested for activity by testing the hydrolysis of benzyl acetate and the acetylation of benzyl alcohol. The test reactions were done using a phosphate buffer (pH 7), and either toluene, EtOAc or MeCN as solvent, in parallel with a control reaction to ensure that hydrolysis did not occur in the absence of the enzyme. The test reactions worked for all the solvents used affording an average yield of benzyl alcohol of 42% in 4 hours. After this a series of reactions were then set up to establish whether any of the 25 selected enzymes would be able to selectively hydrolyse the ester group at the newly formed stereogenic centre of our Passerini product **17**, considering the success achieved by other researchers using this approach.[56] To the best of our knowledge, this is the first time an attempt has been made to enzymatically resolve diastereomeric products of the Passerini reaction. All of the successful enzymatic resolutions reported so far were to resolve enantiomeric products of the Passerini reaction.



Scheme 17: Reagents and Conditions: Lipase enzyme, phosphate buffer pH 7.0, MeCN, 35 °C.

After establishing the activity of the enzymes we had chosen, compound **17** was then subjected to enzymatic kinetic resolution (**Scheme 17**). MeCN was used as the solvent of choice as it is miscible with the buffer and would ensure that both the enzyme and the substrate are in the same phase, which should increase the rate of reaction. This provided the first challenge as the substrate would not dissolve when water miscible solvents were used. Toluene was then used as the solvent for the reaction as it is immiscible with water and dissolved the substrate. To assist in the interphase interactions, a surfactant (Tween, a polyoxyethylene-sorbitan esterified with a long-chain fatty acid) was used. Novozyme 525 was the first enzyme to be tested on our substrate, as it is known to be versatile.



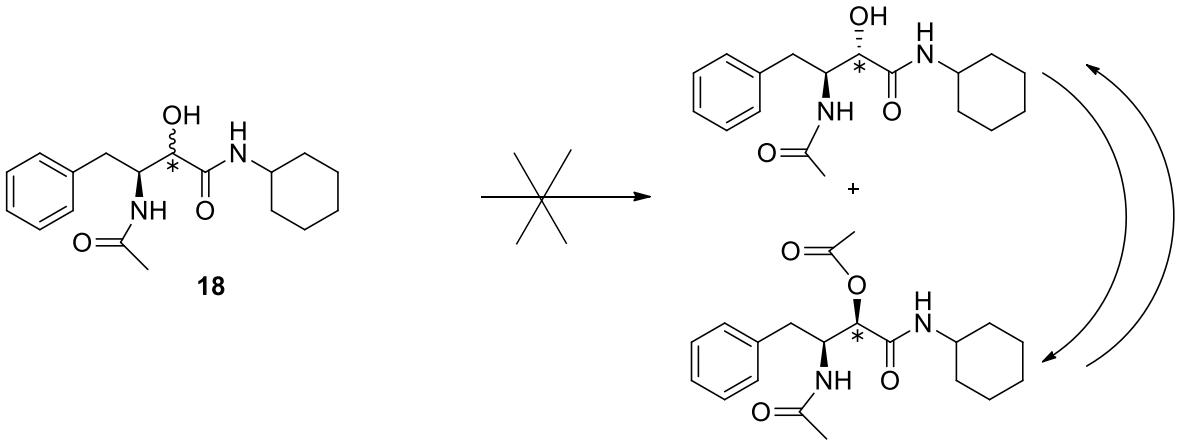
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.59	n.a.	68.135	5.740	1.03	n.a.	BM
2	1.72	n.a.	85.309	10.211	1.84	n.a.	MB
3	3.89	n.a.	57.027	13.522	2.43	n.a.	BMB
4	5.40	n.a.	473.714	151.142	27.17	n.a.	BM
5	6.21	n.a.	675.408	244.734	43.99	n.a.	M
6	7.02	n.a.	333.580	130.974	23.54	n.a.	MB
Total:			1693.172	556.323	100.00	0.000	

Figure 14: HPLC chromatogram of attempted enzymatic hydrolysis of 17.

The reaction was monitored by HPLC where the disappearance of one diastereomeric peak and appearance of another new peak would indicate successful hydrolysis of one diastereomer. There appeared to be formation of a new product by HPLC after 10 days (**Figure 14**) and the reaction was stopped and the products separated by column chromatography. Upon inspection of the ^{1}H NMR spectrum, however, it was evident that the desired product had not formed and instead a reaction between the enzyme and surfactant had occurred, as some signals from Tween were present in the spectrum. Furthermore the d.r. of the diastereomers had not changed, indicating that the desired diastereoselective hydrolysis was not occurring. Enzymes

are usually employed for the selective separation of enantiomers but in this experiment we were trying to separate diastereomers. This might have affected the reactivity and selectivity of the enzymes.

The experiment was then carried out in the absence of Tween, but there was no observable reaction after 14 days, and as a consequence the reaction was abandoned. The procedure was then carried out in different solvents, including EtOAc, Et_2O and DMF with Triton-60 as the surfactant, but still no reaction was observed. A total of 25 lipase enzymes were tested on this substrate but yielded no positive results.



Scheme 18: Reagents and Conditions: Lipase enzyme, vinyl acetate, solvent, 35 °C.

After unsuccessful trials of the hydrolysis reaction, the esterification reaction was tested on compound **18** (**Scheme 18**). Mozhaev *et al.* reported success with the enzymatic acylation of bergenin, a compound with 5 hydroxyl groups.[50] They achieved high selectivity where only one hydroxyl group was acylated, possibly due to steric constraints (**Figure 15**). Based on their findings, we decided to test the acylation reaction on our substrate **18**.

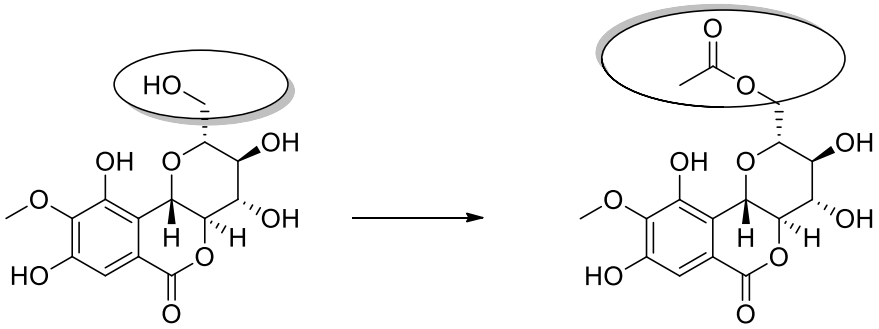
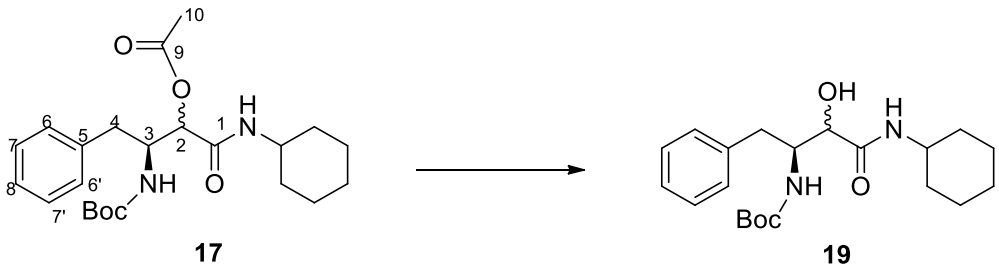


Figure 15: Mono acylation of bergenin.

Once again the reaction was monitored using HPLC but unfortunately no new product formation was observed using either MeCN, EtOAc, toluene or Et_2O as solvent, despite varying the temperature.

In an attempt to probe the reaction and optimise reaction conditions, substrate **17** was modified to form the secondary alcohol **19** (**Scheme 19**). This was achieved by treating **17** with potassium hydroxide in methanol overnight. The procedure yielded the expected product, signified by the loss of the methyl peak in the ^{1}H NMR spectrum at δ 2.13 and δ 2.09 and the appearance of a broad singlet at δ 5.53 for the OH. There was also loss of two C-10 signals in the ^{13}C NMR spectrum at δ 20.85 and δ 20.65. IR also showed a peak for the OH stretch at 3300 cm^{-1}.



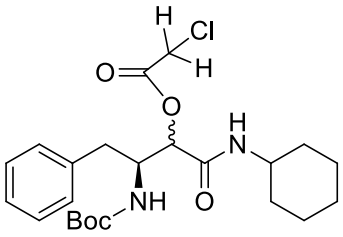
Scheme 19: Reagents and Conditions: 9 e.q. KOH, methanol, overnight, 99%.

Compound **19** was then used in the enzymatic acylation reaction (same reaction conditions as in **Scheme 18**), but unfortunately despite this change, there was no observable reaction with any of the library of enzymes tested. Changing solvents did not appear to have any effect on facilitating the reaction. We suspected that the Boc protecting group, which is a bulky group,

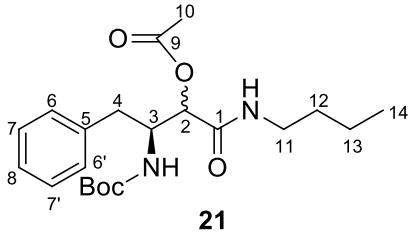
might have provided steric hindrance precluding entry to the enzyme active site since it is very close to the target site.

These results were disappointing indeed as not a single enzyme of the 25 selected showed any activity with the three substrates that had been tested. This led to the conclusion that no substrate binding was occurring at the active site of the enzymes, the substrate was not entering the active site at all or the acetate was not a sufficiently good leaving group in the hydrolysis reaction. We probed further the possibility that the acetate was not a good leaving group. In order to make the acetate a better leaving group, compound **20** was prepared by the Passerini reaction using chloro-acetic acid **6** as the acid component (**Scheme 15**), in a yield of 85% and a d.r. of 1.94:1.00. The NMR spectra of the compound confirmed the formation of the desired product and will be discussed in detail as separated diastereomers at a later stage.

This substrate was then used in the enzymatic hydrolysis reaction (**Scheme 17**) with the hope that the heteroatom would increase the chances of the substrate being hydrolysed. Unfortunately no positive result emanated from this attempt. Upon revisiting work of previously successful applications of the enzymatic kinetic resolution on Passerini products, it was observed that none had attempted to resolve substrates as structurally large as the ones being employed in this work.[63] The second possibility, that the substrate was too large to enter the active site, was thus then explored.



20



21

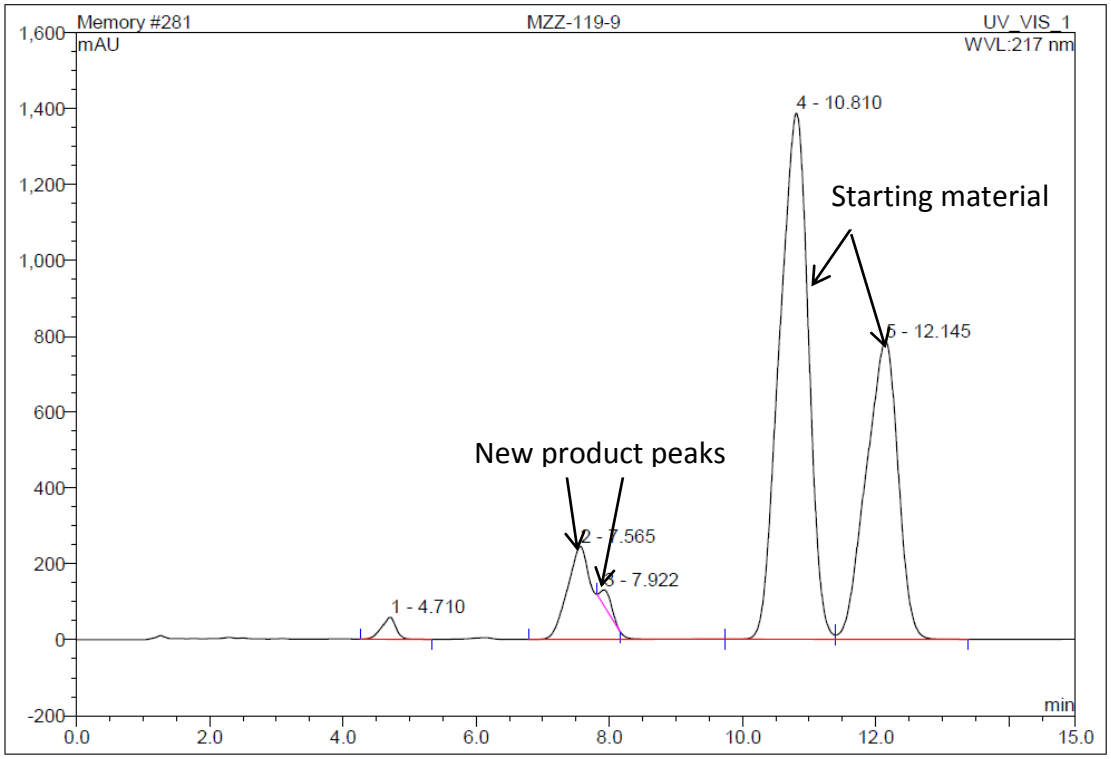
To investigate whether there was a problem with steric hindrance due to the bulky cyclohexyl and phenyl groups inhibiting entry of the substrate into the active site, compound **21** was synthesized where the cyclohexyl isocyanide was replaced with *n*-butyl isocyanide **9** in **Scheme 15**. The compound was recovered in an excellent yield of 96% and a d.r. of 1.72:1.00 which was

slightly lower than that observed for compounds **17** and **20**. The altering of the d.r. was probably as a result of using a smaller, straight chain isocyanide in the Passerini reaction.

Successful preparation of compound **21** was confirmed by the presence of a quartet at δ 0.91 with a coupling constant of 7.2 Hz representing 3 protons in the ^{1}H NMR spectrum for H-14. This is expected from the terminal carbon of the *n*-butyl chain. The methyl group of the ester H-10 was also clearly visible as a multiplet at δ 2.23 – 2.04 integrating for 3 protons. The ^{13}C NMR spectrum also showed the expected two signals for the diastereomeric C-2 signals at δ 74.63 and δ 73.99 and the C-14 signal at δ 13.70. The melting point of the compound was found to be 125-126 °C.

After confirmation of the structure, compound **21** was then used as the substrate in an enzymatic hydrolysis using previously described conditions (**Scheme 17**). The solubility of this substrate was slightly better when water miscible solvents were used. Acetonitrile was then used as solvent of choice for the reactions as literature reports indicate that high yields and selectivity were obtained when this solvent was used. At first, 10 enzymes were tested, Novozyme *525*, Lipase *Rhizopus oryzae, Wheat germ* lipase*,* Lipase *Pseudomonas fluorescens,* Lipase *Candida rugosa, Porcine pancrease* lipase*, Candida antartica* lipase*,* Lipase *Rhizopus arrhizus,* Lipase *Aspergillus sp.* and Lipase *Pseudomonas cepacia*.

From this set, only *Candida antartica* lipase and Lipase *Aspergillus* were found to produce new HPLC peaks after 14 days. The two enzymes were then used again in optimized reactions where the quantity of enzyme used was increased and the temperature varied. Tracking the reaction by HPLC indicated a lack of selectivity as both diastereomers were being hydrolysed, although at different rates (**Figure 16**). This was another setback which required new solutions, as changing the conditions did not provide any remedy to the problem. The reaction was abandoned pending availability of a new library of enzymes.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	4.71	n.a.	57.694	14.687	1.14	n.a.	BMB
2	7.57	n.a.	245.305	124.657	9.71	n.a.	BMB
3	7.92	n.a.	42.049	8.461	0.66	n.a.	Rd
4	10.81	n.a.	1387.035	699.341	54.48	n.a.	bM
5	12.15	n.a.	787.151	436.613	34.01	n.a.	MB
Total:			2519.234	1283.759	100.00	0.000	

Figure 16: HPLC chromatogram for enzymatic resolution of compound 21.

The bio-catalysis route was put on hold pending further investigation into what could be done to optimize the reaction or even to enhance selectivity. It was evident from the use of compound **21** that steric hindrance might be playing a role in the lack of activity, whilst the lack of selectivity may be due to the enzyme not recognizing the two diastereomers as chemically different. However, these findings shed light on what could be done to improve the reactivity and selectivity. Hydrolysable groups could be introduced to the substrate further away from the unresolved centre than the current group with the hope that enzyme hydrolysis might still be stereoselective.[64]

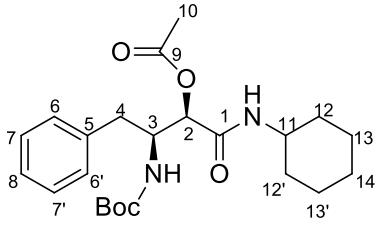
Having failed to resolve the compounds using more environmentally benign catalytic methods, an HPLC preparative system was then employed to separate the Passerini products in small

quantities. This was done in order to facilitate biological testing of the library of compounds that were to be synthesised in their stereochemically pure form, as individual diastereomers could exert different effects in a biological system. The preparative HPLC allows for isolation of the individual diastereomers in quantities that are adequate for further analysis. The instrument utilises a separation column that is 10 times bigger than the analytical column, and allows for larger quantities of sample to be analysed. Fortunately, the analytical method we had developed was transferable to the preparative system, with only a minor adjustment of flow rate which had to be increased to 20 ml/min.

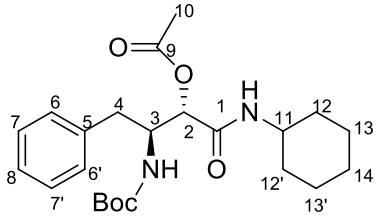
The major problem associated with this method was that several HPLC runs had to be performed in order to collect a reasonable quantity of each product. This meant that large volumes of HPLC-grade solvent were used as at least 10 runs had to be performed for each compound. In addition, this method was found to be successful only with compounds that showed good baseline separation on the C-18 reverse phase analytical column. As a result, not all Passerini products prepared were separated into the individual diastereomers using the preparative HPLC system.

2.4 HPLC separation of 17

Compound **17** was the first to be separated by the preparative HPLC affording **17A** (major diastereomer) and **17B** (minor diastereomer).



17A- major diastereomer *S,R*



17B- minor diastereomer *S,S*

At this stage, we did not know which diastereomer was the major product. However, crystal structures of 2 other separated compounds at a later stage enabled the assignment of the configurations. It was also interesting to note that the minor diastereomer was less soluble than the major diastereomer in most solvents. The identity of **17A** and **17B** was confirmed by

^{1}H NMR and ^{13}C NMR spectroscopy. The H-3 proton appeared as a multiplet at δ 4.38 -4.23 and δ 4.37 – 4.19 for the two different diastereomers **17A** and **17B** respectively. The H-4 protons are also characteristic and they appeared as a doublet at δ 2.83 (*J* = 7.2 Hz) integrating for 2 protons for **17A** and at δ 2.94 (*J* = 7.6) integrating for 2 protons for **17B**. C-2 was also seen as a unique signal in the ^{13}C NMR spectrum with significantly different shifts for the two diastereomers: δ 73.86 for **17A** and δ 74.42 for **17B**. These differences clearly show that there is a difference in the conformational arrangement of these two molecules.

2.5 Synthesis of the cyclohexyl isocyanide series

After the successful separation of the diastereomeric mixture **17**, a range of analogues employing cyclohexyl isocyanide **8** and different carboxylic acids (**2**, **4**, **5**, **6** and **7**) were synthesised (**Figure 17**). The yields for these compounds (**22 – 26**, **Figure 17**) were good, ranging between 85% and 99%. The reactions proceeded as expected with the exception of compound **26**, which precipitated out of solution as it was being formed. This resulted in further purification of the compound being very difficult as it would only dissolve in heated DMSO. It was then partially purified by repeatedly washing with CH_2Cl_2 in an attempt to remove unreacted starting material.

The formation of one diastereomer is favoured and results in an approximately 2:1 ratio of products. Bulky acids did not improve the ratio, while the use of propionic acid **2** slightly decreased the d.r. to 1.87:1.00.

Table 1: d.r. for compounds in the cyclohexyl series.

Compound	17	20	22	23	24	25	26
d.r.	2.14:1.00	1.94:1.00	1.87:1.00	2.01:1.00	2.08:1.00	1.93:1.00	-

The d.r. for compounds **17**, **20** and **22** - **26** are shown in **Table 1**, with the ratio for most compounds being 2:1 except for **17** and **22**. Complete baseline resolution was not achieved during the analysis of compound **23**, and therefore the reported ratio of peaks is an

approximation of the area under the peaks. Due to the insolubility of **26**, HPLC analysis could not be performed to establish the d.r. Furthermore, determination of the d.r. using ^{1}H NMR spectroscopy for this compound was not accurate because of overlapping of signals. However from the d.r. results we could obtain, a general trend is apparent and it can therefore be concluded that use of the chiral *N*-Boc-phenylalaninal has an effect on diastereoselectivity in the Passerini reaction.

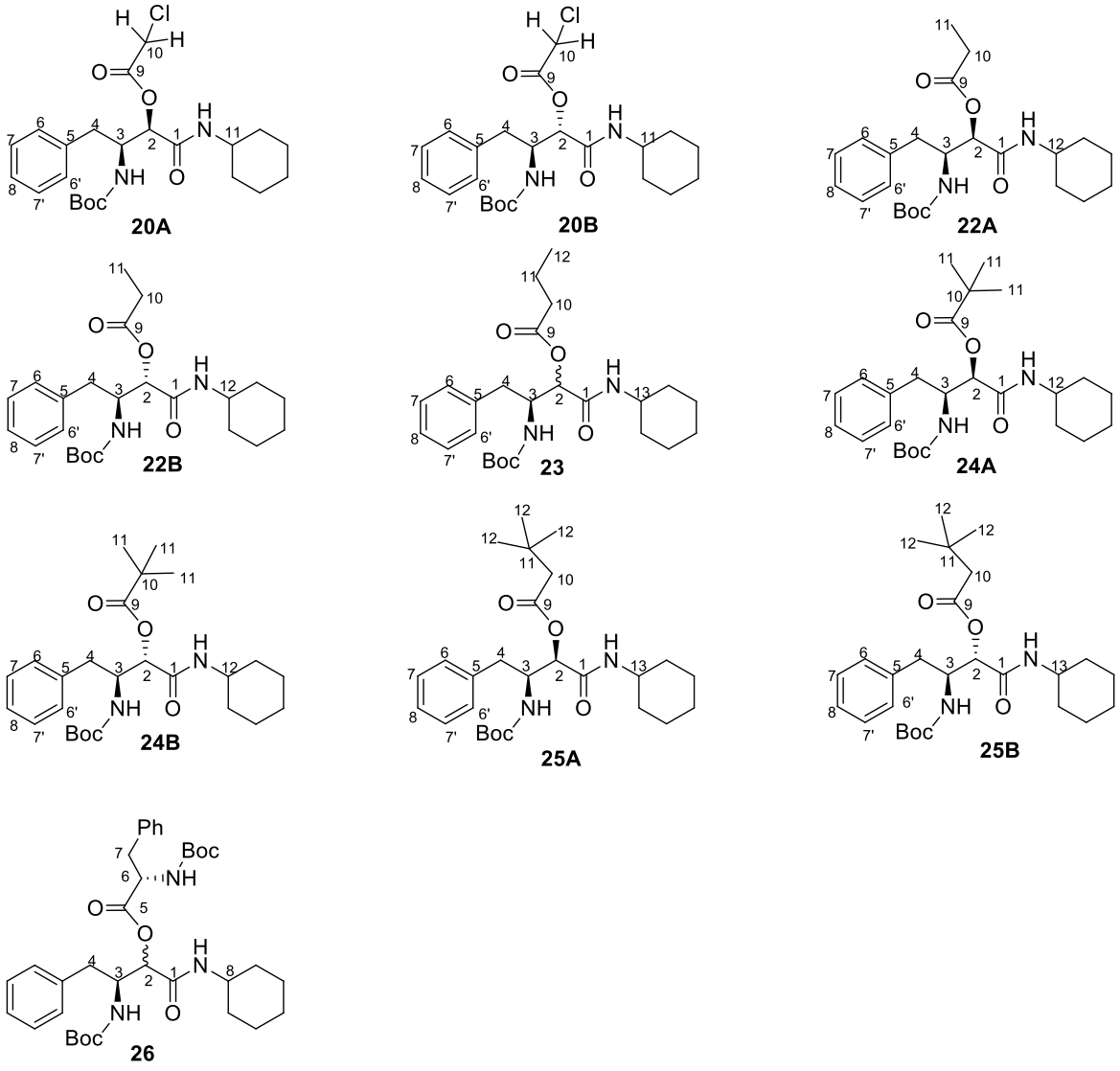


Figure 17: Passerini products from derived from cyclohexyl isocyanide.

After the successful synthesis of the Passerini products utilizing cyclohexyl isocyanide, they were then subjected to preparative HPLC in order to isolate individual diastereomers. Only

compounds **23** and **26** were not separated due to the lack of baseline resolution for **23** and solubility problems for **26**. It was observed that for all the separated analogues, the minor diastereomer **B** was less soluble in all solvents tested than the major diastereomer **A**. It was beginning to appear as a trend and was not unexpected, as diastereomers have been shown to have different chemical and physical properties. In the characterisation of the separated diastereomers, the signals in the NMR spectra for both protons and carbons at positions 2, 3 and 4 for all the compounds were the most significant and will be discussed in more detail.

For both diastereomers (**A** and **B**) for all the compounds (**20** – **26**), the protons at H-3 appeared as a multiplet integrating for one proton. An interesting observation for compound **20** was the signals for the protons at H-10. They appeared as a singlet for **20A** at δ 4.03 integrating for 2 protons and as two doublets at δ 4.18 (*J* = 14.7 Hz) and at δ 4.12 (*J* = 14.7 Hz) for **20B** integrating for two protons. Thus it appears that geminal coupling between H-10 protons occurs in one diastereomer but not the other.

The protons at H-2 appeared as a multiplet at δ 5.30 – 5.10 for **20A**, δ 5.26 – 5.07 for **22A**, δ 5.25 – 5.07 for **24A** and δ 5.22 – 5.00 for **25AB**, in each case integrating for 2 protons due to overlapping with a signal from another proton. The proton-carbon correlation NMR spectra (HSQC) for these compounds showed that the second signal is due to an NH proton as there was no correlation to a second ^{13}C signal. For the minor diastereomers, the H-2 protons did not overlap with the NH signal except in the case of **25B**. This is clear evidence of the conformational differences between the two diastereomers, and strongly supports the assumption that the same diastereomer is formed as a major product in every case. For **20B** the H-2 proton integrates for 1H and appears as a doublet at δ 5.17 (*J* = 3.5 Hz), while for **22B**, **24B** and **25B** it appears as a multiplet at δ 5.23 – 4.98, δ 5.27 – 5.01 and δ 5.26 – 5.01 respectively.

In the ^{13}C NMR spectra of the Passerini products, the C-3 signals for the major diastereomer appear slightly more upfield than the minor diastereomer. This trend is consistent for all the separated diastereomers, while two signals are present in the ^{13}C NMR spectra of unseparated compound **23**. The same trend is also observed for C-2 in the ^{13}C NMR spectra of these compounds (**Table 2**).

Table 2: Chemical shifts for C-2 and C-3 signals.

Compound	20A	20B	22A	22B	23	24A	24B	25A	25B
C-3 δ	52.71	53.09	52.31	52.85	53.36, 52.90	53.08	53.16	52.96	53.37
C-2 δ	75.12	75.96	73.19	73.63	74.05, 73.48	73.28	73.94	73.43	73.85

From these results it can be concluded therefore that the signals at δ 52.90 and δ 73.48 for **23** are due to the major diastereomer whilst the signals at δ 53.36 and δ 74.05 are due to the minor diastereomer. High Resolution Mass Spectrometry (HRMS) results for the compounds were as expected, and the masses for the separated diastereomers were the same. The compounds were also found to have high melting points of between 157 °C – 193 °C.

The Passerini products (both separated and diastereomeric mixtures) were then subjected to the deprotection-acyl migration sequence (**Scheme 16**) in order to achieve the 1,2-amino-hydroxyl functionality of the target compounds (**Figure 18**). In each case the desired product was obtained, and whilst the reaction proceeded smoothly, the resolved diastereomers required more reaction time to go to completion than the diastereomeric mixture had in previous experiments. The reaction was high yielding with overall yields of the reaction ranging between 84% and 96%, with the exception of **27A** and **27B** with yields of 42% and 56% respectively. This low yield can be attributed to the fact that the reaction did not go to completion as starting material was recovered (≈ 35%). The PADAM products were overall less soluble than the Passerini products, possibly owing to the fact that the deprotected product is more likely to have an orderly arrangement in the solid state because of the reduced bulky groups and a more linear structure.

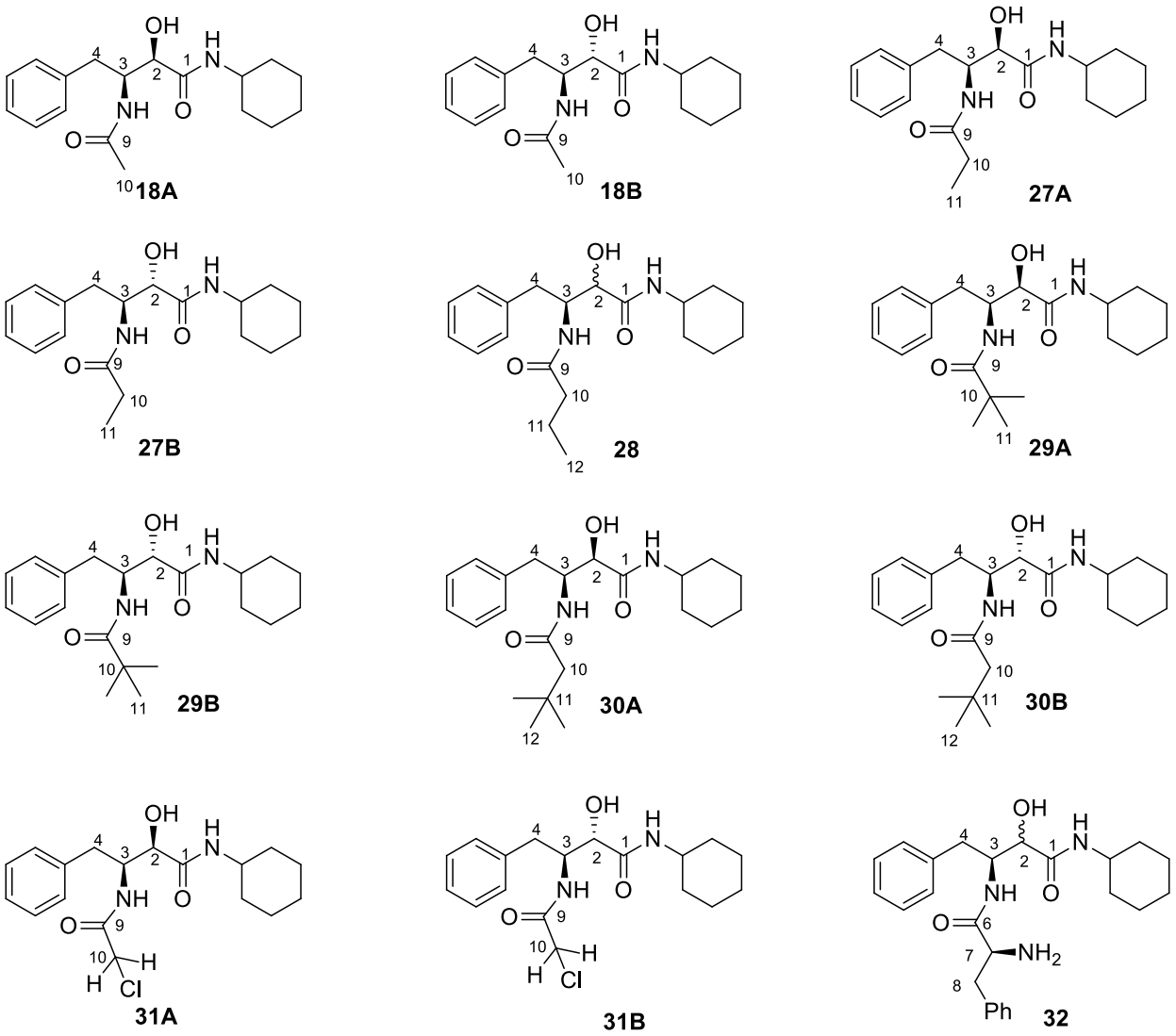


Figure 18: PADAM products for the cyclohexyl series.

The success of the reaction was confirmed by the appearance of a new O-H signal in the IR spectra of the products as shown in **Table 3**. The signal was not as broad as expected in some instances, probably due to the lack of hydrogen bonding due to the possible conformational arrangement of the molecule.

Table 3: Wave number of OH signal in IR spectra of PADAM products of cyclohexyl series.

Compound	18A	18B	26A	26B	27	28A	28B	29A	29B	30A	30B	31
cm^{-1}	3350	3386	3347	3390	3278	3341	3303	3274	3279	3404	3399	3269

It is interesting to note that the O-H signals in the IR spectra for the separated diastereomers do not follow a particular sequence, as was observed with H-2 and C-2 signals in the ^{1}H NMR and

^{13}C NMR spectra. Once again, the HRMS results for all compounds were as expected. In order to ascertain whether racemisation was occurring during the deprotection and acyl migration steps, d.r. analysis was performed for diastereomeric mixtures. The ratio of diastereomers did not change, and hence we can assume that racemisation did not occur during the two reaction steps. The d.r. of compound **17** was 2.14:1.00 and after the deprotection acyl migration sequence to afford **18**, the d.r. was 2.08:1.00. Similarly, the d.r. of compound **23** was 2.01:1.00 and after the PADAM sequence to afford **28**, the product was found to have a d.r. of 2.00:1.00.

The previously observed solubility trend of the diastereomers continued with the PADAM products. The minor product was less soluble in most solvents, although to a greater extent than before. This resulted in the use of different deuturated solvents for NMR spectroscopy, making comparison of the chemical shifts for the separated diastereomers difficult. However the migration of the acyl functionality was proved by the upfield shift of the signal for the H-2 protons in the ^{1}H NMR spectrum of each product. In the case of Passerini products, the H-2 protons appeared in the range of δ 5.00 – 5.30 in the ^{1}H NMR spectra, however in the rearranged products, the H-2 proton appears upfield at δ 4.31 – 3.83 for all the analogues.

For compound **18A**, the H-2 signal appears as a multiplet at δ 4.21 – 3.98 integrating for 2 protons, where the signal overlaps with the H-3 signal. For compound **18B**, the H-2 signal appears as a doublet at δ 4.17 (J = 3.5 Hz). For compound **27A**, the H-2 and H-3 signals are overlapping and appear as a multiplet integrating for 2 protons at δ 4.18 – 4.04 whilst for compound **27B** the H-2 signal is separate appearing at δ 3.90 – 3.83 as a multiplet integrating for 1 proton. H-2 and H-3 signals are at δ 4.30 - 4.04 (2H) for the unresolved compound **28**. For compound **29A** the H-2 proton signal does not overlap with other signals and appears as a singlet at δ 4.09, whilst for compound **29B** the H-2 and H-3 signals are overlapping at δ 4.26 - 4.18. For compounds **30A** and **30B** the H-2 protons appear as multiplets at δ 4.09 – 4.02 and δ 3.98 – 3.91 respectively. The signal due to the H-2 signal for compound **31A** and **31B** also appear as multiplets at δ 4.16 – 4.07 and δ 4.25 – 4.18 respectively, whilst for compound **32** the signal also appears as a multiplet at δ 3.98 – 3.90.

There is no significant change in the chemical shift of the signal due to H-3 in the ^{1}H NMR spectrum after rearrangement, as the structural change occurs three bonds away. In instances where the same solvent was used for performing NMR spectroscopic experiments, the C-2 in the ^{13}C NMR spectrum for the minor product appears more downfield than the major diastereomer, the same trend as was observed for the Passerini products. This was observed for compound **29A** where the C-2 signal appeared at δ 73.62 and the C-3 signal appeared at δ 55.49, while for **29B**, the C-2 signal appeared at δ 75.20 and the C-3 signal appeared at δ 57.58. ^{13}C NMR spectra for compounds **31A** and **31B** were also performed in the same solvent system, and the C-2 signals were observed at δ 72.21 and δ 73.23 respectively and the C-3 signals were observed at δ 55.38 and δ 56.72. Interestingly the signal due to H-10 in the ^{1}H NMR spectrum still appears as a singlet at δ 3.83 for **31A** and two doublets at δ 3.96 and δ 3.90, both with a coupling constant of 15.0 Hz, for **31B**. This shows that despite the acyl migration occurring, the protons are still in the same chemical environment for **31A** but in different environments for **31B**, which shows geminal coupling.

Compound **18B** was successfully crystallised and an X-ray crystal structure was obtained (**Appendix 1**). From the crystal structure we were able to confirm that this minor diastereomer has an *S*,*S* configuration. Analysis of the ^{1}H and ^{13}C NMR spectra for the separated diastereomers showed that all the major diastereomers display similar chemical shifts and splitting patterns, and similarly the minor diastereomers display similar chemical shifts and splitting patterns. From this observation it can be concluded that the diastereomeric selectivity is conserved throughout the cyclohexyl series. It can therefore be deduced that all minor diastereomers have an *S*,*S* configuration, whilst the major diastereomer in each case has an *S*,*R* configuration. The stereochemical integrity at C-3 is maintained throughout the PADAM sequence, as previously shown in similar studies.[65]

2.6 Synthesis of the xylyl series.

After the successful synthesis of the cyclohexyl series of analogues, the same approach was used for the synthesis of the second series of analogues, by substituting cyclohexyl isocyanide (**8**) with xylyl isocyanide (**10**) in the Passerini reaction (**Scheme 15, page 31**). This was done to introduce diversity and structural variation in the target library of compounds and to determine

whether branched isocyanides had an effect on diastereoselectivity in the Passerini reaction. A total of 7 analogues were synthesised (compounds **33** – **39**, **Figure 19**). The reactions were still high yielding, with yields ranging between 82% and 99%, except for compound **39** which was isolated in a yield of 49%. This low yield was a result of product losses during purification of the compound.

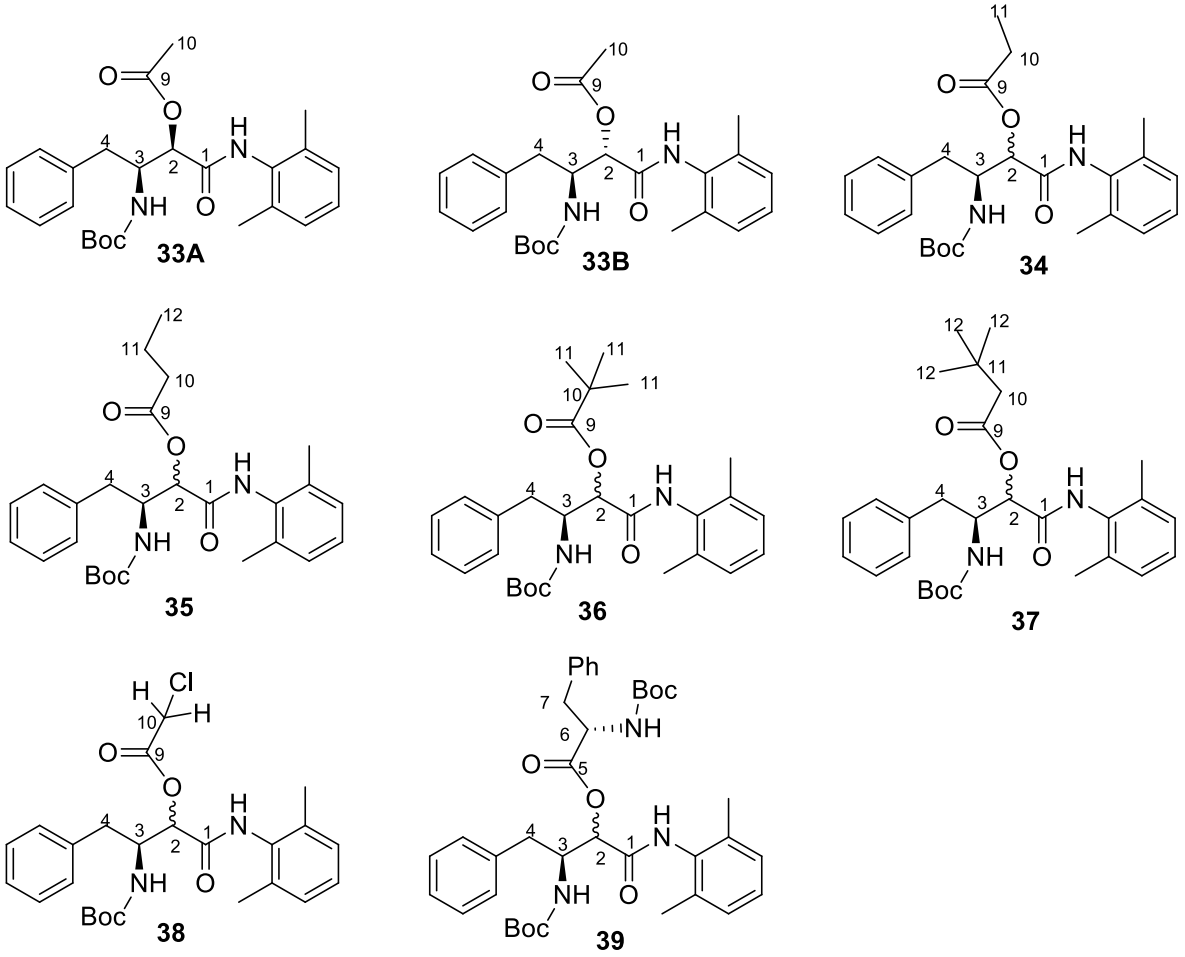


Figure 19: Passerini products derived from xylyl isocyanide.

The solubility of compounds in this series was significantly poorer than that of the previous series. Lack of solubility has been identified as one of the major problems of peptidomimetic analogues, as such a problem is likely to affect the pharmacokinetics of the compounds.[31] Compound **39** precipitated out of solution as it was being synthesised and all attempts to purify the compound by column chromatography or recrystallization were unsuccessful.

Table 4: d.r. for compounds 33 -39.

Compound	33	34	35	36	37	38	39
D.R	1.88:1.00	1.83:1.00	2.06:1.00	1.88:1.00	2.21:1.00	1.72:1.00	1.75:1.00

HPLC analysis was performed on all the compounds shown in **Figure 19**, in order to determine the diastereomeric ratio of the products. Due to the insolubility of the compounds a larger volume of solvent was used and samples were heated in an attempt to ensure complete dissolution prior to performing HPLC analysis. The results of the analysis are shown in **Table 4** and once again indicate a ratio of approximately 2:1 for all products. However, selectivity is shown to differ slightly as the carboxylic acids employed in the reaction change. There is a slight decrease in selectivity when chloro-acetic acid and *N*-Boc-phenylalanine are used (compounds **38** and **39**). The d.r. decreases to 1.72:1.00 and 1.75:1.00 as compared to a selectivity of greater than 1.80:1.00 observed for the other analogues in this series. Despite this slight change in selectivity, it is evident that *N*-Boc-phenylalaninal still has a greater influence over the diastereoselectivity of the Passerini reaction than the carboxylic acid and isocyanide fragments. Using a branched isocyanide did not appear to have any influence whatsoever on the diastereoselectivity of the Passerini reaction in this series.

For all the compounds analysed by HPLC, only compound **33** showed baseline separation in the chromatogram. It was thus the only compound which could be separated on preparative HPLC for collection of the individual diastereomers. Although the solvent system was varied widely, no combination tested was able to give adequate separation of compounds **34** – **39** on the analytical system. The formation of the products was confirmed by NMR and IR spectroscopy and HRMS. IR spectroscopy showed the presence of a strong carbonyl signal from the ester functionality of the compounds, as shown in **Table 5**.

Table 5: IR spectroscopic C=O stretch signal for xylyl Passerini products.

Compound	33A	33B	34	35	36	37	38	39
cm^{-1}	1738	1744	1736	1712	1734	1737	1759	1748

The HRMS results were also as expected, further confirming that the desired products were formed. All expected peaks in the ^{1}H and ^{13}C NMR spectra were observed with the characteristic C-2, C-3 and C-4 signals identified (**Table 6**). The proton spectra showed the

presence of both rotamers and diastereomers. The integration ratio of various signals in the ^{1}H NMR spectra was not the same as the d.r. obtained using HPLC, as evidenced by compounds **34** and **35**. This apparent anomaly points to rotamer effects, a common observation in peptidomimetic compounds.

Table 6: ^{1}H and ^{13}C NMR Chemical shifts for xylyl products.

	H-4	H-3/C-3		H-2/C-2	
	^{1}H NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
33A	δ 2.99 – 2.80 (2H, m)	δ 4.54 – 4.38 (1H, m)	δ 53.19	δ 5.40 – 5.18 (1H, m)	δ 74.14
33B	δ 3.13 (1H, dd, *J* = 14.1, 6.0 Hz), 3.06 – 2.88 (1H, m)	δ 4.43 – 4.34 (1H, m)	δ 53.44	δ 5.36 (1H, d, *J* = 3.9 Hz)	δ 74.93
34	δ 3.18 – 3.09 (0.54H, m, H-4a), 3.05 – 2.80 (1.5H, m, H-4a and H-4b)	δ 4.52 – 4.35 (1H, m)	δ 53.44, δ 53.29	δ 5.43 – 5.28 (0.55H, m, H-2), 5.32 (0.56H, s, H-2)	δ 74.77, δ 73.91
35	δ 3.09 (0.31H, dd, *J* = 14.0, 5.4 Hz, H-4a), 2.99 – 2.52 (1.72H, m, H-4a & 4b)	δ 4.54 – 4.29 (1H, m)	δ 53.29	δ 5.53 – 5.23 (1H, m)	δ 74.77, δ 73.92
36	δ 3.22 – 2.64 (2H, m, H-4)	δ 4.53 – 4.32 (1H, m)	δ 53.55, δ 53.42	δ 5.59 – 5.26 (1H, m)	δ 74.57, δ 73.64
37	δ 3.20 – 2.68 (2H, m)	δ 4.50 – 4.32 (1H, m)	δ 53.57, δ 53.42	δ 5.45 – 5.27 (1H, m)	δ 74.53, δ 73.57
38	δ 3.23 – 2.62 (2H, m)	δ 4.60 – 4.35 (1H, m)	δ 55.84, δ 53.09	δ 5.46 – 5.22 (1H, m)	δ 76.46, δ 75.51
39	δ 3.07 – 2.79 (2H, m)	δ 4.43 – 4.23 (1H, m)	Not confirmed by HSQC	δ 5.22 – 5.12 and 5.06 – 4.94 each 1H	δ 76.19, δ 74.98

It was noteworthy that the ^{13}C NMR spectroscopic signals for C-2 and C-3 for **33B** appeared slightly downfield compared to those of the major product **33A**. This suggests that the same conformational diastereomer is formed as the major product in the xylyl series as was observed for the cyclohexyl isocyanide series. The ^{1}H NMR spectroscopic signals for H-4 in each case are

not as clearly separated as would be expected for diastereomeric protons. The presence of the diastereomers in the unseparated compounds is clearly evident because there are two signals for C-2 in the ^{13}C NMR spectrum for each of the unseparated compounds. For compound **34**, the H-2 signal in the ^{1}H NMR at δ 3.18 – 3.09 correlates to the C-2 signal at δ 73.91 in the HSQC spectrum. This suggests that these signals are for the minor diastereomer. Splitting patterns are distorted because of the likely presence of rotamers and the mixture of diastereomers.

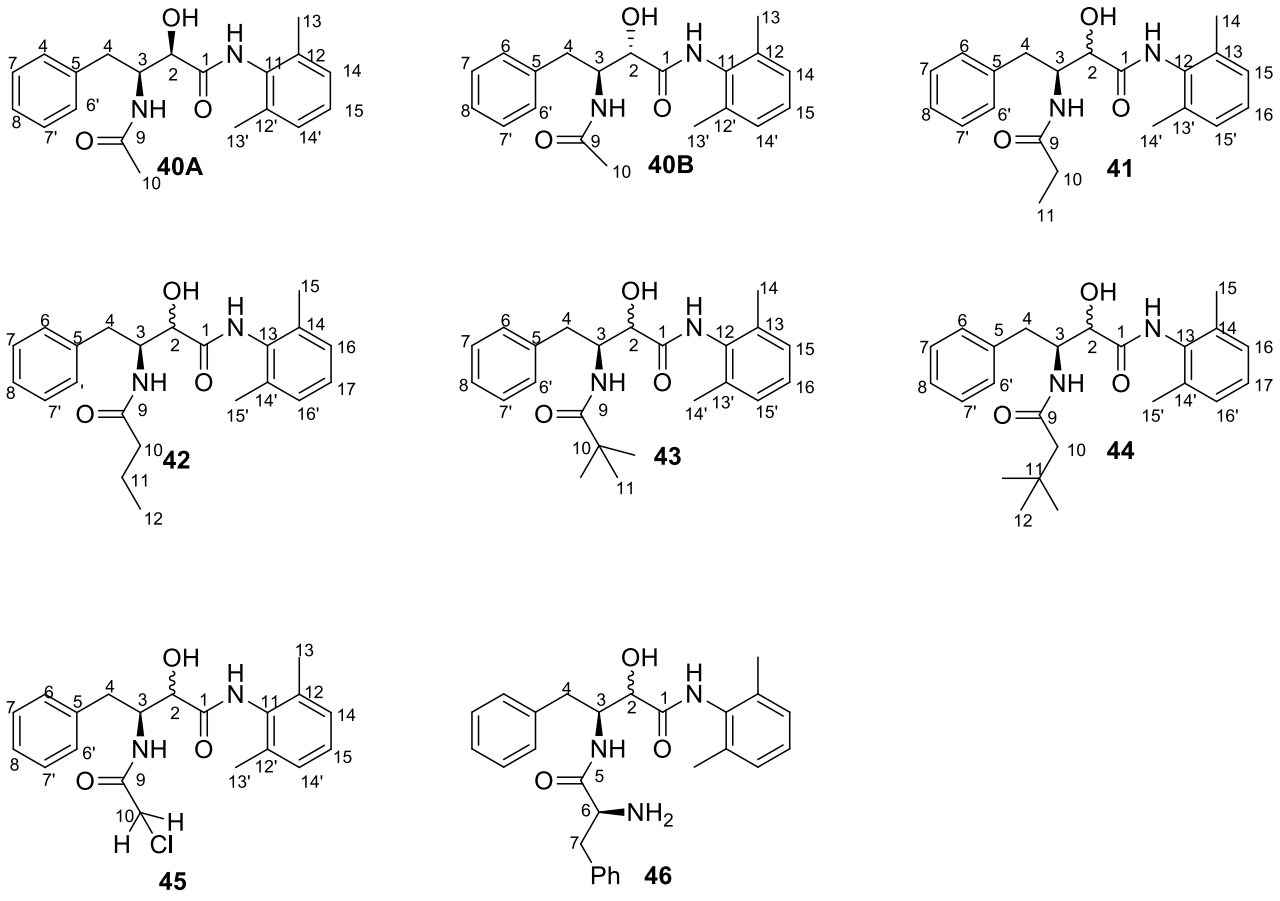


Figure 20: PADAM products for xylyl series.

After the successful synthesis of the Passerini products in this series, we were in a position to subject them to the deprotection acyl-migration sequence. The products of the sequence are shown in **Figure 20**. The reactions proceeded as expected with yields ranging from 70% to 96% and the HRMS results were as expected. Solubility of the compounds remained a problem, with compounds either dissolving only in acidic solution or heated DMSO. However, despite this challenge, NMR spectroscopy was performed for all the compounds.

For compound **40A** IR spectroscopy showed the disappearance of the carbonyl signal at 1738 cm^{-1} for the ester functionality and appearance of an additional amide carbonyl signal. The 2 carbonyl signals were visible at 1658 cm^{-1} and 1617 cm^{-1}. In the ^{1}H NMR spectrum the aromatic protons for the mono substituted phenyl ring appeared as two multiplets at δ 7.25 – 7.17 (4H) and δ 7.13 – 7.08 (1H). This splitting pattern is different to what was observed for the other products in this series where all the aromatic protons appeared together as one multiplet. The aromatic protons of the xylyl ring appeared as a multiplet at δ 7.00 – 6.93. The overlapping of the H-3 proton signal and NH was still observed with compound **40A**, where the signal appears at δ 4.52 – 4.46 as a multiplet integrating for 2 protons. The H-2 proton signal shifts upfield as expected and appears as a doublet at δ 4.06 (*J* = 2.2 Hz). The multiplicity of the signal indicates that it has the proton at H-3 as its neighbour and there is no coupling to OH. The two H-4 protons are clearly in two different environments due to the two observed signals, one at δ 2.89 (dd, *J* = 13.4, 8.1 Hz) and the other at δ 2.82 (dd, *J* = 13.3, 7.4 Hz) each integrating for 1 proton. The Boc signal at δ 1.43 – 1.10 disappeared, showing that N-deprotection had occurred. The aromatic methyl protons appeared as a singlet at δ 2.06 integrating for 6 protons whilst the H-10 signal appeared as a singlet at δ 1.80 integrating for 3 protons.

In the ^{13}C NMR spectrum, the two expected carbonyl signals appeared at δ 172.74 and δ 171.44 and all the expected aromatic carbons were accounted for. Of significance was the disappearance of the Boc carbonyl signal at δ 154.94 and the appearance of the secondary alcohol carbon (C-2) at δ 71.31. The C-3 signal appeared at δ 53.74. The aromatic methyl carbon atoms (C-13 and C-13') appeared as one signal at δ 17.09 and correlate with the proton signal at δ 2.06 in the HSQC spectrum.

Fortunately, NMR spectroscopic analysis for **40B** could be performed in the same solvent as **40A**, and hence the chemical shifts are directly comparable. A difference was observed with the phenyl protons which appeared as a multiplet at δ 7.13 – 7.03 integrating for 5 protons as compared to the separated signals (4H and 1H) observed for **40A**. The H-4 protons appeared as a multiplet at δ 3.02 – 2.88 and the remainder of the signals were the same as for **40A**. In the ^{13}C NMR spectrum, the difference in chemical shifts was evident with the C-3 signal observed at δ 54.11 and δ 53.75 and the C-2 signal at δ 73.58. These observed chemical shifts are consistent

with the observed trend for the Passerini diastereomers. However, there were two signals for C-3 (as above) and C-13 (δ 17.29 and δ 17.09) for compound **40B**, and this was attributed to rotamers. It is noteworthy that the rotamers were only present in the minor diastereomer.

The spectra obtained for compound **41** derived from propionic acid **2** were comparable to those of compound **40**, with the only difference in the ^{1}H NMR spectrum being the extra CH_2 signal which appeared as an overlapping signal with the H-14 signal at δ 2.12 – 1.99 integrating for 8 protons. The rest of the signals for compound **41** were as expected, with the presence of the diastereomers being confirmed by of two H-2 proton signals in the ^{1}H NMR spectrum. These signals appeared as multiplets at δ 4.07 – 3.97 for the major diastereomer and δ 4.23 – 4.17 for the minor diastereomer, in a ratio of approximately 2.7:1.0. The diastereomeric ratio calculated using ^{1}H NMR spectroscopy (2.7:1.0) is much higher than that calculated using HPLC (2:1). HPLC is usually a more accurate method for determining d.r., however in this case complete baseline separation was not achievable and hence the ratios are only an approximate value. The complication presented by the presence of rotamer made using the NMR method for calculating d.r. ill-advised. In the ^{13}C NMR spectrum, C-2 gave rise to two signals for the two diastereomers at δ 74.30 and δ 71.94 whilst C-3 appeared at δ 53.70.

Similarly for compound **42,** C-2 appeared as two signals at δ 74.46 and δ 71.96 whilst C-3 appeared at δ 53.72 and δ 53.67. The H-2 proton gave rise to two doublets in the ^{1}H NMR spectrum for the two diastereomers; one at δ 4.20 (J = 3.3 Hz) for the major diastereomer and the other at δ 4.03 (J = 2.5 Hz) for the minor diastereomer. The ratio of the two signals was approximately 2.7:1.0 which was comparable to the ratio of signals seen for compound **41**.

For compound **43** derived from pivalic acid **4**, the H-2 and H-3 signals overlapped in the ^{1}H NMR spectrum giving rise to a multiplet at δ 4.56 – 4.22, whilst the H-4 signals appeared separate as two multiplets at δ 3.38 – 3.13 and δ 3.12 – 2.81. In the ^{13}C NMR spectrum, the expected C-2 signals appeared at δ 75.54 and δ 73.84, whilst the C-3 signals appeared at δ 57.58 and δ 55.57 for the two diastereomers.

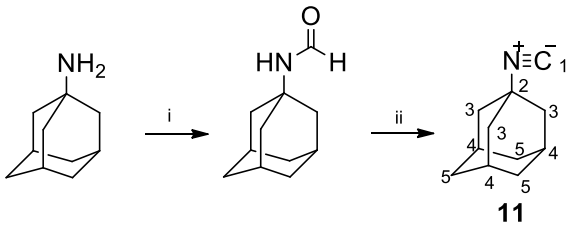
Significant doubling of most signals in the ^{1}H NMR spectrum was observed for compound **44** derived from *t*-butyl acetic acid **5**, because the *tert*-butyl group causes conformational

differences due to its steric bulk. One of the NH signals appeared as two singlets at δ 8.45 for the major diastereomer, and δ 8.25 for the minor diastereomer. Aromatic methyl protons appeared as a multiplet at δ 2.29 – 2.08 integrating for 6 protons. The *tert*-butyl protons appeared as two singlets at δ 1.10 and δ 0.85, with the ratio of protons of approximately 1:2. In the ^{13}C NMR spectrum, the C-3 signals appeared as expected at δ 56.59 and δ 53.86 and the C-2 signals at δ 75.87 and δ 73.67 for the two diastereomers.

Compounds **45** and **46** gave rise to NMR spectra with similar patterns to those observed for compounds **41** – **44**. The *N*-Boc deprotection occurred at both NH's for compound **46**, and this was confirmed by the disappearance of all Boc signals in the ^{1}H NMR spectrum and the appearance of a broad NH_2 signal at δ 1.63 integrating for 2 protons. The migration of the phenylalanine component shows that this approach is versatile and can be used with a wide range of carboxylic acids, including amino acids.

2.7 Synthesis of the adamantyl series

In order to introduce further structural diversity in our library of compounds, we utilised adamantyl isocyanide **11** as one of the components in the Passerini reaction.



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Scheme 20: Reagents and conditions: (i) excess HCOOH, excess Ac_2O (ii) excess $POCl_3$ and Et_3N, dry CH_2Cl_2, 0 °C, 75%.

This approach began with the synthesis of adamantyl isocyanide (**11**) which is readily prepared from adamantylamine as shown in **Scheme 20**. Although adamantyl isocyanide is commercially available, it is very expensive and we chose to prepare it ourselves.[42] The formylation step proceeded smoothly and the identity of the product was confirmed by ^{1}H NMR spectroscopy by the presence of the formyl proton signal at δ 8.38. The formylated adamantyl product was used in its crude form in the subsequent dehydration step without further purification. The work up of the dehydration step must be performed with care to avoid product losses.

Interestingly, signals for both the isocyanide carbon C-1 and C-2 appear as triplets in the ^{13}C NMR spectrum. Crews *et al.* report the same observation and explained this phenomenon as a result of coupling between ^{13}C and ^{14}N as the latter is also an NMR-active nucleus, but it has I = 1 (not I = $^{1}/_{2}$, like proton).[66] This means there are three possible energy levels when under the influence of a magnetic field. The electronic symmetry about the isocyanide ^{14}N nucleus results in a slow quadrupolar relaxation and thus a triplet is observed with coupling constants of approximately 5 Hz. Both the C-1 and C-2 carbons experience this effect giving rise to triplet signals centred at δ 151.59 (*J* = 5.2 Hz) and δ 54.22 (*J* = 11.3 Hz) respectively in the ^{13}C NMR spectrum. The yield was reasonable at 75% and the product had the characteristic pungent isocyanide smell.

With the adamantyl isocyanide in hand we were now in a position to prepare an adamantyl series of Passerini products using *N*-Boc-L-phenylalaninal and the range of carboxylic acids shown in **Figure 11** (**page 21**).

Synthesis of the compounds in this series (**Figure 21**) was successful and the reactions proceeded relatively faster than the cyclohexyl isocyanide and xylyl isocyanide series. Product yields were all above 80% and all the compounds were readily soluble in CH_2Cl_2 and $CHCl_3$. The High Resolution Mass Spectrometry results were as expected for both the diastereomeric mixtures and the separated diastereomers. The melting points of these compounds were generally high, with values above 150°C. The IR spectra were similar to those seen for the previously prepared analogues, with the strong ester carbonyl signal between 1718 cm^{-1} and 1777 cm^{-1} being indicative of the presence of the desired ester functionality.

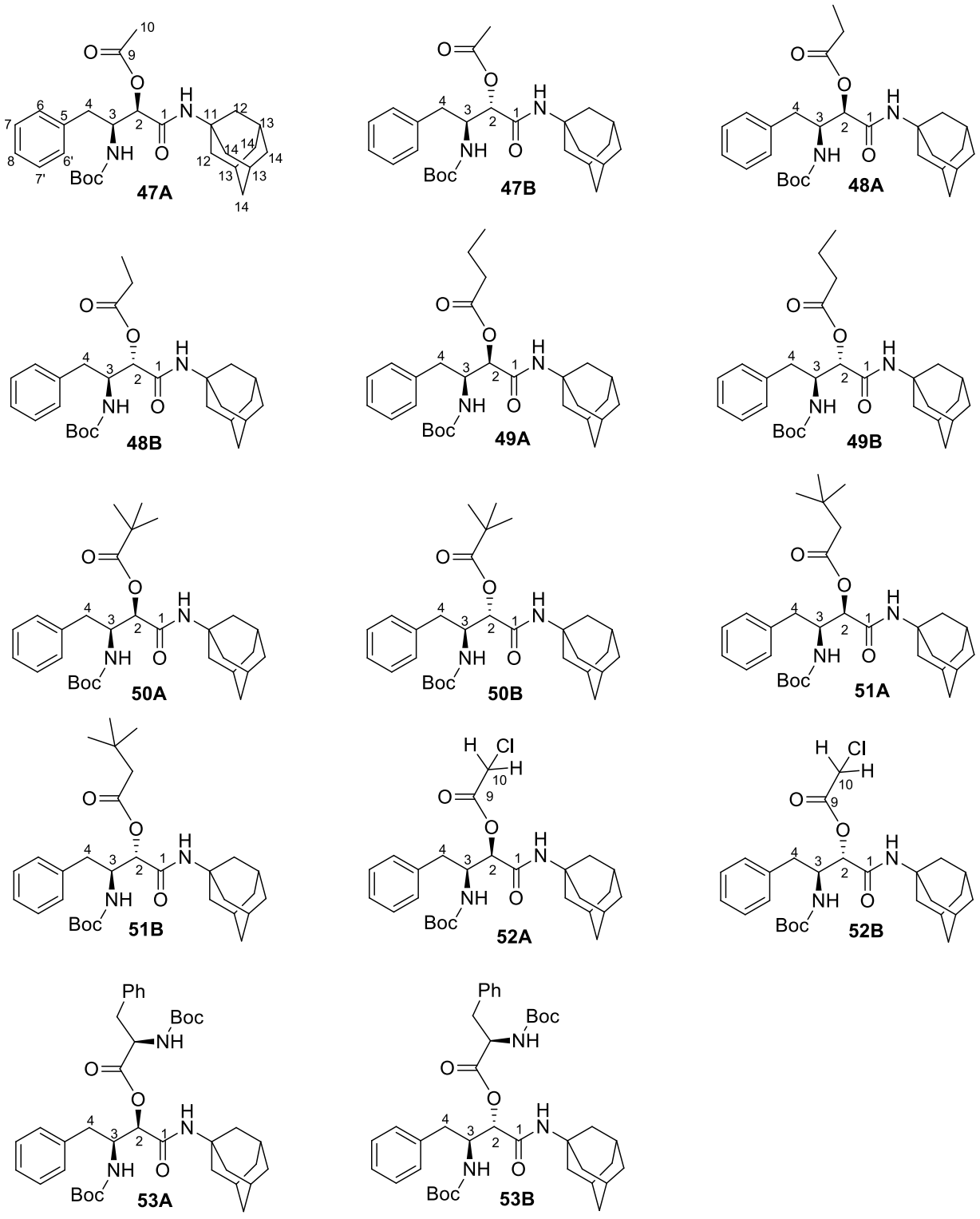


Figure 21: Passerini products derived from adamantyl isocyanide.

Table 7: d.r. for compounds 47 – 53.

Compound	47	48	49	50	51	52	53
D.R	1.96:1.00	1.75:1.00	1.95:1.00	2.12:1.00	2.01:1.00	1.97:1.00	2.49:1.00

The d.r. for the compounds was once again approximately 2:1 with the exception of compounds **48** and **53** (**Table 7**). As was observed for the cyclohexyl and xylyl series prepared previously, the analogues synthesised with propionic acid (**2**) has the lowest d.r. ratio in the series (compound **48**). Use of *N*-Boc-L-phenylalanine (**7**) as the carboxylic acid in the reaction enhanced the formation of the major diastereomer increasing the d.r. to approximately 2.5:1 (compound **53**). Although the change is small, this result sheds some light on how to influence the diastereoselectivity of the Passerini reaction. Baseline separation of diastereomers by HPLC was achieved for all the compounds on the analytical system. This resulted in all of the compounds being separated and collected as single diastereomers with purity of > 99% using preparative HPLC. All the comparable and distinct signals in the ^{1}H NMR and ^{13}C NMR spectra are shown in **Table 8**.

Table 8: Chemical shifts for the Passerini products of the adamantyl series.

	H-4	H-3/C-3		H-2/C-2	
	^{1}H NMR	^{1}H NMR	^{13}C NMR	^{1}NMR	^{13}NMR
47A	δ 2.82 (2H, d, *J* = 7.3 Hz)	δ 4.34 – 4.23 (1H, m)	δ 52.19	δ 5.08 – 4.97 (1H, m)	δ 73.77
47B	δ 2.93 (2H, d, *J* = 7.8 Hz)	δ 4.33 – 4.18 (1H, m)	δ 53.36	δ 5.22 – 5.04 (1H, m)	δ 74.48
48A	δ 2.82 (2H, d, *J* = 7.3 Hz)	δ 4.35 – 4.23 (1H, m)	δ 52.96	δ 5.11 – 5.00 (1H, m)	δ 73.54
48B	δ 2.94 (2H, d, *J* = 7.6 Hz)	δ 4.30 – 4.19 (1H, m)	δ 53.38	δ 5.00 – 4.92(1H, m)	δ 74.27
49A	δ 2.82 (2H, d, *J* = 7.4 Hz)	δ 4.33 – 4.22 (1H, m)	δ 52.98	δ 5.11 – 5.02 (1H, m)	δ 73.48
49B	δ 2.93 (2H, d, *J* = 7.5 Hz)	δ 4.31 – 4.19 (1H, m)	δ 53.36	δ 5.00 – 4.93 (1H, m)	δ 74.14
50A	δ 2.89 – 2.73 (2H, m)	δ 4.37 – 4.18 (1H, m)	δ 53.18	δ 5.18 – 5.03 (1H, m)	δ 73.29
50B	δ 3.02 – 2.87 (2H, m)	δ 4.32 – 4.18 (1H, m)	δ 53.27	δ 4.97 – 4.90 (1H, m)	δ 74.07
51A	δ 2.90 – 2.74 (2H, m)	δ 4.34 – 4.18 (1H, m)	δ 53.04	δ 5.14 – 5.03 (1H, m)	δ 73.50
51B	δ 2.93 (2H, d, *J* = 7.6 Hz)	δ 4.29 – 4.17 (1H, m)	δ 53.42	δ 4.99 – 4.88 (1H, m)	δ 73.92

52A	δ 2.85 (2H, d, *J* = 7.3 Hz)	δ 4.39 – 4.30 (1H, m)	δ 52.77	δ 5.16 – 5.06 (1H, m)	δ 75.01
52B	δ 3.05 – 2.89 (2H, m)	δ 4.35 – 4.25 (1H, m)	δ 53.20	δ 5.14 – 5.01 (1H, m)	δ 75.94
53A	δ 3.13 – 2.95 (2H, m)	δ 4.25 – 4.12 (2H, m) overlapped with H-6	*	δ 5.14 – 5.02 (1H, m)	δ 74.27
53B	δ 3.27 – 3.16 (1H, m) and 3.16 – 2.99 (1H, m)	could not be confirmed by HSQC	*	δ 5.20 – 5.07 (1H, m)	δ 75.74

*Could not be confirmed by HSQC.

The most fascinating trend in these results is the consistency in chemical shifts and multiplicity of signals. This observation also confirmed that the stereochemical identity of the products of the Passerini reaction were the same for the major and minor diastereomer in this series of analogues. The H-2 proton signal in the ^{1}H NMR spectra appeared as multiplets for all compounds in this series. Restricted rotation about the amide bonds is the likely contributing factor for this observation. The coupling constant for the signal due to H-4 was always smaller for the major diastereomers (*J* = 7.3 - 7.4) than the minor diastereomers (*J* = 7.5 - 7.8). This also strongly suggests that therefore similar conformational arrangements at position C-2 and C-3 for the major diastereomers.

The now established trend in the ^{13}C NMR spectra where the C-2 and C-3 signals for the major diastereomer are found slightly upfield when compared to the minor diastereomer, is true for all the analogues in this series. By analogy with the previous series, the major diastereomer has the *S*,*R* configuration and the minor diastereomer has the *S*,*S* configuration. The characteristic H-10 ^{1}H NMR spectroscopic signal for **52A** was evident at δ 4.00 as a singlet integrating for 2 protons and for **52B** the signal appeared as two doublets integrating for 2 protons at δ 4.15 and δ 4.09 with *J* = 14.8 Hz, which is the same trend as was observed for the similar analogues **20A** and **20B**. Other chemical shifts in the ^{1}H and ^{13}C NMR spectra for the analogues in this series were as expected.

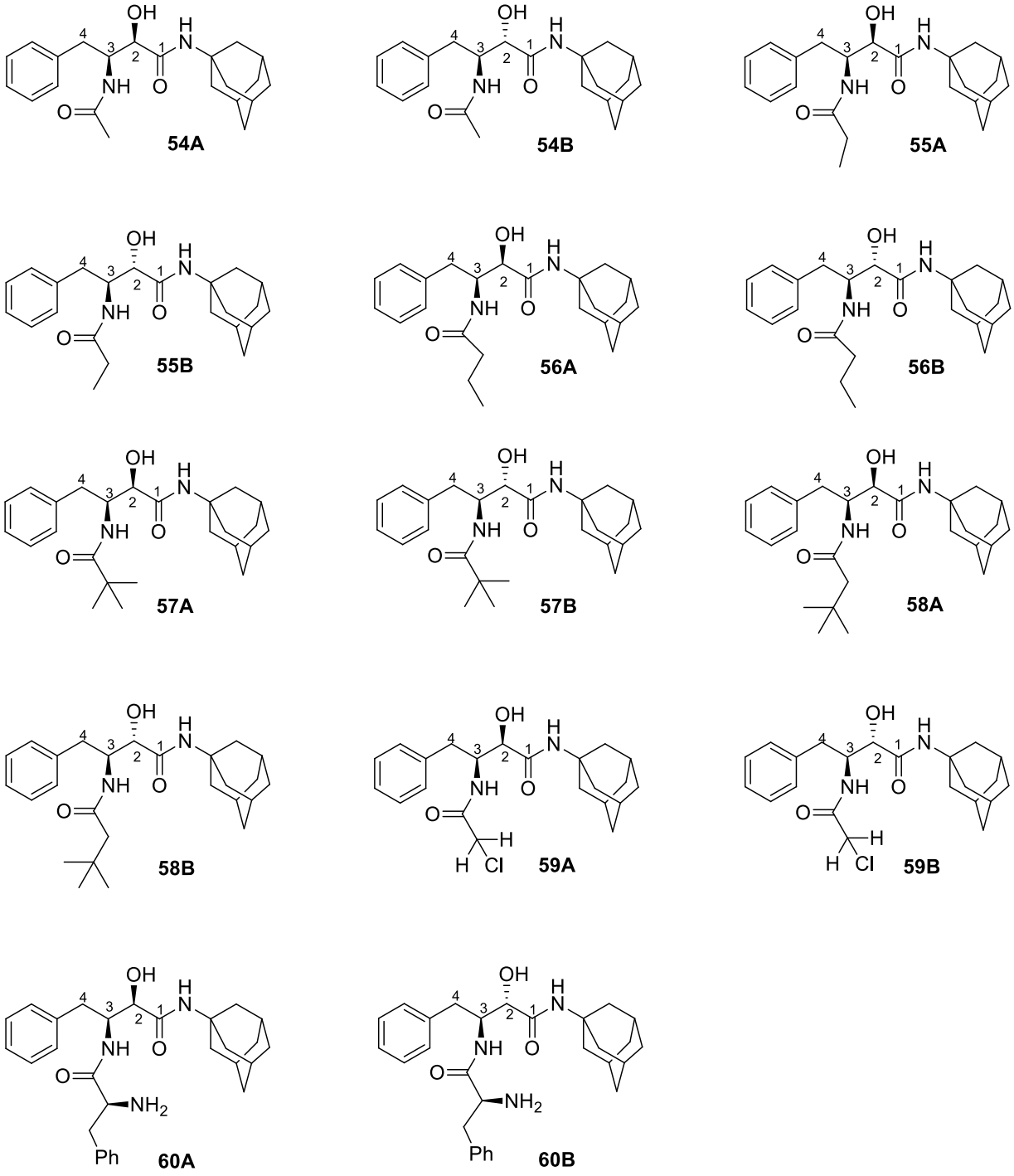


Figure 22: PADAM products for adamantyl series.

The deprotection and acyl migration step was complete in a much shorter time than was observed for the other series of analogues. The products were isolated in high yields of between 70 - 100% (**Figure 22**). Confirmation of formation of the desired alcohol in each case

was confirmed by the appearance of a broad OH signal in the IR spectrum as denoted in **Table 9**.

Table 9: IR OH signals for Adamantyl PADAM products.

	54A	54B	55A	55B	56A	56B	57A	57B	58A	58B	59A	59B	60A	60B
cm^{-1}	3345	3357	3342	3362	3374	3360	3301	3318	3346	3301	3320	3313	3311	3313

The confirmation of the formation of the desired products was further obtained from the ^{1}H and ^{13}C NMR spectra, with the characteristic signals observed as with the previous series. The NMR spectroscopic data is shown in **Table 10**.

Table 10: Chemical shifts for adamantyl PADAM products

	H-4	H-3/C-3		H-2/C-2	
	^{1}H NMR	^{1}H NMR	^{13}C NMR	^{1}NMR	^{13}NMR
54A	δ 3.10 (1H, dd, *J* = 13.7, 6.2 Hz) and 2.92 (1H, dd, *J* = 13.7, 9.2 Hz)	δ 4.14 – 4.01 (1H, m)	δ 55.91	δ 3.97 (1H, dd, *J* = 6.8, 3.4 Hz)	δ 73.22
54B	δ 3.10 – 2.99 (1H, m) and 2.99 – 2.88 (1H, m)	δ 4.26 – 4.13 (1H, m)	δ 57.39, δ 57.29, δ 57.24	δ 4.13 – 4.06 (1H, m)	δ 74.78, δ 74.68
55A	δ 3.08 (1H, dd, *J* = 13.8, 6.2 Hz) and 2.97 (1H, dd, *J* = 13.8, 9.3 Hz)	δ 4.19 – 4.09 (1H, m)	δ 55.74	δ 4.01 – 3.92 (1H, m)	δ 73.52
55B	δ 3.11 (1H, dd, *J* = 14.1, 10.0 Hz,) and 2.99 (1H, dd, *J* = 14.1, 5.6 Hz)	δ 4.21 – 4.14 (1H, m)	δ 57.91	δ 4.14 – 4.09 (1H, m)	δ 75.13
56A	δ 3.11 (1H, dd, *J* = 13.8, 6.2 Hz) and 2.99 – 2.90 (1H, m)	δ 4.20 – 4.09 (1H, m)	δ 55.73	δ 4.01 – 3.93 (1H, m)	δ 73.49
56B	δ 3.13 (1H, dd, *J* = 13.8, 6.2 Hz) and 2.98 – 1.97 (1H ,m)	δ 4.26 – 4.15 (1H, m)	δ 56.48	δ 3.99 (1H, d, *J* = 6.5 Hz)	δ 74.24
57A	δ 3.12 – 2.96 (2H, m)	δ 4.27 – 4.16 (1H, m)	δ 55.46	δ 4.02 – 3.95 (1H, m)	δ 73.85

57B	δ 3.18 (1H, dd, *J* = 14.2, 10.5 Hz) and 3.05 (1H, dd, *J* = 14.1, 5.4 Hz)	δ 4.24 – 4.15 (1H, m)	δ 57.59	δ 4.14 – 4.09 (1H, m)	δ 75.28
58A	δ 3.16 (1H, dd, *J* = 13.8, 6.4 Hz) and 2.92 (1H, dd, *J* = 13.8, 9.3 Hz)	δ 4.24 – 4.15 (1H, m)	δ 55.59	δ 4.00 – 3.92 (1H, m)	δ 73.43
58B	δ 3.14 (1H, dd, *J* = 14.3, 10.3 Hz) and 3.04 (1H, dd, *J* = 14.2, 5.3 Hz)	δ 4.27 – 4.15 (1H, m)	δ 57.77	δ 4.15 – 4.08 (1H, m)	δ 75.36
59A	δ 3.08 (1H, dd, *J* = 13.7, 6.1 Hz) and 2.87 (1H, dd, *J* = 13.5, 8.9 Hz)	δ 4.36 – 4.24 (1H, m)	δ 55.35	δ 4.01 (1H, s, H-2)	δ 72.26
59B	δ 3.09 – 2.95 (2H, m)	δ 4.34 – 4.24 (1H, m)	δ 57.41	δ 4.21 – 4.14 (1H, m)	δ 73.97
60A	3.24 – 3.04 (2H, m, overlapping with H-7a), 3.04 – 2.91 (1H, m, H-4b)	δ 4.34 – 4.23 (1H, m)	*	δ 4.05 – 3.99 and 3.94 – 3.90 (1H, 2xm)	δ 73.38
60B	3.14 – 3.05 (2H, m, overlapping with H-7a), 2.97 (1H, dd, *J* = 14.0, 5.1 Hz, H-4b)	δ 4.24 – 4.13 (2H, m) overlapping with H-2	δ 57.51	δ 4.24 – 4.13 (2H, m) overlapping with H-3	δ 74.93

*Could not be confirmed by HSQC.

The trends observed in the ^{1}H and ^{13}C NMR spectra for the PADAM products of the cyclohexyl and xylyl series of analogues continued in this series without any exceptions. The chemical shifts of the ^{1}H and ^{13}C signals for the major products were slightly more upfield than those of the minor products. In the ^{1}H NMR spectra, the chemical shift for the proton at H-2 shifts upfield when compared to the chemical shift for H-2 of the Passerini products. This is because H-2 is more deshielded by the ester functional group in the Passerini product, when compared to the secondary alcohol of the PADAM product. The H-3 signal remained virtually unchanged, although the splitting pattern was not as expected. This might be due to rotamers resulting from restricted rotation about the amide bonds. This phenomenon was more pronounced for

54B which showed three signals for C-2 and two signals for C-3 in the ^{13}C NMR spectrum. Just as was observed in the cyclohexyl series, the rotamer effect is mainly observed in the NMR spectra for minor diastereomers. Compound **60A** also had two H-2 signals with a ratio of 7:1 which appeared as multiplets at δ 4.05 – 3.99 and δ 3.94 – 3.90 in the ^{1}H NMR spectrum. Both these signals correlated to the signal at δ 73.38 in the ^{13}C NMR spectrum, proving that the signals result from the same proton. The splitting pattern observed for the H-4 protons in the ^{1}H NMR spectrum clearly illustrated the diastereotopic nature of these protons because they couple to one another.

It was very difficult to assign the configuration based on the splitting patterns of the proton at the newly formed stereogenic centre, since the splitting pattern was uneven and inconsistent in the spectra. Only the crystal structure would be able to unequivocally prove the configuration of the diastereomers. We were therefore delighted by the successful crystallization of **55A** as it meant that we were able to obtain a crystal structure from two different series of analogues. This time we were able to crystallise the major diastereomer, and the configuration was confirmed to be the *R*,*S* configuration (**Figure 23**). From this structure it can therefore be seen that the integrity of the stereogenic centre in the starting aldehyde is maintained throughout the reaction sequence.

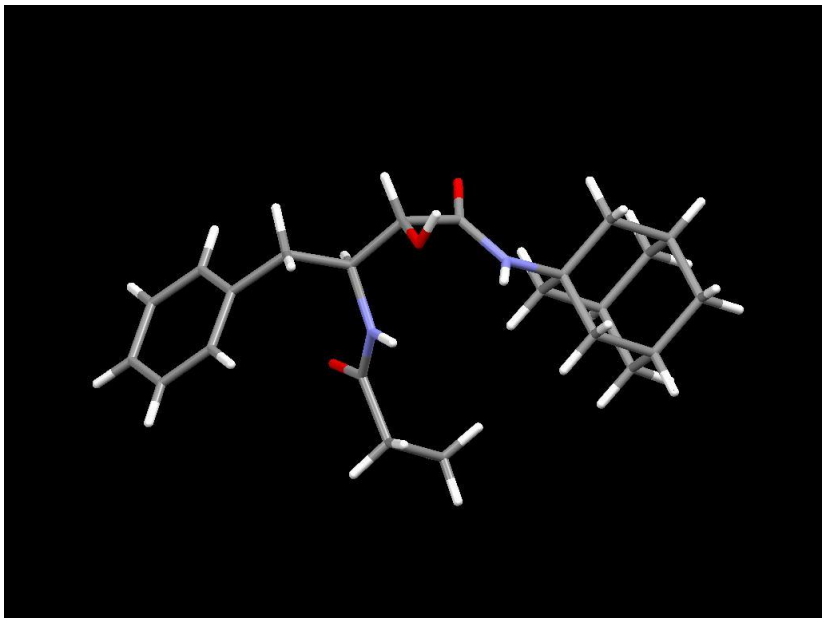
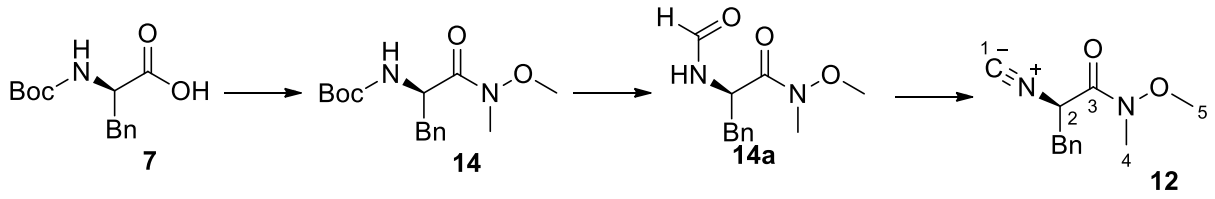


Figure 23: X-ray crystal structure for 55A (data in appendix I).

2.8 Synthesis of phenylalanine isocyanide series.



Scheme 21: Reagents and Conditions:(i) excess HCOOH + Ac_2O (ii) excess $POCl_3$ + Et_3N, dry CH_2Cl_2, 0 °C, 78%.

To test the effect of chiral isocyanides on the stereochemical outcome of the Passerini reaction, chiral isocyanides were synthesised. We chose to use *N*-Boc-L-phenylalanine derived from the naturally occurring amino acid, in our first synthesis of a chiral isocyanide as it was readily available (**Scheme 21**). To this end, Boc-phenylalanine was converted to the Weinreb amide **14**, as we planned to use the Weinreb amide functionality in a post PADAM modification using Grignard reagents. Subsequent *N*-deprotection and formylation was completed in one step, yielding the desired compound **14a** which was used crude in the dehydration step to afford the isocyanide **12** as an oil in a yield of 81%. This isocyanide was less pungent in odour than the simpler isocyanides used before.

Formation of the desired product was confirmed by IR spectroscopy due to the presence of a strong sharp signal at 2143 cm^{-1} characteristic of the isocyanide triple bond. In the ^{1}H NMR spectrum, the loss of the formyl and NH signals was also observed. The characteristic C signal for the isocyanide carbon was also clearly visible at δ 159.28 in the ^{13}C NMR spectrum. The triplet signal that we had observed for the isocyanide carbon in the ^{13}C NMR spectrum for adamantyl isocyanide was not observed for L-phenylalanine isocyanide.

L-phenylalanine isocyanide **12** was then used as a starting material in the synthesis of a new series of analogues using the Passerini reaction (**Scheme 15**). All the compounds prepared using **12** are shown in **Figure 25** (compounds **61** – **63**). The numbering of atoms was kept constant at

the core (C-1 to C-4) but was changed for the other atoms for the purpose of discussion.

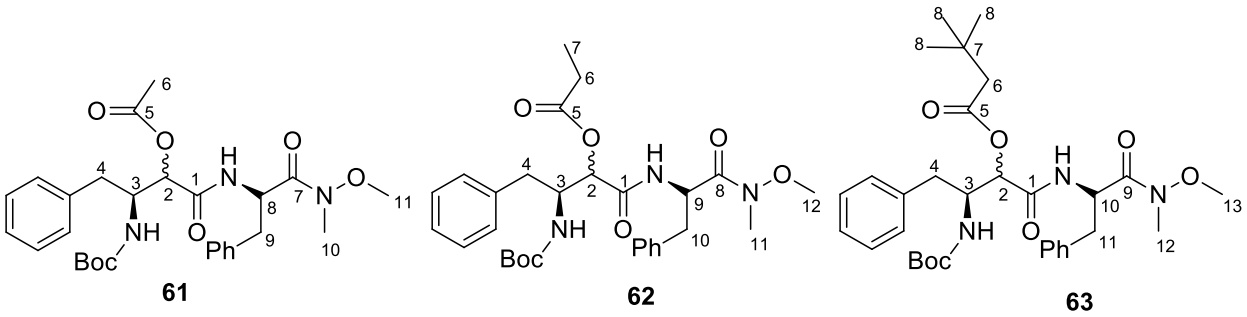


Figure 24: Passerini products for phenylalanine isocyanide series.

The first notable property about this series of compounds was the high solubility of all the compounds when compared to the previously synthesised cyclohexyl, xylyl and adamantyl series. The melting points were also lower when compared to the other analogues, with values of less than 160 °C while HRMS results were as expected (**Table 11**).

Table 11: Melting points, d.r. and HRMS results for compounds in the phenylalanine series.

Compound	61	62	63
D.R	1.52:1.00	1.34:1.00	
m.p./°C	124 - 125	159 - 160	106 – 107
Calculated HRMS	528.2710	542.2866	584.3336
Found HRMS	528.2710	542.2868	584.3338

The d.r. of the compounds was also lower on average, with the largest ratio of approximately 1.5:1.0 observed for compound **61**. Once again the analogue synthesised using propionic acid, compound **62**, had a lower d.r. of 1.34:1.00 as compared to 1.52:1.00 for compound **61**. Although the d.r. values were lower than previously obtained we now had evidence that introduction of a chiral isocyanide could change the d.r. of the products formed. Ideally we would choose to optimise the d.r. in favour of one diastereomer by using an isocyanide derived from unnatural phenylalanine and this will be subject to future work. In this way we would hope to investigate the use of matched aldehyde and isocyanide rather than the mismatch observed in these examples.

Formation of compound **61** was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The phenyl protons were observed as a multiplet at δ 7.40 – 6.99 integrating for 10 protons while the signals for the H-3 and H-8 protons overlapped and appeared as a multiplet at δ 4.46 – 4.15. The methoxy protons H-11 appeared as a multiplet as well at δ 3.83 – 3.58. The multiplet signal observed is due to the diastereomeric signals being closely related and not being resolved into two distinct singlets. The presence of diastereomers is also evident in the ^{13}C NMR spectrum, where there are 8 signals for the four carbonyl carbon atoms present in the compound at δ 171.23, δ 170.88, δ 169.35, δ 168.92, δ 167.55, δ 166.77, δ 155.40 and δ 154.84, with the last two signals belonging to the Boc C=O of each diastereomer. The C-2 signals are also present at δ 74.35 and δ 73.68. The signals for compound **62** were as expected with the characteristic C-2 signals being observed at δ 74.19 and δ 73.51 in the ^{13}C NMR spectrum.

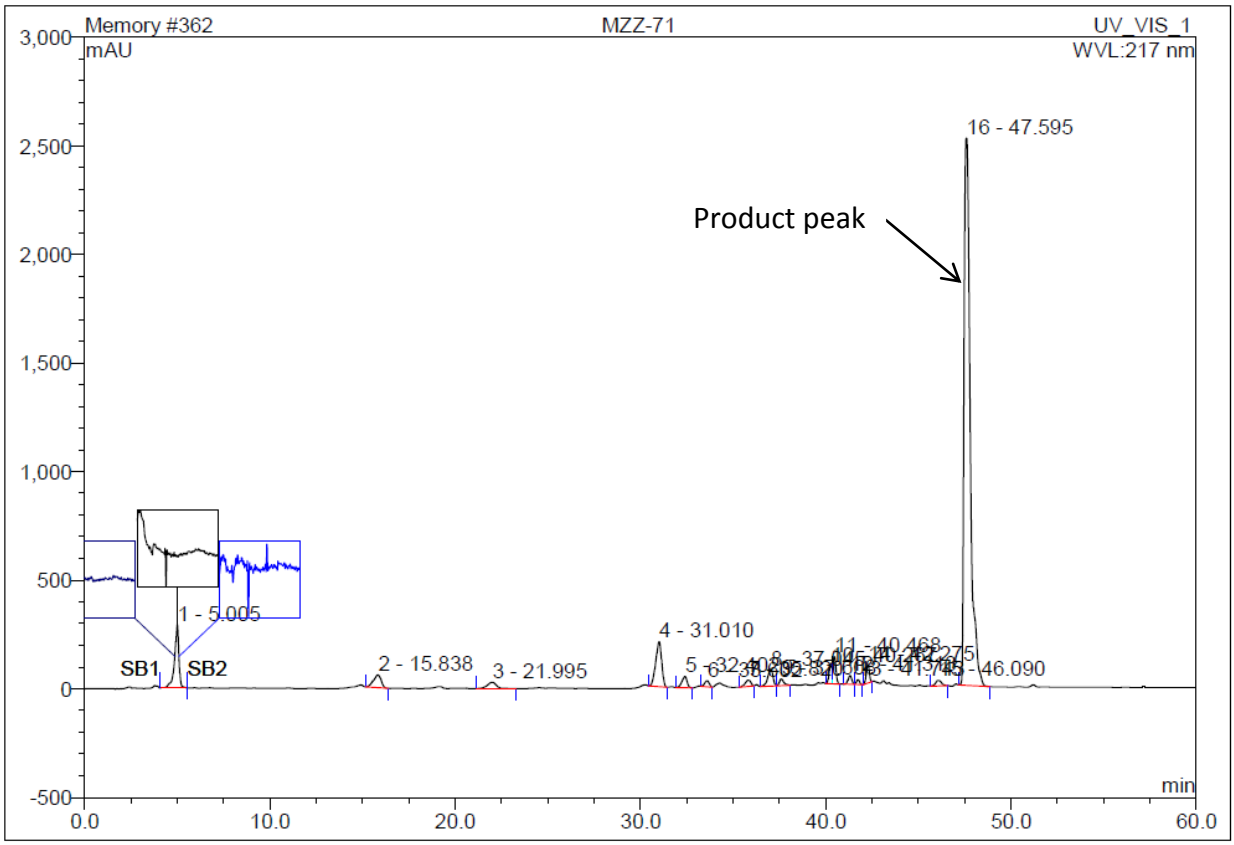
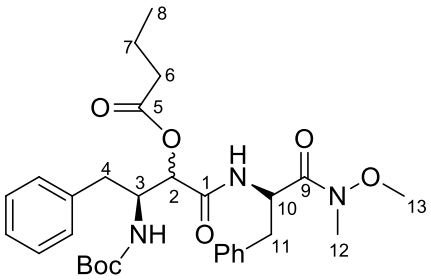


Figure 25: HPLC chromatogram for compound 63.

Surprisingly, synthesis of compound **63** appeared to be selective as only one peak was visible in the HPLC chromatogram as shown in **Figure 25**.

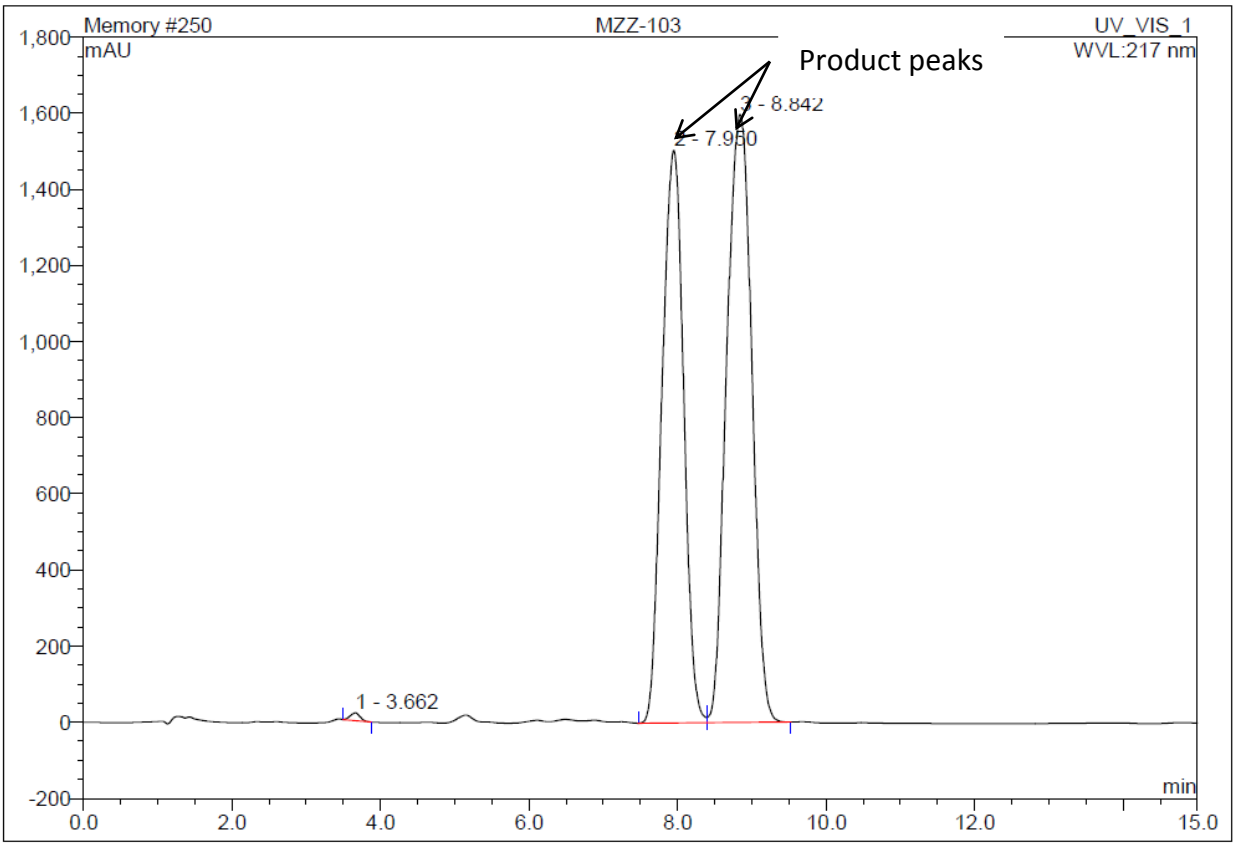
The presence of one diastereomer was also confirmed by the ^{13}C NMR spectrum in which only one C-2 signal was visible at δ 73.21. The doubling up of the ^{13}C NMR spectroscopic signals observed previously with the other analogues was not evident in the ^{13}C NMR spectrum of compound **63**. What could not be established was whether this observation was due to selectivity in the reaction or whether it was due to the unanticipated ability to separate the compounds during column chromatography. Unfortunately the determination of D.R was only performed after purification by column chromatography and not to track the progress of the reaction. This meant that formation of the other diastereomer could not be confirmed. The low yield obtained suggests that separation of diastereomers occurred during purification. This may be the case, as we would expect a higher yield of the single product if the reaction had been selective. Due to time constraints, the reaction was not repeated in order to confirm this hypothesis, but this will be considered for future work.

During the research we came across another method for the Passerini reaction which suggested that, the reaction's selectivity was influenced by thermodynamic factors. Baretto *et al.* reported loss of selectivity of the Passerini reaction when equimolar amounts of starting material were allowed to react under microwave conditions.[67]



64

We decided to synthesise compound **64** to verify this suggestion. The expected product was recovered in a good yield of 83%, with a d.r. of 1.00:0.93 (**Figure 26**). Unfortunately because of the unavailability of the starting material, the reaction could not be repeated using conventional method. However, to test the results of the microwave reaction compound **17** was synthesised again under microwave conditions. The resulting d.r. was 1:1 as compared to the 2:1 previously obtained when conventional heating was used.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	3.66	n.a.	20.556	3.123	0.27	n.a.	BMB
2	7.95	n.a.	1504.924	509.349	44.64	n.a.	BM
3	8.84	n.a.	1597.736	628.471	55.08	n.a.	MB
Total:			3123.216	1140.944	100.00	0.000	

Figure 26: HPLC chromatogram for compound 64.

These results show that the probable pathways for the formation of the two diastereomers become equally possible when sufficient energy is provided to the reaction, indicating that the diastereoselectivity observed is due to an energy difference between the two pathways. One of the pathways is more favourable because it requires less activation energy. Although only two compounds were synthesised in our laboratories using microwave irradiation, we obtained similar results to those reported before by others though they prepared different compounds.[67]

Compounds **61**, **62** and **64** were then subjected to the full PADAM sequence affording products **65** - **67** (**Figure 27**), pending further investigation into the results obtained for compound **63**.

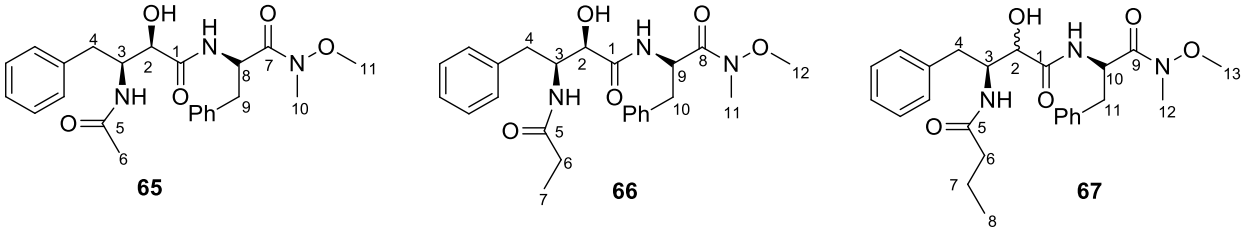


Figure 27: PADAM products of the phenylalanine series.

The NMR spectroscopic data for both compounds **65** and **66** indicated the presence of only one diastereomer although the starting material was a mixture of diastereomers. For compound **65** there was a remarkable difference in the chemical shifts of the signals for the H-3 proton and the H-8 proton in the ^{1}H NMR spectrum. The H-3 proton signal appeared as a multiplet at δ 4.23 – 4.09, whilst the H-8 proton signal appeared as a singlet at δ 5.14. The H-9 protons gave rise to two signals, with one signal overlapping with the H-4 and H-10 signal at δ 3.23 – 2.98 which appeared as a multiplet and the other signal appearing as a doublet of doublets at δ 2.91 with coupling constants of 13.7 Hz and 7.7 Hz and integrating for one proton. There was only one signal in the ^{13}C NMR spectrum for C-2 which appeared at δ 73.40 and similarly one signal for C-3 visible at δ 55.95. There were also only three carbonyl signals, as expected in the spectrum of a single diastereomer. The deprotection-acyl migration step has been shown to be high yielding for the previous series of analogues, but in this instance, the yield was only 53%, which suggests that we had only isolated the major diastereomer from the reaction.

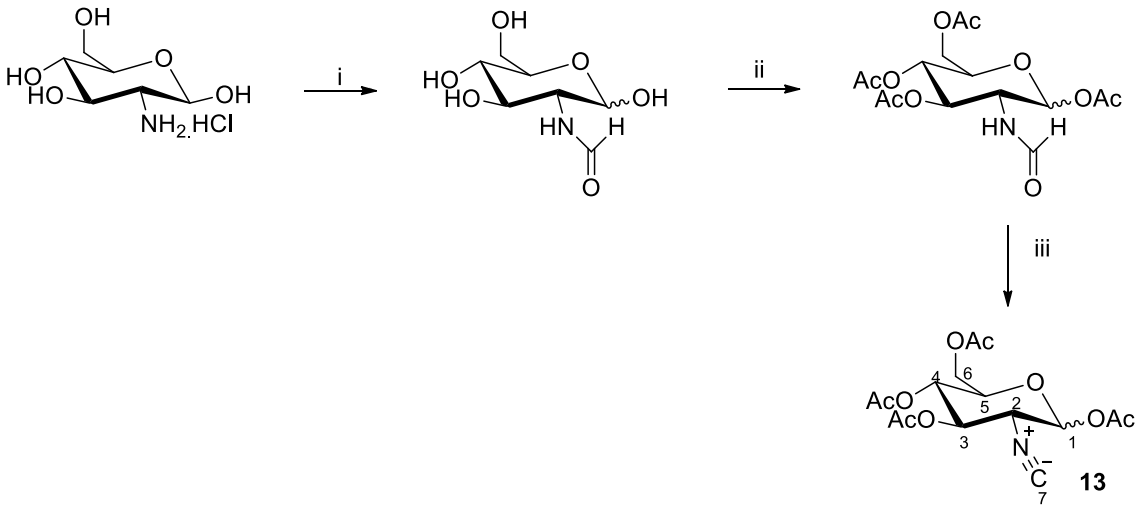
The same signal pattern observed for compound **65** was also observed for compound **66**. In the ^{1}H NMR spectrum, the H-3 signal appeared as a multiplet at δ 4.25 – 4.13 and the H-9 signals also appeared as a multiplet as δ 5.24 – 5.10. The H-4, H-10 and H-11 signals overlapped and appeared as a multiplet at δ 3.21 – 2.85 integrating for 7 protons. HSQC correlation confirmed the multiplet to be due to the H-4, H-10 and H-11 protons. In the ^{13}C NMR spectrum, there were single signals for C-3 at δ 55.56 and for C-2 at δ 73.35. The yield for the deprotection-acyl migration step was also low at 54% and this strongly suggests the isolation of only one diastereomer. The chemical shift of the C-2 and C-3 signals in the ^{13}C NMR spectrum, when compared with the separated diastereomers of previously prepared series, suggests that the diastereomer recovered has the *S*,*R* configuration.

By comparison compound **67** did not show the exclusive presence of one diastereomer as there were signals for both diastereomers in the NMR spectra particularly in the ^{13}C NMR spectrum. There were two signals for C-2, one at δ 74.65 (minor diastereomer) and the other at δ 73.14 (major diastereomer). The yield for the product was 62%, which is slightly higher than the yield obtained observed for the other two compounds in this series.

These findings suggest that separation of the diastereomers occurred during column chromatography, rather than during the reaction. However, the presence of the two diastereomers was not clearly visible on TLC for compounds **65**, **66** or **67**. To verify these findings the reactions will have to be repeated, but due to time constraints this was not done, and will be considered for future work

2.9 Synthesis of the glucose isocyanide series.

The use of a chiral isocyanide derived from phenylalanine in the Passerini reaction showed a slight effect on the diastereoselectivity of the reaction, and this finding had to be probed further. To verify whether the selectivity of the reactions could further be influenced by the use of chiral isocyanides, glucose isocyanide (**13**) which contains multiple stereogenic centres and is sterically hindered, was synthesised (**Scheme 22**).[68]



Scheme 22: Reagents and Conditions:(i) 1 eq Na metal, 4 equiv methyl formate, MeOH, reflux overnight (ii) Ac_2O, pyridine (iii) Excess $POCl_3$, Et_3N, dry CH_2Cl_2, 0 °C, under N_2, 59%.

IR spectroscopy confirmed the formation of the isocyanide by the presence of a characteristic isocyanide triple bond signal at 2153 cm^{-1}. Typically, the synthesis of glucose isocyanide **13** is complicated by the formation of anomers, which are usually separated before the final dehydration step. However, in our synthesis, the separation was unsuccessful and a mixture of anomers was recovered. This was evident in the ^{13}C NMR spectrum where two signals for the anomeric carbon (C-1) were observed, one at δ 91.23 and the other at δ 88.37. In the ^{1}H NMR spectrum there was also evidence of the two anomers with the presence of two anomeric proton signals. Both of the signals appeared as doublets, one at δ 6.41 with J = 3.6 Hz for the major anomer and the other at δ 5.81 with *J* = 8.5 Hz for the minor anomer. The major isomer was determined to be for the equatorial product (β), because the coupling constant of 3.6 Hz corresponds to that expected for an equatorial proton. The ratio of the anomers was calculated to be 3:1. Despite the isocyanide being isolated as an anomeric mixture, it was used as such in the Passerini reaction with the hope that the products arising from the different anomers would be separable either by column chromatography or cystallisation.

A total of 5 compounds (**68** – **69**) were synthesised using this isocyanide, as shown in **Figure 28**.

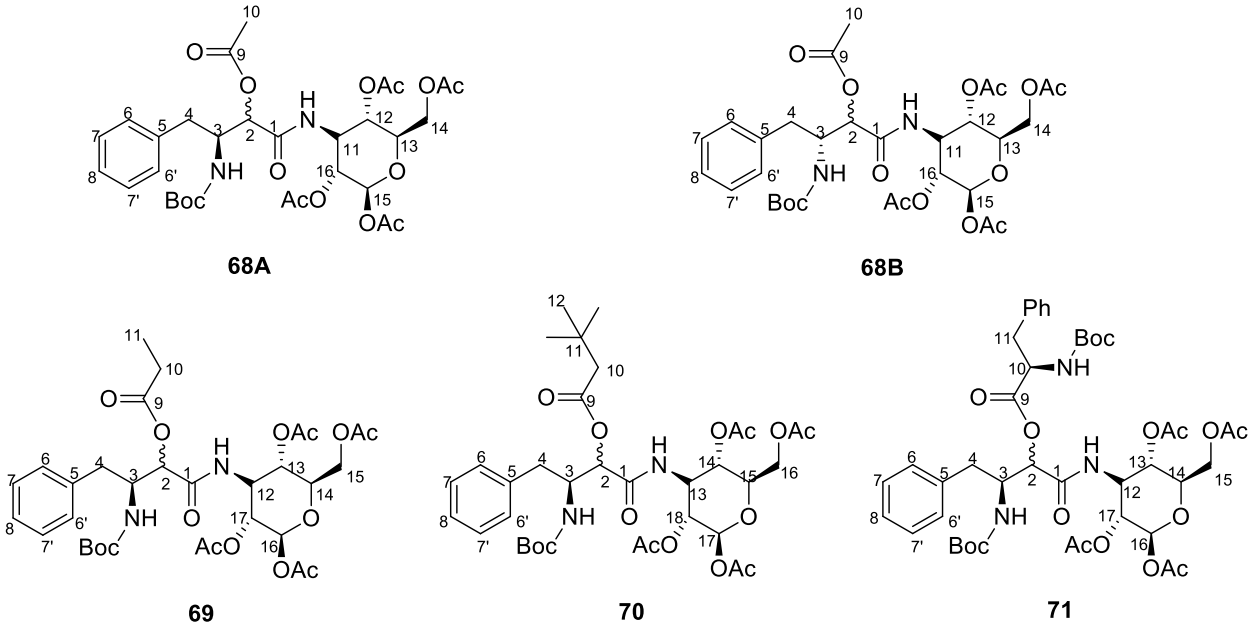


Figure 28: Passerini products for glucose isocyanide series.

The unoptimised yields for the reaction were varied, ranging from 28% to 99%. The product identity in each case was confirmed by ^{1}H and ^{13}C NMR spectroscopy. There was significant doubling up of peaks in the ^{13}C NMR spectra of the products, and the characteristic C-2 and C-3 signals could not be easily distinguished. It was interesting to note that compound **69** synthesised using propionic acid (**2**), had a less complicated spectrum than other analogues in this series. However, the High Resolution Mass Spectrometry results were as expected and the melting points were also higher for this series than those of previously discussed series of analogues (**Table 12**).

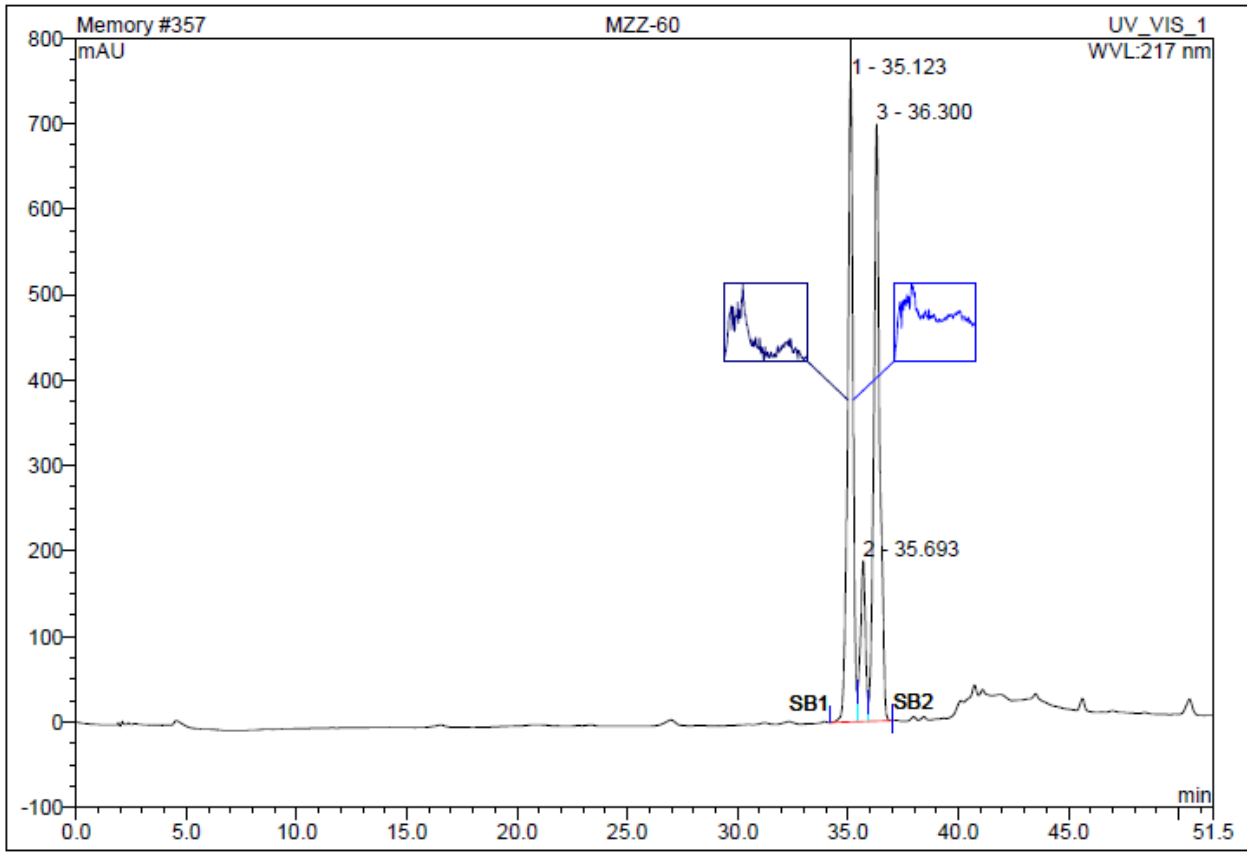
Table 12: Melting points and HRMS results for glucose series.

Compound	68A	68B	69	70	71
m.p./°C	202-204	209-210	181-182	186-187	202-203
Calculated HRMS	667.2714	667.2714	681.2871	723.3340	872.3817
Found HRMS	667.2714	667.2714	681.2874	723.3340	872.3842

Compound **68** was synthesised twice, using both the aldehyde derived from natural and unnatural phenylalanine to establish whether this would have any effect on the diastereoselectivity of the Passerini reaction. Our proposed theory was that the aldehyde was controlling the selectivity via the resolved stereogenic centre, and hence introducing an isocyanide with a resolved stereogenic centre would either increase or decrease the selectivity observed. By using the Boc-phenylalaninal **16**, we hoped to see a significant increase in the formation of one diastereomer, since we assumed that there would be one pathway which would be significantly more favourable. Unfortunately, comparison of the HPLC chromatograms of compounds **68A** and **68B** did not show any of the anticipated results (**Figure 29** and **Figure 30**).

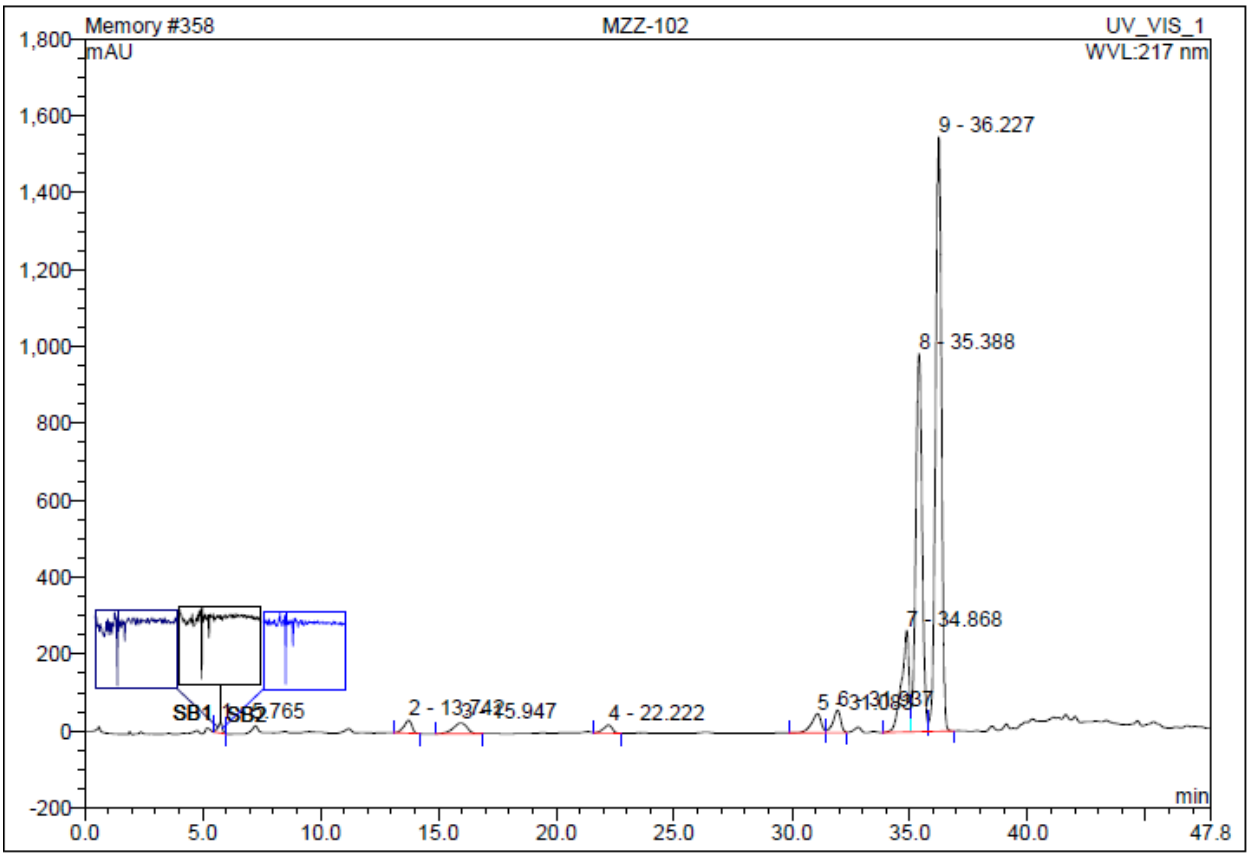
The presence of 3 peaks in the chromatograms of **68A** and **68B** instead of the expected 2 peaks, prevented us from drawing any conclusions about the effect of changing the aldehyde. We had no way of knowing which of the peaks was due to the anticipated diastereomers and which one was due to the presence of anomers. The three product peaks were also observed in the

chromatogram, for compound **71** (**Figure 31**). The only notable difference was the difference in the ratio of the 3 peaks observed in the different chromatograms.



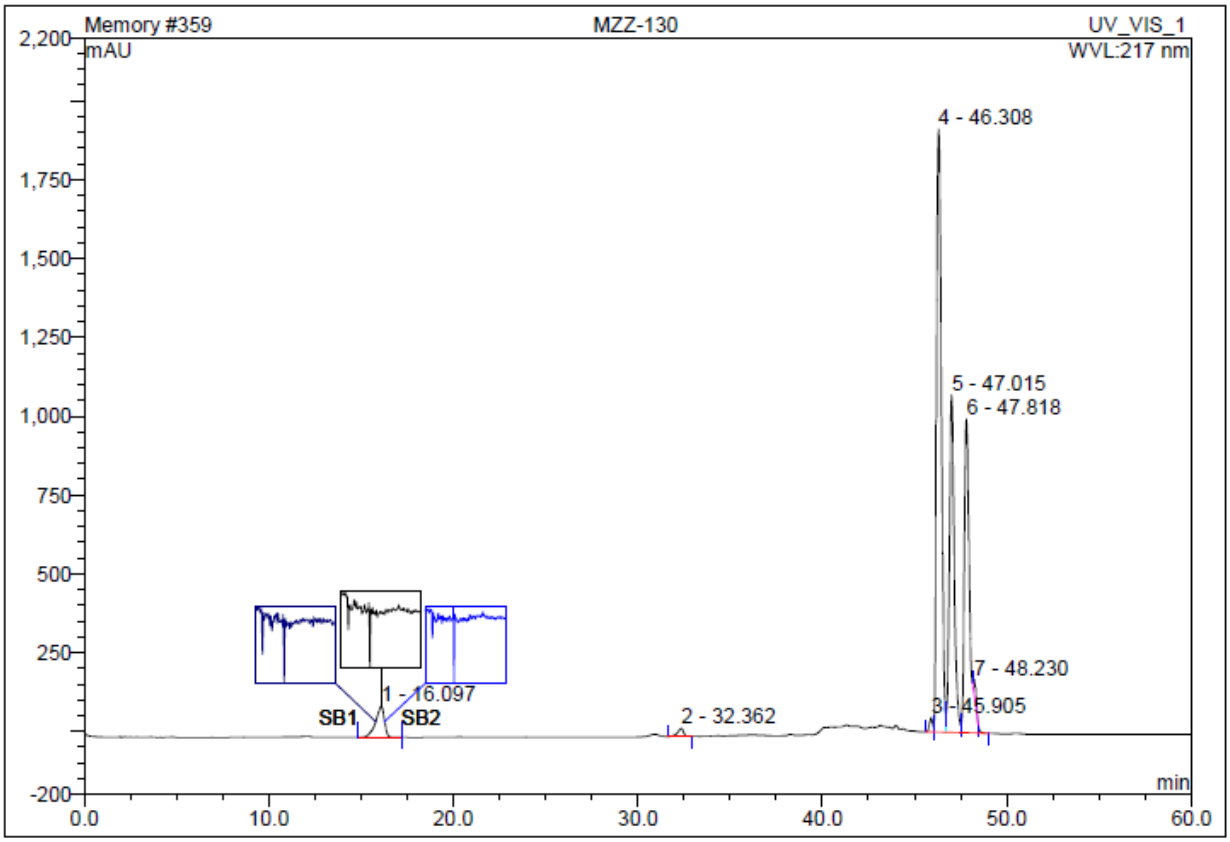
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	35.12	n.a.	754.139	213.758	44.07	n.a.	BM
2	35.69	n.a.	187.969	50.801	10.47	n.a.	M
3	36.30	n.a.	698.305	220.499	45.46	n.a.	MB
Total:			1640.413	485.058	100.00	0.000	

Figure 29: HPLC chromatogram for compound 68A.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5.77	n.a.	26.717	5.877	0.62	n.a.	BMB
2	13.74	n.a.	33.951	13.219	1.39	n.a.	BMB
3	15.95	n.a.	28.047	18.703	1.97	n.a.	BMB
4	22.22	n.a.	20.884	8.946	0.94	n.a.	BMB
5	31.08	n.a.	49.397	23.534	2.48	n.a.	BM
6	31.94	n.a.	57.654	21.554	2.27	n.a.	MB
7	34.87	n.a.	264.138	97.936	10.31	n.a.	BM
8	35.39	n.a.	983.416	306.514	32.27	n.a.	M
9	36.23	n.a.	1545.082	453.458	47.75	n.a.	MB
Total:			3009.286	949.743	100.00	0.000	

Figure 30: HPLC chromatogram for compound 68B.



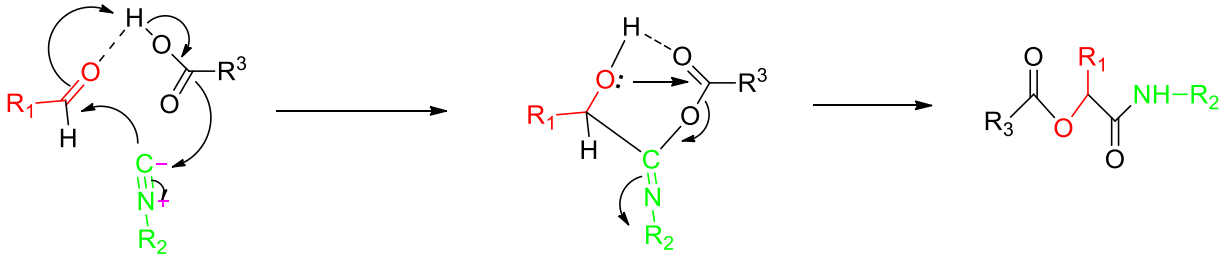
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	16.10	n.a.	98.359	53.851	4.04	n.a.	BMB
2	32.36	n.a.	25.418	9.446	0.71	n.a.	BMB
3	45.91	n.a.	44.123	8.750	0.66	n.a.	BM
4	46.31	n.a.	1911.744	586.827	44.04	n.a.	M
5	47.02	n.a.	1070.361	330.979	24.84	n.a.	M
6	47.82	n.a.	994.718	333.609	25.03	n.a.	MB
7	48.23	n.a.	46.593	9.118	0.68	n.a.	Rd
Total:			4191.315	1332.581	100.00	0.000	

Figure 31: HPLC chromatogram for compound 71.

Because of the presence of too many variables in the Passerini reaction employing glucose isocyanide, the investigation of its effect on diastereoselectivity was suspended pending synthesis of enantiomerically pure isocyanide. The only conclusion that could be drawn from this series of analogues was that glucose isocyanide could be successfully employed in the Passerini reaction.

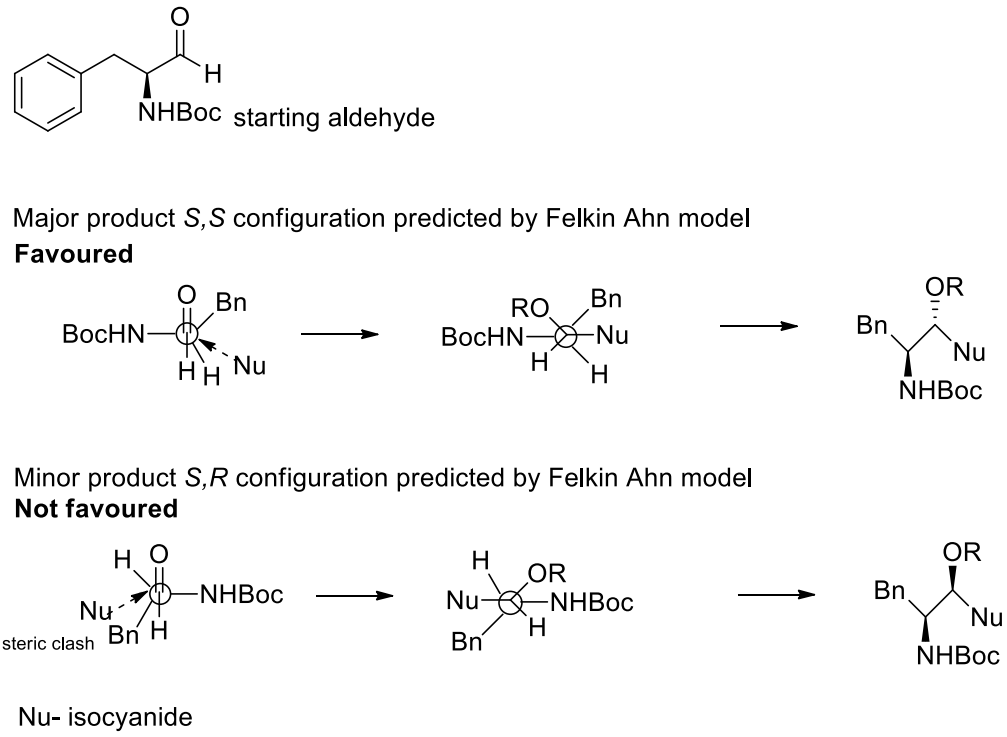
2.10 Proposed mechanism for the Passerini reaction

From the observed results it is clear that the diastereoselectivity of the reaction remained quite consistent throughout the different series, with only minor differences being observed when the Passerini reaction was performed at room temperature in CH_2Cl_2. In each series of compounds prepared, the constant factor was the aldehyde derived from phenylalanine. We therefore suggest that the chiral aldehyde had a more significant influence on the observed diastereoselectivity.



Scheme 23: Mechanism for Passerini reaction.

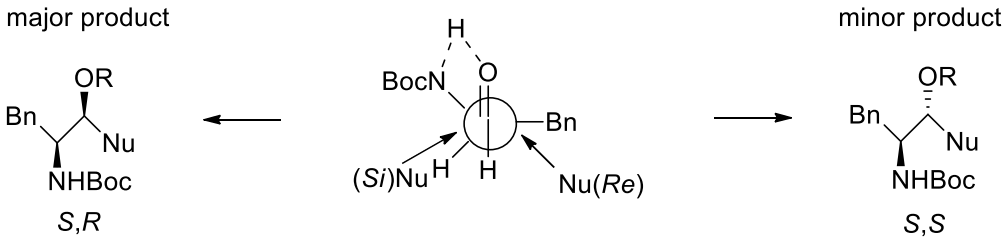
The mechanism for the Passerini reaction is well documented (**Scheme 23**) and is said to be concerted, in the sense that intermediates cannot be isolated to clearly deduce a mechanistic pathway. However, it is likely that the carboxylic acid protonates the carbonyl oxygen of the aldehyde if aprotic solvents are used, making it susceptible to nucleophilic attack by the isocyanide. In our reactions, because the aldehyde bears a stereogenic centre, it can be attacked on one of two diastereotopic faces, the *Re* face or *Si* face, which are distinguished by steric hindrance due to the presence of the NHBoc group. Since the mechanism involves nucleophilic attack on the aldehyde, we initially proposed that a Felkin-Ahn model could explain the selectivity observed (**Scheme 24**). In this case attack at the *Re* face is favoured because this path offers the least steric hindrance due to the placement of the hydrogen and benzyl groups resulting in the *S*,*S* configuration for the major product. The minor product would be formed by the less favoured approach of the Nu on the *Si* face resulting in an *S,R* configuration.



Scheme 24: Felkin Ahn model for the Passerini reaction mechanism.

However, to our surprise, the experimental results we obtained were contrary to that predicted by the Felkin-Ahn model. The experimental results showed that the major diastereomer had an *S*,*R* configuration, whilst the minor diastereomer had an *S*,*S* configuration. These results were baffling indeed and implied that another factor was at play in the diastereoselectivity of the Passerini reaction.

Further analysis revealed that the selectivity that was observed suited a chelation controlled model, although no chelating agent was added to the reaction. Hili disclosed in his PhD thesis that mono-protected amino aldehydes follow a chelation controlled mechanism in nucleophilic addition reactions, which explains the anti Felkin-Anh mechanism observed in our Passerini reaction.[69] From **Scheme 25** it can be observed that chelation control occurs, albeit in the form of hydrogen bonding between the NH and carbonyl oxygen. This changes the bulky group present in the perpendicular position, in turn altering the most favourable pathway. In this model the benzyl group now lies perpendicular to the carbonyl group, hence the isocyanide is more likely to attack via the *Si* face resulting in a product with an *S,R* configuration. This explains the selectivity observed in our Passerini reactions.



Scheme 25: Anti Felkin Anh mechanism.

This proposed mechanism explains our observed results, and confirms that the intramolecular hydrogen bonding between the hydrogen attached to the nitrogen and the carbonyl oxygen of the aldehyde which occurs, prearranges the aldehyde which in turn significantly affects the selectivity of the Passerini reaction at room temperature. The average D.R obtained for all the analogues we synthesised is comparable to those obtained by Banfi *et al*., when they synthesised a library of compounds derived from amino acids.[47] However, they did not determine which was the major and minor diastereomers and they synthesised different types of analogues from the ones that we synthesised.

2.11 Biological testing results of synthesised compounds.

A library of compounds, comprising a few separated diastereomers from the cyclohexyl, xylyl and adamantyl series of peptide mimics were sent for biological testing against HIV (NICD) and malaria (Prof Van Zyl, University of the Witwatersrand, Department of Pharmacy and Pharmacology). Whole cell anti-HIV infectivity assays were performed, but unfortunately none of the compounds showed inhibition (**Table 13**). Lopinavir was used as control and none of the compounds were comparable to the control value as indicated by the IC_{50} values. The lack of activity seems to indicate that the compounds were not entering the cell at all as a diastereomeric mixture of compound **57** had shown activity with an IC_{50} value of 25.0 µM in an enzymatic assay.[42] To further verify this finding, enzyme assays will have to be performed once larger quantities of each product have been made. To establish the toxicity of the compounds, the CC_{50} and CC_{20} tests were performed. The CC_{50} is defined as the concentration of the drug that results in toxicity to 50% of the cells compared with untreated cells and the high values obtained indicate that the tested compounds are quite toxic.

Table 13: Biological results for whole cell HIV assay.

Compound	Toxicity (CC50)µM				Toxicity (CC20)µM				Activity (IC_{50})µM	
	1	2	Average	S.D	1	2	Average	S.D		
18A	100.0	100.0	100.0	0.0	75.7	79.0	77.3	2.4	>77.3	not active
18B	100.0	100.0	100.0	0.0	52.6	80.4	66.5	19.6	>66.5	not active
26A	100.0	100.0	100.0	0.0	59.6	74.0	66.8	10.2	>66.8	not active
26B	96.0	100.0	98.0	2.8	48.1	49.1	48.6	0.7	>48.6	not active
28A	91.2	100.0	95.6	6.2	43.6	34.5	39.0	6.4	>39.0	not active
28B	100.0	100.0	100.0	0.0	81.9	76.5	79.2	3.8	>79.2	not active
29A	100.0	100.0	100.0	0.0	52.3	42.1	47.2	7.2	>47.1	not active
29B	95.8	92.3	94.1	2.5	51.8	34.7	43.3	12.1	>43.2	not active
40A	100.0	100.0	100.0	0.0	51.3	54.3	52.8	2.1	>52.7	not active
40B	89.4	97.3	93.3	5.6	49.8	49.1	49.4	0.5	>49.4	not active
53A	90.7	95.7	93.2	3.5	41.7	38.0	39.8	2.6	>39.8	not active
53B	77.9	76.8	77.3	0.8	29.7	24.3	27.0	3.8	>27.0	not active
54A	78.3	81.2	79.8	2.1	28.2	28.3	28.3	0.1	>28.2	not active
54B	100.0	100.0	100.0	0.0	69.4	77.5	73.4	5.8	>73.4	not active
55A	32.4	37.4	34.9	3.5	12.8	16.4	14.6	2.5	>14.6	not active
55B	21.7	22.0	21.8	0.2	6.0	8.0	7.0	1.4	>6.9	not active
56A	28.5	27.7	28.1	0.6	10.8	12.9	11.8	1.5	>11.8	not active
56B	90.5	74.6	82.5	11.2	20.1	10.6	15.4	6.7	>15.3	not active
57A	65.7	63.6	64.7	1.5	45.2	40.7	43.0	3.2	>42.9	not active
57B	40.2	51.5	45.9	8.0	18.2	21.9	20.0	2.6	>20.0	not active
Lopinavir									0.0010	

Three compounds **50A, 60A and 60B** showed inhibition of parasitic growth in the antimalarial assay (**Table 14**). It is interesting to note that compound **50A** is a Passerini product which has not gone through the amine deprotection and acyl migration sequence. Another noteworthy point is the fact that only products from the adamantyl series showed activity. However, the inhibition values were too high for IC_{50} of the compounds to be performed.

Table 14: Antimalarial assay against FCR-3chloroquine-resistant strain – sybr green I assay; n=3.

Compounds tested at 5µM		
	% parasite growth at 5µM	
Compound	**Average**	**SD**
18A	81.74	11.63
18B	84.94	13.65
27A	85.34	7.74
27B	84.01	10.44

28A	86.78	9.92
40A	82.97	12.74
40B	110.82	13.10
54A	84.53	16.19
54B	87.45	6.70
55A	87.20	10.01
55B	80.90	12.08
56A	86.87	14.16
58A	80.65	8.91
58B	82.57	8.49
50A	63.46	7.75
50B	94.46	7.79
60A	59.68	8.33
60B	58.74	7.56
Dihydroartemisinin (200nM)	50.03	11.55
Dihydroartemisinin (IC50: nM)	0.00269075	0.0003464

The lack of significant biological activity of our compounds implies that we should revisit the design of the compounds, or synthesise a new set of analogues before biologically testing them again.

CHAPTER 3: CONCLUSION

In this research we aimed to synthesise a library of peptidomimetic compounds for biological testing against HIV-1 and malaria. These compounds were synthesised using the Passerini reaction, a multicomponent reaction that couples an aldehyde, isocyanide and carboxylic acid. *N*-Boc-phenylalaninal was used as the aldehyde component for all the analogues synthesised because hydrophobic residues like phenylalanine are present in the currently used HIV-1 protease inhibitors. The Passerini reaction yielded a diastereomeric mixture of products, which were in most cases not separable by purification using silica gel column chromatography. The Passerini product was then subjected to *N*-deprotection which resulted in acyl migration to give the desired compound, using an approach named Passerini Amine Deprotection Acyl Migration sequence (PADAM). The sequence yielded our desired target compounds which had an α-hydroxy-β-amino acid functionality.

Synthesis began with the successful preparation of the aldehyde which was then employed in the preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate, being the Passerini product of *N*-Boc-phenylalaninal, acetic acid and cyclohexyl isocyanide. Deprotection of this diastereomeric mixture resulted in the formation of the PADAM product (3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide.

Both the Passerini and PADAM products were then subjected to kinetic enzymatic resolution in an attempt to resolve the diastereomers. A range of lipase enzymes were employed for both the hydrolysis and acetylation reactions, but success was not achieved. Although other researchers have reported success with the enzymatic resolution of Passerini products, close inspection of the literature showed that enantiomeric mixtures of smaller substrates were successfully resolved, while nothing has been reported for substrates of the size used in this research. The catalytic activity of an enzyme is solely dependent on the substrate entering the active site and the larger the substrate, the less chance it has of fitting in the active site in order to be hydrolysed or acetylated. This reasoning was strongly supported when a smaller substrate (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(butylamino)-1-oxo-4-phenylbutan-2-yl acetate was synthesised by replacing cyclohexyl isocyanide with *n*-butyl isocyanide. After subjecting the

compound to enzymatic hydrolysis, there was an observable reaction, although it lacked selectivity. Several other analogues were synthesised in the hope that variation of the structure would increase the chances of enzymatic resolution, but once again this was not successful. In our research the widely purported versatility of lipase enzymes did not work in our favour. Further investigations regarding enzymatic resolution will have to be performed.

Preparative HPLC was then engaged successfully as an alternative to resolving the diastereomers of the Passerini reaction. Success depended on the ability to attain baseline separation of the diastereomeric peaks when developing the method. Resolution of compound (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate gave pure separated diastereomers, (2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate and (2*S*,3*S*)-3-((*tert*-butoxycarbonyl) amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate. The PADAM sequence was then successfully completed on the separated diastereomers resulting in compounds (2*R*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide and (2*S*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide.

After confirmation of the PADAM sequence and the successful resolution of the diastereomeric products of the Passerini reaction, the first series of analogues was prepared using cyclohexyl isocyanide with propionic acid, butyric acid, pivalic acid, *tert*-butyl acetic acid, chloroacetic acid and *N*-Boc-L-phenylalanine. Determination of the d.r. was performed using HPLC analysis of the Passerini products. The d.r. for analogues in this series ranged from 1.87:1.00 to 2.14:1.00. The ^{13}C NMR spectra for the separated diastereomers exhibited similar patterns of peaks which led us to conclude that the configuration of the major and minor diastereomers was the same in each case. Successful crystallisation of the minor diastereomer (2*S*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide, showed an *S* configuration at the new stereogenic centre. This led us to assign an *S*,*S* configuration to the minor diastereomers and an *S*,*R* configuration to the major diastereomers.

The second series of analogues was then synthesised by replacing cyclohexyl isocyanide with xylyl isocyanide. The d.r. of the products ranged from 1.72:1.00 to 2.21:1.00. The selectivity observed for this series was not significantly different from that observed for the cyclohexyl

series. Only one compound was separable by preparative HPLC in this series. This was a result of observed co-elution of diastereomers of the other analogues. However, the same pattern in the ^{13}C NMR spectra observed in the cyclohexyl series was also observed in the xylyl series of analogues. This suggested that the configuration of the major diastereomers and minor diastereomers was the same as that obtained for the cyclohexyl series.

Synthesis of the third series of analogues employed adamantyl isocyanide. The d.r. of the analogues ranged from 1.75:1.00 to 2.49:1.00. The lowest d.r. was for the analogue synthesised with propionic acid and the highest d.r. was for the analogue synthesised with phenylalanine. The highest d.r. was likely due to the steric hindrance offered by a bulkier phenylalanine and the presence of an additional stereogenic centre. All of the analogues in this series were separable by preparative HPLC yielding pure diastereomers. The observed patterns in the ^{1}H and ^{13}C NMR spectra for the major diastereomers and minor diastereomers were as expected from previous series. By analogy with the cyclohexyl series, the configuration for the major diastereomers was assigned *R,S* and that of minor diastereomers was assigned *S,S*. Successful crystallisation of the major diastereomer (2*R*,3*S*)-*N*-adamantan-1-yl-2-hydroxy-4-phenyl-3-propionamidobutanamide confirmed the configuration of major diastereomers to be *R*,*S*.

In an attempt to significantly affect the selectivity of the Passerini reaction, we incorporated chiral isocyanides into the Passerini reaction. We first synthesised phenylalanine isocyanide from the naturally occurring amino acid. Only three analogues were synthesised in this series because of limited starting materials. The d.r. for the products was 1.52:1.00 for the acetic acid derived analogue and 1.34:1.00 for the propionic acid derived analogue. Once again the propionic acid containing analogue had the lowest d.r. The analogue synthesised using *tert*-butyl acetic acid was recovered as one diastereomer, possibly as a result of separation during purification by silica gel column chromatography. Analogues synthesised from glucose isocyanide will have to be revisited, as the glucose isocyanide was found to be present in both epimeric forms.

In general the d.r. was found to be approximately 2:1 for analogues prepared using carboxylic acids and isocyanides not containing a stereogenic centre, showing the significant role played by the aldehyde in diastereoselectivity. Interestingly, analogues containing propionic acid

always had a lower d.r. for all the series prepared. Only one carboxylic acid with a stereogenic centre was used in the Passerini reaction and the highest diastereoselectivity was observed for this carboxylic acid in the adamantyl series, where a d.r. of 2.49:1.00 was obtained. When an isocyanide with a stereogenic centre was used in the reaction the d.r. was lowered to approximately 1.5:1.00. This led us to suggest that the isocyanide had a mismatch effect when combined with the effect of the aldehyde. However, because of time constraints further investigations into this observation could not be performed. Despite this, the results obtained thus far strongly suggest that the Passerini reaction can be influenced by steric bulk and existing stereogenic centres offered by starting materials.

The Felkin-Ahn chelation model best explains our observed results with respect to the major and minor diastereomers obtained. It suggests that mono-protected amino aldehydes follow a chelation controlled mechanism in nucleophilic addition reactions. This chelation control occurs in the form of hydrogen bonding between the NH and carbonyl oxygen.

X-ray crystallography of compounds (2*R*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenyl butanamide and (2*R*,3*S*)-*N*-adamantan-1-yl-2-hydroxy-4-phenyl-3-propionamidobutanamide, enabled the assignment of the absolute configurations of the diastereomers and assignment of configurations for the other diastereomers was done by analogy. The major diastereomer was identified as that with the *R*,*S* configuration while the minor diastereomer had the *S*,*S* configuration.

Having successfully synthesised our target compounds, 20 stereochemically pure diastereomers were sent for biological testing against both HIV-1 and malaria. In the antimalarial assay three compounds showed inhibition of parasitic growth by 37 – 42% at 5 μM concentration. This is a good starting point for the development of peptidomimetic antimalarials. What is interesting is that all the compounds that showed activity are part of the adamantyl series of analogues. Adamantyl-based compounds are showing increasing utility in the treatment of many different conditions, including viral infections. These promising results provide a basis for further investigations into the synthesis of additional such analogues. HIV-1 biological assays did not yield any promising results.

Future work

Since the PADAM approach has been shown to be effective and versatile in synthesising peptidomimetic compounds, there is a need to find enzymes which can effectively resolve the diastereomers that are formed. The phenylalanine isocyanide series will have to be revisited to conclusively establish the reason for the isolation of individual diastereomers after purification. The synthesised glucose isocyanide was shown to have epimerized, affecting the results observed after the Passerini reaction. There is need to synthesise a single epimer of the glucose isocyanide before using it as a starting material in order to clearly establish its effect on diastereoselectivity. Since the biological testing results were negative for the anti HIV-1 assay, there is a need to establish whether it was because the compounds were not active against the enzyme or whether the compound was not entering the cell. Only one Passerini product was tested for activity against the malaria parasite and showed moderate parasitic inhibition. It will therefore be worthwhile to resynthesize the Passerini compounds and assess their antimalarial activity since focus was only given to the PADAM products.

CHAPTER 4: EXPERIMENTAL PROCEDURES

4.1 General Experimental Procedures

4.1.1 Purification of Solvents and Reagents

All solvents used for reactions and column chromatography were purified by distillation and when required solvents were stored over activated molecular sieves (4Å) under a nitrogen atmosphere. THF and toluene were dried over sodium wire and distilled, with benzophenone as indicator. CH_2Cl_2 and MeCN were distilled from calcium hydride. All other reagents and solvents for HPLC were obtained from commercial sources and used without further purification.

4.1.2 Chromatography

Thin layer chromatography was carried out using aluminium-backed Machery-Nagel ALUGRAMSil G/UV_{254} plates pre-coated with 0.25 mm silica gel 60. Column chromatography was performed on Merck silica gel of particle size 0.035 – 0.070 mm employing different types of solvents. An Agilent 3000 instrument was used for all preparative HPLC on an Agilent prep C18 reverse phase column (21.2x20 mm) with a binary solvent system using gradient elution of MeCN/H_2O and a flow rate of 20 ml/min. For all analytical HPLC a Dionex Ultimate 3000 instrument was used, employing a Luna C18 reverse phase column gradient elution, at a flow rate of 1ml/min with detection at 217 nm.

4.1.3 Spectroscopic and physical data

All melting points were obtained using a Stuart SMP10 melting point apparatus and are uncorrected.

Infrared spectra were obtained using a Bruker Vector 22 spectrometer where measurements were made by directly loading the sample onto the diamond cell. All signals are reported on the wavenumber scale (v/cm^{-1}).

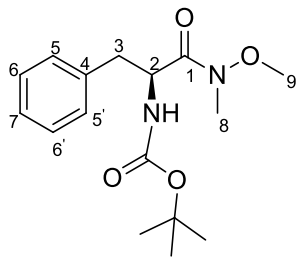
^{1}H NMR and ^{13}C NMR spectroscopy were performed using a Bruker Avance-300 or a Bruker Avance-500 spectrometer at 75 MHz and 125 MHz respectively. Chemical shifts are reported in parts per million and referenced against the internal standard, tetramethyl silane (TMS), which

occurs at zero parts per million downfield and coupling constants are given in Hertz. ^{13}C NMR chemical shifts are reported to 2 decimal places, as this allows for assignment of C signals of diastereomeric products. The difference in diastereomeric signals is only evident at the second decimal place.

High resolution mass spectra (HRMS) were recorded on a Waters Synapt G2. All data was quoted in relative abundance (m/*z*).

4.2 Preparation of (*S*)-*tert*-butyl (1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate 14

Commercially available *N*-Boc-L-phenylalanine (4.00 g, 0.0151 mol) was dissolved in CH_2Cl_2 (40 ml) and CDI (1.2 eq., 2.93 g, 0.0181 mol) was then added in portions, allowing effervescence to cease before each subsequent addition. The reaction was left stirring for 30 min in air before adding *N,O*-dimethylhydroxylamine hydrochloride (1.2 eq., 1.76 g, 0.0181 mol). The mixture was allowed to stir overnight at room temperature in air. The reaction mixture was then diluted with CH_2Cl_2 (90 ml) followed by addition of H_2O (90 ml). The organic layer was separated and further washed with 2M NaOH (90 ml) followed by 1M HCl (90 ml) and then dried over $MgSO_4$. Solvent was removed *in vacuo* and the product was purified using column chromatography (3:7 EtOAc/hex) yielding **14** as clear viscous oil (3.82 g, 82%).

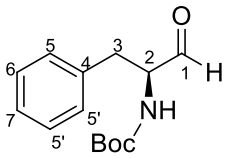


R_f: 0.37 (3:7 EtOAc/hex); **IR**: ν_{max}(cm^{-1}): 3326 (NH), 2960 (CH), 1716 (C=O), 1666 (C=O), 1390 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.37 – 7.12 (5H, m, Ar-H), 5.27 – 5.09 (1H, m, NH), 5.04 – 4.86 (1H, m, H-2), 3.65 (3H, s, H-9), 3.16 (3H, s, H-8), 3.05 (1H, dd, *J* = 13.5, 6.1 Hz, H-3a), 2.95 – 2.80 (1H, m, H-3b), 1.39 (9H, s, $(CH_3)_3C$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.32 (C-1), 155.16 (Boc C=O), 136.62 (C-4), 129.45 (C-6 and C-6'), 128.33 (C-5 and C-5'), 126.73 (C-7), 79.54 ($(CH_3)_3\underline{C}$), 61.50 (C-9), 51.56 (C-2), 38.90 (C-3), 32.03 (C-8), 28.31 ($(\underline{C}H_3)_3C$).

4.3 Preparation of (*S*)-*tert*-butyl (1-oxo-3-phenylpropan-2-yl)carbamate 15

Dry THF was placed in a flame heated 250 ml two neck round-bottomed flask under N_2 followed by addition of *N*-Boc-L-phenylalanine Weinreb amide **14** (3.00 g, 9.73 mmol). The stirring

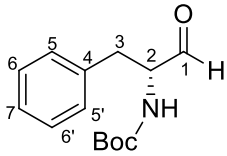
solution was then cooled to 0°C and $LiAlH_4$ (1.3 eq., 0.46 g, 12.16 mmol) was added in one portion. The reaction was allowed to proceed under N_2 for 2 h after which 1M $KHSO_4$ (15 ml) was added slowly whilst stirring vigorously for 20 min. The mixture was then transferred to a separating funnel and washed with EtOAc (90 ml) followed by 1M HCl (90 ml). The organic layer was separated and washed with a saturated solution of $NaHCO_3$ (90 ml) followed by brine (90 ml). The organic layer was then dried over $MgSO_4$ and solvent removed *in vacuo* yielding compound **15** as a white solid (2.15 g, 88%).



R_f = 0.42 (3:7 EtOAc/Hex); **$[\alpha_D]^{20}_{589}$** +44.0 (c 2, CH_2Cl_2), -44.5 (c 2, MeOH); **m.p.**: 83 – 84 °C (lit m.p. 86 °C)[60]; **IR**: $\nu_{max}(cm^{-1})$: 3365 (N-H), 3060 (C=CH), 2848 (C-H), 1728 (C=O), 1687 (C=O), 1250 (C-C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.63 (1H, s, H-1), 7.38 – 7.13 (5H, m, Ar-H), 5.14 – 5.00 (1H, m, NH), 4.52 – 4.35 (1H, m, H-2), 3.11 (2H, d, *J* = 7.2 Hz, H-3), 1.43 (9H, s, $(CH_3)_3C$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 199.44 (C-1), 155.40 (Boc C=O), 135.95 (C-4), 129.32 (C-6 and C-6'), 128.71 (C-5 and C-5'), 127.01 (C-7), 80.11 [$(CH_3)_3\underline{C}$], 60.80 (C-2), 35.38 (C-3), 28.26 [$(\underline{C}H_3)_3C$].

4.4 Preparation of (*R*)-*tert*-butyl (1-oxo-3-phenylpropan-2-yl)carbamate 16

Compound **16** was successfully synthesised from *N*-Boc-D-phenylalanine using the same method as described for compound **15** (1.48 g, 87%).



R_f = 0.42 (3:7 EtOAc/Hex); **$[\alpha_D]^{20}_{589}$** -36.2 (c 2, CH_2Cl_2); **m.p.**: 83 – 84 °C; **IR**: $\nu_{max}(cm^{-1})$ 3365 (N-H), 3060 (C=CH), 2848 (C-H), 1728 (C=O), 1687 (C=O), 1250 (C-C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.61 (1H, s, H-1), 7.37 – 7.12 (5H, m, Ar-H), 5.28 – 4.83 (1H, m, NH), 4.47 – 4.34 (1H, m, H-2), 3.19 – 2.98 (2H, m, H-3), 1.43 (9H, s, $(CH_3)_3$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 199.44 (C-1), 155.38 (Boc C=O), 135.86 (C-4), 129.32 (C-6 and C-6'), 128.73 (C-5 and C-5'), 127.04 (C-7), 80.15 [$(CH_3)_3\underline{C}$], 60.78 (C-2), 35.41 (C-3), 28.26 [$(\underline{C}H_3)_3C$].

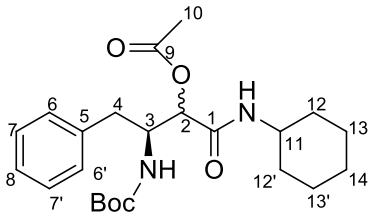
4.5 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate 17

N-Boc-L-phenylalaninal **15** (1.00 g, 4.01 mmol) was dissolved in CH_2Cl_2 (9 ml) followed by drop wise addition of cyclohexyl isocyanide **8** (492 µl, 4.01 mmol) and acetic acid **1** (271 µl, 4.01 mmol). The reaction mixture was allowed to stir at room temperature in air for 3 d. Excess solvent was then removed *in vacuo* and the product was purified by normal phase silica gel column chromatography (elution $CHCl_3$/MeOH 1:0 to 49:1). This afforded compound **17** (1.56 g, 93%) as a white solid, as a mixture of diastereomers. This product was then analysed by HPLC to establish the d.r. Compound **17** (1 mg) was dissolved in 1 ml of MeCN after which 25 µl of the solution was manually injected onto the column. An example of the program that was used to separate the diastereomers in order to obtain maximum baseline separation is given below.

Pressure. Lower Limit = 0 mbars
Pressure. Upper Limit = 350 mbars
Maximum Flow Ramp Down = 6.000
Rise_Time = 2.00
UV_VIS_1.Wavelength = 217
Flowrate = 1.000 ml/min
% acetonitrile = 50.0
% water = 50.0
@ 2.00 min Flow = 1.000 ml/min
@2.00 min % acetonitrile Gradient Start = 50.0, End = 60.0, Duration = 2.00
@2.00 min % water Gradient Start = 50.0, End = 40.0, Duration = 2.00
@4.00 min Flow = 1.000 ml/min
@4.00 min % acetonitrile Gradient Start = 60.0, End = 70.0, Duration = 4.00
@4.00 min % water Gradient Start = 40.0, End = 30.0, Duration = 4.00
@8.00 min Flow = 1.000 ml/min
@8.00 min % acetonitrile Gradient Start = 70.0, End = 80.0, Duration = 4.00
@8.00 min % water Gradient Start = 30.0, End = 20.0, Duration = 4.00
@12.00 min Flow = 1.000 ml/min

@12.00 min % acetonitrile = 80.0
@12.00 min % water = 20.0
@20.00 min stop batch

All the other methods used for separating diastereomers were based on the slight modification of the above method.



R_f = 0.50 (49:1 $CHCl_3$/MeOH); **D.R** = 2.14:1.00; **IR** (solid) v_{max} (cm^{-1}): 3352 (NH), 2932 (C-H), 2854 (C-H), 1745 (C=O), 1686 (C=O), 1649 (C=O), 1523 (C=C), 1226 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.14 (5H, m, Ar-H), 6.01 – 5.90 (0.33H, m, NH), 5.83 – 5.75 (0.60H, m, NH), 5.28 – 4.90 (2H, m, NH and H-2), 4.35 – 4.22 (1H, m, H-3), 3.85 – 3.71 (1H, m, H-11), 2.94 (1H, d, *J* = 7.5 Hz, H-4a), 2.83 (1H, d, *J* = 7.2 Hz, H-4b), 2.13 (1H, s, H-10), 2.09 (2H, s, H-10), 1.96 – 1.84 (2H, m) and 1.76 – 1.57 (4H, m, cyclohexyl-CH_2), 1.43 – 1.29 (9H, m, $(CH_3)_3C$), 1.23 – 1.07 (4H, m, cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.80, 169.09, 166.86 and 166.59 (C=O), 155.50 and 154.94 (Boc C=O), 137.35 and 137.27 (C-5), 129.21 (Ar-C), 129.13 (Ar-C), 128.46 (Ar-C), 126.57 (Ar-C), 126.55 (Ar-C), 79.48 [$(CH_3)_3\underline{C}$], 74.32 and 73.73 (C-2), 53.27 and 52.79 (C-3), 48.29 and, 48.18 (C-11), 38.05 and, 37.27 (C-4), 32.88, 32.85 and 32.73 (C-12 and 12'), 28.23 [$(\underline{C}H_3)_3C$], 25.40 and 24.71 (C-13, 13' and C-14), 20.85 and 20.65 (C-10).

Synthesis of compound **18** is explained in section **4.11** as separated diastereomers.

4.6 Enzymatic kinetic resolution

4.6.1 Enzyme test reactions (hydrolysis reaction)

Benzyl acetate (50 mg, 0.33 mmol) was dissolved in toluene (2 ml) and 0.01M sodium phosphate buffer pH 7.5 (5 ml) and allowed to stir at a controlled temperature of 35°C. Lipase enzyme (50 mg or 10 µl) was then added and reaction was allowed to proceed for 3 h after which TLC was performed to determine whether or not the active enzyme had successfully hydrolysed the substrate. A control experiment was set up without the enzyme, to ascertain and confirm that hydrolysis was not occurring in the absence of the enzyme.

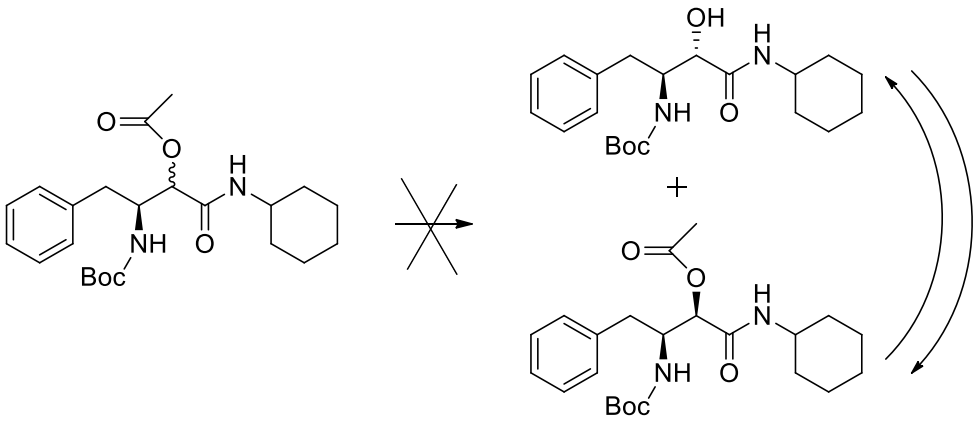
4.6.2 Enzyme test reactions (acylation reaction)

Benzyl alcohol (50 µl, 0.44 mmol) was dissolved in MeCN (5 ml) to which vinyl acetate (1.5 eq., 135 µl, 0.66 mmol) was added. The temperature of the stirring solution was adjusted and controlled at 35 °C, after which lipase enzyme (50 mg or 10 µl) was added. The reaction was allowed to proceed for 3 h after which TLC was performed to determine whether or not the enzyme had successfully catalysed the acetylation reaction. A control experiment was set without the enzyme to ensure that no reaction occurred in the absence of the enzyme. A total of 30 enzymes were tested and 25 were found to be active against the test substrate.

List of active enzymes

1. Novozyme 525
2. Porcine pancreas lipase
3. *Lipozyme* TL IM (*Novozyme*)
4. Lipase *AYS Amano* (LAYW045145)
5. Lipase *Penicillium roqueforti*
6. Lipase *AK-D Amanas*
7. Lipase *AH-D Amano*
8. Lipase *CR Analytical*
9. *Lipozyme RM* Lux 0111(25)
10. Wheat germ lipase
11. Lipase *Candida rugosa*
12. Lipase *Rhizopus oryzae*
13. Grist brocades
14. *Alcalase* 24
15. *Novozym* 425
16. *Lipolase* 100T
17. SP398 LAN00052
18. Lipase *AK* C7 solution No 54-001
19. Esterase *Rhizopus*
20. *Candida Antarctica* lipase
21. Lipase *Rhizopus arrhizus*
22. Lipase *Aspergillus s.p*
23. Lipase *Pseudomonas cepacia*
24. Lipase *Pseudomonas fluorescens*
25. Novozym 388

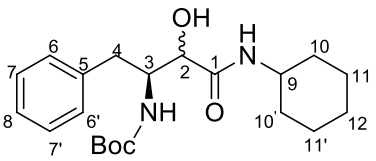
4.6.3 Attempted enzymatic kinetic resolution of compound 17



Compound **17** (50 mg, 0.12 mmol) was dissolved in toluene (5 ml) followed by the addition of phosphate buffer pH 7.5 (20 ml). The reaction temperature of the reaction mixture was adjusted and controlled at 35 °C. A few drops of Tween, a surfactant, were added followed by the enzyme substrate. The reaction was allowed to proceed in air for 28 d whilst tracking for hydrolysis of the substrate using HPLC analysis. There was no observable reaction for all active enzymes and the experiment was abandoned. The same procedure was applied for compounds **20** and **21**, but evidence of enzymatic hydrolysis was only evident with compound **21**.

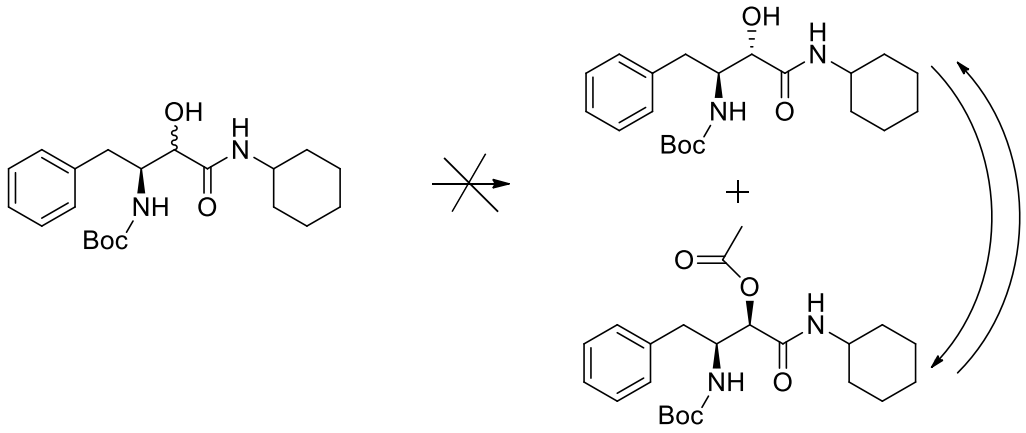
4.7 Preparation of *tert*-butyl ((2*S*)-4-(cyclohexylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)carbamate 19

Compound **17** (400 mg, 0.96 mmol) was dissolved in MeOH (4 ml) followed by addition of KOH (9 eq., 482 mg, 8.60 mmol). The reaction mixture was left stirring in air at room temperature overnight. After completion of the reaction, H_2O (10 ml) was added, followed by 6N HCl (5 ml). The product was then extracted using EtOAc (2x10 ml) and dried over $MgSO_4$. Excess solvent was removed in *vacuo.* Purification of the product by column chromatography (49:1 $CHCl_3$/MeOH) afforded compound **19** (355 mg, 99%) as a white flaky solid.



R_f = 0.47 (49:1 $CHCl_3$:MeOH); **d.r.** = 2.10:1.00; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.40 – 7.14 (5H, m, Ar-H), 6.85 (0.35H, d, *J* = 8.4 Hz, NH), 6.69 (0.71H, d, *J* = 8.4 Hz, NH), 5.53 (1H, br-s, OH), 5.16 (0.70H, d, *J* = 8.4 Hz, NH), 4.98 (0.36H, d, *J* = 7.2 Hz, NH), 4.12 – 3.91 (2H, m, H-2 and H-3), 3.86 – 3.68 (1H, m, H-9), 3.16 – 2.79 (2H, m, H-4), 1.97 – 1.78 (2H, m) and 1.78 – 1.53 (3H, m, cyclohexyl-CH_2), 1.48 – 1.31 (14H, m, $(CH_3)_3C$ and cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.52 and 170.68 (C-1), 157.38 (Boc C=O), 138.06 and 138.17 (C-5), 129.34 (C-7 and C-7'), 128.51 (C-6 and C-6'), 126.55 (C-8), 80.58 and 80.23 [$(CH_3)_3\underline{C}$], 75.05 and 73.59 (C-2), 55.60 (C-3), 48.08 and 47.90 (C-9), 35.56 (C-4), 33.56, 33.23, 33.12 and 32.82 (cyclohexyl-CH_2), 28.23 [$(\underline{C}H_3)_3C$], 25.50, 24.80 and 24.76 (cyclohexyl-CH_2).

4.8 Attempted enzymatic esterification of 19

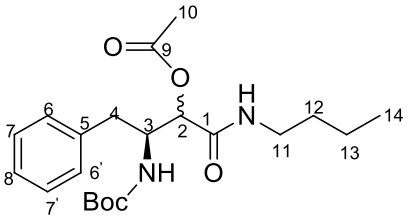


Compound **19** (50 mg, 0.13 mmol) was dissolved in MeCN (5 ml) followed by the addition of vinyl acetate (5 eq., 61 µl, 0.66 mmol). The temperature of the reaction was adjusted and controlled at 35 °C after which the lipase enzyme was added. The reaction was allowed to proceed in air whilst tracking for acylation of the substrate by HPLC analysis. After 14 d there was no observable reaction for all active enzymes and the experiment was abandoned. The reaction was also attempted on a diastereomeric mixture of compound **18** and there was still no evidence of enzymatic acylation of the substrate.

Synthesis of compound **20** is explained in section **4.12**, after diastereomeric separation.

4.9 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(butylamino)-1-oxo-4-phenylbutan-2-yl acetate 21

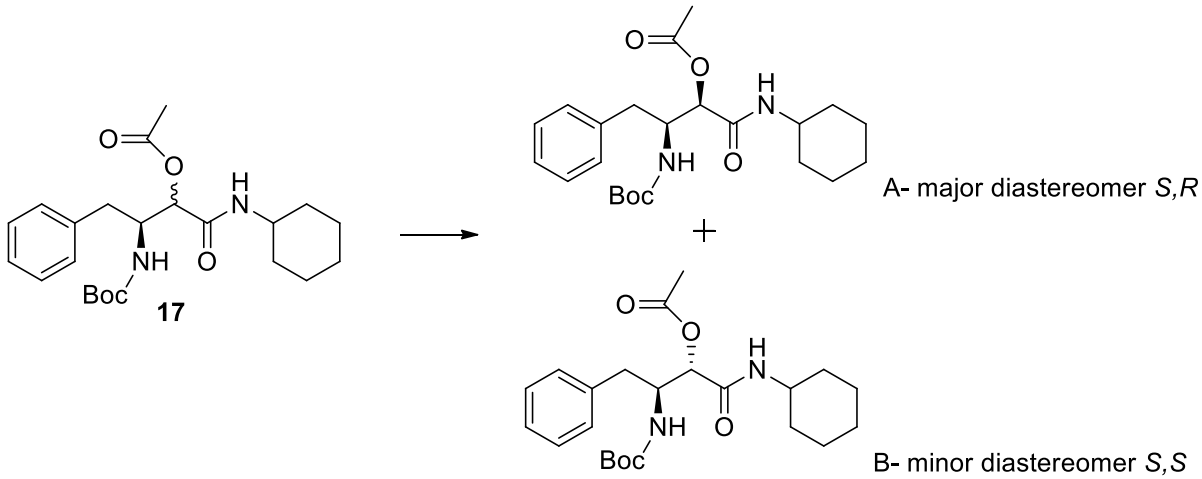
N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), *n*-butyl isocyanide **9** (210 µl, 2.01 mmol) and acetic acid **1** (135 µl, 2.01 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment was carried out as described in **4.5** yielding compound **21** (759 mg, 96%) as a white solid.



R_f = 0.31 (1:1 EtOAc/Hex); **d.r.** = 1.72:1.00; **m.p.** 125 - 126°C; **IR** $\nu_{max}(cm^{-1})$: 3341 (NH), 3030 (C=CH), 1750 (C=O), 1686 (C=O), 1656 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.35 – 7.12 (5H, m, Ar-H), 6.57 – 6.33 (1H, m, NH), 5.44 – 5.22 (1H, m, H-2), 5.21 – 5.07 (1H, m, NH), 4.44 – 4.24 (1H, m, H-3), 3.35 – 3.11 (2H, m, H-4), 3.04 – 2.61 (2H, m, H-11), 2.23 – 2.04 (3H, m, H-10), 1.58 – 1.19 (13H, m, H-12, H-13 and $(CH_3)_3C$), 0.91 (3H, q, *J* = 7.2 Hz, H-14); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.84, 169.32, 167.92 and 167.62 (C=O), 155.52

and 155.10 (Boc C=O), 137.37 (C-5), 129.28 and 129.22 (C-7 and C-7'), 128.45 and 128.41 (C-6 and C-6'), 126.56 and 126.53 (C-8), 79.43 [$(CH_3)_3\underline{C}$], 74.63 and 73.99 (C-2), 53.20 (C-3), 52.87 (C-3), 39.14 (C-11), 39.08 (C-11), 37.23 (C-4), 28.25 [$(\underline{C}H_3)_3C$], 20.84 (C-10), 20.68 (C-10), 20.02 (C-12), 19.98 (C-13), 13.70 (C-14); **HRMS** (m/*z*), calculated for $C_{21}H_{33}N_2O_5$: 393.2389, found $(M + H)^+$: 393.2393.

4.10 HPLC Separation of compound 17



Compound **17** (150 mg) was dissolved in HPLC grade MeCN (5 ml) and 250 µl of this solution was injected onto the C-18 column for each separation. The diastereomers were separated using gradient elution (5:5-8:2 MeCN/H_2O) and collected separately. The full method is shown below and it was adjusted for separation of other compounds.

- Pump

Channel	Solvent Name	Percentage %
Channel A	water	40.0
Channel B	acetonitrile	60.0
Stoptime Mode:		Time set
Stoptime:		20.00 min

Gradient Timetable

Time min	A %	B %	Flow mL/min	Pressure bar
7.00	40.0	60.0	20	---
10.00	22.0	78.0	20	---

- DAD (G1315D)

Peakwidth: >0.013 min (0.25 s response time) (20 Hz)

Slit: 4 nm
UV Lamp Required: Yes
Vis Lamp Required: Yes

Signal table

Use Sig.	Signal	Wavelength nm	Bandwidth nm	Use Ref.
Yes	Signal A	217	4	No

Prepare Mode
Margin for negative Absorbance: 100
Spectrum Range WL from: 190 nm
Spectrum Range WL to: 700 nm
Spectrum Step: 2.0 nm
Spectrum Store: All

Valve Position: Port 1 -> 2
Temperature: 20.00 °C
Timetable

- Prep. Sampler (G2260A)

Injection Mode: Standard injection
Injection Volume: 100.00 µL

- Fraction Collector (G1364B)

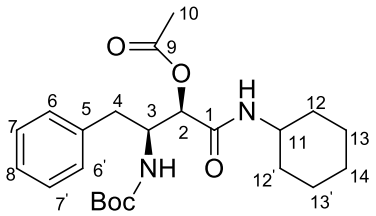
Peak Detector Mode: at least one peak detected
Maximum fill volume: 13.50 mL

Detector type with serial number	Unit	Up Slope	Down Slope	Threshold	Upper Threshold	Mode
G1315D:DEAAX04653	mAU	2.00	5.00	25.000	4000.000	Threshold

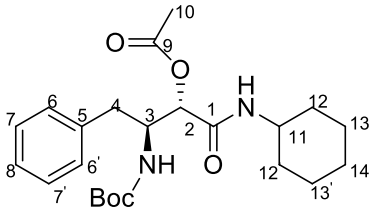
Timetable

Time	Function	Parameter
15.00	Change Trigger Mod.	Trigger Mode: Peak-based with a maximum peak duration of 1.7 min
@20.00	stop batch	

Products were then extracted from eluent using $CHCl_3$ and dried over $MgSO_4$. Excess solvent was removed *in vacuo* yielding compounds **17A** (66 mg) and **17B** (38 mg) as white solids.



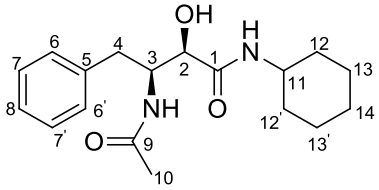
(2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate 17A. **m.p.** 162 – 163 °C; **IR** (solid) ν_{max}(cm^{-1}): 3341 (NH), 3290 (NH), 2922 (C-H), 2851 (C-H), 1735 (C=O), 1686 (C=O), 1652 (C=O), 1257 (C-O), 1168 (C-C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.39 – 7.14 (5H, m, Ar-H), 5.93 – 5.75 (1H, m, NH), 5.28 – 5.02 (2H, m, NH and H-2), 4.38 – 4.23 (1H, m, H-3), 3.86 – 3.68 (1H, m, H-11), 2.83 (2H, d, *J* = 7.2 Hz, H-4), 2.10 (3H, s, H-10), 1.98 – 1.81 (2H, m) and 1.79 – 1.52 (5H, m, cyclohexyl-CH_2), 1.35 – 1.04 (12H, m, $(CH_3)_3C$ and cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.15 and 166.95 (C=O), 155.03 (Boc C=O), 137.34 (C-5), 129.28 (C-7 and C-7'), 128.51 (C-6 and C-6'), 126.61 (C-8), 79.61 [$(CH_3)_3\underline{C}$], 73.86 (C-2), 52.88 (C-3), 48.35 (C-11), 38.14 (C-4), 32.78 and 32.92 (cyclohexyl-CH_2), 28.24 [$(\underline{C}H_3)_3C$], 25.46 and 24.77 (cyclohexyl-CH_2), 20.69 (C-10); **HRMS** (m/*z*), calculated for $C_{23}H_{35}N_2O_5$: 419.2546, found $(M + H)^+$: 419.2537.



(2*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl acetate 17B. **m.p.** 164 - 165°C; **IR** (solid) ν_{max}(cm^{-1}): 3370 (NH), 2933 (C-H), 2854 (C-H), 1749 (C=O), 1685 (C=O), 1648 (C=O), 1222 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.38 – 7.14 (5H, m, Ar-H), 5.99 (1H, d, *J* = 8.3 Hz, NH), 5.26 – 4.93 (2H, m, NH and H-2), 4.37 – 4.19 (1H, m, H-3), 3.88 – 3.71 (1H, m, H-11), 2.94 (2H, d, *J* = 7.6 Hz, H-4), 2.14 (3H, s, H-10), 1.99 – 1.82 (2H, m) and 1.81 – 1.52 (5H, m, cyclohexyl-CH_2), 1.36 – 1.01 (12H, m, $(CH_3)_3C$ and cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.89 and 166.65 (C=O), 155.57 (Boc C=O), 137.42 (C-5), 129.19 (C-7 and C-7'), 128.51 (C-6 and C-6'), 126.63 (C-8), 79.56 [$(CH_3)_3\underline{C}$], 74.42 (C-2), 53.38 (C-3), 48.26 (C-11), 37.30 (C-4), 32.91 (cyclohexyl-CH_2), 28.28 [$(\underline{C}H_3)_3C$], 25.46 and 24.75 (cyclohexyl-CH_2), 20.89 (C-10); **HRMS** (m/*z*), calculated for $C_{23}H_{35}N_2O_5$: 419.2546, found $(M + H)^+$: 419.2532.

4.11 Preparation of (2*R*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide 18A

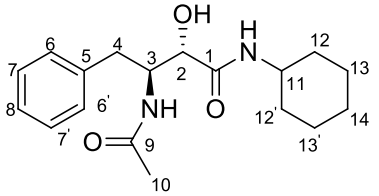
Compound **17A** (90 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (1 ml) and TFA (10 eq., 166 µl, 2.15 mmol) was added. The reaction mixture was stirred at room temperature in air for 3 h after which the solvent and most of the TFA were removed *in vacuo*. The residue was then redissolved in CH_2Cl_2 (1 ml) and Et_3N (10 eq., 298 µl, 2.15 mmol) and the reaction mixture was left stirring at room temperature in air for 1 h. Excess solvent and most of the Et_3N were removed *in vacuo* and the product was purified by column chromatography (49:1 $CHCl_3$/MeOH) affording **18A** (59 mg, 84%) as a white solid.



R_f = 0.53 (49:1 $CHCl_3$/MeOH); **m.p.** 159-160 °C; **IR** (solid) ν_{max}(cm^{-1}): 3350 (OH), 3249 (NH), 2931 (C-H), 2853 (C-H), 1689 (C=O), 1635 (C=O), 1528 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.40 – 7.09 (6H, m, Ar-H and NH), 6.98 (1H, d, *J* = 8.4 Hz) and 6.48 (1H, d, *J* = 6.4 Hz, NH and OH), 4.21 – 3.98 (2H, m, H-2 and H-3), 3.85 – 3.63 (1H, m, H-11), 3.12 (1H, dd, *J* = 13.7, 6.0 Hz, H-4a), 3.01 – 2.75 (1H, m, H-4b), 1.97 – 1.78 (4H, m, cyclohexyl-CH_2), 1.77 – 1.51 (3H, m, cyclohexyl-CH_2), 1.46 – 1.01 (5H, m, cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.29 and 171.94 (C=O), 138.24 (C-5), 129.15 (C-7 and C-7'), 128.46 (C-6 and C-6'), 126.54 (C-8), 73.21 (C-2), 56.10 (C-3), 48.04 (C-11), 35.51 (C-4), 33.10, 32.57, 25.46 and 24.77 (cyclohexyl-CH_2), 22.90 (C-10); **HRMS** (m/*z*), calculated for $C_{18}H_{27}N_2O_3$: 319.2022, found $(M + H)^+$: 319.2014.

4.12 Preparation of (2*S*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide 18B

Compound **18B** was synthesized using the same procedure as described in **4.11**. Compound **17B** (50 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (1 ml), followed by the addition of TFA (10 eq., 92 µl, 1.19 mmol) and Et_3N (10 eq., 165 µl, 1.19 mmol). Purification of the reaction mixture yielded compound **18B** (36 mg, 95%) as a white precipitate.

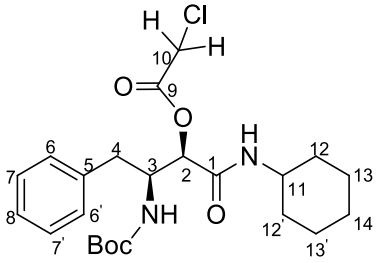


R_f = 0.47 (49:1 $CHCl_3$/MeOH); **m.p.** 238 – 239 °C ; **IR** ν_{max}(cm^{-1}): 3386 (OH), 3277 (NH), 2918 (C-H), 2850 (C-H), 1645 (C=O), 1570 (C=C); **^{1}H NMR** (300 MHz, CD_3OD) δ 8.01 (0.16H, d, *J* = 8.9 Hz, NH), 7.74 (0.65H, d, *J* = 8.4 Hz, NH), 7.36 – 7.14 (5H, m, Ar-H),

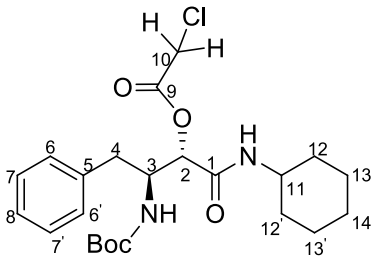
4.56 – 4.43 (1H, m, H-3), 4.17 (1H, d, *J* = 3.5 Hz, H-2), 3.84 – 3.61 (1H, m, H-11), 2.76 (2H, d, *J* = 7.3 Hz, H-4), 1.98 – 1.73 (6H, m, H-10 and cyclohexyl-CH_2), 1.73 – 1.57 (1H, m, cyclohexyl-CH_2), 1.57 – 1.11 (6H, m, cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, CD_3OD) δ 174.65 and 171.53 (C=O), 138.38 (C-5), 128.70 (C-7 and C-7'), 127.76 (C-6 and C-6'), 125.81 (C-8), (72.99 C-2), 53.93 (C-3), 33.80 (C-4), 32.19, 25.08 and 24.60 (cyclohexyl-CH_2), 20.97 (C-10); **HRMS** (m/*z*), calculated for $C_{18}H_{27}N_2O_3$: 319.2022, found $(M + H)^+$: 319.2015.

4.13 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino) -1-oxo-4-phenylbutan-2-yl 2-chloroacetate 20

N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), cyclohexyl isocyanide **8** (247 µl, 2.01 mmol) and chloroacetic acid **5** (189 mg, 2.01 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4**.**5**. The reaction yielded compound **20** (776 mg, 85%) as a white solid being a mixture of diastereomers. R_f = 0.53 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.94:1.00. This mixture was then separated by preparative HPLC as described in **4.10** to give two clean separated diastereomers.



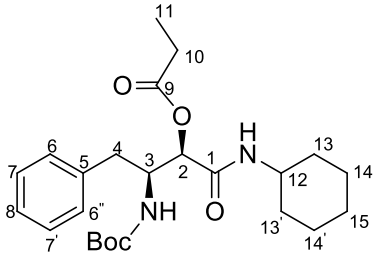
(2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl 2-chloroacetate 20A. m.p. 184 -185 °C; **IR** (solid) ν_{max}(cm^{-1}): 3351 (NH), 3313 (NH), 2923 (C-H), 2552 (C-H), 1767 (C=O), 1687 (C=O), 1646 (C=O), 700 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.26 (2H, m, H-7 and 7'), 7.24 – 7.16 (3H, m, H-6, H-6' and H-8), 6.11 – 5.95 (1H, m, NH), 5.30 – 5.10 (2H, m, NH and H-2), 4.42 – 4.31 (1H, m, H-3), 4.03 (2H, s, H-10), 3.82 – 3.71 (1H, m, H-11), 2.85 (2H, d, *J* = 7.1 Hz, H-4), 1.97 – 1.82 (2H, m, cyclohexyl-CH_2), 1.75 – 1.64 (2H, m, cyclohexyl-CH_2), 1.64 – 1.56 (1H, m, cyclohexyl-CH_2), 1.41 – 1.09 (14H, m, $(CH_3)_3C$ and cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.10 and 165.59 (C=O), 154.99 (Boc C=O), 137.10 (C-5), 129.23 (C-7 and C-7'), 128.57 (C-6 and C-6'), 126.71 (C-8), 79.73 [$(CH_3)_3\underline{C}$], 75.12 (C-2), 52.71 (C-3), 48.46 (C-11), 40.46 (C-10), 38.05 (C-4), 32.83 and 32.65 (cyclohexyl-CH_2), 28.25 [$(\underline{C}H_3)_3C$], 25.41 and 24.66 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{23}H_{34}ClN_2O_5$: 453.2156, found, $(M + H)^+$: 453.2153.



(2*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl 2-chloroacetate 20B. 193-194 °C; **IR** (solid) ν_{max}(cm^{-1}): 3373 (NH), 3330 (NH), 2931 (C-H), 2854 (C-H), 1767 (C=O), 1682 (C=O), 1650 (C=O), 1167 (C-O), 736 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 – 7.25 (2H, m, H-7 and 7'), 7.25 – 7.16 (3H, m, H-6, 6' and H-8), 6.41 (1H, d, *J* = 8.1 Hz, NH), 5.17 (1H, d, *J* = 3.5 Hz, H-2), 4.39 – 4.24 (1H, m, H-3), 4.18 (1H, d, *J* = 14.7 Hz, H-10), 4.12 (1H, d, *J* = 14.7 Hz, H-10) 3.86 – 3.74 (1H, m, H-11,), 3.05 – 2.82 (2H, m, H-4), 1.97 – 1.83 (2H, m, cyclohexyl-CH_2), 1.77 – 1.66 (2H, m, cyclohexyl-CH_2), 1.66 – 1.56 (1H, m, cyclohexyl-CH_2), 1.47 – 1.02 (14H, m, $(CH_3)_3C$ and cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.15 and 165.80 (C=O), 155.69 (Boc C=O), 137.14 (C-5), 129.13 (C-7 and 7'), 128.57 (C-6 and 6'), 126.74 (C-8), 79.94 [$(CH_3)_3\underline{C}$], 75.96 (C-2), 53.09 (C-3), 48.27 (C-11), 40.72 (C-10), 36.71 (C-4), 32.80 and 29.71 (cyclohexyl-CH_2), 28.26 Boc ($(\underline{C}H_3)_3C$), 25.44 and 24.64 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{23}H_{34}ClN_2O_5$: 453.2156, found, $(M + H)^+$: 453.2156.

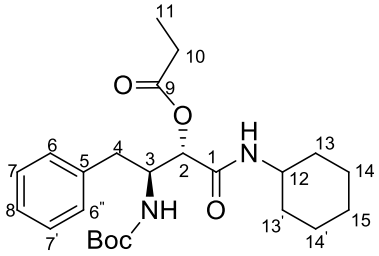
4.14 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino) -1-oxo-4-phenylbutan-2-yl propionate 22

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), cyclohexyl isocyanide **8** (148 µl, 1.20 mmol) and propionic acid **2** (90 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. The reaction yielded compound **22** (360 mg, 69%) as a white solid, being a mixture of diastereomers. **R_f** = 0.55 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.87:1.00. The diastereomeric mixture of compound **22** was then separated on preparative HPLC as described in **4.10** to give two clean separated diastereomers.



(2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl propionate 22A. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.34 – 7.12 (5H, m, Ar-H), 5.91 (1H, d, *J* = 8.1 Hz, NH), 5.26 – 5.07 (2H, m, NH and H-2), 4.37 – 4.25 (1H, m, H-3), 3.86 – 3.68 (1H, m, H-12), 2.82 (2H, d, *J* = 7.0 Hz, H-4), 2.49 – 2.26 (2H, m, H-10), 1.97 – 1.81 (2H, m, cyclohexyl-CH_2), 1.76 – 1.53 (3H, m, cyclohexyl-CH_2), 1.46 – 1.05

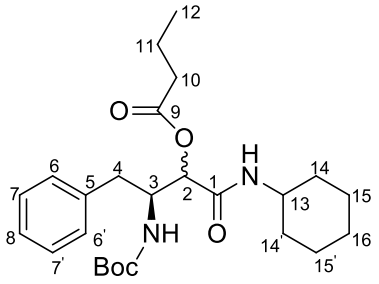
(17H, m, $(CH_3)_3C$, H-11 and cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.55 and 167.03 (C=O), 154.96 (Boc C=O), 137.34 (C-5), 129.24 (C-7 and C-7'), 128.42 (C-6 and C-6'), 126.53 (C-8), 79.44 [$(CH_3)_3\underline{C}$], 73.19 (C-2), 52.31 (C-3), 48.23 (C-12), 38.06 (C-4), 32.85 (C-10), 32.71 (cyclohexyl-CH_2), 28.19 [$(\underline{C}H_3)_3C$], 27.35, 25.42 and 24.69 (cyclohexyl-CH_2), 9.03 (C-11).



(2*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl propionate 22B. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.27 – 7.07 (5H, m, Ar-H), 5.92 (1H, d, *J* = 8.3 Hz, NH), 5.23 – 4.98 (1H, m, H-2), 4.94 (1H, d, *J* = 9.2 Hz, NH), 4.29 – 4.11 (1H, m, H-3), 3.81 – 3.63 (1H, m, H-12), 2.87 (2H, d, *J* = 7.5 Hz, H-4), 2.34 (2H, q, *J* = 7.5 Hz, H-10), 1.98 – 1.74 (2H, m, cyclohexyl-CH_2), 1.71 – 1.46 (3H, m, cyclohexyl-CH_2), 1.39 – 0.93 (17H, m, $(CH_3)_3C$), H-11 and cyclohexyl-CH_2); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.14 and 165.71 (C=O), 154.47 (Boc C=O), 136.41 (C-5), 128.17 (C-7 and C-7'), 127.45 (C-6 and C-6'), 125.57 (C-8), 78.40 [$(CH_3)_3\underline{C}$], 73.63 (C-2), 52.85 (C-3), 47.13 (C-12), 36.40 (C-4), 31.85 (cyclohexyl-CH_2), 28.67 [$(\underline{C}H_3)_3C$], 27.24 (cyclohexyl-CH_2), 26.46 (C-10), 24.43 (cyclohexyl-CH_2), 9.93 (C-11).

4.15 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino) -1-oxo-4-phenylbutan-2-yl butyrate 23

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), cyclohexyl isocyanide **8** (148 µl, 1.20 mmol) and butyric acid **3** (110 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment was carried out as described in **4.5** yielding compound **23** (270 mg, 51%) as a white solid being a mixture of diastereomers.

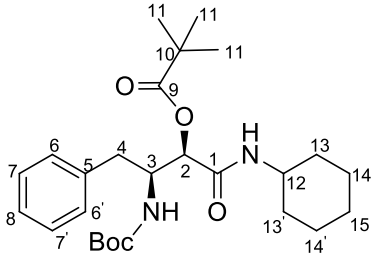


R_f = 0.58 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.32:1.00; **m.p.** 156 – 157 °C; **IR** ν_{max}(cm^{-1}): 3350 (NH), 2933 (C-H), 2854 (C-H), 1738 (C=O), 1686 (C=O), 1650 (C=O), 1251 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.38 – 7.12 (5H, m, Ar-H), 5.98 (0.39H, d, *J* = 8.2 Hz, NH), 5.82 (0.53H, d, *J* = 8.2 Hz, NH), 5.28 – 4.94 (2H, m, NH and H-2), 4.37 – 4.20 (1H, m, H-3), 3.87 – 3.69 (1H, m, H-13), 2.94 (1H, d, *J* = 7.1 Hz, H-4a), 2.82 (1H, d, *J* = 7.1

Hz, H-4b), 2.46 – 2.22 (2H, m, H-10), 1.99 – 1.80 (2H, m, cyclohexyl-CH_2), 1.79 – 1.54 (5H, m, H-11 and cyclohexyl-CH_2), 1.46 – 1.08 (14H, m, $(CH_3)_3C$ and cyclohexyl-CH_2), 1.04 – 0.92 (3H, m, H-12); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.42, 171.70, 167.05 and 166.72 (C=O), 155.62 and 154.97 (Boc C=O), 137.35 (C-5), 129.29 (C-7 and C-7'), 128.49 (C-6 and C-6'), 126.61 (C-8), 79.48 [$(CH_3)_3\underline{C}$], 74.05 and 73.48 (C-2), 53.36 and 52.90 (C-3), 48.25 and 48.16 (C-13), 38.10 and 37.41 (C-4), 36.02 and 35.91 (C-10), 32.94 and 32.78 (cyclohexyl-CH_2), 28.27 [$(\underline{C}H_3)_3C$], 25.45 and 24.72 (cyclohexyl-CH_2), 18.41 and 18.33 (C-11), 13.66 and 13.63 (C-12); **HRMS** (m/*z*), calculated for $C_{25}H_{39}N_2O_5$: 447.2859, found $(M + H)^+$: 447.2855.

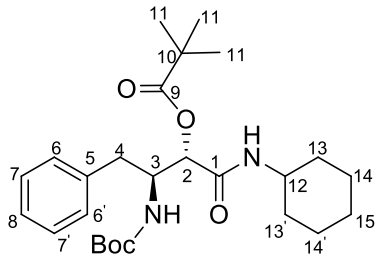
4.16 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino) -1-oxo-4-phenylbutan-2-yl pivalate 24

N-Boc-L-phenylalaninal **15** (250 mg, 1.00 mmol), cyclohexyl isocyanide **8** (123 µl, 1.00 mmol) and pivalic acid **4** (102 µl, 1.00 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. Purification of the product yielded compound **24** (450 mg, 97%), as a white solid, as a mixture of diastereomers. **R_f** = 0.36 (49:1 $CHCl_3$/MeOH); **d.r.** = 2.08:1.00. This mixture was then separated on preparative HPLC as described in **4.10** to give two separated diastereomers.



(2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl pivalate 24A. **m.p.** 159 – 161 °C; **IR** (solid) ν_{max}(cm^{-1}): 3341 (NH), 3265 (NH), 3030 (C=CH), 2928 (C-H), 1653 (C=O), 1619 (C=O), 1185 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.30 – 7.24 (2H, m, H-7 and H-7'), 7.23 – 7.12 (3H, m, H-6, H-6' and H-8), 5.83 (1H, d, *J* = 8.2 Hz, NH), 5.25 – 5.07 (2H, m, NH and H-2), 4.37 – 4.21 (1H, m, H-3), 3.84 – 3.71 (1H, m, H-12), 2.90 – 2.74 (2H, m, H-4), 1.96 – 1.82 (2H, m, cyclohexyl-CH_2), 1.74 – 1.52 (3H, m, cyclohexyl-CH_2), 1.42 – 1.06 (23H, m, $(CH_3)_3C$, cyclohexyl-CH_2 and H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.38 and 167.20 (C=O), 154.95 (Boc C=O), 137.25 (C-5), 129.30 (C-7 and C-7'), 128.48 (C-6 and C-6'), 126.59 (C-8), 79.48 ($(CH_3)_3\underline{C}$), 73.28 (C-2), 53.08 (C-3), 48.07 (C-12), 38.87 (C-10), 37.98 (C-4), 32.87 (cyclohexyl-CH_2), 28.25 ($(\underline{C}H_3)_3C$), 27.07 (C-11), 25.41 and

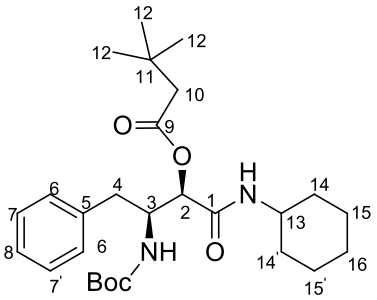
24.58 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{26}H_{41}N_2O_5$: 461.3015, found $(M + H)^+$: 461.3008.



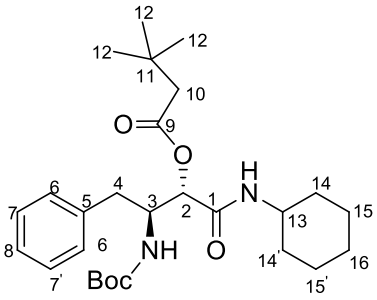
(2*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl pivalate 24B. **m.p.** 180 – 181 °C; **IR** $\nu_{max}(cm^{-1})$: 3303 (NH), 3065 (C=CH), 2926 (C-H), 1644 (C=O), 1526 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 – 7.25 (2H, m, H-7 and H-7'), 7.24 – 7.13 (3H, m, H-6, H-6', H-8), 5.97 (1H, d, *J* = 8.3 Hz, NH), 5.27 – 5.01 (1H, m, H-2), 4.98 (1H, d, *J* = 9.4 Hz, NH), 4.34 – 4.19 (1H, m, H-3), 3.85 – 3.70 (1H, m, H-12), 3.02 – 2.69 (2H, m, H-4), 1.97 – 1.80 (2H, m, cyclohexyl-CH_2), 1.74 – 1.65 (2H, m, cyclohexyl-CH_2), 1.65 – 1.52 (1H, m, cyclohexyl-CH_2), 1.44 – 1.02 (23H, s, $((CH_3)_3C)$, cyclohexyl-CH_2 and H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 177.05 and 166.83 (C=O), 155.35 (Boc C=O), 137.32 (C-5), 129.31 (C-7 and C-7'), 128.49 (C-6 and C-6'), 126.62 (C-8), 79.37 $[(CH_3)_3\underline{C}]$, 73.94 (C-2), 53.16 (C-3), 47.94 (C-12), 38.87 (C-10), 37.57 (C-4), 32.85 (cyclohexyl-CH_2), 28.29 $[(\underline{C}H_3)_3C]$, 27.08 (C-11), 25.43 and 24.58 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{26}H_{41}N_2O_5$: 461.3015, found, $(M + H)^+$: 461.3017.

4.17 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino) -1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 25

N-Boc-L-phenylalaninal **15** (600 mg, 2.40 mmol), cyclohexyl isocyanide **8** (296 µl, 2.40 mmol) and *tert*-butyl acetic acid **5** (306 µl, 2.40 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment was carried out as described in **4.5**. This yielded compound **25** (1.07 g, 94%), as white solid, being a mixture of diastereomers. $\mathbf{R_f}$ = 0.53 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.93:1.00. This diastereomeric mixture was then separated on preparative HPLC as described in **4.10** to give two separated diastereomers.



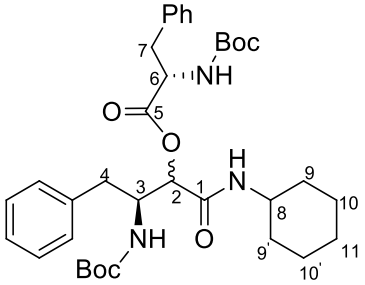
(2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 25A. **m.p.** 162-163 °C; **IR** (solid) v_{max}(cm^{-1}): 3328 NH), 3032 (C=CH), 1742 (C=O), 1682 (C=O), 1652 (C=O), 1169 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.22 – 7.16 (2H, m, H-7 and H-7'), 7.16 – 7.06 (3H, m, H-6, H-6' and H-8), 5.82 (1H, d, *J* = 8.1 Hz, NH), 5.22 – 5.00 (2H, m, NH and H-2), 4.26 – 4.16 (1H, m, H-3), 3.76 – 3.63 (1H, m, H-13), 2.83 – 2.65 (2H, m, H-4), 2.28 – 2.13 (2H, m, H-10), 1.89 – 1.75 (2H, m, cyclohexyl-CH_2), 1.68 – 1.57 (2H, m, cyclohexyl-CH_2), 1.57 – 1.49 (1H, m, cyclohexyl-CH_2), 1.37 – 0.93 (23H, m, ($\underline{C}H_3)_3C$, cyclohexyl-CH_2 and H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.33 and 167.10 (C=O), 154.95 (Boc C=O), 137.38 (C-5), 129.30 (C-7 and C-7'), 128.45 (C-6 and C-6'), 126.53 (C-8), 79.42 [$(CH_3)_3\underline{C}$], 73.43 (C-2), 52.96 (C-3), 48.25 (C-13), 47.51 (C-12), 37.93 (C-4), 32.95 and 32.77 (cyclohexyl-CH_2), 29.69 (C-12), 28.26 [($\underline{C}H_3)_3C$], 25.44 and 24.74 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{27}H_{43}N_2O_5$: 475.3172, found, $(M + H)^+$: 475.3163.



(2*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexylamino)-1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 25B. **m.p.** 169-170 °C; **IR** v_{max}(cm^{-1}): 3290 (NH), 2930 (C-H), 1738 (C=O), 1366 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.25 (2H, m, H-7 and H-7'), 7.25 – 7.15 (3H, m, H-6, H-6' and H-8), 6.00 (1H, d, *J* = 8.2 Hz, NH), 5.26 – 5.01 (2H, m, NH and H-2), 4.31 – 4.19 (1H, m, H-3), 3.84 – 3.73 (1H, m, H-13), 2.99 – 2.71 (2H, m, H-4), 2.34 – 2.22 (2H, m, H-10), 1.97 – 1.83 (3H, m, cyclohexyl-CH_2), 1.75 – 1.65 (2H, m, cyclohexyl-CH_2), 1.65 – 1.57 (1H, m, cyclohexyl-CH_2), 1.41 – 1.03 (23H, m, $(CH_3)_3C$, cyclohexyl-CH_2 and H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.18 and 166.78 (C=O), 155.42 (Boc C=O), 137.42 (C-5), 129.18 (C-7 and C-7'), 128.51 (C-6 and C-6'), 126.61 (C-8), 79.41 [$(CH_3)_3\underline{C}$], 73.85 (C-2), 53.37 (C-3), 48.21 (C-13), 47.81 (C-10), 38.76 (C-4), 32.89 and 30.98 (cyclohexyl-CH_2), 29.70 (C-12), 28.30 (($\underline{C}H_3)_3C$), 25.45 and 24.71 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{27}H_{43}N_2O_5$: 475.3172, found, $(M + H)^+$: 475.3161.

4.18 Preparation of (2*S*)-(3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-(cyclohexyl amino)-1-oxo-4-phenylbutan-2-yl 2-((*tert*-butoxycarbonyl)amino)-3-phenyl propanoate 26

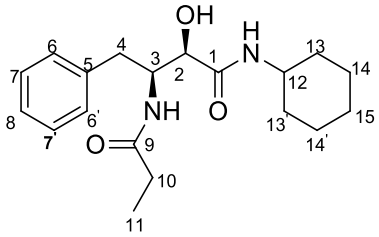
N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), cyclohexyl isocyanide **8** (246 µl, 2.01 mmol) and *N*-Boc-*L*-phenylalanine **7** (533 mg, 2.01 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment was carried out as described in **4.5** but product precipitated out of solution during the experiment. Addition of CH_2Cl_2 (20 ml) did not dissolve the product. Purification of the product was then achieved by washing with CH_2Cl_2 to remove the starting material. This afforded compound **26** (1.25 g, 100%) as a white solid which was a mixture of diastereomers.



R_f = 0.58 (49:1 $CHCl_3$/MeOH); **m.p.** 190 – 192 °C; **IR** ν_{max}(cm^{-1}): 3348 (NH), 3029 (C=CH), 1752 (C=O), 1689 (C=O), 1653 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45 – 6.98 (10H, m, Ar-H), 6.97 – 6.80 (1H, m, NH), 5.49 – 4.81 (2H, m, H-2 and NH), 4.64 – 4.12 (2H, m, H-3 and H-6), 3.81 – 3.64 (1H, m, H-8), 3.24 – 2.95 (2H, m, H-4), 2.93 – 2.38 (2H, m, H-7), 1.95 – 1.79 (2H, m, cyclohexyl-CH_2) 1.78 – 1.55 (3H, m, cyclohexyl-CH_2), 1.51 – 1.02 (23H, m, 2x$(CH_3)_3C$ and cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.14, 170.76, 167.00 and 166.17 (C=O), 155.71 and 154.77 (Boc C=O), 137.49 (Ar-C), 135.45 (Ar-C), 129.42 (Ar-C), 129.24 (Ar-C), 129.08 (Ar C), 129.02 (Ar C), 128.43 (Ar C), 128.41 (Ar C), 128.24 (Ar C), 127.59 (Ar C), 126.54 (Ar C), 126.35 (Ar-C), 80.75 [$(CH_3)_3\underline{C}$], 79.14 ($(CH_3)_3\underline{C}$), 74.11 (C-2), 55.74, 54.87 and 52.60 (C-3 and C-8), 48.68, 37.61 and 37.15 (C-4 and C-7), 32.79, 32.64, 32.50 (cyclohexyl-CH_2), 28.39 and 28.29 Boc [$(\underline{C}H_3)_3C$], 25.55, 25.51, 25.09 and 24.99 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{35}H_{50}N_3O_7$: 624.3679, found, $(M + H)^+$: 624.3653.

4.19 Preparation of (2*R*,3*S*)-*N*-cyclohexyl-2-hydroxy-4-phenyl-3-propion amidobutanamide 27A

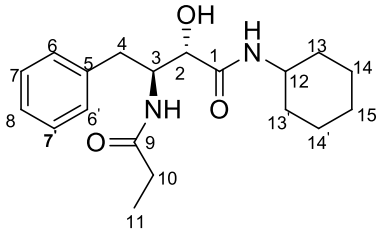
Compound **22A** (76 mg, 0.18 mmol), TFA (136 µl, 1.77 mmol) and Et_3N (245 µl, 1.77 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11** to yield compound **27A** (25 mg, 42%) as a white solid.



R_f = 0.32 (49:1 $CHCl_3$/MeOH); **m.p.** = 130 – 131 °C; **IR** ν_{max}(cm^{-1}): 3347 (OH), 3322 (NH), 3236 (NH), 2934 (C-H), 2855 (C-H), 1730 (C=O), 1676 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.34 – 7.14 (5H, m, Ar-H), 7.09 – 6.92 (2H, m, NH and OH), 6.57 (1H, d, *J* = 6.7 Hz, NH), 4.18 – 4.04 (2H, m, H-2 and H-3), 3.80 – 3.63 (1H, m, H-12), 3.11 (1H, dd, *J* = 13.7, 6.0 Hz, H-4a), 3.03 – 2.88 (1H, m, H-4b), 2.08 (2H, q, *J* = 7.6 Hz, H-10), 1.92 – 1.54 (5H, m, cyclohexyl-CH_2), 1.46 – 1.07 (5H, m, cyclohexyl-CH_2), 1.01 (3H, t, *J* = 7.6 Hz, H-11); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 176.08 and 171.96 (C=O), 138.27 (C-5), 129.19 (C-7 and C-7'), 128.42 (C-6 and C-6'), 126.52 (C-8), 73.41 (C-2), 55.92 (C- 3), 48.03 (C-12), 35.45 (C-4), 33.16 (cyclohexyl-CH_2), 32.62 (cyclohexyl-CH_2), 29.43 (C-10), 25.44 (cyclohexyl-CH_2), 24.79 (cyclohexyl-CH_2), 9.87 (C-11); **HRMS** (m/*z*), calculated for $C_{19}H_{29}N_2O_3$: 333.2178, found $(M + H)^+$: 333.2175.

4.20 Preparation of (2*S*,3*S*)-*N*-cyclohexyl-2-hydroxy-4-phenyl-3-propion amidobutanamide 27B

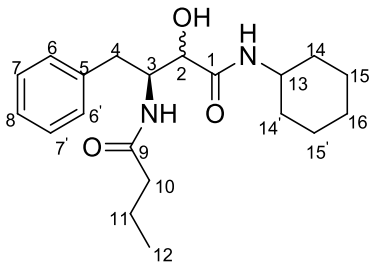
Compound **22B** (57 mg, 0.13 mmol), TFA (101 µl, 1.31 mmol) and Et_3N (181 µl, 1.31 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11** to yield compound **27B** (24 mg, 56%).



R_f = 0.32 (49:1 $CHCl_3$/MeOH); **m.p.** 154 – 155 °C; **IR** ν_{max}(cm^{-1}): 3390 (OH), 3297 (NH), 2978 (C-H), 2850 (C-H), 1634 (C=O); **^{1}H NMR** (300 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.8 Hz, NH or OH), 7.44 (1H, d, *J* = 8.5 Hz, NH or OH) 7.25 – 7.01 (5H, m, Ar), 5.80 (1H, d, *J* = 6.0 Hz, NH or OH), 4.28 – 4.16 (1H, m, H-3), 3.90 – 3.83 (1H, m, H-2), 3.54 (1H, s, H-12), 2.68 – 2.48 (2H, m, H-4), 1.92 (2H, q, *J* = 7.5 Hz, H-10), 1.72 – 1.55 (3H, m, cyclohexyl-CH_2), 1.55 – 1.36 (2H, m, cyclohexyl-CH_2), 1.31 – 1.13 (5H, m, cyclohexyl-CH_2), 0.79 (3H, t, *J* = 7.6 Hz, H-11); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 172.50 and 170.59 (C=O), 139.20 (C-5), 128.95 (C-7 and C-7'), 127.88 (C-6 and C-6'), 125.77 (C-8), 73.24 (C-2), 53.05 (C-3), 47.16 (C-12), 33.94 (C-4), 32.30 and 32.07 (cyclohexyl-CH_2), 28.41 (C-10), 25.08 and 24.64 (cyclohexyl-CH_2), 9.83 (C-11); **HRMS** (m/*z*), calculated for $C_{19}H_{29}N_2O_3$: 333.2178, found, $(M + H)^+$: 333.2173.

4.21 Preparation of (3*S*)-3-butyramido-*N*-cyclohexyl-2-hydroxy-4-phenyl butanamide 28

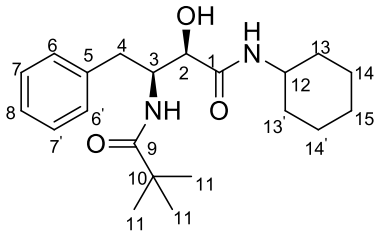
Compound **23** (250 mg, 0.54 mmol), TFA (418 µl, 5.43 mmol) and Et_3N (743 µl, 5.43 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11** to yield compound **28** (65 mg, 36%) as a white solid.



R_f = 0.50 (49:1 $CHCl_3$/MeOH); **m.p.** 182 – 183 °C; **IR** v_{max}(cm^{-1}): 3278 (OH), 2930 (C-H), 1642 (C=O), 1124 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.34 – 7.15 (5H, m, Ar-H), 7.01 (0.34H, d, *J* = 8.5 Hz, NH), 6.93 (0.60H, d, *J* = 8.4 Hz, NH), 6.74 (0.60H, d, *J* = 8.0 Hz, NH), 6.47 (0.54H, d, *J* = 6.9 Hz, NH), 6.24 (0.34H, d, *J* = 6.7 Hz, OH), 6.07 (0.33H, d, *J* = 5.1 Hz, OH), 4.30 – 4.04 (2H, m, H-2 and H-3), 3.85 – 3.62 (1H, m, H-13), 3.21 – 2.94 (2H, m, H-4), 2.15 – 2.00 (2H, m, H-10), 1.98 – 1.44 (7H, m, cyclohexyl-CH_2 and H-11), 1.44 – 1.08 (5H, m, cyclohexyl-CH_2), 0.94 – 0.78 (3H, m, H-12); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 176.11, 175.28, 171.80 and 170.86 (C=O), 138.19 and 137.86 (C-5), 129.20 and 128.62 (C-7 and C-7'), 128.47 (C-6 and C-6'), 126.71 and 126.57 (C-8), 74.93 and 73.55 (C-2), 57.72 and 56.06 (C-3), 48.04 and 47.96 (C-13), 38.29 and 38.13 (C-4), 35.72 and 35.57 (C-10), 33.21, 33.15, 32.80, 32.69, 25.46 and 24.80 (cyclohexyl-CH_2), 19.07 (C-11), 13.63 and 13.56 (C-12); **HRMS** (m/*z*), calculated for $C_{20}H_{31}N_2O_3$: 347.2335, found $(M + H)^+$: 347.2330.

4.22 Preparation of (2*R*,3*S*)-*N*-cyclohexyl-2-hydroxy-4-phenyl-3-pivalamido butanamide 29A

Compound **24A** (50 mg, 0.11 mmol), TFA (84 µl, 1.09 mmol) and Et_3N (151 µl, 1.09 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11** to yield compound **29A** (37 mg, 93%) as a white solid.

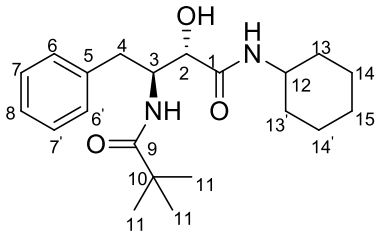


R_f = 0.37 (49:1 $CHCl_3$/MeOH); **m.p.** 182 – 183 °C; **IR** v_{max}(cm^{-1}): 3341 (OH), 3264 (NH), 3030 (C=CH), 1653 (C=O), 1619 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.36 – 7.21 (2H, m, H-7 and H-7'), 7.21 – 7.11 (3H, m, H-6, H-6' and H-8), 6.97 (1H, d, *J* = 8.5 Hz, NH), 6.78 (1H, d, *J* = 8.1 Hz, NH), 6.56 (1H, br-s, OH), 4.32 – 4.20 (1H, m, H-3), 4.09 (1H, s, H-2), 3.84 – 3.65 (1H, m, H-12), 3.10 (1H, dd, *J* = 13.8, 5.7 Hz, H-4a), 3.02 – 2.93 (1H, m, H-4b), 1.94 – 1.78 (2H, m, cyclohexyl-CH_2), 1.77 – 1.65 (2H, m, cyclohexyl-CH_2),

1.65 – 1.52 (1H, m, cyclohexyl-CH_2), 1.44 – 1.28 (3H, m, cyclohexyl-CH_2), 1.25 – 1.09 (2H, m, cyclohexyl-CH_2), 1.08 – 0.92 (9H, m, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 180.65 and 171.82 (C=O), 138.27 (C-5), 129.26 (C-7 and C-7'), 128.35 (C-6 and C-6'), 126.47 (C-8), 73.62 (C-2), 55.49 (C-3), 47.96 (C-12), 38.63 (C-10), 35.28 (C-4), 33.32 and 32.77 (cyclohexyl-CH_2), 27.39 C-11, 27.18 (C-11), 25.45 and 24.84 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{21}H_{33}N_2O_3$: 361.2491, found, $(M + H)^+$: 361.2488.

4.23 Preparation of (2*S*,3*S*)-*N*-cyclohexyl-2-hydroxy-4-phenyl-3-pivalamido butanamide 29B

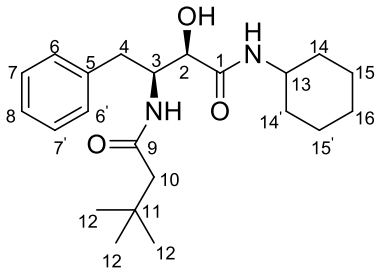
Compound **24B** (50 mg, 0.11 mmol), TFA (84 µl, 1.09 mmol) and Et_3N (151 µl, 1.09 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11** to yield compound **29B** (26 mg, 65%) as a white precipitate.



R_f = 0.45 (49:1 $CHCl_3$/MeOH); **m.p.** 123 – 125 °C; **IR** ν_{max}(cm^{-1}): 3303 (OH), 3029 (C=CH), 1644 (C=O), 1152 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.40 – 7.26 (2H, m, H-7 and H-7'), 7.24 – 7.13 (3H, m, H-6, H-6' and H-8), 6.94 (1H, d, *J* = 8.5 Hz, NH), 6.14 (1H, br-s, OH), 5.97 (1H, d, *J* = 6.3 Hz, NH), 4.26 – 4.18 (2H, m, H-2 and H-3), 3.84 – 3.69 (1H, m, H-12), 3.21 (1H, dd, *J* = 14.1, 10.5 Hz, H-4a), 3.05 (1H, dd, *J* = 14.0, 5.3 Hz, H-4b), 1.98 – 1.80 (2H, m, cyclohexyl-CH_2), 1.77 – 1.65 (2H, m, cyclohexyl-CH_2), 1.65 – 1.52 (1H, m, cyclohexyl-CH_2), 1.44 – 1.09 (5H, m, cyclohexyl-CH_2), 1.03 (9H, s, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 181.77 and 170.74 (C=O), 137.81 (C-5), 129.16 (C-7 and C-7'), 128.66 (C-6 and C-6'), 126.78 (C-8), 75.20 (C-2), 57.58 (C-3), 47.91 (C-12), 38.61 (C-10), 35.81 (C-4), 33.35 and 32.85 (cyclohexyl-CH_2), 27.35 (C-11), 25.47 and 24.84 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{21}H_{33}N_2O_3$: 361.2491, found, $(M + H)^+$: 361.2487.

4.24 Preparation of (2*R*,3*S*)-*N*-cyclohexyl-3-(3,3-dimethylbutanamido)-2-hydroxy-4-phenylbutanamide 30A

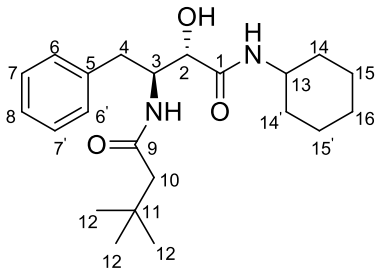
Compound **25A** (75 mg, 0.16 mmol), TFA (122 µl, 1.58 mmol) and Et_3N (151 µl, 1.58 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11** to yield compound **30A** (51 mg, 86%) as a white solid.



R_f = 0.37 (49:1 $CHCl_3$/MeOH); **m.p.** 129 – 130 °C; **IR** $\nu_{max}(cm^{-1})$: 3274 (OH), 3088 (C=CH), 2926 (C-H), 1729 (C=O), 1643 (C=O), 1256 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.17 (5H, m, Ar-H), 6.89 (1H, d, *J* = 8.3 Hz), 6.47 (0.9H, d, *J* = 7.8 Hz) and 6.39 (1H, d, *J* = 7.1 Hz) and 6.31 (0.1H, d, *J* = 7.0 Hz, 2xNH and OH) 4.27 – 4.13 (1H, m, H-3), 4.09 – 4.02 (1H, m, H-2), 3.77 – 3.64 (1H, m, H-13), 3.21 – 3.12 (1H, m, H-4a), 3.05 (1H, dd, *J* = 13.8, 9.1 Hz, H-4b), 1.98 (1H, d, *J* = 13.0 Hz) and 1.93 (1H, d, *J* = 13.0 Hz, H-10), 1.91 – 1.77 (2H, m, cyclohexyl-CH_2), 1.76 – 1.64 (3H, m, cyclohexyl-CH_2), 1.47 – 1.00 (5H, m, cyclohexyl-CH_2), 0.92 (9H, s, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.26 and 171.78 (C=O), 138.05 (C-5), 129.22 (C-7 and C-7'), 128.54 (C-6 and C-6'), 126.63 (C-8), 73.68 (C-2), 56.17 (C-3), 50.18 (C-10), 48.13 (C-13), 35.64 (C-4), 33.03 and 32.75 (cyclohexyl-CH_2), 30.71 (C-11), 29.67 (C-12), 25.44 and 24.77 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{22}H_{35}N_2O_3$: 375.2648, found, $(M + H)^+$: 375.2640.

4.25 Preparation of (2*S*,3*S*)-*N*-cyclohexyl-3-(3,3-dimethylbutanamido)-2-hydroxy-4-phenylbutanamide 30B

Compound **25B** (50 mg, 0.11 mmol), TFA (81 µl, 1.05 mmol), Et_3N (146 µl, 1.05 mmol) and CH_2Cl_2 (1 ml) were reacted together as described in **4.11** yielding compound **30B** (30 mg, 73%) as a white solid.

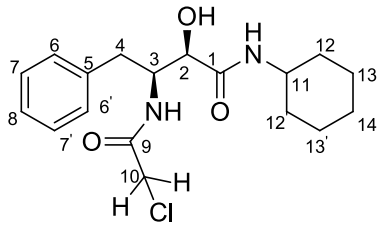


R_f = 0.37 (49:1 $CHCl_3$/MeOH); **m.p.** 130 – 132 °C; **IR** $\nu_{max}(cm^{-1})$: 3279 (OH), 3092 (NH), 2930 (C-H), 2859 (C-H), 1636 (C=O); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 7.59 (1H, d, *J* = 8.7 Hz, NH), 7.50 (1H, d, *J* = 8.4 Hz, NH), 7.25 – 7.19 (2H, m, H-7 and H-7'), 7.18 – 7.10 (3H, m, H-6, H-6' and H-8), 5.90 (0.1H, d, *J* = 5.8 Hz) and 5.83 (0.9H, d, *J* = 5.8 Hz, OH), 4.40 – 4.31 (1H, m, H-3), 3.98 – 3.91 (1H, m, H-2), 3.61 (1H, s, H-13), 3.10 (2H, d, *J* = 7.7 Hz, H-4), 2.70 – 2.62 (1H, m, H-4a), 2.59 – 2.54 (1H, m, H-4b), 1.78 – 1.61 - 1.49 (5H, m, cyclohexyl-CH_2), 1.35 – 1.06 (5H, m, cyclohexyl-CH_2), 0.79 (9H, s, H-12); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.16 and 170.94 (C=O), 139.68 (C-5), 129.47 (C-7 and 7'), 128.37 (C-6 and 6'), 126.27 (C-8), 73.86 (C-2), 53.50 (C-3), 49.15 (C-10), 47.69 (C-13), 34.28 (C-4), 32.81 and

32.59 (cyclohexyl-CH_2), 30.74 (C-11), 30.06 (C-12), 25.59 and 25.22 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{22}H_{35}N_2O_3$: 375.2648, found, $(M + H)^+$: 375.2644.

4.26 Preparation of (2*R*,3*S*)-3-(2-chloroacetamido)-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide 31A

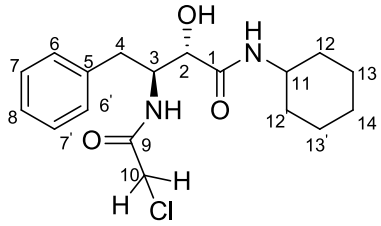
Compound **20A** (52 mg, 0.11 mmol), TFA (98 µl, 1.14 mmol) and Et_3N (177 µl, 1.14 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **31A** (35 mg, 88%) as a white solid.



R_f = 0.47 (49:1 $CHCl_3$/MeOH); **m.p.** 169 – 170 °C; **IR** (solid) v_{max} (cm^{-1}): 3404 (OH), 3298 (NH), 3032 (C=CH), 1661 (C=O), 1614 (C=O), 892 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.69 (1H, d, *J* = 9.0 Hz, NH), 7.31 – 7.23 (2H, m, H-7 and H-7'), 7.23 – 7.15 (3H, m, H-6, H-6' and H-8), 6.84 (1H, d, *J* = 9.2 Hz, NH), 5.59 (1H, d, *J* = 6.0 Hz, OH), 4.39 – 4.24 (1H, m, H-3), 4.16 – 4.07 (1H, m, H-2), 3.83 (2H, s, H-10), 3.79 – 3.69 (1H, m, H-11), 3.09 (1H, dd, *J* = 13.7, 5.8 Hz, H-4a), 2.84 (1H, dd, *J* = 13.7, 9.3 Hz, H-4b), 1.91 – 1.80 (2H, m, cyclohexyl-CH_2), 1.77 – 1.66 (2H, m, cyclohexyl-CH_2), 1.66 – 1.56 (1H, m, cyclohexyl-CH_2), 1.44 – 1.27 (2H, m, cyclohexyl-CH_2), 1.24 – 1.07 (3H, m, cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.25 and 167.23 (C=O), 137.60 (C-5), 129.19 (C-7 and C-7'), 128.50 (C-6 and C-6'), 126.69 (C-8), 72.21 (C-2), 55.38 (C-3), 48.10 (C-11), 42.30 (C-10), 35.63 (C-4), 33.11, 32.68, 25.39 and 24.78 (cyclohexyl-CH_2); **HRMS** (m/z), calculated for $C_{18}H_{26}ClN_2O_3$: 353.1632, found, $(M + H)^+$: 353.1624.

4.27 Preparation of (2*S*,3*S*)-3-(2-chloroacetamido)-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide 31B

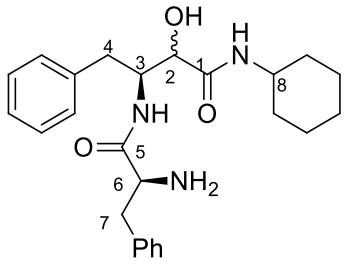
Compound **20B** (28 mg, 0.06 mmol), TFA (48 µl, 0.61 mmol) and Et_3N (85 µl, 0.61 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **31B** (20 mg, 90%) as a white solid.



R_f = 0.50 (49:1 $CHCl_3$/MeOH); **m.p.** 209 – 210 °C; **IR** $\nu_{max}(cm^{-1})$: 3399 (OH), 3280 (NH), 3028 (C=CH), 1658 (C=O), 1618 (C=O), 893 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) 7.32 – 7.26 (3H, m, Ar-H), 7.25 – 7.10 (5H, m, Ar-H, 2xNH and OH), 4.34 – 4.25 (1H, m, H-3), 4.25 – 4.18 (1H, m, H-2), 3.96 (1H, d, *J* = 15.0 Hz) and 3.90 (1H, d, *J* = 15.0 Hz, H-10), 3.83 – 3.71 (1H, m, H-11), 3.00 – 2.87 (2H, m, H-4), 1.94 – 1.83 (2H, m, cyclohexyl-CH_2), 1.80 – 1.68 (2H, m, cyclohexyl-CH_2), 1.68 – 1.58 (1H, m, cyclohexyl-CH_2), 1.44 – 1.32 (2H, m, cyclohexyl-CH_2), 1.31 – 1.04 (3H, m, cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.12 and 167.96 (C=O), 137.30 (C-5), 129.14 (C-7 and C-7'), 128.67 (C-6 and C-6'), 126.83 (C-8), 73.23 (C-2), 56.72 (C-3), 48.27 (C-11), 42.25 (C-10), 34.82 (C-4), 33.01, 32.85, 25.46 and 24.83 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{18}H_{26}ClN_2O_3$: 353.1632, found, $(M + H)^+$: 353.1628.

4.28 Preparation of (3*S*)-3-((*S*)-2-amino-3-phenylpropanamido)-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide 32

Compound **28** (200 mg, 0.32 mmol), TFA (247 µl, 3.21 mmol) and Et_3N (445 µl, 3.21 mmol) in CH_2Cl_2 (3 ml) were reacted together as described in **4.11**. The reaction yielded compound **32** (92 mg, 68%) as a white solid which was a mixture of diastereomers.

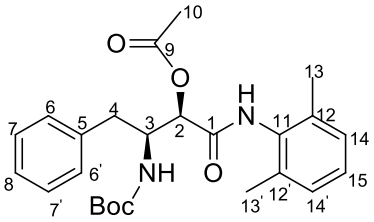


R_f = 0.41 (49:1 $CHCl_3$/MeOH); **m.p.** 149 – 150 °C; **IR** (solid): 3269 (OH), 3027 (C=CH), 2930 (C-H), 1671 (C=O), 1641 (C=O), 1641 (C=O), 1198 (C-O); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.64 (0.60H, d, *J* = 8.8 Hz) and 8.48 (0.37H, d, *J* = 8.8 Hz) and 7.63 (0.61H, d, *J* = 8.4 Hz) and 7.49 (0.37H, d, *J* = 8.3 Hz, 2xNH), 7.35 – 7.09 (10H, m, Ar-H), 6.37 (0.38H, d, *J* = 5.7 Hz) and 6.24 (0.61H, d, *J* = 5.8 Hz, OH), 4.45 – 4.35 (0.62H, m) and 4.32 – 4.21 (0.42H, m, H-3), 4.08 (0.39H, dd, *J* = 8.9, 4.0 Hz, H-6), 3.98 – 3.90 (1H, m, H-2), 3.85 – 3.80 (0.40H, m, H-6), 3.67 – 3.31 (2H, m, H-4), 3.19 – 3.12 (1H, m, H-8), 2.97 – 2.57 (2H, m, H-7), 2.51 (2H, s, NH_2), 1.79 – 1.46 (5H, m, cyclohexyl-CH_2), 1.37 – 1.01 (5H, m, cyclohexyl-CH_2); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.14, 170.90, 168.62 and 168.41 (C=O), 139.35 (Ar-C), 138.98 (Ar-C), 135.68 (Ar-C), 135.66 (Ar-C), 130.00 (Ar-C), 129.95 (Ar-C), 129.70 (Ar-C), 129.45 (Ar-C), 128.93 (Ar-C), 128.90 (Ar-C), 128.83 (Ar-C), 128.62 (Ar-C), 127.48 (Ar-C), 126.73 (Ar-C), 126.48 (Ar-C), 73.40 (C-2), 70.45 (C-2), 54.23 (C-3), 54.12 (C-3), 53.83 (C-6), 47.80 (C-8), 47.76 (C-8), 37.93 (C-

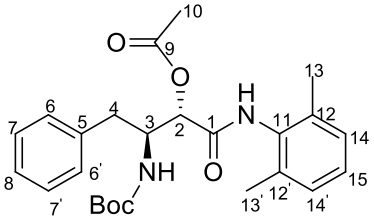
4), 37.82 (C-4), 37.34 (C-7), 34.40 (C-7), 32.77 and 32.69, 32.49, 25.56, 25.21, 25.16 and 24.97 (cyclohexyl-CH_2); **HRMS** (m/*z*), calculated for $C_{25}H_{34}N_3O_3$: 424.2600 found, $(M + H)^+$: 424.2597.

4.29 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethyl phenyl)amino)-1-oxo-4-phenylbutan-2-yl acetate 33

N-Boc-L-phenylalaninal **15** (307 mg, 1.23 mmol), xylyl isocyanide **10** (161 mg, 1.23 mmol) and acetic acid **1** (83 µl, 1.23 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. The reaction yielded compound **33** (534 mg, 99%), a yellow solid, as a mixture of diastereomers. $\mathbf{R_f}$ = 0.53 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.88:1.00. This diastereomeric mixture was then separated using preparative HPLC as described in **4.10** to give two clean separated diastereomers.



(2*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethylphenyl)amino)-1-oxo-4-phenylbutan-2-yl acetate 33A. **m.p.** 176 – 177 °C; **IR** ν_{max} (cm^{-1}): 3314 (NH), 3256 (NH), 2918 (C-H), 2849 (C-H), 1738 (C=O), 1692 (C=O), 1676 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.35 – 7.14 (5H, m, Ar-H), 7.14 – 6.97 (3H, m, Ar-H), 5.40 – 5.18 (1H, m, H-2), 5.18 – 5.05 (1H, m, NH), 4.54 – 4.38 (1H, m, H-3), 2.99 – 2.80 (2H, m, H-4), 2.26 – 2.09 (9H, m, H-10, H-13 and H-13'), 1.43 – 1.10 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.37 and 166.42 (C=O), 154.97 (Boc C=O), 137.07 (C-11), 135.49 (C-5), 132.63 (C-12 and 12'), 129.36 (Ar-C), 128.61 (Ar-C), 128.23 (Ar-C), 127.65 (Ar-C), 126.75 (Ar-C), 79.70 [$(CH_3)_3\underline{C}$], 74.14 (C-2), 53.19 (C-3), 38.56 (C-4), 28.26 ($(\underline{C}H_3)_3C$), 20.67 (C-10), 18.38 (C-13 and 13'); **HRMS** (m/*z*), calculated for $C_{25}H_{33}N_2O_5$: 441.2389, found, $(M + H)^+$: 441.2380.

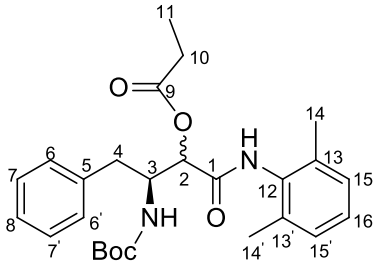


(2*S*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethylphenyl)amino)-1-oxo-4-phenylbutan-2-yl acetate 33B. **m.p.** 166 – 167 °C; **IR** ν_{max} (cm^{-1}): 3317 (NH), 3030 (C=CH), 2920 (C-H), 1744 (C=O), 1690 (C=O), 1676 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52 (1H, s, NH), 7.33 – 7.17 (5H, m, Ar-H), 7.15 – 7.01 (3H, m, Ar-H), 5.36 (1H, d, *J* = 3.9 Hz, H-2), 4.95 (1H, d, *J* = 9.2 Hz, NH), 4.43 – 4.34 (1H, m, H-3), 3.13 (1H, dd, *J* = 14.1, 6.0 Hz, H-

4a), 3.06 – 2.88 (1H, m, H-4b), 2.23 (6H, s, H-13 and 13'), 2.21 – 2.13 (3H, m, H-10), 1.41 – 1.17 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.85 and 166.29 (C=O), 155.52 Boc (C=O), 137.31, 135.24 and 132.72 (C-5, C-12 and 12'), 129.24 (Ar-C), 128.64 (Ar-C), 128.54 (Ar-C), 128.33 (Ar-C), 128.26 (Ar-C), 127.67 (Ar-C), 126.77 (Ar-C), 126.68 (Ar-C), 79.68 [$(CH_3)_3\underline{C}$], 74.93 (C-2), 53.44 (C-3), 37.39 (C-4), 28.25 [$(\underline{C}H_3)_3C$], 20.87 (C-10), 18.53 and 18.39 (C-13 and C-13'); **HRMS** (m/*z*), calculated for $C_{25}H_{33}N_2O_5$: 441.2389, found, $(M + H)^+$: 441.2376.

4.30 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethyl phenyl)amino)-1-oxo-4-phenylbutan-2-yl propionate 34

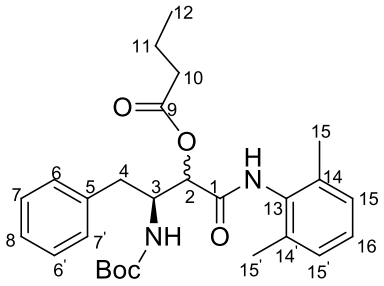
N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), xylyl isocyanide **10** (263 mg, 2.01 mmol) and propionic acid **2** (150 µl, 2.01 mmol) were dissolved in of CH_2Cl_2 (5 ml). The experiment was carried out as described in **4.5** yielding compound **34** (908 mg, 99%) as a white solid which was a mixture of diastereomers and rotamers.



R_f = 0.62 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.83:1.00; **m.p.** 154 – 155 °C; **IR** ν_{max}(cm^{-1}): 3328 (NH), 3296 (NH), 2976 (C-H), 2933 (C-H), 1736 (C=O), 1686 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.34 – 7.16 (5H, m, Ar-H), 7.14 – 7.01 (3H, m, Ar-H), 5.43 – 5.28 (1H, m, H-2), 5.12 (0.57H, d, *J* = 9.5 Hz) and 4.99 (0.40H, d, *J* = 9.3 Hz, NH), 4.52 – 4.35 (1H, m, H-3), 3.18 – 3.09 (0.54H, m, H-4a), 3.05 – 2.80 (1.5H, m, H-4a and H-4b), 2.59 – 2.40 (2H, m, H-10), 2.25 – 2.10 (6H, m, H-14 and 14'), 1.40 – 1.15 (12H, m, $(CH_3)_3C$ and H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.25, 172.84, 166.54 and 166.41 (C=O), 155.51 and 155.00 (Boc C=O), 137.34 (Ar-C), 137.11 (Ar-C, 135.50 (Ar-C), 135.23 (Ar-C), 132.78 (Ar-C), 132.65 (Ar-C), 129.37 (Ar-C), 129.25 (Ar-C), 128.61 (Ar-C), 128.53 (Ar-C), 128.32 (Ar-C), 128.25 (Ar-C), 127.66 (Ar-C), 127.64 (Ar-C), 126.75 (Ar-C), 126.66 (Ar-C), 79.74 and 79.64 [$(CH_3)_3\underline{C}$], 74.77 and 73.91 (C-2), 53.44 and 53.29 (C-3), 38.55 and 37.48 (C-4), 28.25 ($(\underline{C}H_3)_3C$), 27.51 and 27.41 (C-10), 18.54, 18.39 (C-14 and C-14'), 9.10 and 8.94 (C-11); **HRMS** (m/*z*), calculated for $C_{26}H_{35}N_2O_5$: 455.2546, found, $(M + H)^+$: 455.2541.

4.31 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethyl phenyl)amino)-1-oxo-4-phenylbutan-2-yl butyrate 35

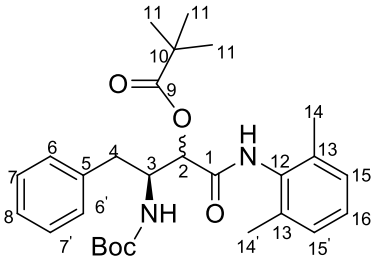
N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), xylyl isocyanide **10** (158 mg, 1.20 mmol) and butyric acid **3** (110 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment was carried out as described in **4.5** yielding compound **35** (460 mg, 82%) as a white solid which was a mixture of diastereomers.



R_f = 0.63 (49:1 $CHCl_3$/MeOH); **d.r.** = 2.06:1.00; **m.p.** 170 – 171 °C; **IR** ν_{max}(cm^{-1}): 3339 (NH), 3257 (NH), 3029 (C=CH), 2932 (C-H), 1712 (C=O), 1687 (C=O), 1654 (C=O) 1390 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.74 (0.29H, s, NH), 7.61 (0.16H, s, NH), 7.38 (0.46H, s, NH), 7.33 – 7.13 (5H, m, Ar-H), 7.11 – 6.87 (3H, m, Ar-H), 5.53 – 5.23 (1H, m, H-2), 5.21 – 5.00 (1H, m, NH), 4.54 – 4.29 (1H, m, H-3), 3.09 (0.31H, dd, *J* = 14.0, 5.4 Hz, H-4a), 2.99 – 2.52 (1.72H, m, H-4a and 4b), 2.51 – 2.32 (2H, m, H-10), 2.24 – 2.01 (6H, m, H-15 and 15'), 1.80 – 1.60 (2H, m, H-11), 1.44 – 1.02 (9H, m, $(CH_3)_3C$), 1.05 – 0.92 (3H, m, H-12); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.49, 172.04, 166.59 and 166.46 (C=O), 155.43 and 155.02 (Boc C=O), 137.38 (Ar-C), 137.17 (Ar-C), 135.51 (Ar-C), 135.27 (Ar-C), 132.91 (Ar-C), 132.79 (Ar-C), 129.39 (Ar-C), 129.29 (Ar-C), 128.54 (Ar-C), 128.45 (Ar-C), 128.25 (Ar-C), 128.18 (Ar-C), 127.56 (Ar-C), 127.54 (Ar-C), 126.67 (Ar-C), 126.57 (Ar-C), 79.60 [$(CH_3)_3\underline{C}$], 74.77 and 73.92 (C-2), 53.29 (C-3), 38.53 and 37.43 (C-4), 35.96 and 35.81 (C-10), 28.23 [$(\underline{C}H_3)_3C$], 18.50, 18.37 and 18.28 (C-15 and C-15'), 13.65 (C-12); **HRMS** (m/*z*), calculated for $C_{27}H_{37}N_2O_5$: 466.2702, found, $(M + H)^+$: 469.2702.

4.32 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethyl phenyl)amino)-1-oxo-4-phenylbutan-2-yl pivalate 36

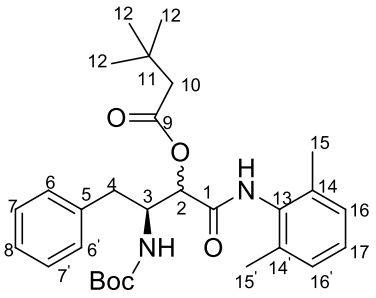
N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), xylyl isocyanide **10** (158 mg, 1.20 mmol) and pivalic acid **4** (123 mg, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment was carried out as described in **4.5** yielding compound **36** (575 mg, 99%) as an off white precipitate which was a mixture of diastereomers.



R_f = 0.65 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.88:1.00; **m.p.** 206 – 207 °C; **IR** ν_{max}(cm^{-1}): 3266 (NH), 2975 (C-H), 2931 (C-H), 1734 (C=O), 1690 (C=O), 1663 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.62 (0.56H, s) and 7.46 (0.31H, s, NH), 7.37 – 7.16 (5H, m, Ar-H), 7.15 – 6.97 (3H, m, Ar-H), 6.45 – 6.28 (0.28H, m, NH) 5.59 – 5.26 (1H, m, H-2), 5.20 – 5.00 (0.65H, m, NH), 4.53 – 4.32 (1H, m, H-3), 3.22 – 2.64 (2H, m, H-4), 2.32 – 2.06 (6H, m, H-14 and 14'), 1.46 – 1.05 (18H, m, $(CH_3)_3C$ and H-11); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 177.16, 176.76 and 166.59 (C=O), 155.40 (Boc C=O), 137.34 (Ar-C), 137.15 (Ar-C), 135.47 (Ar-C), 135.18 (Ar-C), 132.88 (Ar-C), 132.74 (Ar-C), 129.40 (Ar-C), 129.28 (Ar-C), 128.59 (Ar-C), 128.49 (Ar-C), 128.29 (Ar-C), 128.22 (Ar-C), 127.61 (Ar-C), 127.58 (Ar-C), 126.73 (Ar-C), 126.62 (Ar-C), 79.68 ($(CH_3)_3\underline{C}$), 79.45 [$(CH_3)_3\underline{C}$], 74.57 and 73.64 (C-2), 53.55 and 53.42 (C-3), 38.96 (C-10), 38.36 and 37.68 (C-4), 28.25 ($(\underline{C}H_3)_3C$), 27.19, 27.11 and 27.08 (C-11), 18.55 and 18.39 (C-14 and C-14'); **HRMS** (m/*z*), calculated for $C_{28}H_{39}N_2O_5$: 483.2859, found, $(M + H)^+$: 483.2857.

4.33 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethyl phenyl)amino)-1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 37

N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), xylyl isocyanide **10** (263 mg, 2.01 mmol) and *tert*-butyl acetic acid **5** (255 µl, 2.01 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment was carried out as described in **4.5** yielding compound **37** (936 mg, 94%) as a white solid which was a mixture of diastereomers.

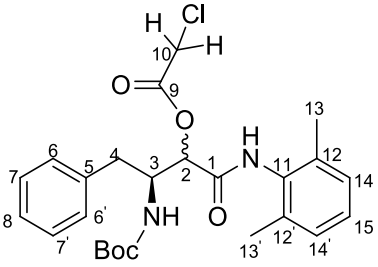


R_f = 0.63 (49:1 $CHCl_3$/MeOH); **d.r.** = 2.21:1.00; **m.p.** 213 – 214 °C; **IR** ν_{max}(cm^{-1}): 3337 (NH), 3275 (NH), 3030 (C=CH), 2960 (C-H), 2868, 1737 (C=O), 1679 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.59 (0.3H, br-s, NH), 7.28 (0.2H, br-s, NH), 7.46 – 7.14 (5H, m, Ar-H), 7.14 – 7.00 (3H, m, Ar-H), 5.49 (0.2H, br-s, NH), 5.45 – 5.27 (1H, m, H-2), 5.11 (0.52H, d, *J* = 9.4 Hz, NH), 4.97 (0.31H, d, *J* = 9.0 Hz, NH), 4.50 – 4.32 (1H, m, H-3), 3.20 – 2.68 (2H, m, H-4), 2.52 – 2.31 (2H, m, H-10), 2.28 – 2.10 (6H, m, H-15 and H-15'), 1.47 – 1.17 (9H, m, $(CH_3)_3C$), 1.16 – 1.03 (9H, m, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.20, 170.66, 166.59 and 166.45 (C=O), 155.40 and 154.98 (Boc C=O), 137.38 (Ar-C), 137.18 (Ar-C), 135.52 (Ar-C), 135.29 (Ar-C), 132.85 (Ar-C), 132.66 (Ar-C), 129.38 (Ar-C),

129.24 (Ar-C), 128.61 (Ar-C), 128.53 (Ar-C), 128.31 (Ar-C), 128.25 (Ar-C), 127.65 (Ar-C), 127.61 (Ar-C), 126.73 (Ar-C), 126.65 (Ar-C), 79.65 [$(CH_3)_3\underline{C}$], 74.53 and 73.57 (C-2), 53.57 and 53.42 (C-3), 47.78 and 47.46 (C-10), 38.42 and 37.26 (C-4), 30.95 (C-11), 29.66 and 29.59 (C-12), 29.64 (C-12), 28.26 ($(\underline{C}H_3)_3C$), 18.57 and 18.42 (C-15 and C-15'); **HRMS** (m/*z*), calculated for $C_{29}H_{41}N_2O_5$: 497.3015, found, $(M + H)^+$: 497.3015.

4.34 Preparation of (3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethyl phenyl)amino)-1-oxo-4-phenylbutan-2-yl 2-chloroacetate 38

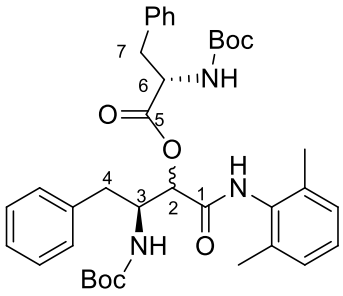
N-Boc-L-phenylalaninal **15** (262 mg, 1.05 mmol), xylyl isocyanide **10** (138 mg, 1.05 mmol) and chloroacetic acid **5** (99 mg, 1.05 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment was carried out as described in **4.5** yielding compound **38** (423 mg, 85%) as a white precipitate which was a mixture of diastereomers.



R_f = 0.54 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.72:1.00; **m.p.** 161 – 162 °C; **IR** $\nu_{max}(cm^{-1})$: 3328 (NH), 3241 (NH), 3027 (C=CH), 2978 (C-H), 1759 (C=O), 1689 (C=O), 1663 (C=O), 762 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.82 (0.26H, s) and 7.53 (0.45H, s, NH), 7.41 – 7.15 (5H, m, Ar-H), 7.14 – 6.91 (3H, m, Ar-H), 5.46 – 5.22 (1H, m, H-2), 5.22 – 4.88 (1H, m, NH), 4.60 – 4.35 (1H, m, H-3), 4.26 – 3.89 (2H, m, H-10), 3.23 – 2.62 (2H, m, H-4), 2.24 – 2.06 (6H, m, H-13 and 13'), 1.43 – 1.02 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.52, 171.24, 166.13, 165.98, 165.85 and 165.58 (C=O), 155.60 and 154.99 (Boc C=O), 137.06 (Ar-C), 136.88 (Ar-C), 135.53 (Ar-C), 135.24 (Ar-C), 135.18 (Ar-C), 132.70 (Ar-C), 132.60 (Ar-C), 129.34 (Ar-C), 129.29 (Ar-C), 129.19 (Ar-C), 128.64 (Ar-C), 128.55 (Ar-C), 128.52 (Ar-C), 128.32 (Ar-C), 128.24 (Ar-C), 128.10 (Ar-C), 127.72 (Ar-C), 127.69 (Ar-C), 126.82 (Ar-C), 126.73 (Ar-C), 79.90 [$(CH_3)_3\underline{C}$], 76.46 and 75.51 (C-2), 55.84 and 53.09 (C-3), 40.65 and 40.35 (C-10), 28.24 [$(\underline{C}H_3)_3C$], 18.53 and 18.37 (C-13 and 13'); **HRMS** (m/*z*), calculated for $C_{25}ClH_{32}N_2O_4$: 475.2000, found, $(M + H)^+$: 475.2006.

4.35 Preparation of (2*S*)-(3*S*)-3-((*tert*-butoxycarbonyl)amino)-1-((2,6-dimethylphenyl)amino)-1-oxo-4-phenylbutan-2-yl 2-((*tert*-butoxycarbonyl) amino)-3-phenylpropanoate 39

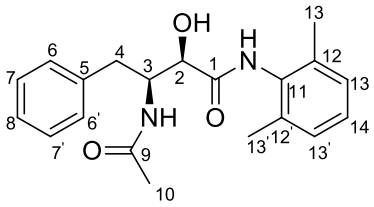
N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), xylyl isocyanide **10** (263 mg, 2.01 mmol) and *N*-Boc-L-phenylalanine **7** (533 mg, 2.01 mmol) were dissolved in CH_2Cl_2 (10 ml). A white precipitate was formed after 1 d of stirring. The reaction product was cleaned by filtration, washing with CH_2Cl_2 and drying *in vacuo* yielding compound **39** (630 mg, 49%) which was a mixture of diastereomers.



d.r. = 1.75:1.00; **m.p.** 197 – 199 °C; **IR** ν_{max}(cm^{-1}): 3354 (NH), 2980 (C-H), 1748 (C=O), 1688 (C=O), 1366 (C-O); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.39 (0.48H, s, NH), 9.25 (0.55H, s, NH), 7.52 (0.52H, d, *J* = 8.4 Hz, NH), 7.46 (0.50H, d, *J* = 8.2 Hz, NH), 7.39 – 7.27 (5H, m, Ar-H), 7.27 – 7.16 (5H, m, Ar-H), 7.14 – 6.99 (3H, m, Ar-H), 6.86 (0.56H, d, *J* = 9.4 Hz, NH), 6.67 (0.51H, d, *J* = 9.7 Hz, NH), 5.22 – 5.12 (0.64H, m) and 5.06 – 4.94 (0.65H, m, H-2), 4.63 – 4.55 (0.61H, m), 4.55 – 4.46 (0.65H, m) and 4.43 – 4.23 (1H, m, H-3 and H-6), 3.32 – 3.19 (1H, m, H-7a), 3.07 – 2.79 (2H, m, H-4), 2.76 – 2.65 (1H, m, H-7b), 2.16 (3H, s) and 2.14 – 2.03 (3H, m, 2xCH_3), 1.39 – 1.24 (18H, m, 2x$(CH_3)_3$C); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.04, 171.90, 166.28 and 166.23 (C=O), 156.25, 156.23 and 155.31 (Boc C=O), 138.56 (Ar-C), 138.25 (Ar-C), 138.16 (Ar-C), 137.95 (Ar-C), 136.06 (Ar-C), 135.86 (Ar-C), 134.66 (Ar-C), 134.60 (Ar-C), 129.72 (Ar-C), 129.70 (Ar-C), 129.68 (Ar-C), 129.60 (Ar-C), 128.66 (Ar-C), 128.56 (Ar-C), 128.21 (Ar-C), 128.08 (Ar-C), 127.31 (Ar-C), 127.25 (Ar-C), 126.96 (Ar-C), 126.89 (Ar-C), 126.76 (Ar-C), 126.62 (Ar-C), 79.06, 78.97 and 78.54 [$(CH_3)_3\underline{C}$], 76.19 and 74.98 (C-2), 55.74, 55.50, 53.50 and 53.23 (C-2 and C-6), 38.50, 36.98, 36.64 and 35.72 (C-4 and C-7), 28.59 and 28.48 [$(\underline{C}H_3)_3$C], 18.56 and 18.44 (CH_3x2); **HRMS** (m/*z*), calculated for $C_{37}H_{38}N_3O_7$: 646.3492, found $(M + H)^+$: 646.3482.

4.36 Preparation of (2*R*,3*S*)-3-acetamido-*N*-(2,6-dimethylphenyl)-2-hydroxy-4-phenylbutanamide 40A

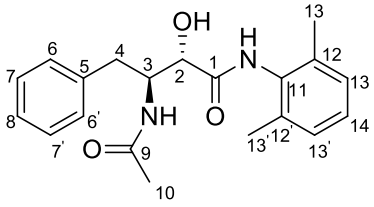
Compound **33A** (100 mg, 0.23 mmol), TFA (175 µl, 2.27 mmol) and Et_3N (315 µl, 2.27 mmol) in CH_2Cl_2 (1.5 ml) were reacted together as described in **4.11**. The reaction yielded compound **40A** (63 mg, 81%) as a white solid.



R_f = 0.35 (49:1 $CHCl_3$/MeOH); **m.p.** 238 – 248 °C; **IR** ν_{max}(cm^{-1}): 3064 (NH), 2924 (C-H), 2854 (C-H), 1658 (C=O), 1617 (C=O); **^{1}H NMR** (500 MHz, CD_3OD) δ 7.25 – 7.17 (4H, m, Ar-H), 7.13 – 7.08 (1H, m, Ar-H), 7.00 – 6.93 (3H, m, Ar-H), 4.52 – 4.46 (2H, m, NH and H-3), 4.06 (1H, d, *J* = 2.2 Hz, H-2), 2.89 (1H, dd, *J* = 13.4, 8.1 Hz, H-4a), 2.82 (1H, dd, *J* = 13.3, 7.4 Hz, H-4b), 2.06 (6H, s, H-13 and 13'), 1.80 (3H, s, H-10); **^{13}C NMR** (126 MHz, CD_3OD) δ 172.74 and 171.44 (C=O), 138.12, 135.45 and 133.65 (C-5, C-12 and 12'), 129.13 (Ar-C), 128.07 (Ar-C), 127.63 (Ar-C), 127.01 (Ar-C), 126.14 (Ar-C), 71.31 (C-2), 53.74 (C-3), 37.64 (C-4), 21.39 (C-10), 17.09 (C-13 and 13'); **HRMS** (m/*z*), calculated for $C_{20}H_{25}N_2O_3$: 341.1865, found, $(M + H)^+$: 341.1854.

4.37 Preparation of (2*S*,3*S*)-3-acetamido-N-(2,6-dimethylphenyl)-2-hydroxy-4-phenylbutanamide 40B

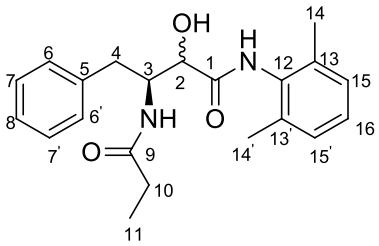
Compound **33B** (40 mg, 0.091 mmol), TFA (70 µl, 0.91 mmol) and Et_3N (126 µl, 0.91 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **40B** (21 mg, 70%) as a white solid.



R_f = 0.37 (49:1 $CHCl_3$/MeOH); **m.p.** 223 – 224 °C; **IR** ν_{max}(cm^{-1}): 3316 (OH), 2909 (C-H), 2851 (C-H), 1745 (C=O); **^{1}H NMR** (500 MHz, CD_3OD) δ 7.34 – 7.15 (5H, m, Ar-H), 7.13 – 7.03 (3H, m, Ar-H), 4.64 – 4.56 (3H, m, 2xNH and H-3), 4.39 (1H, d, *J* = 3.5 Hz, H-2), 3.02 – 2.88 (2H, m, H-4), 2.25 (6H, s, H-13 and 13'), 2.18 – 2.14 (3H, m, H-10); **^{13}C NMR** (126 MHz, CD_3OD) δ 172.74, 172.11, 171.76 and 171.44 (C=O), 138.44 (Ar-C), 138.12 (Ar-C), 135.45 (Ar-C), 135.41 (Ar-C), 133.66 (Ar-C), 128.80 (Ar-C), 127.91 (Ar-C), 127.74 (Ar-C), 127.63 (Ar-C), 127.04 (Ar-C), 126.14 (Ar-C), 125.97 (Ar-C), 73.58 (C-2), 54.11 and 53.75 (C-3), 34.17 (C-4), 21.39 and 21.09 (C-10), 17.29 and 17.09 (C-13 and C-13'); **HRMS** (m/*z*), calculated for $C_{20}H_{25}N_2O_5$: 341.1865 found, $(M + H)^+$: 341.1856.

4.38 Preparation of (3*S*)-*N*-(2,6-dimethylphenyl)-2-hydroxy-4-phenyl-3-propionamidobutanamide 41

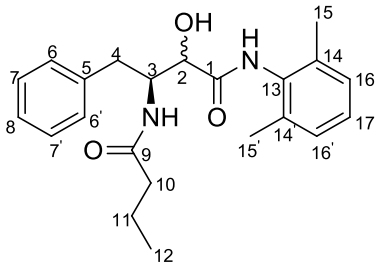
Compound **34** (450 mg, 0.99 mmol), TFA (763 µl, 9.90 mmol) and Et_3N (1.37 ml, 9.90 mmol) in CH_2Cl_2 (3 ml) were reacted together as described in **4.11**. The reaction yielded compound **41** (351 mg, 98%) as an off white solid which was a mixture of diastereomers.



R_f = 0.48 (49:1 $CHCl_3$/MeOH); **m.p.** 150 – 151 °C; **IR** ν_{max}(cm^{-1}): 3318 (OH), 3245 (NH), 3027 (C-H), 1664 (C=O), 1619 (C=O); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.29 (0.24H, s, NH), 9.10 (0.66H, s, NH), 7.77 (0.23H, d, *J* = 8.7 Hz, NH), 7.55 (0.71H, d, *J* = 8.9 Hz, NH), 7.38 – 7.14 (5H, m, Ar-H), 7.13 – 6.98 (3H, m, Ar-H), 6.29 (0.66H, br-s, OH), 6.24 – 6.14 (0.29H, m, OH), 4.45 – 4.30 (1H, m, H-3), 4.23 – 4.17 (0.27H, m, H-2), 4.07 – 3.97 (0.74H, m, H-2), 2.96 – 2.74 (2H, m, H-4), 2.12 – 1.99 (8H, m, H-10, H-14 and H-14'), 0.96 – 0.84 (3H, m, H-11); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 173.18, 173.02, 171.21 and 171.10 (C=O), 139.79 (Ar-C), 139.29 (Ar-C), 135.77 (Ar-C), 135.73 (Ar-C), 129.77 (Ar-C), 129.46 (Ar-C), 128.65 (Ar-C), 128.46 (Ar-C), 128.07 (Ar-C), 127.98 (Ar-C), 126.84 (Ar-C), 126.57 (Ar-C), 74.30 and 71.94 (C-2), 53.70 (C-3), 46.23 (C-10), 37.96 and 34.68 (C-4), 28.96 and 28.93 (C-10), 18.71 and 18.52 (C-14 and C-14'), 10.35 and 10.14 (C-11); **HRMS** (m/*z*), calculated for $C_{21}H_{27}N_2O_3$: 355.2022, found, $(M + H)^+$: 355.2019.

4.39 Preparation of (3*S*)-3-butyramido-*N*-(2,6-dimethylphenyl)-2-hydroxy-4-phenylbutanamide 42

Compound **35** (100 mg, 0.21 mmol), TFA (164 µl, 2.13 mmol) and Et_3N (295 µl, 2.13 mmol) in CH_2Cl_2 (2 ml) were reacted together as described in **4.11**. The reaction yielded compound **42** (58 mg, 74%) as a white solid which was a mixture of diastereomers.

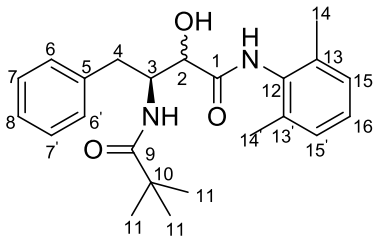


R_f = 0.42 (49:1 $CHCl_3$/MeOH); **m.p.** 242 – 244 °C; **IR** ν_{max}(cm^{-1}): 3264 (NH), 3028 (C=CH), 2960 (NH), 1662 (C=O), 1649 (C=O), 1375 (C-O); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.30 (0.79H, s, NH), 9.11 (0.26H, s, NH), 7.79 (0.72H, d, *J* = 8.8 Hz, NH), 7.56 (0.26H, d, *J* = 8.9 Hz, NH), 7.36 – 7.13 (5H, m, Ar-H), 7.12 – 7.00 (3H, m, Ar-H), 6.25 (1H, s, OH), 4.49 – 4.32 (1H, m, H-3), 4.20 (0.72H, d, *J* = 3.3 Hz, H-2), 4.03 (0.26H, d, *J* = 2.5 Hz, H-2), 2.96 – 2.75 (2H, m, H-4), 2.18 and 2.10 (6H, 2xs, H-15 and H-15'), 2.08 – 1.96 (2H,

m, H-10), 1.50 – 1.35 (2H, m, H-11), 0.79 and 0.72 (3H, 2t, *J* = 7.4 Hz, H-12); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.22, 172.18, 171.20 and 171.12 (C=O), 139.78 (Ar-C), 139.28 (Ar-C), 135.76 (Ar-C), 135.73 (Ar-C), 135.50 (Ar-C), 135.35 (Ar-C), 129.75 (Ar-C), 129.44 (Ar-C), 128.64 (Ar-C), 128.44 (Ar-C), 128.07 (Ar-C), 127.98 (Ar-C), 126.84 (Ar-C), 126.82 (Ar-C), 126.55 (Ar-C), 126.33 (Ar-C), 74.46 and 71.96 (C-2), 53.72 and 53.67 (C-3), 37.92 and 37.80 (C-4), 34.60 (C-10), 19.07 and 18.96 (C-11), 18.71 and 18.55 (C-15 and C-15'), 14.10 and 13.94 (C-12); **HRMS** (m/*z*), calculated for $C_{22}H_{29}N_2O_3$: 369.2178, found $(M + H)^+$: 369.2171.

4.40 Preparation of (3*S*)-*N*-(2,6-dimethylphenyl)-2-hydroxy-4-phenyl-3-pivalamidobutanamide 43

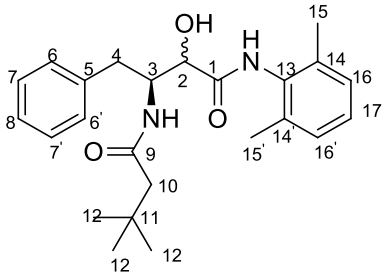
Compound **36** (150 mg, 0.31 mmol), TFA (240 µl, 3.12 mmol) and Et_3N (432 µl, 3.12 mmol) in CH_2Cl_2 (2 ml) were reacted together as described in **4.11**. The reaction yielded compound **43** (114 mg, 96%) as an off white solid which was a mixture of diastereomers.



R_f = 0.50 (49:1 $CHCl_3$/MeOH); **m.p.** 196 – 197 °C; **IR** ν_{max}(cm^{-1}): 3352 (OH), 3238 (NH), 3028 (C=C-H), 2968 (C-H), 1654 (C=O), 1637 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.54 (0.47H, s, NH), 8.49 (0.53H, s, NH), 7.45 – 7.15 (5H, m, Ar-H), 7.15 – 6.87 (3H, m, Ar-H), 6.70 – 6.50, 6.35 – 6.20 and 5.53 – 5.02 (2H, m) NH and OH, 4.56 – 4.22 (2H, m, H-2 and H-3), 3.38 – 3.13 (1H, m, H-4a), 3.12 – 2.81 (1H, m, H-4b), 2.30 – 2.03 (6H, m, H-14 and 14'), 1.09 – 0.73 (9H, m, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 181.86, 180.97, 171.48 and 170.31 (C=O), 138.10 (Ar-C), 137.92 (Ar-C), 134.98 (Ar-C), 134.96 (Ar-C), 133.23 (Ar-C), 133.18 (Ar-C), 129.34 (Ar-C), 129.28 (Ar-C), 129.16 (Ar-C), 128.60 (Ar-C), 128.58 (Ar-C), 128.40 (Ar-C), 128.23 (Ar-C), 128.21 (Ar-C), 128.18 (Ar-C), 128.16 (Ar-C), 128.10 (Ar-C), 127.57 (Ar-C), 126.74 (Ar-C), 126.71 (Ar-C), 75.54 and 73.84 (C-2), 57.58 and 55.57 (C-3), 38.68 and 38.66 (C-10), 35.42 and 34.90 (C-4), 27.36 and 27.31 (C-11), 18.62 and 18.58 (C-14 and C-14'); **HRMS** (m/*z*), calculated for $C_{23}H_{31}N_2O_3$: 383.2335, found, $(M + H)^+$: 383.2331.

4.41 Preparation of (3*S*)-3-(3,3-dimethylbutanamido)-*N*-(2,6-dimethyl phenyl)-2-hydroxy-4-phenylbutanamide 44

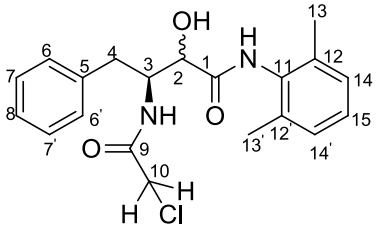
Compound **37** (450 mg, 0.90 mmol), TFA (694 µl, 9.01 mmol) and Et_3N (1.25ml, 9.01mmol) in CH_2Cl_2 (3 ml) were reacted together as described in **4.11**. The reaction yielded compound **44** (325 mg, 91%) as a white solid which was a mixture of diastereomers.



R_f = 0.55 (49:1 $CHCl_3$/MeOH); **m.p**. 183 – 184 °C; **IR** v_{max}(cm^{-1}): 3256 (NH), 3062 (C=C-H), 2954 (C-H), 1660 (C=O), 1640 (C=O), 1365 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.45 (0.72H, s, NH), 8.25 (0.28H, s, NH), 7.47 – 7.15 (5H, m, Ar-H), 7.14 – 6.94 (3H, m, Ar-H), 4.29 – 4.10 (1H, m, H-2 and H-3), 3.68 – 3.50 (0.28H, m) and 3.38 (0.74H, dd, *J* = 13.7, 7.8 Hz) and 3.23 – 3.08 (0.46H, m) and 2.96 (0.76H, dd, *J* = 13.8, 7.8 Hz) and 2.62 (0.29H, dd, *J* = 13.6, 10.1 Hz, H-4), 2.29 – 2.08 (6H, m, H-15 and H-15'), 1.84 (1H, d, *J* = 12.8 Hz, H-10a), 1.76 (1H, d, *J* = 12.8 Hz, H-10b), 1.10 and 0.85 (9H, 2 x s, H-12); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.89, 171.91 and 171.28 (C=O), 137.96 (Ar-C), 135.25 (Ar-C), 135.13 (Ar-C), 133.24 (Ar-C), 129.34 (Ar-C), 129.26 (Ar-C), 128.75 (Ar-C), 128.58 (Ar-C), 128.25 (Ar-C), 128.16 (Ar-C), 127.43 (Ar-C), 127.28 (Ar-C), 126.76 (Ar-C), 126.67 (Ar-C), 75.87 and 73.67 (C-2), 56.59 and 53.86 (C-3), 49.82 and 47.80 (C-10), 39.71 and 35.57 (C-4), 30.99 and 30.80 (C-11), 29.69, 29.64 and 29.52 (C-12), 18.74, 18.53 and 18.41 (C-15 and C-15'); **HRMS** (m/*z*), calculated for $C_{24}H_{33}N_2O_3$: 399.2491, found, $(M + H)^+$: 399.2486.

4.42 Preparation of (3*S*)-3-(2-chloroacetamido)-*N*-(2,6-dimethylphenyl)-2-hydroxy-4-phenylbutanamide 45

Compound **38** (150 mg, 0.32 mmol), TFA (243 µl, 3.16 mmol) and Et_3N (438 µl, 3.16 mmol) in CH_2Cl_2 (3 ml) were reacted together as described in **4.11**. The reaction yielded compound **45** (78 mg, 76%) as a mixture of oil and white precipitate which was a mixture of diastereomers.

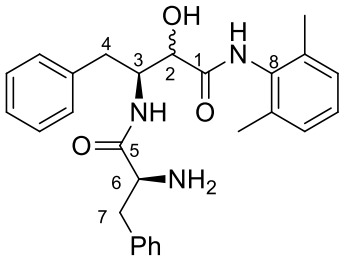


R_f = 0.42 (49:1 $CHCl_3$/MeOH); **m.p.** 216 – 217 °C; **IR** v_{max}(cm^{-1}): 3333 (OH), 3068 (C=CH), 2973 (C-H), 1657 (C=O), 768 (C-Cl); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.31 (0.21H, s), 9.08 (0.46H, s), 8.23 – 8.17 (0.10H, m) and 7.94 (0.31H, d, *J* = 9.3 Hz) NH and OH, 7.31 – 7.07 (5H, m, Ar-H), 7.05 – 6.91 (3H, m, Ar-H), 6.40 (0.48H, d, *J* = 6.0 Hz) and 6.24 (0.21H, d, *J* = 6.1 Hz) NH, 4.38 – 4.29 (1H, m, H-3), 4.18 – 4.13 and 4.01 – 3.88 (3H, 2xm, H-2 and H-10), 2.85

(0.70H, dd, *J* = 13.2, 7.8 Hz, H-4a), 2.78 – 2.74 (0.54H, m, H-4b), 2.69 (0.66H, dd, *J* = 13.2, 7.1 Hz, H-4b 2.10 and 2.02 (4H, 2xs, H-13 and 13'); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 170.94, 170.91, 170.87, 165.78, 165.61 and 165.54 (C=O), 139.27 (Ar-C), 138.79 (Ar-C), 135.81 (Ar-C), 135.74 (Ar-C), 129.77 (Ar-C), 129.39 (Ar-C), 128.78 (Ar-C), 128.63 (Ar-C), 128.11 (Ar-C), 128.01 (Ar-C), 126.95 (Ar-C), 126.92 (Ar-C), 126.76 (Ar-C), 126.56 (Ar-C), 73.72, 71.21 and 71.12 (C-2), 54.20, 54.09 and 54.06 (C-3), 43.13 and 43.09 (C-10), 37.87 and 34.53 (C-4), 18.73 and 18.58 (C-13 and C-13'); **HRMS** (m/*z*), calculated for $C_{20}ClH_{24}N_2O_3$: 375.1475, found $(M + H)^+$: 375.1469.

4.43 Preparation of (3*S*)-3-((*S*)-2-amino-3-phenylpropanamido)-*N*-(2,6-dimethylphenyl)-2-hydroxy-4-phenylbutanamide 46

Compound **39** (500 mg, 0.78 mmol), TFA (600 µl, 7.77 mmol) and Et_3N (1.08 ml, 7.77 mmol) in CH_2Cl_2 (3 ml) were reacted together as described in **4.11**. The reaction yielded compound **46** (320 mg, 92%) as a white precipitate which was a mixture of diastereomers.



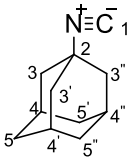
R_f = 0.49 (49:1 $CHCl_3$/MeOH); **m.p**. 167 – 168 °C; **IR** ν_{max}(cm^{-1}): 3308 (OH), 3031 (C=C-H), 1654 (C=O), 1359 (C-O); **^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.37 (0.55H, s, NH), 9.20 (0.49H, s, NH), 8.03 (0.39H, d, *J* = 9.4 Hz, NH), 7.94 (0.56H, d, *J* = 9.2 Hz, NH), 7.43 – 7.11 (10H, m, Ar-H), 7.10 – 6.98 (3H, m, Ar-H), 6.47 (0.48H, d, *J* = 5.6 Hz, OH), 6.27 (0.57H, d, *J* = 5.8 Hz, OH), 4.57 – 4.32 (1H, m, H-3), 4.27 – 4.13 and 4.09 – 3.99 (1H, 2xm, H-2), 3.01 – 2.66 and 2.48 – 2.30 (4H, 2xm) H-4 and H-7, 2.18 (3H, s, CH_3), 2.08 (3H, s, CH_3), 1.63 (2H,br-s, NH_2); **^{13}C NMR** (75 MHz, DMSO-d_6) δ 173.83, 173.31, 170.68 and 170.43 (C=O), 138.75 (Ar-C), 138.51 (Ar-C), 135.30 (Ar-C), 135.22 (Ar-C), 134.89 (Ar-C), 129.34 (Ar-C), 129.29 (Ar-C), 129.17 (Ar-C), 129.09 (Ar-C), 128.22 (Ar-C), 128.12 (Ar-C), 128.08 (Ar-C), 128.04 (Ar-C), 127.59 (Ar-C), 127.44 (Ar-C), 126.39 (Ar-C), 126.00 (Ar-C), 73.46 and 70.72 (C-2), 56.21, 55.99 and 52.64 (C-3 and C-6), 37.61 and 34.48 (C-4 and C-7), 18.21 (CH_3), 18.04 (CH_3); **HRMS** (m/*z*), calculated for $C_{27}H_{32}N_3O_3$: 446.2444, found $(M + H)^+$: 446.2441.

4.44 Preparation of 1-isocyanoadamantane 11

Commercially available adamantylamine (1.00 g, 6.61 mmol), excess formic acid (5 ml) and excess acetic anhydride (5 ml) were added together and left to stir under N_2 overnight. The

reaction was then worked up in brine (200 ml) and solid Na_2CO_3 (≈ 50 g) was added in small portions waiting for effervescence to stop before each addition. The product was then extracted with EtOAc (2 x 100 ml) and dried over $MgSO_4$. After filtration the solvent was removed *in vacuo* yielding a white precipitate (crude – 1.38 g).

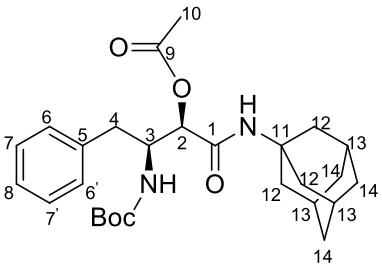
Crude adamantyl formamide (1.38 g, ≈7.14 mmol) was dissolved in anhydrous CH_2Cl_2 (40 ml) and cooled to 0 °C whilst stirring under N_2. Excess $POCl_3$ (5 ml) was then added gradually followed by excess Et_3N (15 ml). After 30 min of stirring the reaction was allowed to warm to room temperature and was left to stir overnight. The reaction was then quenched by slowly adding a saturated aqueous solution of $NaHCO_3$ (150 ml) in ice and solid Na_2CO_3 (≈ 20 g) until effervescence had stopped. The product was then extracted using CH_2Cl_2 (100 ml) and dried over Na_2SO_4. Excess solvent was removed *in vacuo*. Column chromatography (3:7 EtOAc/Hex) afforded **11** as a light yellow precipitate (805 mg, 75%) with a distinct isocyanide smell.[42]



R_f = 0.33 (3:7 EtOAc/Hex) visualised using iodine; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.16 – 2.06 (3H, m, H-4, 4’ and 4’’), 2.06 – 1.98 (6H, m, H-3, 3’ and 3’’), 1.76 – 1.60 (6H, m, H-5, 5’ and 5’’); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 151.59 (t, $J_{C\text{-}N}$ = 5.2 Hz, C-1), 54.22 (t, $J_{C\text{-}N}$ = 11.3 Hz, C-2), 43.58 (C-3, 3’ and 3’), 35.50 (C-5, 5’ and 5’), 28.73 (C-4, 4’ and 4’).

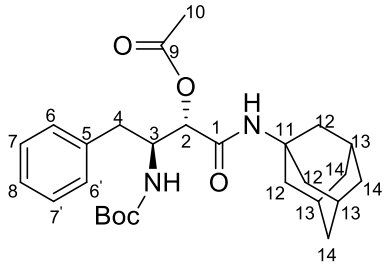
4.45 Preparation of (3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl acetate 47

N-Boc-L-phenylalaninal **15** (500 mg, 2.01 mmol), adamantyl isocyanide **11** (220 mg, 2.01 mmol) and acetic acid **1** (162 µl, 2.01 mmol) were dissolved in CH_2Cl_2 (5 ml) and the experiment was carried out as described in **4.5**. The reaction yielded compound **47** (637 mg, 100%) as a white flaky solid, as a mixture of diastereomers. **R_f** = 0.54 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.96:1.00. This diastereomeric mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl acetate 47A.

m.p. 156 – 157 °C; **IR** ν_{max} (cm^{-1}): 3284 (NH), 3085 (C=C-H), 2908 (C-H), 1756 (C=O), 1719 (C=O), 1655 (C=O), 1365 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.25 (2H, m, H-7 and H-7'), 7.24 – 7.15 (3H, m, H-6, H-6' and H-8), 5.60 (1H, s, NH), 5.20 (1H, d, *J* = 9.6 Hz, NH), 5.08 – 4.97 (1H, m, H-2), 4.34 – 4.23 (1H, m, H-3), 2.82 (2H, d, *J* = 7.3 Hz, H-4), 2.19 – 2.03 (6H, m, H-10 and H-13), 1.99 (6H, s, H-12), 1.72 – 1.60 (6H, m, H-14), 1.40 – 1.19 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.09 and 166.76 (C=O), 154.97 (Boc C=O), 137.38 (C-5), 129.24 (C-7 and C-7'), 128.47 (C-6 and C-6'), 126.56 (C-8), 79.45 [$(CH_3)_3\underline{C}$], 73.77 (C-2), 52.91 (C-3), 52.35 (C-11), 41.36 (C-12), 38.16 (C-14), 36.24 (C-4), 29.38 (C-13), 28.29 [$(\underline{C}H_3)_3C$], 20.70 (C-10); **HRMS** (m/*z*), calculated for $C_{27}H_{39}N_2O_5$: 471.2859, found $(M + H)^+$: 471.2856.

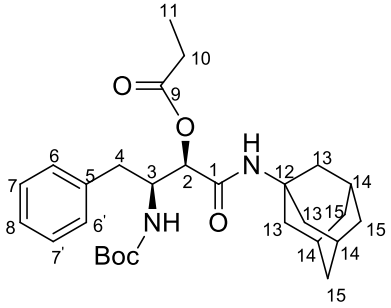


(2*S*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl acetate 47B. **m.p.** 156 – 157 °C; **IR** (solid) ν_{max} (cm^{-1}): 3299 (NH), 2915 (C-H), 1748 (C=O), 1696 (C=O), 1669 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.25 (2H, m, H-7 and H-7'), 7.24 – 7.16 (3H, m, H-6, H-6' and H-8), 5.77 (1H, s, NH), 5.22 – 5.04 (1H, m, H-2), 4.97 – 4.82 (1H, m, NH), 4.33 – 4.18 (1H, m, H-3), 2.93 (2H, d, *J* = 7.8 Hz, H-4), 2.20 – 2.05 (6H, m, H-13 and H-10), 2.04 – 2.00 (6H, m, H-12), 1.73 – 1.60 (6H, m, H-14), 1.42 – 1.20 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.90 and 166.59 (C=O), 155.56 (Boc C=O), 137.44 (C-5), 129.17 (C-7 and C-7'), 128.49 (C-6 and C-6',) 126.60 (C-8), 79.45 [$(CH_3)_3\underline{C}$], 74.48 (C-2), 53.36 (C-3), 52.38 (C-11), 41.43 (C-12), 37.34 (C-4), 36.26 (C-14), 29.70 (C-13), 28.32 [$(\underline{C}H_3)_3C$], 20.91 (C-10); **HRMS** (m/*z*), calculated for $C_{27}H_{39}N_2O_5$: 471.2859, found $(M + H)^+$: 471.2854.

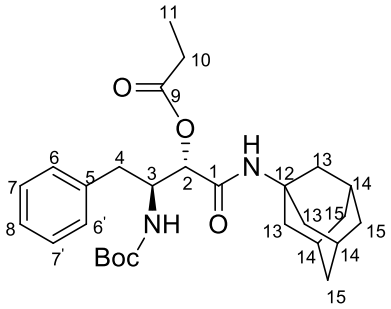
4.46 Synthesis of (3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl propionate 48

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), adamantyl isocyanide **11** (193 mg, 1.20 mmol) and propionic acid **3** (90 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. The reaction yielded compound **48** (594 mg, 100%) as a white flaky solid being a mixture of diastereomers. $\mathbf{R_f}$ = 0.61 (5:5 EtOAc/Hex); **d.r.** = 1.75:1.00. This

diastereomeric mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



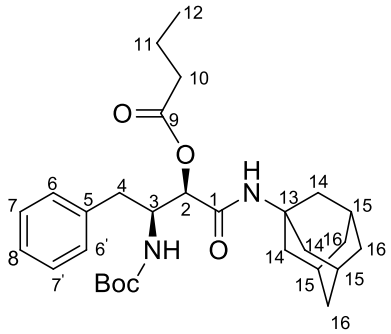
(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl propionate 48A. **m.p.** 117 – 118 °C; **IR** ν_{max} (cm^{-1}): 3386 (NH), 3290 (NH), 2977 (C-H), 1718 (C=O), 1685 (C=O), 1656 (C=O), 1364 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 – 7.24 (2H, m, Ar-H), 7.24 – 7.11 (3H, m, Ar-H), 5.62 (1H, s, NH), 5.22 (1H, d, *J* = 9.6 Hz, NH), 5.11 – 5.00 (1H, m, H-2), 4.35 – 4.23 (1H, m, H-3), 2.82 (2H, d, *J* = 7.3 Hz, H-4), 2.47 – 2.26 (2H, m, H-10), 2.07 (3H, s, H-14), 1.99 (6H, s, H-13), 1.67 (6H, s, H-15), 1.40 – 1.04 (12H, m, $(CH_3)_3C$ and H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.40 and 166.95 (C=O), 154.95 (Boc C=O), 137.44 (C-5), 129.25 (C-7 and C-7'), 128.44 (C-6 and C-6'), 126.52 (C-8), 79.40 [$(CH_3)_3\underline{C}$], 73.54 (C-2), 52.96 (C-3), 52.29 (C-12), 41.39 (C-13), 38.11 (C-4), 36.24 (C-15), 29.69 (C-14), 28.29 [$(\underline{C}H_3)_3C$], 27.38 (C-10), 9.05 (C-11); **HRMS** (m/*z*), calculated for $C_{28}H_{41}N_2O_5$: 485.3015, found $(M + H)^+$: 485.3007.



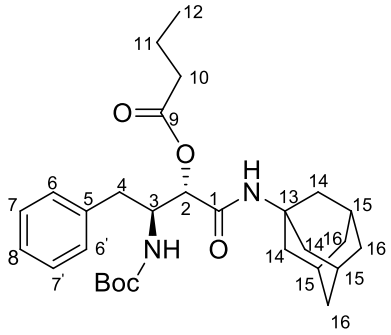
(2*S*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl propionate 48B. **m.p.** 125 – 126 °C; **IR** ν_{max} (cm^{-1}): 3333 (NH), 2916 (C-H), 1747 (C=O), 1692 (C=O), 1664 (C=O), 1365 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.25 (2H, m, Ar-H), 7.25 – 7.17 (3H, m, Ar-H), 5.75 (1H, s, NH), 5.06 (1H, d, *J* = 9.1 Hz, NH), 5.00 – 4.92 (1H, m, H-2), 4.30 – 4.19 (1H, m, H-3), 2.94 (2H, d, *J* = 7.6 Hz, H-4), 2.50 – 2.35 (2H, m, H-10), 2.14 – 2.05 (3H, m, H-14), 2.05 – 1.94 (6H, m, H-13), 1.73 – 1.61 (6H, m, H-15), 1.45 – 1.10 (14H, m, $(CH_3)_3C$ and H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.19 and 166.70 (C=O), 155.52 (Boc C=O), 137.46 (C-5), 129.19 (C-7 and C-7'), 128.48 (C-6 and C-6'), 126.59 (C-8), 79.38 [$(CH_3)_3\underline{C}$], 74.27 (C-2), 53.38 (C-3), 52.32 (C-12), 41.45 (C-13), 37.49 (C-4), 36.26 (C-15), 29.38 (C-14), 28.32 [$(\underline{C}H_3)_3C$], 27.50 (C-10), 8.94 (C-11); **HRMS** (m/*z*), calculated for $C_{28}H_{41}N_2O_5$: 485.3015, found $(M + H)^+$: 485.3011.

4.47 Synthesis of (3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl butyrate 49

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), adamantyl isocyanide **11** (193 mg, 1.20 mmol) and butyric acid **3** (110 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. The reaction yielded compound **49** (522 mg, 87%) as a mixture of diastereomers. $\mathbf{R_f}$ = 0.65 (3:7 EtOAc/Hex); **d.r.** = 1.95:1.00. This diastereomeric mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl butyrate 49A. m.p. 108 – 109 °C; **IR** ν_{max} (cm^{-1}): 3286 (NH), 3067 (C=CH), 1710 (C=O), 1691 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.24 (2H, m, Ar-H), 7.23 – 7.13 (3H, m, Ar-H), 5.62 (1H, s, NH), 5.21 (1H, d, *J* = 9.6 Hz, NH), 5.11 – 5.02 (1H, m, H-2), 4.33 – 4.22 (1H, m, H-3), 2.82 (2H, d, *J* = 7.4 Hz, H-4), 2.42 – 2.23 (2H, m, H-10), 2.07 (3H, s, H-15), 1.99 (6H, s, H-14), 1.76 – 1.60 (8H, m, H-11 and H-16), 1.41 – 1.18 (9H, m, $(CH_3)_3C$), 1.06 – 0.92 (3H, m, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.57 and 166.99 (C=O), 154.93 (Boc C=O), 137.45 (C-5), 129.27 (C-7 and C-7'), 128.44 (C-6 and C-6'), 126.51 (C-8), 79.38 [$(CH_3)_3\underline{C}$], 73.48 (C-2), 52.98 (C-3), 52.28 (C-13), 41.40 (C-14), 38.10 (C-4), 36.25 (C-16), 35.91 (C-10), 29.38 (C-15), 28.29 [$(\underline{C}H_3)_3C$], 18.38 (C-11), 13.70 (C-12); **HRMS** (m/*z*), calculated for $C_{29}H_{43}N_2O_5$: 499.3172, found $(M + H)^+$: 499.3169.

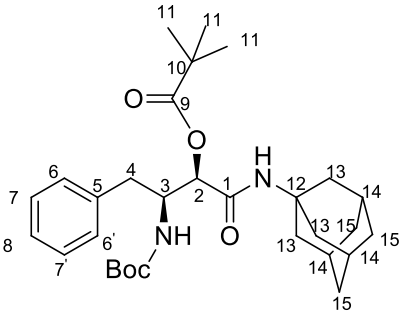


(2*S*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl butyrate 49B. m.p. 139 – 140 °C; **IR** ν_{max} (cm^{-1}): 3316 (NH), 2908 (C-H), 1745 (C=O), 1693 (C=O), 1665 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.25 (2H, m, Ar-H), 7.25 – 7.16 (3H, m, Ar-H), 5.73 (1H, s, NH), 5.07 (1H, d, *J* = 9.4 Hz, NH), 5.00 – 4.93 (1H, m, H-2), 4.31 – 4.19 (1H, m, H-3), 2.93 (2H, d, *J* = 7.5 Hz, H-4), 2.45 – 2.27 (2H, m, H-10), 2.12 – 2.05 (3H, m, H-15), 2.05 – 1.95 (6H, m, H-14), 1.75 – 1.60 (8H, m, H-11 and H-16), 1.44 – 1.15 (9H, m, $(CH_3)_3C$),

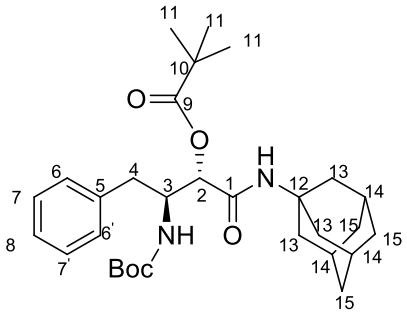
1.04 – 0.93 (3H, m, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.42 and 166.70 (C=O), 155.48 (Boc C=O), 137.45 (C-5), 129.19 (C-7 and C-7'), 128.49 (C-6 and C-6'), 126.60 (C-8), 79.36 [$(CH_3)_3\underline{C}$], 74.14 (C-2), 53.36 (C-3), 52.32 (C-13), 41.44 (C-14), 37.49 (C-4), 36.26 (C-16), 36.03 (C-10), 29.70 (C-15), 28.32 [$(\underline{C}H_3)_3C$], 18.32 (C-11), 13.65 (C-12); **HRMS** (m/*z*), calculated for $C_{29}H_{43}N_2O_5$: 499.3172, found $(M + H)^+$: 499.3169

4.48 Preparation of (3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl pivalate 50

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), adamantyl isocyanide **11** (193 mg, 1.20 mmol) and pivalic acid **4** (123 mg, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. The experiment yielded compound **50** (564 mg, 92%) as a white solid, being a mixture of diastereomers. $\mathbf{R_f}$ = 0.67 (49:1 $CHCl_3$/MeOH); **d.r.** = 2.12:1.00. This mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



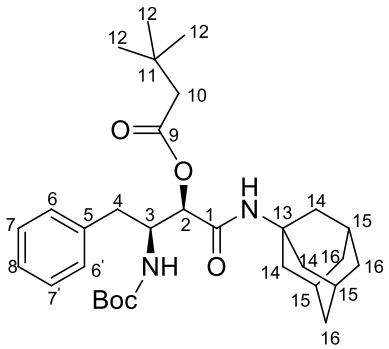
(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl pivalate 50A. m.p. 147 – 148 °C; **IR** ν_{max} (cm^{-1}): 3417 (NH), 3351 (NH), 2964 (C-H), 1742 (C=O), 1717 (C=O), 1677 (C=O), 1530 (C=C); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.11 (5H, m, Ar-H), 5.63 (1H, s, NH), 5.24 (1H, d, *J* = 9.7 Hz, NH), 5.18 – 5.03 (1H, m, H-2), 4.37 – 4.18 (1H, m, H-3), 2.89 – 2.73 (2H, m, H-4), 2.07 (3H, s, H-14), 1.99 (6H, s, H-13), 1.67 (6H, s, H-15), 1.40 – 1.15 (18H, m, H-11 and $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.09 and 167.27 (C=O), 154.91 (Boc C=O), 137.43 (C-5), 129.32 (C-7 and C-7'), 128.45 (C-6 and C-6'), 126.53 (C-8), 79.36 [$(CH_3)_3\underline{C}$], 73.29 (C-2), 53.18 (C-3), 52.22 (C-12), 41.48 (C-13), 38.79 (C-10), 37.96 (C-4), 36.24 (C-15), 29.69 (C-14), 28.29 [$(\underline{C}H_3)_3C$], 27.05 (C-11); **HRMS** (m/*z*), calculated for $C_{30}H_{45}N_2O_5$: 513.3328, found $(M + H)^+$: 513.3326.



(2*S*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl pivalate 50B. m.p. 132 – 133 °C; **IR** ν_{max} (cm^{-1}): 3342 (NH), 3306 (NH), 2912 (C-H), 2852 (C-H), 1734 (C=O), 1691 (C=O), 1663 (C=O), 1547 (C=C); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 – 7.16 (5H, m, Ar-H), 5.74 (1H, s, NH), 4.99 (1H, d, *J* = 9.4 Hz, NH), 4.97 – 4.90 (1H, m, H-2), 4.32 – 4.18 (1H, m, H-3), 3.02 – 2.87 (2H, m, H-4), 2.09 (3H, s, H-14), 2.00 (6H, s, H-13), 1.69 (6H, s, H-15), 1.42 – 1.13 (18H, m, H-11 and $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.93 and 166.79 (C=O), 155.35 (Boc C=O), 137.43 (C-5), 129.23 (C-7 and C-7'), 128.49 (C-6 and C-6'), 126.60 (C-8), 79.29 [$(CH_3)_3\underline{C}$], 74.07 (C-2), 53.27 (C-3), 52.21 (C-12), 41.51 (C-13), 38.82 (C-10), 37.73 (C-4), 36.26 (C-15), 29.39 (C-14), 28.34 [$(\underline{C}H_3)_3C$], 27.04 (C-11); **HRMS** (m/*z*), calculated for $C_{30}H_{45}N_2O_5$: 513.3328, found $(M + H)^+$: 513.3322.

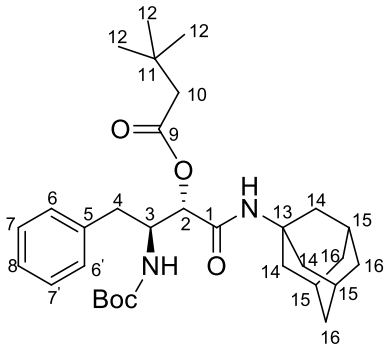
4.49 Synthesis of (3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 51

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), adamantyl isocyanide **11** (193 mg, 1.20 mmol) and *tert*-butyl acetic acid **5** (153 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment proceeded as described in **4.5**. Purification of the product yielded compound **51** (588 mg, 93%) as a mixture of diastereomers. $\mathbf{R_f}$ = 0.47 (49:1 $CHCl_3$/MeOH); **d.r.** = 2.01:1.00. The diastereomeric mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 51A. m.p. 150 – 151 °C; **IR** ν_{max} (cm^{-1}): 3405 (NH), 3302 (NH), 2909 (C-H), 2850 (C-H), 1735 (C=O), 1720 (C=O), 1677 (C=O), 1535 (C=C); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.30 – 7.24 (2H, m, Ar-H), 7.23 – 7.14 (3H, m, Ar-H), 5.66 (1H, s, NH), 5.22 (1H, d, *J* = 9.6 Hz, NH), 5.14 – 5.03 (1H, m, H-2), 4.34 – 4.18 (1H, m, H-3), 2.90 – 2.74 (2H, m, H-4), 2.21 (2H, s, H-10), 2.07 (3H, s, H-15), 2.03 – 1.91 (6H, m, H-14), 1.74 – 1.61 (6H, m, H-16), 1.41 – 1.18 (9H, m, $(CH_3)_3C$), 1.07 (9H, s, H-12); **^{13}C NMR** (126

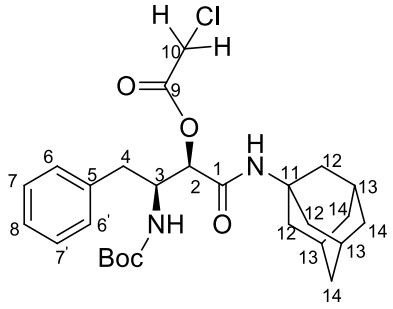
MHz, $CDCl_3$) δ 170.17 and 167.12 (C=O), 154.90 (Boc C=O), 137.53 (C-5), 129.31 (C-7 and C-7'), 128.43 (C-6 and C-6'), 126.49 (C-8), 79.33 [$(CH_3)_3\underline{C}$], 73.50 (C-2), 53.04 (C-3), 52.32 (C-13), 47.57 (C-10), 41.44 (C-14), 37.88 (C-4), 36.26 (C-16), 30.96 (C-11), 29.69 (C-12), 29.40 (C-15), 28.30 [$(\underline{C}H_3)_3C$]; **HRMS** (m/*z*), calculated for $C_{31}H_{47}N_2O_5$: 527.3485, found $(M + H)^+$: 527.3484.



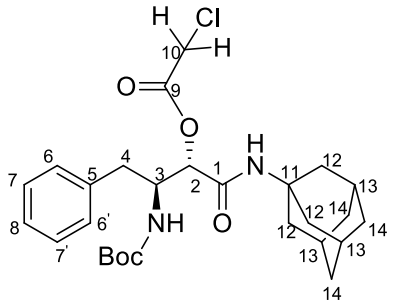
(2S,3S)-1-adamantan-1-ylamino-3-((tert-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl 3,3-dimethylbutanoate 51B. m.p. 144 – 145 °C; **IR** ν_{max} (cm^{-1}): 3267 (NH), 3087 (C=CH), 2908 (C-H), 1735 (C=O), 1668 (C=O), 1653 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.25 (2H, m, Ar-H), 7.25 – 7.16 (3H, m, Ar-H), 5.72 (1H, s, NH), 5.17 (1H, d, *J* = 9.0 Hz, NH), 4.99 – 4.88 (1H, m, H-2), 4.29 – 4.17 (1H, m, H-3), 2.93 (2H, d, *J* = 7.6 Hz, H-4), 2.35 – 2.19 (2H, m, H-10), 2.12 – 2.05 (3H, m, H-15), 2.05 – 1.94 (6H, m, H-14), 1.74 – 1.60 (6H, m, H-16), 1.44 – 1.19 (9H, m, $(CH_3)_3C$), 1.05 (9H, s, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.23 and 166.77 (C=O), 155.44 (Boc C=O), 137.50 (C-5), 129.18 (C-7 and C-7'), 128.52 (C- and C-6'), 126.61 (C-8), 79.29 [$(CH_3)_3\underline{C}$], 73.92 (C-2), 53.42 (C-3), 52.37 (C-13), 47.88 (C-10), 41.44 (C-14), 37.61 (C-4), 36.27 (C-16), 31.00 (C-11), 29.70 (C-12), 29.39 (C-15), 28.35 [$(\underline{C}H_3)_3C$]; **HRMS** (m/*z*), calculated for $C_{31}H_{47}N_2O_5$: 527.3485, found $(M + H)^+$: 527.3484.

4.50 Synthesis of (3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl 2-chloroacetate 52

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), adamantyl isocyanide **11** (193 mg, 1.20 mmol) and chloro-acetic acid **6** (113 mg, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment proceeded as described in **4.5**. The reaction yielded compound **52** (495 mg, 82%) as a mixture of diastereomers. $\mathbf{R}_f$ = 0.27 (1:1 EtOAc/Hex); **d.r.** = 1.97:1.00. This diastereomeric mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



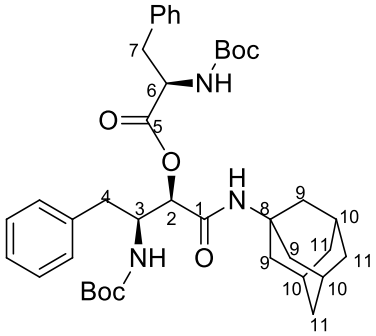
(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl)amino)-1-oxo-4-phenylbutan-2-yl 2-chloro acetate 52A. m.p. 143 – 144 °C; **IR** ν_{max} (cm^{-1}): 3281 (NH), 3064 (C=CH), 1777 (C=O), 1725 (C=O), 745 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.33 – 7.26 (2H, m, Ar-H), 7.25 – 7.14 (3H, m, Ar-H), 5.73 (1H, s, NH), 5.29 – 5.17 (1H, m, NH), 5.16 – 5.06 (1H, m, H-2), 4.39 – 4.30 (1H, m, H-3), 4.00 (2H, s, H-10), 2.85 (2H, d, *J* = 7.3 Hz, H-4), 2.07 (3H, s, H-13), 2.03 – 1.93 (6H, m, H-12), 1.72 – 1.62 (6H, m, H-14), 1.45 – 1.27 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.94 and 165.43 (C=O), 154.95 (Boc C=O), 137.20 (C-5), 129.23 (C-7 and C-7'), 128.55 (C-6 and C-6'), 126.67 (C-8), 79.66 [$(CH_3)_3\underline{C}$], 75.01 (C-2), 52.77 (C-3), 52.58 (C-11), 41.35 (C-10), 40.43 (C-12), 38.09 (C-4), 36.23 (C-14), 29.38 (C-13), 28.29 [$(\underline{C}H_3)_3C$]; **HRMS** (m/*z*), calculated for $C_{27}H_{38}ClN_2O_5$: 505.2469, found $(M + H)^+$: 505.2461.



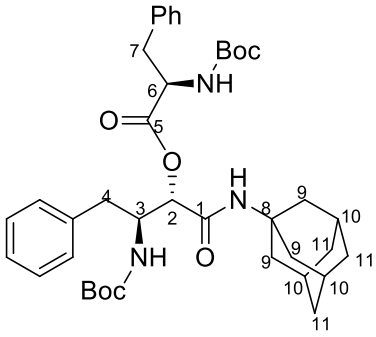
(2*S*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl-2-chloroacetate 52B. m.p. 80 – 81 °C; **IR** ν_{max} (cm^{-1}): 3285 (NH), 3064 (C=C-H), 1770 (C=O), 1725 (C=O), 745 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.34 – 7.17 (5H, m, Ar-H), 5.87 (1H, s, NH), 5.14 – 5.01 (1H, m, H-2), 4.90 (1H, d, *J* = 9.1 Hz, NH), 4.35 – 4.25 (1H, m, H-3), 4.15 (1H, d, *J* = 14.8 Hz, H-10a), 4.09 (1H, d, *J* = 14.8 Hz, H-10b), 3.05 – 2.89 (2H, m, H-4), 2.13 – 2.06 (3H, m, H-13), 2.06 – 1.97 (6H, m, H-12), 1.74 – 1.65 (6H, m, H-14), 1.38 (9H, s, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.96 and 165.67 (C=O), 155.53 (Boc C=O), 137.18 (C-5), 129.17 (C-7 and C-7'), 128.55 (C-6 and C-6'), 126.72 (C-8), 79.68 [$(CH_3)_3\underline{C}$], 75.94 (C-2), 53.20 (C-3), 52.53 (C-11), 41.44 (C-12), 40.70 (C-10), 37.10 (C-4), 36.24 (C-14), 29.39 (C-13), 28.32 ($(\underline{C}H_3)_3C$); **HRMS** (m/z), calculated for $C_{27}H_{38}ClN_2O_5$: 505.2469, found $(M + H)^+$: 505.2469.

4.51 Synthesis of (2*S*)-(3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl 2-((*tert*-butoxycarbonyl)amino)-3-phenyl propanoate 53

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), adamantyl isocyanide **11** (193 mg, 1.20 mmol) and *N*-Boc-phenylalanine **7** (318 mg, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml) and the experiment was carried out as described in **4.5**. The reaction yielded compound **53** (632 mg, 78%) as a mixture of diastereomers. $\mathbf{R_f}$ = 0.27 (1:1 EtOAc/Hex); **d.r.** = 2.49:1.00. This mixture was then separated using preparative HPLC as detailed in **4.10** to give two clean separated diastereomers.



(2*S*)-(2*R*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl-2-((*tert*-butoxycarbonyl) amino)-3-phenylpropanoate 53A. m.p. 80 – 81 °C; **IR** ν_{max} (cm^{-1}): 3342 (NH), 2977 (C-H), 2909 (C-H), 1743 (C=O), 1692 (C=O), 1365 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.41 – 7.00 (10H, m, Ar-H), 6.42 – 6.27 (1H, m, NH), 5.22 (1H, d, *J* = 9.9 Hz, NH), 5.14 – 5.02 (1H, m, H-2), 5.02 – 4.94 (1H, m, NH), 4.37 – 4.25 and 4.25 – 4.12 (2H, 2xm, H-3 and H-6), 3.13 – 2.95 (2H, m, H-4), 2.61 – 2.47 and 2.35 – 2.25 (2H, 2xm, H-7) 2.12 – 1.94 (9H, m, H-9 and H-10), 1.66 (6H, s, H-11), 1.49 – 1.10 (18H, m, 2x$(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.08 and 166.94 (C=O), 155.50 and 154.74 (Boc C=O), 137.51 (Ar-C), 135.51 (Ar-C), 129.39 (Ar-C), 129.15 (Ar-C), 129.09 (Ar-C), 128.96 (Ar-C), 128.26 (Ar-C), 127.51 (Ar-C), 126.37 (Ar-C), 80.55 [$(CH_3)_3\underline{C}$], 79.15 [$(CH_3)_3\underline{C}$], 74.27 (C-2), 55.50 and 52.80 (C-3 and C-6), 52.52 (C-8), 41.17 (C-9), 37.73 and 37.27 (C-4 and C-7), 36.37 (C-11), 29.44 (C-10), 28.32 and 28.25 [$(\underline{C}H_3)_3C$]; **HRMS** (m/z), calculated for $C_{39}H_{54}N_3O_7$: 676.3962, found $(M + H)^+$: 676.3954.

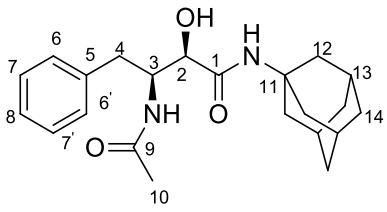


(2*S*)-(2*S*,3*S*)-1-adamantan-1-ylamino-3-((*tert*-butoxycarbonyl) amino)-1-oxo-4-phenylbutan-2-yl-2-((*tert*-butoxycarbonyl) amino)-3-phenylpropanoate 53B. m.p. 132 – 133 °C; **IR** ν_{max} (cm^{-1}): 3343 (NH), 2908 (C-H), 1690 (C=O), 1657 (C=O), 1518 (C=C); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.39 – 7.09 (10H, m, Ar-H), 6.45 – 6.27 (m) and 5.98 (1H, s, NH), 5.20 – 5.07 (1H, m, H-2), 5.02 – 4.76 (2H, m, 2xNH), 4.61 – 4.49 (1H, m) and 4.41 – 4.21 (1H, m) H-3 and H-6, 3.27 – 3.16 (1H, m, H-4a), 3.16 – 2.99 (1H, m, H-4b), 2.97 – 2.85 (1H, m, H-7a), 2.85 – 2.71 (1H, m, H-7b), 2.14 –

1.91 (9H, m, H-9 and H-10), 1.74 – 1.60 (6H, m, H-11), 1.47 – 1.17 (18H, m, 2x$(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.72 and 166.07 (C=O), 155.35 (Boc C=O), 137.45 (Ar-C), 135.89 (Ar-C), 129.32 (Ar-C), 129.23 (Ar-C), 128.99 (Ar-C), 128.78 (Ar-C), 128.45 (Ar-C), 128.28 (Ar-C), 127.20 (Ar-C), 126.56 (Ar-C), 80.29 [$(CH_3)_3\underline{C}$], 79.50 [$(CH_3)_3\underline{C}$], 75.74 (C-2), 54.70 and 53.45 (C-3 and C-6), 52.43 (C-8), 41.30 (C-9), 37.50 and 36.64 (C-4 and C-7), 36.31 (C-11), 29.42 (C-10), 28.37 and 28.26 [$(\underline{C}H_3)_3C$], **HRMS** (m/*z*), calculated for $C_{39}H_{54}N_3O_7$: 676.3962, found $(M + H)^+$: 676.3962.

4.52 Synthesis of (2*R*,3*S*)-3-acetamido-*N*-adamantan-1-yl-2-hydroxy-4-phenyl butanamide 54A

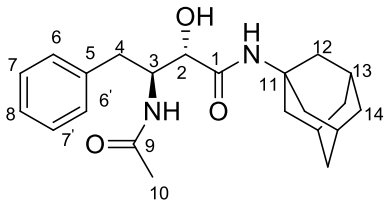
Compound **47A** (70 mg, 0.15 mmol), TFA (115 µl, 1.49 mmol) and Et_3N (207 µl, 1.49 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **54A** (51 mg, 93%) as a white solid.



R_f = 0.39 (49:1 $CHCl_3$/MeOH); **m.p.** 196 – 197 °C; **IR** ν_{max}(cm^{-1}): 3345 (OH), 3314 (NH), 3031 (C=CH), 1748 (C=O), 1312 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.34 – 7.15 (5H, m, Ar-H), 7.05 (1H, d, *J* = 8.2 Hz, NH), 6.72 (1H, s, OH), 6.34 (1H, d, *J* = 6.8 Hz, NH), 4.14 – 4.01 (1H, m, H-3), 3.97 (1H, dd, *J* = 6.8, 3.4 Hz, H-2), 3.10 (1H, dd, *J* = 13.7, 6.2 Hz, H-4a), 2.92 (1H, dd, *J* = 13.7, 9.2 Hz, H-4b), 2.07 (3H, s, H-13), 2.02 – 1.95 (6H, m, H-12), 1.87 (3H, s, H-10), 1.67 (6H, s, H-14); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.08 and 171.87 (C=O),138.26 (C-5), 129.15 (C-7 and C-7'), 128.44 (C-6 and C-6'), 126.52 (C-8), 73.22 (C-2), 55.91 (C-3), 51.76 (C-11), 41.42 (C-12), 36.29 (C-4), 35.58 (C-14), 29.38 (C-13), 23.00 (C-10); **HRMS** (m/*z*), calculated for $C_{22}H_{31}N_2O_3$: 371.2335, found $(M + H)^+$: 371.2331.

4.53 Synthesis of (2*S*,3*S*)-3-acetamido-*N*-adamantan-1-yl-2-hydroxy-4-phenyl butanamide 54B

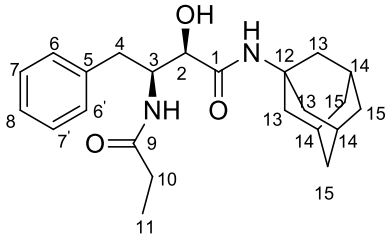
Compound **47B** (60 mg, 0.13 mmol), TFA (98 µl, 1.27 mmol) and Et_3N (176 µl, 1.27 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **54B** (47 mg, 99%) as a white solid.



R_f = 0.33 (49:1 $CHCl_3$/MeOH); **m.p.** 217 – 218 °C; **IR** ν_{max}(cm^{-1}): 3357 (OH), 3324 (NH), 3103 (NH), 2902 (C-H), 1606 (C=O), 1344 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.34 – 7.15 (6H, m, Ar-H and NH), 6.73 (0.56H, s, NH), 6.61 (0.44H, s) and 5.91 – 5.82 (2H, m) NH and OH, 4.26 – 4.13 (1H, m, H-3), 4.13 – 4.06 (1H, m, H-2), 3.10 – 2.99 (1H, m, H-4a), 2.99 – 2.88 (1H, m, H-4b), 2.13 – 1.96 (9H, m, H-12 and H-13), 1.93 – 1.86 (3H, m, H-10), 1.70 (6H, s, H-14); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.59, 172.51,170.75 and 170.69 (C=O), 138.36 (C-5), 129.28 (C-7 and C-7'), 128.51 and 128.41 (C-6 and C-6'), 126.52 (C-8), 74.78 and 74.68 (C-2), 57.39, 57.29 and 57.24 (C-3), 51.62 and 51.40 (C-11), 41.64 and 41.61 (C-12), 36.39 (C-14), 35.33 35.29 and 35.24 (C-4), 29.46 (C-13), 22.88 and 22.83 (C-10); **HRMS** (m/*z*), calculated for $C_{22}H_{31}N_2O_3$: 371.2335, found $(M + H)^+$: 371.2331.

4.54 Preparation of (2*R*,3*S*)-*N*-adamantan-1-yl-2-hydroxy-4-phenyl-3-propionamidobutanamide 55A

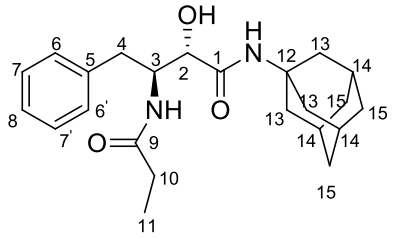
Compound **48A** (60 mg, 0.12 mmol), TFA (96 µl, 1.24 mmol) and Et_3N (172 µl, 1.24 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **55A** (47 mg, 99%) as a white solid.



R_f = 0.46 (49:1 $CHCl_3$/MeOH); **m.p.** 185 – 186 °C; **IR** ν_{max}(cm^{-1}): 3342 (OH), 3292 (NH), 2906 (C-H), 1750 (C=O), 1360 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28 – 7.22 (2H, m, Ar-H), 7.21 – 7.16 (3H, m, Ar-H), 6.74 (1H, d, *J* = 8.1 Hz), 6.65 (1H, s) and 6.22 (1H, s) 2xNH and OH, 4.19 – 4.09 (1H, m, H-3), 4.01 – 3.92 (1H, m, H-2), 3.08 (1H, dd, *J* = 13.8, 6.2 Hz, H-4a), 2.97 (1H, dd, *J* = 13.8, 9.3 Hz, H-4b), 2.15 – 2.03 (5H, m, H-10 and H-14), 1.98 (6H, s, H-13), 1.67 (6H, s, H-15), 1.02 (3H, t, *J* = 7.6 Hz, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.82 and 171.84 (C=O), 138.35 (C-5), 129.23 (C-7 and C-7'), 128.44 (C-6 and C-6'), 126.53 (C-8), 73.52 (C-2), 55.74 (C-3), 51.81 (C-12), 41.57 (C-13), 36.38 (C-15), 35.76 (C-4), 29.54 (C-10), 29.49 (C-14), 9.91 (C-11); **HRMS** (m/*z*), calculated for $C_{23}H_{33}N_2O_3$: 385.2491, found $(M + H)^+$: 385.2487.

4.55 Preparation of (2*S*,3*S*)-*N*-adamantan-1-yl-2-hydroxy-4-phenyl-3-propion amidobutanamide 55B

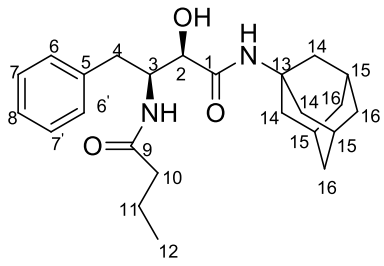
Compound **48B** (40 mg, 0.083 mmol), TFA (56 µl, 0.91 mmol) and Et_3N (114 µl, 0.91 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **55B** (28 mg, 88%) as a white solid.



R_f = 0.49 (49:1 $CHCl_3$/MeOH); **m.p.** 214 – 215 °C; **IR** v_{max}(cm^{-1}): 3362 (OH), 3264 (NH), 2908 (C-H), 1760 (C=O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.31 – 7.24 (2H, m, Ar-H), 7.24 – 7.16 (3H, m, Ar-H), 6.71 (1H, s), 5.95 (1H, d, *J* = 6.8 Hz) and 5.86 (1H, d, *J* = 5.2 Hz) 2xNH and OH, 4.21 – 4.14 (1H, m, H-3), 4.14 – 4.09 (1H, m, H-2), 3.11 (1H, dd, *J* = 14.1, 10.0 Hz, H-4a), 2.99 (1H, dd, *J* = 14.1, 5.6 Hz, H-4b), 2.18 – 1.94 (11H, m, H-10, H-13 and H-14), 1.69 (6H, s, H-15), 1.06 (3H, t, *J* = 7.6 Hz, H-10); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.66 and 170.78 (C=O), 138.01 (C-5), 129.19 (C-7 and C-7'), 128.66 (C-6 and C-6'), 126.73 (C-8), 75.13 (C-2), 57.91 (C-3), 51.75 (C-12), 41.68 (C-13), 36.41 (C-15), 35.72 (C-4), 29.52 (C-14), 29.46 (C-10), 9.76 (C-11); **HRMS** (m/*z*), calculated for $C_{23}H_{33}N_2O_3$: 385.2491, found $(M + H)^+$: 385.2486.

4.56 Preparation of (2*R*,3*S*)-*N*-adamantan-1-yl-3-butyramido-2-hydroxy-4-phenylbutanamide 56A

Compound **49A** (80 mg, 0.16 mmol), TFA (123 µl, 1.60 mmol) and Et_3N (222 µl, 1.60 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **56A** (60 mg, 94%) as a white solid.

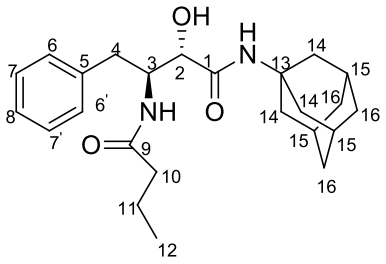


R_f = 0.44 (49:1 $CHCl_3$/MeOH); **m.p.** 109 – 110 °C; **IR** v_{max}(cm^{-1}): 3374 (OH), 3284 (NH), 3064 (C=C-H), 2907 (C-H), 1645 (C=O), 1360 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28 – 7.21 (2H, m, Ar-H), 7.21 – 7.15 (3H, m, Ar-H), 6.95 (1H, s, OH), 6.78 – 6.70 (1H, m, NH), 6.50 – 6.38 (1H, m, NH), 4.20 – 4.09 (1H, m, H-3), 4.01 – 3.93 (1H, m, H-2), 3.11 (1H, dd, *J* = 13.8, 6.2 Hz, H-4a), 2.99 – 2.90 (1H, m, H-4b), 2.14 – 2.02

(5H, m, H-10 and H-15), 1.99 (6H, s, H-14), 1.67 (6H, s, H-16), 1.58 – 1.45 (2H, m, H-11), 0.84 (3H, t, *J* = 7.3 Hz, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.06 and 171.89 (C=O), 138.29 (C-5), 129.17 (C-7 and C-7'), 128.40 (C-6 and C-6'), 126.47 (C-8), 73.49 (C-2), 55.73 (C-3), 51.71 (C-13), 41.44 (C-14), 38.24 (C-4), 36.30 (C-16), 35.53 (C-10), 29.38 (C-15), 19.08 (C-11), 13.69 (C-12); **HRMS** (m/*z*), calculated for $C_{24}H_{35}N_2O_3$: 399.2648, found $(M + H)^+$: 399.2645.

4.57 Preparation of (2*S*,3*S*)-*N*-adamantan-1-yl-3-butyramido-2-hydroxy-4-phenylbutanamide 56B

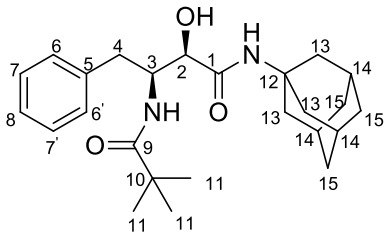
Compound **49B** (70 mg, 0.14 mmol), TFA (108 µl, 1.40 mmol) and Et_3N (194 µl, 1.40 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **56B** (57 mg, 100%) as a white solid.



R_f = 0.49 (49:1 $CHCl_3$/MeOH); **m.p.** 151 – 152 °C; **IR** ν_{max}(cm^{-1}): 3360 (OH), 3266 (NH), 3064 (C-H), 2924 (C-H), 1660 (C=O), 1603 (C=C), 1370 (C-O); ; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.25 (2H, m, Ar-H), 7.21 – 7.13 (3H, m, Ar-H) , 5.73 (1H, s, NH), 4.96 (1H, d, *J* = 3.9 Hz, NH), 4.26 – 4.15 (1H, m, H-3), 3.99 (1H, d, *J* = 6.5 Hz, H-2), δ 3.13 (1H, dd, *J* = 13.8, 6.2 Hz, H-4a), 2.98 – 1.97 (1H ,m , H-4b), 2.36 (2H, t, *J* = 7.4 Hz, H-10), 2.12 – 2.05 (3H, m, H-15), 2.01 (6H, m, H-14), 1.71 – 1.68 (8H, m, H-11 and H-16), 0.97 (3H, q, *J* = 7.1 Hz, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.42 and 166.70 (C=O), 137.45 (C-5), 129.19 (C-7 and C-7'), 128.49 (C-6 and C-6'), 126.60 (C-8), 74.24 (C-2), 56.48 (C-3), 52.32 (C-13), 41.44 (C-14), 37.49 (C-4), 36.26 (C-16), 36.03 (C-10), 29.70 (C-15), 18.32 (C-11), 13.65 (C-12); **HRMS** (m/*z*), calculated for $C_{24}H_{35}N_2O_3$: 399.2648, found $(M + H)^+$: 399.2643.

4.58 Preparation of (2*R*,3*S*)-*N*-adamantan-1-yl-2-hydroxy-4-phenyl-3-pivalamidobutanamide 57A

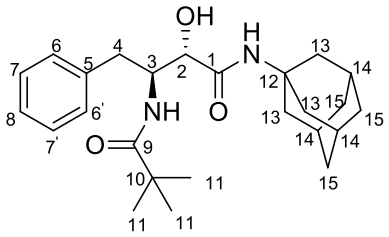
Compound **50A** (90 mg, 0.18 mmol), TFA (136 µl, 1.76 mmol) and Et_3N (244 µl, 1.76 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The experiment yielded compound **57A** (51 mg, 70%) as a white solid.



R_f = 0.62 (49:1 $CHCl_3$/MeOH); **m.p.** 239 – 240 °C; **IR** v_{max}(cm^{-1}): 3301 (OH), 2907 (C-H), 1737 (C=O), 1651 (C-O), 1345 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28 – 7.22 (2H, m, Ar-H), 7.21 – 7.15 (3H, m, Ar-H), 6.75 – 6.67 (1H, m), 6.67 – 6.54 (1H, m) and 6.48 – 6.39 (1H, m, 2xNH and OH), 4.27 – 4.16 (1H, m, H-3), 4.02 – 3.95 (1H, m, H-2), 3.12 – 2.96 (2H, m, H-4), 2.07 (3H, s, H-14), 1.99 (6H, s, H-13), 1.67 (6H, s, H-15), 1.05 (9H, s, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 180.67 and 171.73 (C=O), 138.26 (C-5), 129.25 (C-7 and C-7'), 128.37 (C- and C-6'), 126.49 (C-8), 73.85 (C-2), 55.46 (C-3), 51.69 (C-12), 41.52 (C-13), 38.65 (C-10) 36.30 (C-15), 35.52 (C-4), 29.38 (C-14), 27.48 (C-11); **HRMS** (m/*z*), calculated for $C_{25}H_{37}N_2O_3$: 413.2804, found $(M + H)^+$: 413.2798.

4.59 Preparation of (2*S*,3*S*)-*N*-adamantan-1-yl-2-hydroxy-4-phenyl-3-pival amidobutanamide of 57B

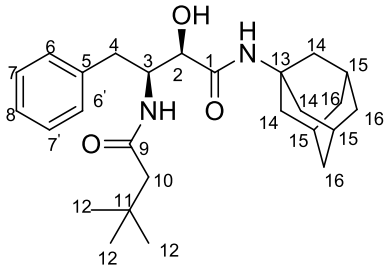
Compound **50B** (45 mg, 0.087 mmol), TFA (67 µl, 0.87 mmol) and Et_3N (121 µl, 0.87 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **57B** (26 mg, 70%) as a white solid.



R_f = 0.54 (49:1 $CHCl_3$/MeOH); **m.p.** 61 – 63 °C; **IR** v_{max}(cm^{-1}): 3318 (OH), 3248 (NH), 2961 (C-H), 1664 (C=O), 1636 (C=O), 1358 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.34 – 7.17 (5H, m, Ar-H), 6.75 (1H, s, NH), 5.97 (1H, d, *J* = 6.6 Hz, NH), 4.24 – 4.15 (1H, m, H-3), 4.14 – 4.09 (1H, m, H-2), 3.18 (1H, dd, *J* = 14.2, 10.5 Hz, H-4a), 3.05 (1H, dd, *J* = 14.1, 5.4 Hz, H-4b), 2.13 – 2.05 (3H, m, H-14), 2.05 – 1.91 (6H, m, H-13), 1.73 – 1.60 (6H, m, H-15), 1.05 (9H, s, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 181.67 and 170.79 (C=O), 137.89 (C-5), 129.36 (C-7 and C-7'), 128.65 (C-6 and C-6'), 126.75 (C-8), 75.28 (C-2), 57.59 (C-3), 51.65 (C-12), 41.62 (C-13), 38.62 (C-10), 36.27 (C-15), 35.97 (C-4), 29.41 (C-14), 27.40 (C-11); **HRMS** (m/*z*), calculated for $C_{25}H_{37}N_2O_3$: 413.2804, found $(M + H)^+$: 413.2802.

4.60 Preparation of (2*R*,3*S*)-*N*-adamantan-1-yl-3-(3,3-dimethylbutanamido)-2-hydroxy-4-phenylbutanamide 58A

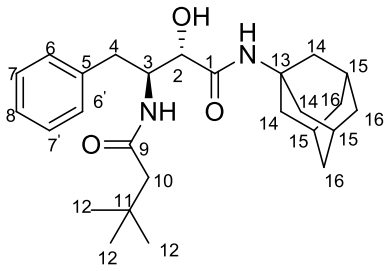
Compound **51A** (75 mg, 0.14 mmol), TFA (110 µl, 1.42 mmol) and Et_3N (197 µl, 1.42 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **58A** (50 mg, 82%) as a white solid.



R_f = 0.44 (49:1 $CHCl_3$/MeOH); **m.p.** 172 – 173 °C; **IR** v_{max}(cm^{-1}): 3346 (OH), 3290 (NH), 3088 (C=CH), 2906 (C-H), 1660 (C=O), 1360 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28 – 7.13 (5H, m, Ar-H), 6.97 (1H, d, *J* = 8.2 Hz), 6.77 (1H, s) and 6.57 (1H, d, *J* = 6.5 Hz, 2xNH and OH), 4.24 – 4.15 (1H, m, H-3), 4.00 – 3.92 (1H, m, H-2), 3.16 (1H, dd, *J* = 13.8, 6.4 Hz, H-4a), 2.92 (1H, dd, *J* = 13.8, 9.3 Hz, H-4b), 2.11 – 2.04 (3H, m, H-15), 2.04 – 1.91 (8H, m, H-10 and H-14), 1.70 – 1.63 (6H, m, H-16), 0.90 (9H, s, H-12), **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.84 and 171.96 (C=O), 138.25 (C-5), 129.19 (C-7 and C-7'), 128.41 (C-6 and C-6'), 126.45 (C-8), 73.43 (C-2), 55.59 (C-3), 51.73 (C-13), 50.02 (C-10), 41.47 (C-14), 36.30 (C-16), 35.49 (C-4), 30.74 (C-11), 29.73 (C-12), 29.38 (C-15); **HRMS** (m/*z*), calculated for $C_{26}H_{39}N_2O_3$: 427.2961, found $(M + H)^+$: 427.2955.

4.61 Preparation of (2*S*,3*S*)-*N*-adamantan-1-yl-3-(3,3-dimethylbutanamido)-2-hydroxy-4-phenylbutanamide 58B

Compound **51B** (31 mg, 0.059 mmol), TFA (45 µl, 0.59 mmol) and Et_3N (82 µl, 0.59 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **58B** (27 mg, 100%) as a white solid.

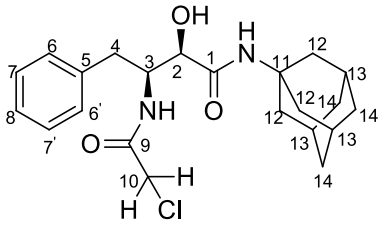


R_f = 0.46 (49:1 $CHCl_3$/MeOH); **m.p.** 195 – 196 °C; **IR** v_{max}(cm^{-1}): 3301 (OH), 3285 (NH), 2906 (C-H), 2851 (C-H), 1654 (C=O), 1638 (C=O), 1360 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.14 (5H, m, Ar-H), 6.76 (1H, s) and 6.07 – 5.95 (2H, m, 2xNH and OH), 4.27 – 4.15 (1H, m, H-3), 4.15 – 4.08 (1H, m, H-2), 3.14 (1H, dd, *J* = 14.3, 10.3 Hz. H-4a), 3.04 (1H, dd, *J* = 14.2, 5.3 Hz, H-4b), 2.04 (3H, m, H-15), 2.04 – 1.89 (8H, m, H-10 and H-14), 1.73 – 1.63 (6H, m, H-16), 0.88 (9H, s, H-12); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.87 and 170.82 (C=O), 137.91 (C-5), 129.11 (C-7 and C-7'), 128.67 (C-6 and C-6'), 126.71 (C-8), 75.36 (C-2), 57.77 (C-3), 51.68 (C-13), 50.07 (C-10), 41.53 (C-14), 36.32 (C-16), 35.94 (C-4),

30.81 (C-11), 29.63 (C-12), 29.40 (C-15); **HRMS** (m/*z*), calculated for $C_{26}H_{39}N_2O_3$: 427.2961, found $(M + H)^+$: 427.2962.

4.62 Preparation of (2*R*,3*S*)-*N*-adamantan-1-yl-3-(2-chloroacetamido)-2-hydroxy-4-phenylbutanamide 59A

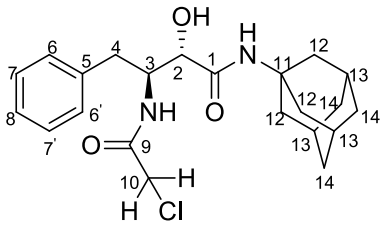
Compound **52A** (60 mg, 0.12 mmol), TFA (92 µl, 1.19 mmol) and Et_3N (165 µl, 1.19 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **59A** (42 mg, 88%) as a white solid.



R_f = 0.44 (49:1 $CHCl_3$/MeOH); **m.p.** 141 – 142 °C; **IR** $\nu_{max}(cm^{-1})$: 3320 (OH), 3280 (NH), 3064 (C=C-H), 2907 (C-H), 1649 (C=O), 1360 (C-O), 749 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.51 (1H, d, *J* = 8.7 Hz, NH), 7.31 – 7.24 (2H, m, Ar-H), 7.24 – 7.17 (3H, m, Ar-H), 6.50 (1H, s, NH), 5.37 (1H, s, OH), 4.36 – 4.24 (1H, m, H-3), 4.01 (1H, s, H-2), 3.86 (2H, s, H-10), 3.08 (1H, dd, *J* = 13.7, 6.1 Hz, H-4a), 2.87 (1H, dd, *J* = 13.5, 8.9 Hz, H-4b), 2.13 – 2.05 (3H, m, H-13), 1.99 (6H, s, H-12), 1.68 (6H, s, H-14); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.06 and 167.15 (C=O), 137.61 (C-5), 129.22 (C-7 and C-7'), 128.52 (C-6 and C-6'), 126.69 (C-8), 72.26 (C-2), 55.35 (C-3), 52.00 (C-11), 42.34(C-10), 41.40 (C-12), 36.23 (C-14), 35.87 (C-4), 29.36 (C-13); **HRMS** (m/*z*), calculated for $C_{22}H_{30}ClN_2O_3$: 405.1945, found $(M + H)^+$: 405.1945.

4.63 Preparation of (2*S*,3*S*)-*N*-adamantan-1-yl-3-(2-chloroacetamido)-2-hydroxy-4-phenylbutanamide 59B

Compound **52B** (42 mg, 0.083 mmol), TFA (64 µl, 0.83 mmol) and Et_3N (115 µl, 0.83 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **59B** (25 mg, 77%) as a white solid.

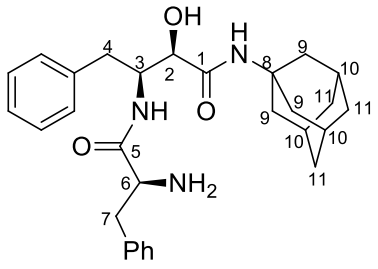


R_f = 0.47 (49:1 $CHCl_3$/MeOH); **m.p.** 223 – 224 °C; **IR** (solid) $\nu_{max}(cm^{-1})$: 3313 (OH), 2906 (C-H), 1648 (C=C), 1361 (C-O), 794 (C-Cl); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.25 (2H, m, Ar-H), 7.25 – 7.17 (3H, m, Ar-H), 6.97 (1H, d, *J* = 7.5 Hz), 6.64 (1H, s) and 5.07 (1H, d, *J* = 5.2 Hz, 2xNH and OH), 4.34 – 4.24 (1H, m, H-3), 4.21 – 4.14 (1H, m, H-2),

3.98 – 3.88 (2H, m, H-10), 3.09 – 2.95 (2H, m, H-4), 2.13 – 2.06 (3H, m, H-13), 2.06 – 1.98 (6H, m, H-12), 1.77 – 1.66 (6H, m, H-14); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.07 and 168.05 (C=O), 137.39 (C-5), 129.18 (C-7 and C-7'), 128.67 (C-6 and C-6'), 126.82 (C-8), 73.97 (C-2), 57.41 (C-3), 51.94 (C-11), 42.29 (C-10), 41.51 (C-12), 36.27 (C-14), 35.40 (C-4), 29.39 (C-13); **HRMS** (m/*z*), calculated for $C_{22}H_{30}ClN_2O_3$: 405.1945, found $(M + H)^+$: 405.1945.

4.64 Preparation of (2*R*,3*S*)-*N*-adamantan-1-yl-3-((*S*)-2-amino-3-phenyl propanamido)-2-hydroxy-4-phenylbutanamide 60A

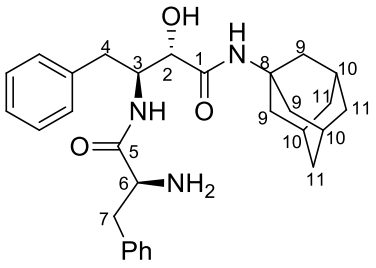
Compound **53A** (100 mg, 0.15 mmol), TFA (114 µl, 1.48 mmol) and Et_3N (205 µl, 1.48 mmol) in CH_2Cl_2 (2 ml) were reacted together as described in **4.11**. The reaction yielded compound **60A** (59 mg, 84%) as a white solid.



R_f = 0.29 (49:1 $CHCl_3$/MeOH); **m.p.** 65 – 66 °C; **IR** ν_{max}(cm^{-1}): 3311 (OH), 3028 (C=CH), 2907 (C-H), 1648 (C=O), 1345 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.96 (0.83H, d, *J* = 8.3 Hz, NH), 7.67 (0.13H, d, *J* = 8.9 Hz, NH), 7.34 – 7.11 (10H, m, Ar-H), 6.60 (0.89H, s, NH), 6.53 (0.13H, s, NH), 6.10 (1H, br-s, OH), 4.34 – 4.23 (1H, m, H-3), 4.05 – 3.99 (0.89H, m, H-2), 3.94 – 3.90 (0.13H, m, H-2), 3.49 (1H, dd, *J* = 10.4, 3.7 Hz, H-6), 3.24 – 3.04 (2H, m, H-4a and H-7a), 3.04 – 2.91 (1H, m, H-4b), 2.36 (1H, dd, *J* = 13.8, 10.3 Hz, H-7b), 2.11 – 2.03 (3H, m, H-10), 2.03 – 1.93 (6H, m, H-9), 1.71 – 1.61 (6H, m, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.31 and 171.40 (C=O), 138.19 (Ar-C), 137.75 (Ar-C), 129.35 (Ar-C), 129.29 (Ar-C), 129.10 (Ar-C), 128.77 (Ar-C), 128.66 (Ar-C), 128.44 (Ar-C), 128.40 (Ar-C), 126.89 (Ar-C), 126.86 (Ar-C), 126.57 (Ar-C), 126.54 (Ar-C), 73.38 (C-2), 56.51 and 55.20 (C-3 and C-6), 51.79 (C-8), 41.50 (C-9), 41.01 (C-7), 36.29 (C-11), 35.95 (C-4), 29.39 (C-10); **HRMS** (m/*z*), calculated for $C_{29}H_{38}N_3O_3$: 476.2913, found $(M + H)^+$: 476.2918.

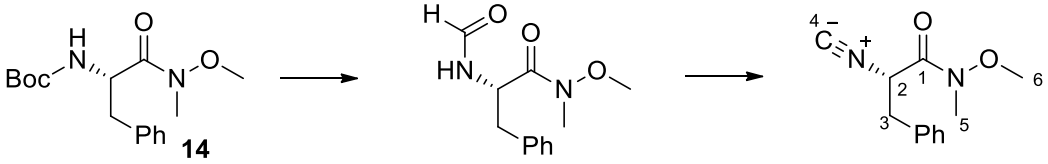
4.65 Preparation of (2*S*,3*S*)-*N*-adamantan-1-yl-3-((*S*)-2-amino-3-phenylpropanamido)-2-hydroxy-4-phenylbutanamide 60B

Compound **53A** (40 mg, 0.059 mmol), TFA (46 µl, 0.59 mmol) and Et_3N (82 µl, 0.59 mmol) in CH_2Cl_2 (1 ml) were reacted described together as in **4.11**. The reaction yielded compound **60B** (25 mg, 89%) as a white solid.



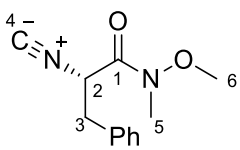
R_f = 0.31 (49:1 $CHCl_3$/MeOH); **m.p.** 60 – 62 °C; **IR** $\nu_{max}(cm^{-1})$: 3313 (OH), 3028 (C=C-H), 2906 (C-H), 1654 (C=O), 1603 (C=O), 1360 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.74 (1H, d, *J* = 7.1 Hz, NH), 7.33 – 7.09 (10H, m, Ar-H), 6.74 (1H, s, NH), 4.24 – 4.13 (2H, m, H-2 and H-3), 3.57 – 3.49 (1H, m, H-6), 3.14 – 3.05 (2H, m, H-4a and H-7a), 2.97 (1H, dd, *J* = 14.0, 5.1 Hz, H-4b), 2.49 (1H, dd, *J* = 13.8, 9.2 Hz, H-7b), 2.12 – 1.96 (9H, m, H-9 and H-10), 1.75 – 1.63 (6H, m, H-11); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 176.95 and 170.48 (C=O), 138.17 (Ar-C), 137.22 (Ar-C), 129.22 (Ar-C), 129.18 (Ar-C), 128.77 (Ar-C), 128.53 (Ar-C), 126.99 (Ar-C), 126.60 (Ar-C), 74.93 (C-2), 57.51 (C-3), 56.08 (C-6), 51.63 (C-8), 41.56 (C-9), 40.77 (C-7) 36.32 (C-11), 35.03 (C-4), 29.42 (C-10); HRMS (m/z), calculated for $C_{29}H_{38}N_3O_3$: 476.2913, found $(M + H)^+$: 476.2917.

4.66 Preparation of (*S*)-2-isocyano-*N*-methoxy-*N*-methyl-3-phenyl propanamide 12



14

Excess formic acid (20 ml) and excess acetic anhydride (5 ml) were added to compound **14** (3.15 g, 15.10 mmol) and left to stir under N_2. TFA (4 eq., 464 µl, 60.4 mmol) was added dropwise and the reaction was left stirring at room temperature for 4 h whilst being monitored by TLC. The reaction was worked up as described in **4.44** yielding a crude formylated product as a viscous colourless oil (2.76 g, 78%). Crude product (2.76 g, ≈ 11.66 mmol) was dissolved in anhydrous CH_2Cl_2 (10 ml) under N_2 at 0°C, followed by slow addition of excess $POCl_3$ (5 ml) and excess Et_3N (15 ml). The experiment was completed using the same procedure as that described in **4.2** yielding **12** (2.06 g, 81%), as a brown oil with a slight distinct smell.



R_f = 0.29 (3:7 EtOAc/Hex); **$[\alpha_D]^{20}_{589}$** +2.13 (c 2, $CHCl_3$); **IR**: $\nu_{max}(cm^{-1})$: 3033 (C=C-H), 2940 (C-H), 2143 (CΞN), 1676 (C=O); **^{1}H NMR** (500 MHz, CDCl3) δ 7.32 – 7.29 (5H, m, Ar-H), 4.85 – 4.77 (1H, m, H-2), 3.66 (3H, s, H-6), 3.25 – 3.22 (4H, m, H-5 and H-3a), 3.10 (1H, q, *J* = 8.5Hz, H-3b); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.88 C-

1), 159.28 (C-4), 135.26 (Ar-C), 129.33 (Ar-C), 129.00 (Ar-C), 128.27 (Ar-C), 61.66 (C-6), 55.45 (C-2), 38.70 (C-3), 32.53 (C-5).

4.67 Synthesis (5*S*,9*S*)-5,9-dibenzyl-3,13,13-trimethyl-4,7,11-trioxo-2,12-dioxa-3,6,10-triazatetradecan-8-yl acetate 61

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), phenylalanine isocyanide **12** (250 mg, 1.20 mmol) and acetic acid **1** (81 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment proceeded as described in **4.5** yielding compound **61** (283 mg, 45%) as a white solid which was a mixture of diastereomers

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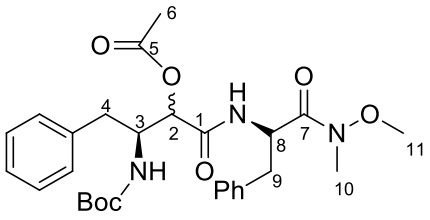
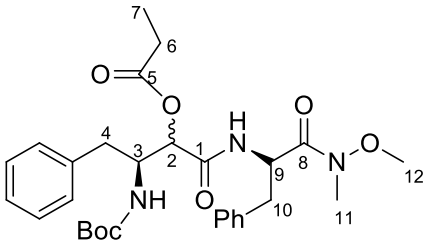


R_f = 0.54 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.52:1.00; **m.p.** 124 – 125 °C; **IR** ν_{max}(cm^{-1}): 3354 (NH), 2917 (C-H), 1747 (C=O), 1690 (C=O), 1605 (C=C), 1366 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.40 – 6.99 (10H, m, Ar-H), 6.92 – 6.57 (1H, m, NH), 5.43 – 4.95 (2H, m, NH and H-2), 4.46 – 4.15 (2H, m, H-3 and H-8), 3.83 – 3.58 (3H, m, H-11), 3.33 – 3.14 (3H, m, H-10), 3.14 – 2.39 (4H, m H-4 and H-9), 2.22 – 1.95 (3H, m, H-6), 1.44 – 1.20 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.23, 170.88, 169.35, 168.92, 167.55 and 166.77 (C=O), 155.40 and 154.84 (Boc C=O), 137.28 (Ar-C), 135.82 (Ar-C), 129.54 (Ar-C), 129.49 (Ar-C), 129.33 (Ar-C), 129.24 (Ar-C), 129.17 (Ar-C), 128.77 (Ar-C), 128.58 (Ar-C), 128.46 (Ar-C), 128.43 (Ar-C), 128.36 (Ar-C), 127.14 (Ar-C), 127.04 (Ar-C), 126.53 (Ar-C), 126.48 (Ar-C), 79.42 [$(CH_3)_3\underline{C}$], 74.35 and 73.68 (C-2), 61.77 and 61.66 (C-11), 52.66 (C-3), 52.54, 50.08 and 49.83 (C-8), 38.69, 38.53, 37.91 and 36.96 (C-4 and C-9), 32.16 (C-10), 28.44 and 28.25 [$(\underline{C}H_3)_3C$], 20.86 and 20.55 (C-6); **HRMS** (m/*z*), calculated for $C_{28}H_{38}N_3O_7$: 528.2710, found $(M + H)^+$: 528.2710.

4.68 Synthesis of (5*S*,9*S*)-5,9-dibenzyl-3,13,13-trimethyl-4,7,11-trioxo-2,12-dioxa-3,6,10-triazatetradecan-8-yl propionate 62

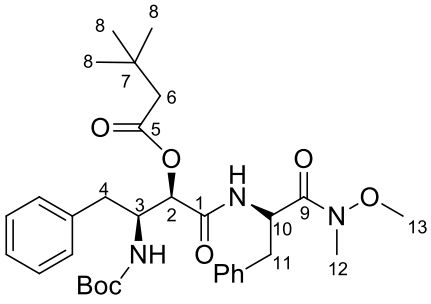
N-Boc-L-phenylalaninal **15** (250 mg, 1.00 mmol), phenylalanine isocyanide **12** (218 mg, 1.00 mmol) and propionic acid **2** (83 µl, 1.00 mmol) were dissolved in of CH_2Cl_2 (3 ml). The experiment proceeded as in **4.5** yielding **62** (544 mg, 100%) as a white solid which was a mixture of diastereomers.



R_f = 0.55 (1:1 EtOAc/Hex); **d.r.** = 1.34:1.00; **m.p.** 159 – 160 °C; **IR** v_{max}(cm^{-1}): 3372 (NH), 3064 (C-H), 1738 (C=O), 1691 (C=O), 1656 (C=O), 1523 (C=C); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.48 – 6.69 (11H, m, Ar-H and NH), 5.49 – 4.98 (3H, m, NH, H-2 and H-9), 4.58 – 4.15 (1H, m, H-3), 3.84 – 3.52 (3H, m, H-12), 3.41 – 2.10 (9H, m, H-4, H-6, H-10 and H-11), 1.60 – 0.99 (12H, m, $(CH_3)_3C$ and H-7); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.67, 172.37, 170.99, 167.75 and 166.95 (C=O), 155.34 and 154.90 (Boc C=O), 137.35 (Ar-C), 136.30 (Ar-C), 135.86 (Ar-C), 135.27 (Ar-C), 129.52 (Ar-C), 129.48 (Ar-C), 129.31 (Ar-C), 129.26 (Ar-C), 129.19 (Ar-C), 128.74 (Ar-C), 128.54 (Ar-C), 128.45 (Ar-C), 128.39 (Ar-C), 128.31 (Ar-C), 127.09 (Ar-C), 126.96 (Ar-C), 126.48 (Ar-C), 126.43 (Ar-C), 79.36 [$(CH_3)_3\underline{C}$], 74.19 and 73.51 (C-2), 61.60 (C-12), 52.74 and 50.18 (C-3), 49.87 (C-9), 38.69, 38.50, 37.98, 37.77 and 37.01 (C-4 and C-10), 32.16 (C-11), 28.23 [$(\underline{C}H_3)_3C$], 27.44 and 27.25 (C-6), 8.93 and 8.83 (C-7); **HRMS** (m/*z*), calculated for $C_{29}H_{40}N_3O_7$: 542.2866, found $(M + H)^+$: 542.2868.

4.69 Synthesis of (5*S*,9*S*)-5,9-dibenzyl-3,13,13-trimethyl-4,7,11-trioxo-2,12-dioxa-3,6,10-triazatetradecan-8-yl 3,3-dimethylbutanoate 63

N-Boc-L-phenylalaninal **15** (400 mg, 1.60 mmol), phenylalanine isocyanide **13** (349 µl, 1.60 mmol) and tert-butyl isocyanide **4** (204 µl, 1.60 mmol) were dissolved in CH_2Cl_2 (3 ml). The experiment proceeded as described in **4.5** yielding compound **63** (239 mg, 26%) as a white solid that appeared as a single diastereomer.

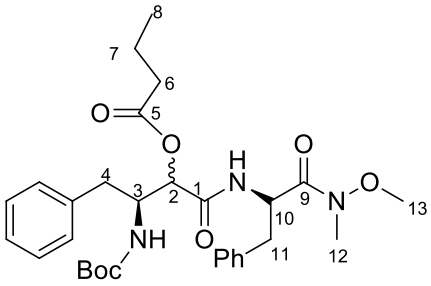


R_f = 0.54 (49:1 $CHCl_3$/MeOH); **d.r.** = 1 peak; **m.p.** 106 – 107 °C; **IR** (solid) v_{max}(cm^{-1}): 3309 (NH), 3029 (C-H), 1649 (C=O), 1604 (C=C), 1365 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.38 – 7.02 (10H, m, Ar-H), 6.87 – 6.70 (1H, m, NH), 5.42 – 4.91 (3H, m, NH, H-2 and H-10), 4.40 – 4.17 (1H, m, H-3), 3.78 – 3.59 (3H, m, H-13), 3.22 – 2.59 (7H, m, H-4, H-11 and H-12), 2.26 (2H, s, H-6), 1.32 (9H, s, $(CH_3)_3C$), 1.13 – 0.70 (9H, m, H-8); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.00, 170.58, 170.15 and 167.78 (C=O), 154.83 (Boc C=O), 137.37 (Ar-C), 135.89 (Ar-C), 129.45 (Ar-C), 129.28 (Ar-C), 128.44 (Ar-C), 128.40 (Ar-C), 126.99 (Ar-C), 126.46 (Ar-C), 79.34 [$(CH_3)_3\underline{C}$], 73.21 (C-2), 61.62 (C-13), 52.86 and 50.15 (C-3 and C-10), 47.32 (C-6), 38.57 and 37.96 (C-4 and C-11),

32.15 (C-12), 30.80 (C-7), 29.72 (C-8), 29.57 (C-8), 28.24 [(*C*H_3)$_3$C]; **HRMS** (m/*z*), calculated for $C_{32}H_{44}N_3O_7$: 584.3336, found $(M + H)^+$: 584.3338.

4.70 Synthesis of (5*S*,9*S*)-5,9-dibenzyl-3,13,13-trimethyl-4,7,11-trioxo-2,12-dioxa-3,6,10-triazatetradecan-8-yl butyrate 64

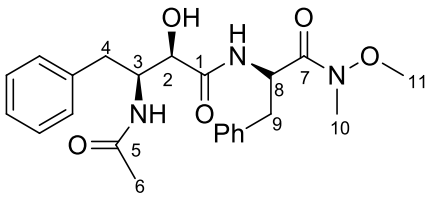
N-Boc-L-phenylalaninal **15** (150 mg, 0.60 mmol), phenylalanine isocyanide **12** (131 mg, 0.60 mmol) and butyric acid **3** (55 µl, 0.60 mmol) were combined and the reaction was heated under microwave conditions at 120 °C, at 60 W for 7 min. The reaction was repeated 3 times and the product from all the reactions was combined and purified by column chromatography as detailed in **4.5**. The experiment yielded compound **64** (832 mg, 83%) as a white solid which was a mixture of diastereomers.



R_f = 0.54 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.00:0.93; **m.p.** 135 – 136 °C; **IR** ν_{max}(cm^{-1}): 3384 (NH), 2966 (C-H), 1736 (C=O), 1689 (C=O), 1654 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.37 – 7.00 (10H, m, Ar-H), 6.96 – 6.64 (1H, m, NH), 5.44 – 5.03 (3H, m, NH, H-2 and H-10), 4.60 – 4.16 (1H, m, H-3), 3.81 – 3.61 (3H, m, H-13), 3.30 – 2.12 (9H, m,H-4, H-6, H-11 and H-12), 1.76 – 1.50 (2H, m, H-7), 1.45 – 1.11 (9H, m, $(CH_3)_3C$), 1.03 – 0.81 (3H, m, H-8); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.87, 171.53, 167.74 and 166.94 (C=O), 155.30 (Boc C=O), 154.85 (Boc C=O), 137.33 (Ar-C), 136.27 (Ar-C), 135.84 (Ar-C), 129.53 (Ar-C), 129.47 (Ar-C), 129.27 (Ar-C), 129.20 (Ar-C), 128.55 (Ar-C), 128.42 (Ar-C), 128.40 (Ar-C), 128.33 (Ar-C), 127.10 (Ar-C), 126.98 (Ar-C), 126.46 (Ar-C), 79.39 [$(CH_3)_3\underline{C}$], 74.05 and 73.36 (C-2), 61.72 and 61.63 (C-13), 52.73, 50.15 and 49.84 (C-3 and C-10), 38.52, 37.87 and 37.05 (C-4 and C-11), 35.96 and 35.76 (C-6), 32.16 (C-12), 28.25 [(*C*H_3)$_3$C], 18.25 and 18.15 (C-7), 13.64 (C-8); **HRMS** (m/*z*), calculated for $C_{30}H_{42}N_3O_7$: 556.3023, found $(M + H)^+$: 556.3030.

4.71 Synthesis of (2*R*,3*S*)-3-acetamido-2-hydroxy-*N*-(*S*)-1-(methoxy (methyl)amino)-1-oxo-3-phenylpropan-2-yl)-4-phenylbutanamide 65

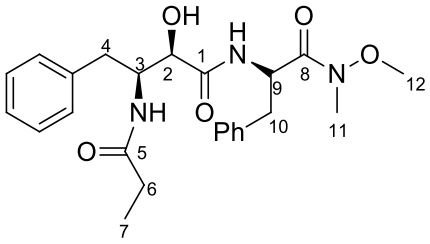
Compound **61** (100 mg, 0.19 mmol), TFA (146 µl, 1.89 mmol) and Et_3N (262 µl, 1.89 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **65** (43 mg, 53%) as a yellow oil which appeared as a single diastereomer.



R_f = 0.43 (49:1 $CHCl_3$/MeOH); **IR** $\nu_{max}(cm^{-1})$: 3332 (OH), 3029 (C=C-H), 1642 (C=O), 1518 (C=C), 1374 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.37 (1H, d, *J* = 8.3 Hz, NH), 7.32 – 7.13 (10H, m, Ar-H), 6.27 (1H, d, *J* = 8.1 Hz, NH), 5.77 (1H, s, OH), 5.14 (1H, s, H-8), 4.23 – 4.09 (1H, m, H-3), 4.05 (1H, s, H-2), 3.65 (3H, s, H-11), 3.23 – 2.98 (6H, m, H-4a, H-9a and H-10), 2.91 (1H, dd, *J* = 13.7, 7.7 Hz, H-9b), 1.87 (3H, s, H-6); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.70, 172.36 and 171.54 (C=O), 138.04 (Ar-C), 136.30 (Ar-C), 129.26 (Ar-C), 129.19 (Ar-C), 128.56 (Ar-C), 128.53 (Ar-C), 127.01 (Ar-C), 126.60 (Ar-C), 73.40 (C-2), 61.47 (C-11), 55.95 (C-3), 50.48 (C-8), 38.02 (C-9), 35.90 (C-4), 32.16 (C-10), 23.12 (C-6). **HRMS** (m/*z*), calculated for $C_{23}H_{30}N_3O_5$: 428.2185, found $(M + H)^+$: 428.2177.

4.72 Synthesis of (2*R*,3*S*)-2-hydroxy-*N*-(*S*)-1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)-4-phenyl-3-propionamidobutanamide 66

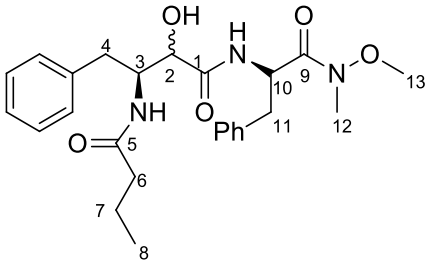
Compound **62** (100 mg, 0.18 mmol), TFA (142 µl, 1.85 mmol) and Et_3N (256 µl, 1.85 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The reaction yielded compound **66** (44 mg, 54%) as a light brown oil which appeared as a single diastereomer.



R_f = 0.33 (49:1 $CHCl_3$/MeOH); **IR** $\nu_{max}(cm^{-1})$: 3346 (OH), 3028 (C=C-H), 2974 (C-H), 1643 (C=O), 1389 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.48 (1H, d, *J* = 8.4 Hz, NH), 7.32 – 7.12 (10H, m, Ar-H), 6.39 (1H, d, *J* = 8.2 Hz) and 6.08 (1H, d, *J* = 6.6 Hz) NH and OH, 5.24 – 5.10 (1H, m, H-9), 4.25 – 4.13 (1H, m, H-3), 4.09 – 4.00 (1H, m, H-2), 3.61 (3H, s, H-12), 3.21 – 2.85 (7H, m, H-4, H-10 and H-11), 2.13 – 2.02 (2H, m, H-6), 1.00 (3H, t, *J* = 7.6 Hz, H-7); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.79 (C=O), 172.66 (C=O), 171.47 (C=O), 138.09 (Ar-C), 136.30 (Ar-C), 129.27 (Ar-C), 129.19 (Ar-C), 128.51 (Ar-C), 128.42 (Ar-C), 126.97 (Ar-C), 126.49 (Ar-C), 73.35 (C-2), 61.45 (C-12), 55.56 (C-3), 50.30 (C-9), 38.15 and 35.75 (C-4 and C-10), 32.08 (C-11), 29.47 (C-6), 9.70 (C-7); **HRMS** (m/*z*), calculated for $C_{24}H_{32}N_3O_5$: 442.2342, found $(M + H)^+$: 442.2342.

4.73 Synthesis of (3*S*)-3-butyramido-2-hydroxy-*N*-(*S*)-1-(methoxy(methyl) amino)-1-oxo-3-phenylpropan-2-yl)-4-phenylbutanamide 67

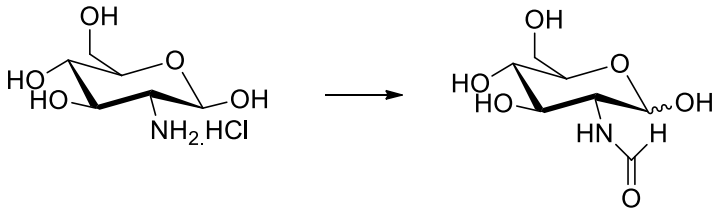
Compound **64** (130 mg, 0.23 mmol), TFA (234 µl, 2.33 mmol) and Et_3N (323 µl, 2.33 mmol) in CH_2Cl_2 (1 ml) were reacted together as described in **4.11**. The procedure yielded compound **67** (62 mg, 59%) as a light brown oil that appeared as predominantly one diastereomer.



R_f = 0.50 (49:1 $CHCl_3$/MeOH); **IR** (oil) v_{max}(cm^{-1}): 3328 (OH), 3063 (C=C-H), 2963 (C-H), 1643 (C=O), 1517 (C=C), 1389 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.68 – 7.49 (1H, m, NH), 7.31 – 7.11 (10H, m, Ar-H), 6.56 – 6.41 (1H, m) and 6.12 – 5.88 (1H, m) NH and OH, 5.35 – 5.13 (1H, m, H-10), 4.34 – 4.17 (1H, m, H-3), 4.10 – 4.01 (1H, m, H-2), 3.59 (3H, s, H-13), 3.25 – 2.79 (7H, m, H-4, H-11 and H-12), (2H, t, *J* = 7.4 Hz, H-6), 1.56 – 1.40 (2H, m, H-7), 0.80 (3H, t, *J* = 7.4 Hz, H-8); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.66, 172.56, 171.51 and 171.44 (C=O), 138.09 (Ar-C), 136.29 (Ar-C), 129.30 (Ar-C), 129.27 (Ar-C), 129.23 (Ar-C), 129.20 (Ar-C), 129.18 (Ar-C), 129.16 (Ar-C), 128.48 (Ar-C), 128.40 (Ar-C), 126.95 (Ar-C), 126.45 (Ar-C), 74.65 and 73.14 (C-2), 61.45 (C-13), 55.22 (C-3), 50.21 (C-10), 38.30, 38.28, 38.18 (C-4 and C-11), 35.80 (C-6), 32.06 (C-12), 18.95 (C-7), 13.59 and 13.52 (C-8); **HRMS** (m/*z*), calculated for $C_{25}H_{34}N_3O_5$: 456.2498, found $(M + H)^+$: 456.2500.

4.74 Preparation of 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-isocyano-*D*-glucopyranose 13 [68]

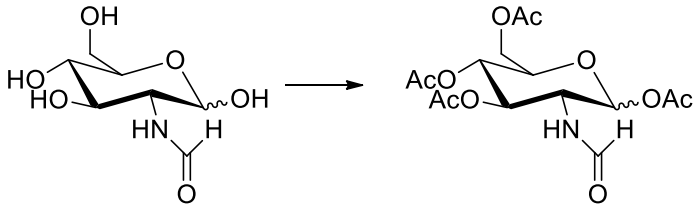
4.74.1 Formylation of glucosamine



Na metal (0.533 g, 23.19 mmol) was dissolved in MeOH (49 ml) and glucosamine hydrochloride (5.00 g, 23.186 mmol) was added to the solution ensuring complete dissolution by rigorous stirring. Methyl formate (4 eq., 5.14 ml, 83.469 mmol) was added and the reaction was left to

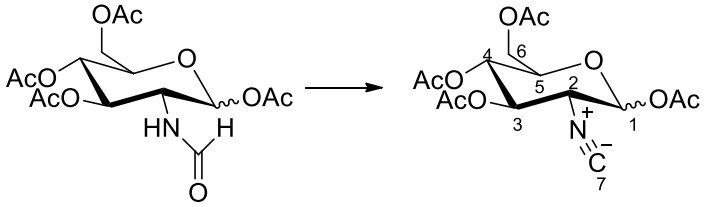
stir under reflux at 40 °C overnight. Excess solvent and methyl formate were removed *in vacuo* and crude formylated glucosamine (4.80 g) was used in the next step.

4.74.2 Acyl protection of hydroxyl groups

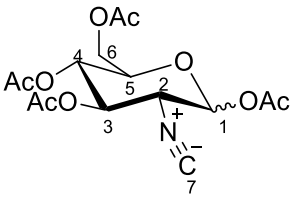


Crude formylated glucosamine (4.80 g, 23.186 mmol) was dissolved in pyridine (25 ml) and acetic anhydride (25 ml) was added. The reaction was allowed to proceed for 4 h after which the acetic anhydride was quenched using MeOH and removed *in vacuo*. Excess pyridine was then removed as an azeotropic mixture with toluene, yielding a white precipitate. ^{1}H NMR spectroscopy showed the expected acylated product $\mathbf{R_f}$ 0.29 (3:7 EtOAc/Hex) and the crude mixture was used in the next step.

4.74.3 Dehydration of glucose formamide



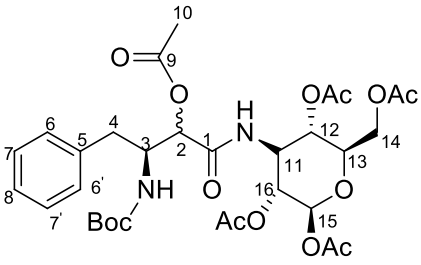
Crude acylated glucose formamide (8.69 g, 23.19 mmol) was dissolved in anhydrous CH_2Cl_2 (40 ml) under N_2 and cooled down to 0 °C. $POCl_3$ (3 eq., 6.48 ml, 69.56 mmol) was slowly added followed by Et_3N (8 eq., 58.0 ml, 0.42 mol) and the experiment was completed as described in **4.4**. Column chromatography yielded **13** as a white solid being an anomeric mixture (4.79 g, 59%).



R_f = 0.29 (3:7 EtOAc/Hex); **$[\alpha_D]^{20}_{589}$** +420.7 (c 0.375, $CHCl_3$) (lit +149 (c 0.375, $CHCl_3$))[68]; **m.p.** 114 – 115 °C (lit m.p. 113 °C)[68], **IR** (solid): $\nu_{max}(cm^{-1})$: 2961 (C-H), 2915 (C-H), 2153 (C≡N), 1750 (C=O), 1738 (C=O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.41 (0.79H, d, *J* = 3.6 Hz, H-1), 5.81 (0.26H, d, *J* = 8.5 Hz, H-1), 5.55 (0.75H, dd, *J* = 10.6, 9.4 Hz, H-3), 5.40 (0.28H, dd, *J* = 10.5, 9.4 Hz, H-3), 5.91 – 4.95 (1H, m, H-4), 4.38 – 4.25 (1H, m, H-6a), 4.19 – 4.08 (1H, m, H-5), 4.07 (0.55H, d, *J* = 2.3 Hz) and 4.05 – 4.00 (0.69H, m, H-6b), 3.99 (0.34H, d, *J* = 3.6 Hz, H-2), 3.90 (0.23H, ddd, *J* = 10.1, 4.4, 2.1 Hz, H-2), 3.82 (0.24H, dd, *J* = 10.5, 8.5 Hz, H-2), 2.25 (3H, s, CH_3), 2.13 (3H, s, CH_3), 2.10 – 2.06 (3H, m, CH_3), 2.06 – 2.01 (3H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.39, 169.68, 169.44, 169.42, 169.35, 168.37 and 168.29 (C=O), 162.19 and 162.00 (C-7), 91.23 and 88.37 (C-1), 73.00 , 71.98, 70.07, 69.87(C-3, C-4 and C-5) , 67.19 and 61.19 (C-6), 56.10 and 54.79 (C-2), 20.61, 20.49 and 20.41 (CH_3x3).

4.75 Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-4-(3*S*)-2-acetoxy-3-((*tert*-butoxycarbonyl)amino)-4-phenylbutanamido)-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2,3,5-triyl triacetate 68A

N-Boc-L-phenylalaninal **15** (335 mg, 1.34 mmol), glucose isocyanide **13** (400 mg, 1.34 mmol) and acetic acid **1** (91 µl, 1.34 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment proceeded as described in **4.5** yielding compound **68A** (207 mg, 28%) as a white solid as a mixture of at least 3 diastereomers.

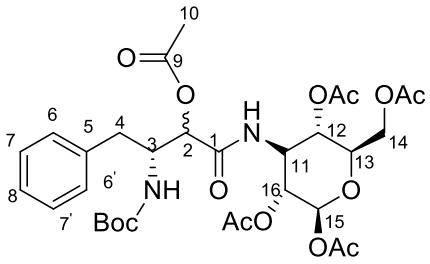


R_f = 0.42 (49:1 $CHCl_3$/MeOH); **m.p.** 202 – 204 °C; **IR** (solid) $\nu_{max}(cm^{-1})$: 3370 (NH), 2982 (C-H), 1744 (C=O), 1691 (C=O), 1674 (C=O), 1520 (C=C), 1367 (C-O); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.32 – 7.24 (2H, m, Ar-H), 7.24 – 7.10 (3H, m, Ar-H), 6.60 – 6.44 (0.5H, m, NH), 6.44 – 6.12 (1.5H, m, NH and H-15), 5.32 – 4.70 (4H, m, NH, H-2, H-12 and H-16), 4.48 – 4.15 (3H, m, H-3, H-11 and H-14a), 4.15 – 3.93 (2H, m, H-13 and H-14b), 3.00 – 2.60 (2H, m, H-4), 2.26 – 1.97 (15H, m, H-10 and CH_3x4), 1.33 (9H, s, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.20 (C=O)), 170.63, 170.61, 169.68, 169.38, 169.25, 169.08, 169.06, 169.02, 168.62, 168.37, 167.99 and 167.91 (C=O), 155.49 and 154.98 (Boc C=O), 137.01 (C-5), 136.96 (C-5), 129.25 (Ar-C), 129.19 (Ar-C), 129.13 (Ar-C), 129.10

(Ar-C), 128.59 (Ar-C), 128.55 (Ar-C), 128.48 (Ar-C), 126.75 (Ar-C), 126.70 (Ar-C), 126.65 (Ar-C), 92.24 (C-15), 90.09 (C-15), 89.84 (C-15), 79.77 [$(CH_3)_3\underline{C}$], 74.36 (C-2), 73.81 (C-2), 72.96 (C-O), 72.87 (C-O), 71.84 (C-O), 70.33 (C-O), 70.13 (C-O), 69.90 (C-O), 69.77 (C-O), 67.25 (C-O), 61.55 (C-O), 53.00, 52.66, 52.06 and 51.49 (C-3 and C-11), 37.50 (C-4), 36.87 (C-4), 28.22 [$(\underline{C}H_3)_3C$], 20.98 (CH_3), 20.71 (CH_3), 20.68 (CH_3), 20.66 (CH_3), 20.54 (CH_3), 20.50 (CH_3); **HRMS** (m/*z*), calculated for $C_{31}H_{43}N_2O_{14}$: 667.2714, found $(M + H)^+$: 667.2714.

4.76 Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-4-((3*R*)-2-acetoxy-3-((*tert*-butoxycarbonyl) amino)-4-phenylbutanamido)-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2,3,5-triyl triacetate 68B

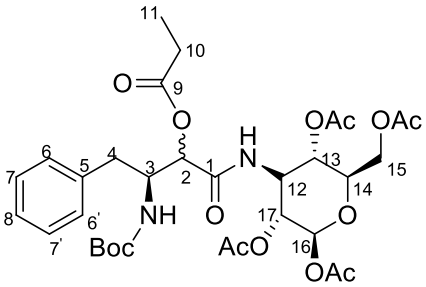
N-Boc-D-phenylalaninal **16** (335 mg, 1.34 mmol), glucose isocyanide **13** (400 mg, 1.34 mmol) and acetic acid **1** (91 µl, 1.34 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment proceeded as described in **4.5** yielding compound **68B** (900 mg, 100%) as a white precipitate.



R_f = 0.59 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.00:1.28; **m.p.** 209 – 210 °C; **IR** (solid) $\nu_{max}(cm^{-1})$: 3365 (NH), 2982 (C-H), 1741 (C=O), 1688 (C=O), 1676 (C=O), 1518 (C=C), 1366 (C-O), 702; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.40 – 7.08 (5H, m, Ar-H), 6.94 – 6.83 and 6.51 – 6.34 (1H, m, NH), 6.31 – 6.09 (1H, m, H-15), 5.40 – 4.82 (4H, m, H-2, H-12, H-16 and NH), 4.51 – 3.92 (5H, m, H-3, H-11, H-13 and H-14), 3.19 – 2.61 (2H, m, H-4), 2.28 – 1.91 (15H, m, H-10 and CH_3x4), 1.55 – 1.16 (9H, m, $(CH_3)_3C$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.45, 170.65, 170.63, 169.44, 169.15, 169.06, 168.67, 168.52, 168.42 and 168.01 (C=O), 155.53, 155.41 and 155.01 (Boc C=O), 136.85 and 135.97 (C-5), 129.30 (Ar-C), 129.24 (Ar-C), 129.14 (Ar-C), 129.07 (Ar-C), 128.70 (Ar-C), 128.57 (Ar-C), 128.53 (Ar-C), 128.50 (Ar-C), 128.45 (Ar-C), 127.00 (Ar-C), 126.73 (Ar-C), 126.70 (Ar-C), 126.66 (Ar-C), 90.27 (C-15), 89.84 (C-15), 80.09 [$(CH_3)_3\underline{C}$], 79.79 ($(CH_3)_3\underline{C}$), 74.51 (C-2), 73.86 (C-2), 70.27 (C-O), 70.05 (C-O), 69.85 (C-O), 69.74 (C-O), 69.69 (C-O), 67.55 (C-O), 67.20 (C-O), 61.58 (C-14), 60.79 (C-O), 52.68 and 51.88 (C-3), 35.32 (C-4), 28.26 [$(\underline{C}H_3)_3C$], 28.20 [$(\underline{C}H_3)_3C$], 20.67 (CH_3), 20.64 (CH_3), 20.62 (CH_3), 20.60 (CH_3), 20.51 (CH_3); **HRMS** (m/*z*), calculated for $C_{31}H_{43}N_2O_{14}$: 667.2714, found $(M + H)^+$: 667.2714.

4.77 Synthesis of (2S,3R,4R,5S,6R)-6-(acetoxymethyl)-4-((3S)-3-((tert-butoxy carbonyl)amino)-4-phenyl-2-(propionyloxy)butanamido)tetrahydro-2H-pyran-2,3,5-triyl triacetate 69

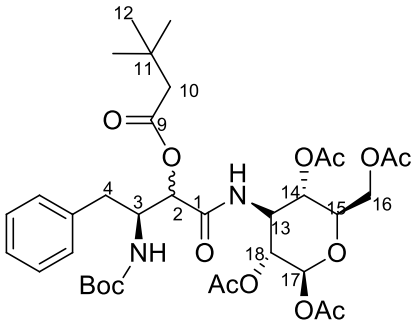
N-Boc-L-phenylalaninal **15** (250 mg, 1.00 mmol), glucose isocyanide **13** (357 mg, 1.00 mmol) and propionic acid **2** (83 µl, 1.00 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment proceeded as described in **4.5** yielding compound **69** (680 mg, 99%) as a white solid.



R_f = 0.42 (49:1 $CHCl_3$/MeOH); **d.r.** = 2.04:1.00; **m.p.** 181-182°C; **IR** ν_{max}(cm^{-1}): 3370 (NH), 2982 (C-H), 1748 (C=O), 1688 (C=O), 1673 (C=O), 1521 (C=C), 1367 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.37 – 7.09 (5H, m, Ar-H), 6.62 – 6.12 (2H, m, NH and H-16), 5.39 – 4.61 (4H, m, NH, H-2 H-13 and H-17), 4.53 – 3.94 (5H, m, H-3 H-12, H-14 and H-15), 3.01 – 2.60 (2H, m, H-4), 2.60 – 2.31 (2H, m, H-10), 2.24 – 1.91 (12H, m, CH_3x4), 1.33 (9H, s, $(CH_3)_3C$), 1.25 – 1.03 (3H, m, H-11); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 173.01, 172.82, 170.61, 169.12, 169.07, 168.56 and 168.09 (C=O), 155.46 and 154.98 (Boc C=O), 137.02 (C-5), 129.21 (Ar-C), 129.16 (Ar-C), 128.58 (Ar-C), 128.48 (Ar-C), 126.75 (Ar-C), 126.65 (Ar-C), 90.13 (C-16), 79.93 [$(CH_3)_3\underline{C}$], 74.21, 73.67 and 73.00 (C-2), 71.89 (C-O), 70.35 (C-O), 70.18 (C-O), 69.92 (C-O), 69.79 (C-O), 67.35 (C-O), 61.59 (C-15), 53.03, 51.89 and 51.56 (C-3 and C-9), 36.97 (C-4), 28.22 [$(\underline{C}H_3)_3C$], 27.28 (C-10), 27.23 (C-10), 20.93 (CH_3), 20.67 (CH_3), 20.64 (CH_3), 20.52 (CH_3), 8.97 (C-11), 8.66 (C-11); **HRMS** (m/*z*), calculated for $C_{32}H_{45}N_2O_{14}$: 681.2871, found $(M + H)^+$: 681.2874.

4.78 Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-6-(acetoxymethyl)-4-((3*S*)-3-((*tert*-butoxy carbonyl)amino)-2-((3,3-dimethylbutanoyl)oxy)-4-phenylbutanamido) tetrahydro-2*H*-pyran-2,3,5-triyl triacetate 70

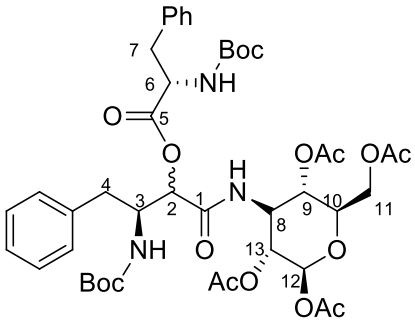
N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), glucose isocyanide **13** (429 mg, 1.20 mmol) and *tert*-butyl acetic acid **5** (153 µl, 1.20 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment proceeded as described in **4.5** yielding compound **70** (427 mg, 50%) as a white solid as a mixture of at least 3 diastereomers.



R_f = 0.55 (49:1 $CHCl_3$/MeOH); **m.p** 186 – 187 °C; **IR** v_{max}(cm^{-1}): 3371 (NH), 2967 (C-H), 1743 (C=O), 1689 (C=O), 1518 (C=C), 1321 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.46 – 7.10 (5H, m, Ar-H), 6.65 – 6.16 (2H, m, NH and H-17), 5.60 – 4.73 (4H, m, NH, H-2, H-14 and H-18), 4.59 – 3.78 (5H, m, H-3, H-13, H-15 and H-16), 3.00 – 2.51 (2H, m, H-4), 2.47 – 1.88 (14H, m, H-10 and CH_3x4), 1.56 – 1.19 (9H, m, $(CH_3)_3C$), 1.19 – 0.81 (9H, m, H-12); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 175.88, 170.90, 170.65, 170.64, 169.29, 169.10, 168.65 and 168.18 (C=O), 155.35 and 155.09 (Boc C=O), 137.08 (C-5), 129.32 Ar C, 129.22 (Ar-C), 129.15 (Ar-C), 128.54 (Ar-C), 128.47 (Ar-C), 126.70 (Ar-C), 126.42 (Ar-C), 90.11 (C-17), 79.85 and 79.64 [$(CH_3)_3\underline{C}$], 73.44 and 72.87 (C-2), 70.27 (C-O), 69.73 (C-O), 67.98 (C-O), 67.37 (C-O), 61.56 (C-16), 52.99 and 51.57(C-3 and C-13), 47.68 (C-10), 47.52 (C-10), 47.28 (C-10), 37.42 (C-4), 30.89 (C-11), 30.72 (C-11), 30.52 (C-11), 29.66 (C-12), 29.58 (C-12), 28.35 [$(\underline{C}H_3)_3C$], 28.19 ($(\underline{C}H_3)_3C$), 20.80 (CH_3), 20.67 (CH_3), 20.65 (CH_3), 20.52 (CH_3); **HRMS** (m/*z*), calculated for $C_{35}H_{51}N_2O_{14}$: 723.3340, found (M + H^+): 723.3340.

4.79 Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-6-(acetoxymethyl)-4-((3*S*)-3-((*tert*-Butoxy carbonyl)amino)-2-(((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanoyl) oxy)-4-phenylbutanamido)tetrahydro-2*H*-pyran-2,3,5-triyl triacetate 71

N-Boc-L-phenylalaninal **15** (300 mg, 1.20 mmol), glucose isocyanide **13** (429 mg, 1.20 mmol) and *N*-Boc-L-phenylalanine **7** (318 mg, 1.20 mmol) were dissolved in CH_2Cl_2 (5 ml). The experiment was carried out as described in **4.5** yielding compound **71** (743 mg, 71%) as a white solid as a mixture of at least 3 diastereomers.



R_f = 0.42 (49:1 $CHCl_3$/MeOH); **d.r.** = 1.77:1.00:1.00; **m.p.** 202 – 203 °C; **IR** v_{max}(cm^{-1}): 3371 (NH), 2967 (C-H), 1743 (C=O), 1689 (C=O), 1518 (C=C), 1366 (C-O); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.54 – 6.87 (10H, m, Ar-H), 6.88 – 6.60 (1H, m, NH), 6.44 – 6.18 (1H, m, H-12), 5.58 – 4.72 (5H, m, NHx2 , H-2 H-9and H-13), 4.70 – 3.45 (6H, m, H-3, H-6, H-8, H-10 and H-11), 3.34 – 3.09 and 3.09 – 2.27 (4H, 2xm, H-4 and H-7), 2.24 – 1.79 (12H, m, CH_3x4), 1.63 – 1.13 (18H, m,

$(CH_3)_3Cx2$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.91, 170.61, 169.38, 169.22, 169.18, 168.70 and 167.86 (C=O), 155.59 (Boc C=O), 154.72 (Boc C=O), 136.92 (Ar-C), 135.58 (Ar-C), 129.34 (Ar-C), 129.26 (Ar-C), 129.19 (Ar-C), 129.14 (Ar-C), 129.01 (Ar-C), 128.88 (Ar-C), 128.76 (Ar-C), 128.45 (Ar-C), 127.44 (Ar-C), 127.15 (Ar-C), 126.64 (Ar-C), 126.38 (Ar-C), 91.92 and 90.08 (C-12), 80.63 [$(CH_3)_3\underline{C}$], 80.24 [$(CH_3)_3\underline{C}$], 79.78 [$(\underline{C}H_3)_3C$], 72.64 (C-O), 70.52 (C-O), 70.25 (C-O), 69.68 (C-O), 69.48 (C-O), 68.35 (C-O), 67.72 (C-O), 61.60 (C-11), 52.70, 52.30, 51.73 and 51.52 (C-3 and C-8), 37.73 (C-4), 28.51 and 28.36 [$(\underline{C}H_3)_3C$], 20.94 (CH_3), 20.76 (CH_3), 20.73 (CH_3), 20.72 (CH_3), 20.65 (CH_3), 20.57 (CH_3), 20.54 (CH_3); **HRMS** (m/*z*), calculated for $C_{43}H_{58}N_3O_{16}$: 872.3817, found $(M + H)^+$: 872.3842.

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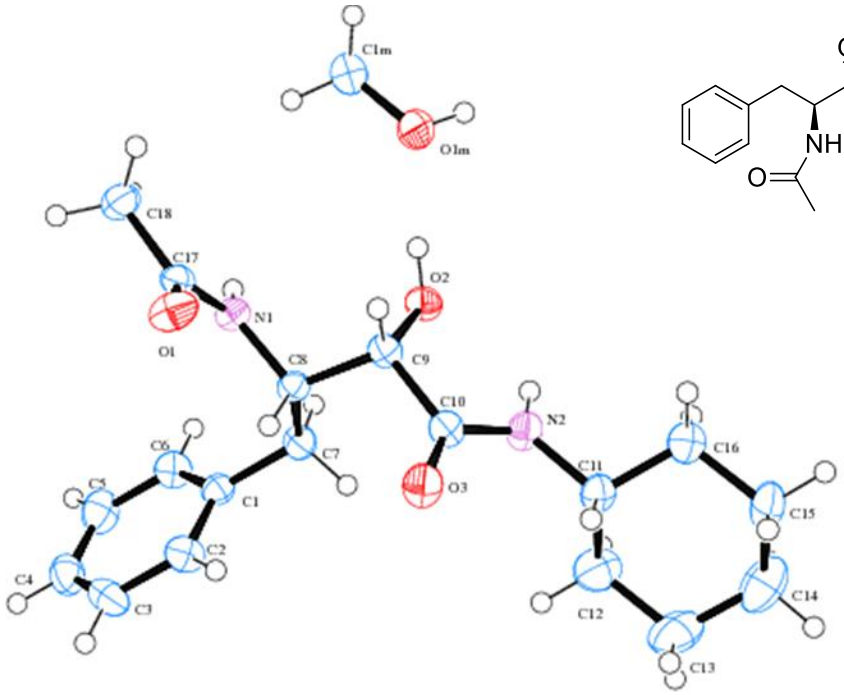
APPENDIX I-CRYSTALLOGRAPHIC DATA

1. X-ray crystallography data for (2*S*,3*S*)-3-acetamido-*N*-cyclohexyl-2-hydroxy-4-phenylbutanamide 18B



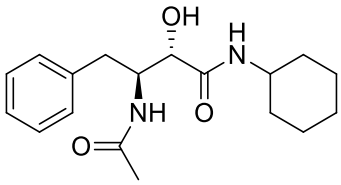


Table 1. Crystal data and structure refinement.

Identification code	14a_mzz113b_p	
Empirical formula	C19 H30 N2 O4	
Formula weight	350.45	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.4416(4) Å	a= 90°.
	b = 13.1342(11) Å	b= 90°.
	c = 23.4165(18) Å	g = 90°.
Volume	1981.2(3) $Å^3$	
Z	4	
Density (calculated)	1.175 Mg/m^3	

Absorption coefficient	0.082 mm^{-1}
F(000)	760
Crystal size	0.61 x 0.12 x 0.07 mm^3
Theta range for data collection	3.28 to 28.00°.
Index ranges	-8<=h<=8, -15<=k<=17, -30<=l<=26
Reflections collected	14036
Independent reflections	2750 [R(int) = 0.0434]
Completeness to theta = 28.00°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9943 and 0.9516
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2750 / 0 / 241
Goodness-of-fit on F^2	1.029
Final R indices [I>2sigma(I)]	R1 = 0.0428, wR2 = 0.0914
R indices (all data)	R1 = 0.0622, wR2 = 0.0988
Absolute structure parameter	10(10)
Largest diff. peak and hole	0.216 and -0.168 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3). U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	14716(3)	5476(2)	167(1)	27(1)
C(2)	13207(3)	6139(2)	-38(1)	34(1)
C(3)	13646(4)	6818(2)	-474(1)	40(1)
C(4)	15607(4)	6843(2)	-714(1)	41(1)
C(5)	17119(4)	6193(2)	-514(1)	42(1)
C(6)	16685(3)	5515(2)	-75(1)	34(1)
C(7)	14217(3)	4753(1)	648(1)	28(1)
C(8)	13679(3)	5304(1)	1205(1)	23(1)
C(9)	12794(3)	4576(2)	1656(1)	26(1)
C(10)	10703(3)	4166(2)	1458(1)	27(1)
C(11)	8580(3)	2607(2)	1363(1)	29(1)
C(12)	8452(4)	2190(2)	759(1)	49(1)
C(13)	6394(4)	1627(2)	677(1)	59(1)
C(14)	6122(5)	786(2)	1107(1)	62(1)
C(15)	6294(4)	1198(2)	1713(1)	49(1)
C(16)	8328(4)	1772(2)	1802(1)	42(1)
C(17)	15415(3)	6705(2)	1698(1)	26(1)
C(18)	17427(3)	7125(2)	1922(1)	36(1)
N(1)	15512(2)	5814(1)	1426(1)	25(1)
N(2)	10513(3)	3155(1)	1469(1)	32(1)
O(1)	13750(2)	7160(1)	1768(1)	38(1)
O(2)	14177(2)	3762(1)	1769(1)	31(1)
O(3)	9329(2)	4750(1)	1305(1)	38(1)
C(1M)	18140(4)	4470(2)	2833(1)	48(1)
O(1M)	16225(3)	3982(1)	2743(1)	48(1)

Table 3. Bond lengths [Å] and angles [°].

C(1)-C(6)	1.391(3)
C(1)-C(2)	1.391(3)
C(1)-C(7)	1.506(3)
C(2)-C(3)	1.384(3)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.384(3)
C(3)-H(3)	0.9500
C(4)-C(5)	1.378(3)
C(4)-H(4)	0.9500
C(5)-C(6)	1.387(3)
C(5)-H(5)	0.9500
C(6)-H(6)	0.9500
C(7)-C(8)	1.532(2)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(1)	1.453(2)
C(8)-C(9)	1.535(3)
C(8)-H(8)	1.0000
C(9)-O(2)	1.416(2)
C(9)-C(10)	1.523(3)
C(9)-H(9)	1.0000
C(10)-O(3)	1.225(2)
C(10)-N(2)	1.334(3)
C(11)-N(2)	1.459(2)
C(11)-C(16)	1.511(3)
C(11)-C(12)	1.520(3)
C(11)-H(11)	1.0000
C(12)-C(13)	1.530(3)
C(12)-H(12A)	0.9900
C(12)-H(12B)	0.9900
C(13)-C(14)	1.503(4)
C(13)-H(13A)	0.9900
C(13)-H(13B)	0.9900

C(14)-C(15)	1.524(4)
C(14)-H(14A)	0.9900
C(14)-H(14B)	0.9900
C(15)-C(16)	1.525(3)
C(15)-H(15A)	0.9900
C(15)-H(15B)	0.9900
C(16)-H(16A)	0.9900
C(16)-H(16B)	0.9900
C(17)-O(1)	1.239(2)
C(17)-N(1)	1.333(2)
C(17)-C(18)	1.503(3)
C(18)-H(18A)	0.9800
C(18)-H(18B)	0.9800
C(18)-H(18C)	0.9800
N(1)-H(1)	0.84(2)
N(2)-H(2)	0.84(2)
O(2)-H(2B)	0.86(3)
C(1M)-O(1M)	1.406(3)
C(1M)-H(1M1)	0.9800
C(1M)-H(1M2)	0.9800
C(1M)-H(1M3)	0.9800
O(1M)-H(1M)	0.8400

C(6)-C(1)-C(2)	118.20(18)
C(6)-C(1)-C(7)	121.55(18)
C(2)-C(1)-C(7)	120.24(17)
C(3)-C(2)-C(1)	121.02(19)
C(3)-C(2)-H(2A)	119.5
C(1)-C(2)-H(2A)	119.5
C(4)-C(3)-C(2)	120.1(2)
C(4)-C(3)-H(3)	119.9
C(2)-C(3)-H(3)	119.9
C(5)-C(4)-C(3)	119.5(2)
C(5)-C(4)-H(4)	120.3
C(3)-C(4)-H(4)	120.3
C(4)-C(5)-C(6)	120.5(2)

C(4)-C(5)-H(5)	119.8
C(6)-C(5)-H(5)	119.8
C(5)-C(6)-C(1)	120.7(2)
C(5)-C(6)-H(6)	119.7
C(1)-C(6)-H(6)	119.7
C(1)-C(7)-C(8)	112.70(15)
C(1)-C(7)-H(7A)	109.1
C(8)-C(7)-H(7A)	109.1
C(1)-C(7)-H(7B)	109.1
C(8)-C(7)-H(7B)	109.1
H(7A)-C(7)-H(7B)	107.8
N(1)-C(8)-C(7)	109.68(14)
N(1)-C(8)-C(9)	110.15(14)
C(7)-C(8)-C(9)	112.02(15)
N(1)-C(8)-H(8)	108.3
C(7)-C(8)-H(8)	108.3
C(9)-C(8)-H(8)	108.3
O(2)-C(9)-C(10)	110.29(15)
O(2)-C(9)-C(8)	111.44(14)
C(10)-C(9)-C(8)	109.85(15)
O(2)-C(9)-H(9)	108.4
C(10)-C(9)-H(9)	108.4
C(8)-C(9)-H(9)	108.4
O(3)-C(10)-N(2)	124.31(18)
O(3)-C(10)-C(9)	120.41(17)
N(2)-C(10)-C(9)	115.28(16)
N(2)-C(11)-C(16)	109.54(17)
N(2)-C(11)-C(12)	112.48(17)
C(16)-C(11)-C(12)	111.39(19)
N(2)-C(11)-H(11)	107.7
C(16)-C(11)-H(11)	107.7
C(12)-C(11)-H(11)	107.7
C(11)-C(12)-C(13)	109.72(18)
C(11)-C(12)-H(12A)	109.7
C(13)-C(12)-H(12A)	109.7
C(11)-C(12)-H(12B)	109.7

C(13)-C(12)-H(12B)	109.7
H(12A)-C(12)-H(12B)	108.2
C(14)-C(13)-C(12)	111.9(2)
C(14)-C(13)-H(13A)	109.2
C(12)-C(13)-H(13A)	109.2
C(14)-C(13)-H(13B)	109.2
C(12)-C(13)-H(13B)	109.2
H(13A)-C(13)-H(13B)	107.9
C(13)-C(14)-C(15)	110.7(2)
C(13)-C(14)-H(14A)	109.5
C(15)-C(14)-H(14A)	109.5
C(13)-C(14)-H(14B)	109.5
C(15)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	108.1
C(14)-C(15)-C(16)	111.4(2)
C(14)-C(15)-H(15A)	109.3
C(16)-C(15)-H(15A)	109.3
C(14)-C(15)-H(15B)	109.3
C(16)-C(15)-H(15B)	109.3
H(15A)-C(15)-H(15B)	108.0
C(11)-C(16)-C(15)	111.02(18)
C(11)-C(16)-H(16A)	109.4
C(15)-C(16)-H(16A)	109.4
C(11)-C(16)-H(16B)	109.4
C(15)-C(16)-H(16B)	109.4
H(16A)-C(16)-H(16B)	108.0
O(1)-C(17)-N(1)	121.88(17)
O(1)-C(17)-C(18)	121.49(17)
N(1)-C(17)-C(18)	116.62(17)
C(17)-C(18)-H(18A)	109.5
C(17)-C(18)-H(18B)	109.5
H(18A)-C(18)-H(18B)	109.5
C(17)-C(18)-H(18C)	109.5
H(18A)-C(18)-H(18C)	109.5
H(18B)-C(18)-H(18C)	109.5
C(17)-N(1)-C(8)	122.42(16)

C(17)-N(1)-H(1)	117.6(14)
C(8)-N(1)-H(1)	119.9(14)
C(10)-N(2)-C(11)	124.45(17)
C(10)-N(2)-H(2)	114.4(16)
C(11)-N(2)-H(2)	121.0(16)
C(9)-O(2)-H(2B)	110.0(17)
O(1M)-C(1M)-H(1M1)	109.5
O(1M)-C(1M)-H(1M2)	109.5
H(1M1)-C(1M)-H(1M2)	109.5
O(1M)-C(1M)-H(1M3)	109.5
H(1M1)-C(1M)-H(1M3)	109.5
H(1M2)-C(1M)-H(1M3)	109.5
C(1M)-O(1M)-H(1M)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$). The anisotropic displacement factor exponent takes the form: $-2p^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	32(1)	26(1)	22(1)	-4(1)	-2(1)	-1(1)
C(2)	31(1)	40(1)	30(1)	1(1)	2(1)	4(1)
C(3)	50(1)	39(1)	31(1)	6(1)	-4(1)	8(1)
C(4)	56(1)	37(1)	30(1)	2(1)	4(1)	-10(1)
C(5)	40(1)	45(1)	39(1)	-2(1)	12(1)	-7(1)
C(6)	32(1)	35(1)	35(1)	-1(1)	2(1)	3(1)
C(7)	32(1)	24(1)	27(1)	-3(1)	0(1)	1(1)
C(8)	21(1)	23(1)	26(1)	-1(1)	-1(1)	2(1)
C(9)	23(1)	27(1)	28(1)	2(1)	1(1)	4(1)
C(10)	21(1)	31(1)	29(1)	3(1)	4(1)	4(1)
C(11)	23(1)	30(1)	35(1)	1(1)	3(1)	2(1)
C(12)	42(1)	70(2)	35(1)	-6(1)	6(1)	-5(1)
C(13)	55(2)	85(2)	37(1)	-22(1)	-2(1)	-13(2)
C(14)	63(2)	46(2)	77(2)	-23(1)	0(2)	-16(1)
C(15)	55(1)	37(1)	54(2)	4(1)	-1(1)	-20(1)
C(16)	45(1)	36(1)	44(1)	4(1)	-6(1)	-7(1)
C(17)	29(1)	28(1)	22(1)	2(1)	-3(1)	1(1)
C(18)	33(1)	41(1)	34(1)	-1(1)	-5(1)	-8(1)
N(1)	19(1)	26(1)	30(1)	-2(1)	0(1)	4(1)
N(2)	21(1)	30(1)	47(1)	4(1)	-1(1)	3(1)
O(1)	34(1)	36(1)	45(1)	-16(1)	-8(1)	10(1)
O(2)	24(1)	31(1)	37(1)	5(1)	-5(1)	6(1)
O(3)	22(1)	33(1)	58(1)	7(1)	-1(1)	6(1)
C(1M)	55(1)	44(1)	44(1)	11(1)	-7(1)	-13(1)
O(1M)	59(1)	31(1)	55(1)	12(1)	-23(1)	-7(1)

Table 5. Torsion angles [°].

C(6)-C(1)-C(2)-C(3)	0.3(3)
C(7)-C(1)-C(2)-C(3)	179.07(18)
C(1)-C(2)-C(3)-C(4)	0.3(3)
C(2)-C(3)-C(4)-C(5)	-0.6(3)
C(3)-C(4)-C(5)-C(6)	0.2(3)
C(4)-C(5)-C(6)-C(1)	0.3(3)
C(2)-C(1)-C(6)-C(5)	-0.6(3)
C(7)-C(1)-C(6)-C(5)	-179.39(18)
C(6)-C(1)-C(7)-C(8)	115.2(2)
C(2)-C(1)-C(7)-C(8)	-63.6(2)
C(1)-C(7)-C(8)-N(1)	-67.79(19)
C(1)-C(7)-C(8)-C(9)	169.58(15)
N(1)-C(8)-C(9)-O(2)	-65.96(19)
C(7)-C(8)-C(9)-O(2)	56.41(19)
N(1)-C(8)-C(9)-C(10)	171.50(14)
C(7)-C(8)-C(9)-C(10)	-66.13(18)
O(2)-C(9)-C(10)-O(3)	-175.49(17)
C(8)-C(9)-C(10)-O(3)	-52.3(2)
O(2)-C(9)-C(10)-N(2)	4.7(2)
C(8)-C(9)-C(10)-N(2)	127.96(18)
N(2)-C(11)-C(12)-C(13)	-180.0(2)
C(16)-C(11)-C(12)-C(13)	56.6(3)
C(11)-C(12)-C(13)-C(14)	-56.9(3)
C(12)-C(13)-C(14)-C(15)	56.2(3)
C(13)-C(14)-C(15)-C(16)	-54.9(3)
N(2)-C(11)-C(16)-C(15)	178.62(18)
C(12)-C(11)-C(16)-C(15)	-56.3(2)
C(14)-C(15)-C(16)-C(11)	55.0(3)
O(1)-C(17)-N(1)-C(8)	-1.5(3)
C(18)-C(17)-N(1)-C(8)	177.80(17)
C(7)-C(8)-N(1)-C(17)	145.11(17)
C(9)-C(8)-N(1)-C(17)	-91.2(2)
O(3)-C(10)-N(2)-C(11)	-7.3(3)
C(9)-C(10)-N(2)-C(11)	172.47(17)

C(16)-C(11)-N(2)-C(10)	-135.7(2)
C(12)-C(11)-N(2)-C(10)	99.8(2)

Symmetry transformations used to generate equivalent atoms:

Table 6. Hydrogen bonds [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1)...O(3)#1	0.84(2)	2.00(2)	2.843(2)	175(2)
N(2)-H(2)...O(2)	0.84(2)	2.11(2)	2.588(2)	116.0(19)
O(2)-H(2B)...O(1M)	0.86(3)	1.79(3)	2.650(2)	174(2)
O(1M)-H(1M)...O(1)#2	0.84	1.82	2.653(2)	174.9

Symmetry transformations used to generate equivalent atoms:

#1 x+1,y,z #2 -x+3,y-1/2,-z+1/2

2. X-ray crystallographic data for (2*R*,3*S*)-*N*-adamantanyl-3-butyramido-2-hydroxy-4-phenylbutanamide 55A

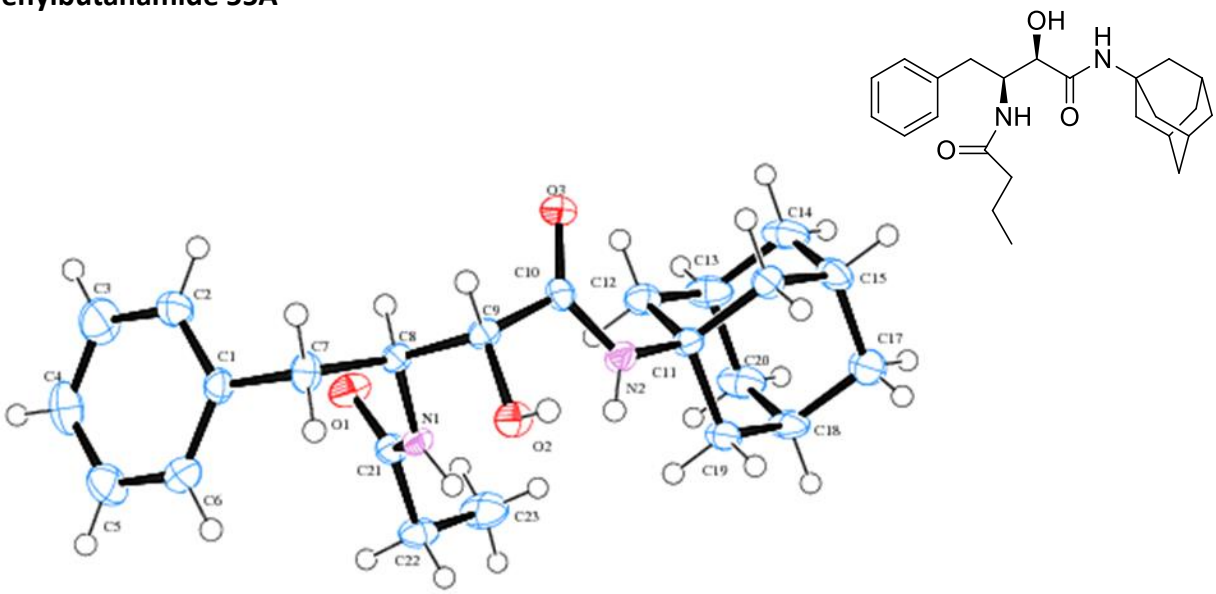


Table 1. Crystal data and structure refinement.

Identification code	14a_mzz155a_p	
Empirical formula	C23 H32 N2 O3	
Formula weight	384.51	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 7.3110(4) Å	□= 90°.
	b = 10.8229(6) Å	□= 90°.
	c = 26.0551(15) Å	□ = 90°.
Volume	2061.6(2) $Å^3$	
Z	4	
Density (calculated)	1.239 Mg/m^3	
Absorption coefficient	0.082 mm^{-1}	
F(000)	832	
Crystal size	0.47 x 0.11 x 0.09 mm^3	

Theta range for data collection	3.36 to 28.00°.
Index ranges	-9<=h<=7, -13<=k<=14, -34<=l<=28
Reflections collected	13871
Independent reflections	2842 [R(int) = 0.0375]
Completeness to theta = 28.00°	99.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9927 and 0.9626
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2842 / 0 / 266
Goodness-of-fit on F^2	1.069
Final R indices [I>2sigma(I)]	R1 = 0.0507, wR2 = 0.1307
R indices (all data)	R1 = 0.0571, wR2 = 0.1343
Absolute structure parameter	10(10)
Largest diff. peak and hole	0.326 and -0.204 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3). U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	1897(4)	2279(3)	4141(1)	23(1)
C(2)	916(5)	3350(3)	4214(1)	34(1)
C(3)	-815(5)	3326(4)	4434(1)	44(1)
C(4)	-1566(5)	2220(4)	4592(1)	40(1)
C(5)	-592(5)	1144(3)	4521(1)	42(1)
C(6)	1137(5)	1180(3)	4302(1)	35(1)
C(7)	3763(4)	2323(3)	3892(1)	25(1)
C(8)	3665(4)	2401(2)	3302(1)	18(1)
C(9)	5578(4)	2582(2)	3074(1)	19(1)
C(10)	5386(4)	3015(2)	2514(1)	21(1)
C(11)	5093(4)	2240(2)	1609(1)	21(1)
C(12)	3284(4)	2912(3)	1511(1)	28(1)
C(13)	2895(5)	2953(3)	934(1)	36(1)
C(14)	4464(6)	3633(3)	662(1)	41(1)
C(15)	6265(5)	2960(3)	760(1)	36(1)
C(16)	6645(4)	2919(3)	1339(1)	26(1)
C(17)	6113(6)	1648(3)	546(1)	39(1)
C(18)	4569(5)	960(3)	816(1)	33(1)
C(19)	4970(4)	921(2)	1395(1)	26(1)
C(20)	2771(5)	1625(3)	728(1)	38(1)
C(21)	1094(4)	1207(3)	2949(1)	22(1)
C(22)	464(4)	-1(3)	2715(1)	28(1)
C(23)	-25(6)	173(3)	2154(1)	43(1)
N(1)	2865(3)	1284(2)	3083(1)	19(1)
N(2)	5463(3)	2118(2)	2165(1)	23(1)
O(1)	25(3)	2076(2)	3007(1)	29(1)
O(2)	6520(3)	1456(2)	3123(1)	23(1)
O(3)	5161(3)	4117(2)	2423(1)	27(1)

Table 3. Bond lengths [Å] and angles [°].

C(1)-C(2)	1.376(4)
C(1)-C(6)	1.378(4)
C(1)-C(7)	1.511(4)
C(2)-C(3)	1.390(5)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.380(5)
C(3)-H(3)	0.9500
C(4)-C(5)	1.377(5)
C(4)-H(4)	0.9500
C(5)-C(6)	1.387(5)
C(5)-H(5)	0.9500
C(6)-H(6)	0.9500
C(7)-C(8)	1.541(3)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(1)	1.460(3)
C(8)-C(9)	1.533(4)
C(8)-H(8)	1.0000
C(9)-O(2)	1.405(3)
C(9)-C(10)	1.538(4)
C(9)-H(9)	1.0000
C(10)-O(3)	1.228(3)
C(10)-N(2)	1.332(3)
C(11)-N(2)	1.478(3)
C(11)-C(16)	1.524(4)
C(11)-C(12)	1.530(4)
C(11)-C(19)	1.535(3)
C(12)-C(13)	1.533(4)
C(12)-H(12A)	0.9900
C(12)-H(12B)	0.9900
C(13)-C(14)	1.536(5)
C(13)-C(20)	1.536(4)
C(13)-H(13)	1.0000
C(14)-C(15)	1.526(5)

C(14)-H(14A)	0.9900
C(14)-H(14B)	0.9900
C(15)-C(17)	1.530(4)
C(15)-C(16)	1.536(4)
C(15)-H(15)	1.0000
C(16)-H(16A)	0.9900
C(16)-H(16B)	0.9900
C(17)-C(18)	1.524(5)
C(17)-H(17A)	0.9900
C(17)-H(17B)	0.9900
C(18)-C(20)	1.516(5)
C(18)-C(19)	1.537(4)
C(18)-H(18)	1.0000
C(19)-H(19A)	0.9900
C(19)-H(19B)	0.9900
C(20)-H(20A)	0.9900
C(20)-H(20B)	0.9900
C(21)-O(1)	1.231(3)
C(21)-N(1)	1.343(3)
C(21)-C(22)	1.515(4)
C(22)-C(23)	1.518(4)
C(22)-H(22A)	0.9900
C(22)-H(22B)	0.9900
C(23)-H(23A)	0.9800
C(23)-H(23B)	0.9800
C(23)-H(23C)	0.9800
N(1)-H(1)	0.86(3)
N(2)-H(2)	0.84(4)
O(2)-H(2B)	0.77(4)

C(2)-C(1)-C(6)	118.3(3)
C(2)-C(1)-C(7)	120.2(3)
C(6)-C(1)-C(7)	121.4(3)
C(1)-C(2)-C(3)	121.0(3)
C(1)-C(2)-H(2A)	119.5
C(3)-C(2)-H(2A)	119.5

C(4)-C(3)-C(2)	120.1(3)
C(4)-C(3)-H(3)	119.9
C(2)-C(3)-H(3)	119.9
C(5)-C(4)-C(3)	119.2(3)
C(5)-C(4)-H(4)	120.4
C(3)-C(4)-H(4)	120.4
C(4)-C(5)-C(6)	120.2(3)
C(4)-C(5)-H(5)	119.9
C(6)-C(5)-H(5)	119.9
C(1)-C(6)-C(5)	121.1(3)
C(1)-C(6)-H(6)	119.4
C(5)-C(6)-H(6)	119.4
C(1)-C(7)-C(8)	112.8(2)
C(1)-C(7)-H(7A)	109.0
C(8)-C(7)-H(7A)	109.0
C(1)-C(7)-H(7B)	109.0
C(8)-C(7)-H(7B)	109.0
H(7A)-C(7)-H(7B)	107.8
N(1)-C(8)-C(9)	108.6(2)
N(1)-C(8)-C(7)	111.3(2)
C(9)-C(8)-C(7)	110.6(2)
N(1)-C(8)-H(8)	108.8
C(9)-C(8)-H(8)	108.8
C(7)-C(8)-H(8)	108.8
O(2)-C(9)-C(8)	107.5(2)
O(2)-C(9)-C(10)	113.3(2)
C(8)-C(9)-C(10)	108.9(2)
O(2)-C(9)-H(9)	109.0
C(8)-C(9)-H(9)	109.0
C(10)-C(9)-H(9)	109.0
O(3)-C(10)-N(2)	125.5(2)
O(3)-C(10)-C(9)	119.5(2)
N(2)-C(10)-C(9)	115.0(2)
N(2)-C(11)-C(16)	111.0(2)
N(2)-C(11)-C(12)	111.3(2)
C(16)-C(11)-C(12)	109.7(2)

N(2)-C(11)-C(19)	106.4(2)
C(16)-C(11)-C(19)	109.0(2)
C(12)-C(11)-C(19)	109.3(2)
C(11)-C(12)-C(13)	109.7(3)
C(11)-C(12)-H(12A)	109.7
C(13)-C(12)-H(12A)	109.7
C(11)-C(12)-H(12B)	109.7
C(13)-C(12)-H(12B)	109.7
H(12A)-C(12)-H(12B)	108.2
C(12)-C(13)-C(14)	109.1(3)
C(12)-C(13)-C(20)	109.0(2)
C(14)-C(13)-C(20)	109.4(3)
C(12)-C(13)-H(13)	109.8
C(14)-C(13)-H(13)	109.8
C(20)-C(13)-H(13)	109.8
C(15)-C(14)-C(13)	109.8(2)
C(15)-C(14)-H(14A)	109.7
C(13)-C(14)-H(14A)	109.7
C(15)-C(14)-H(14B)	109.7
C(13)-C(14)-H(14B)	109.7
H(14A)-C(14)-H(14B)	108.2
C(14)-C(15)-C(17)	108.7(3)
C(14)-C(15)-C(16)	109.5(3)
C(17)-C(15)-C(16)	110.1(3)
C(14)-C(15)-H(15)	109.5
C(17)-C(15)-H(15)	109.5
C(16)-C(15)-H(15)	109.5
C(11)-C(16)-C(15)	109.4(2)
C(11)-C(16)-H(16A)	109.8
C(15)-C(16)-H(16A)	109.8
C(11)-C(16)-H(16B)	109.8
C(15)-C(16)-H(16B)	109.8
H(16A)-C(16)-H(16B)	108.2
C(18)-C(17)-C(15)	109.8(3)
C(18)-C(17)-H(17A)	109.7
C(15)-C(17)-H(17A)	109.7

C(18)-C(17)-H(17B)	109.7
C(15)-C(17)-H(17B)	109.7
H(17A)-C(17)-H(17B)	108.2
C(20)-C(18)-C(17)	109.9(3)
C(20)-C(18)-C(19)	109.1(3)
C(17)-C(18)-C(19)	109.0(3)
C(20)-C(18)-H(18)	109.6
C(17)-C(18)-H(18)	109.6
C(19)-C(18)-H(18)	109.6
C(11)-C(19)-C(18)	110.0(2)
C(11)-C(19)-H(19A)	109.7
C(18)-C(19)-H(19A)	109.7
C(11)-C(19)-H(19B)	109.7
C(18)-C(19)-H(19B)	109.7
H(19A)-C(19)-H(19B)	108.2
C(18)-C(20)-C(13)	109.9(3)
C(18)-C(20)-H(20A)	109.7
C(13)-C(20)-H(20A)	109.7
C(18)-C(20)-H(20B)	109.7
C(13)-C(20)-H(20B)	109.7
H(20A)-C(20)-H(20B)	108.2
O(1)-C(21)-N(1)	122.2(3)
O(1)-C(21)-C(22)	121.0(2)
N(1)-C(21)-C(22)	116.7(3)
C(21)-C(22)-C(23)	110.6(2)
C(21)-C(22)-H(22A)	109.5
C(23)-C(22)-H(22A)	109.5
C(21)-C(22)-H(22B)	109.5
C(23)-C(22)-H(22B)	109.5
H(22A)-C(22)-H(22B)	108.1
C(22)-C(23)-H(23A)	109.5
C(22)-C(23)-H(23B)	109.5
H(23A)-C(23)-H(23B)	109.5
C(22)-C(23)-H(23C)	109.5
H(23A)-C(23)-H(23C)	109.5
H(23B)-C(23)-H(23C)	109.5

C(21)-N(1)-C(8)	122.6(2)
C(21)-N(1)-H(1)	117(2)
C(8)-N(1)-H(1)	120(2)
C(10)-N(2)-C(11)	126.6(2)
C(10)-N(2)-H(2)	113(3)
C(11)-N(2)-H(2)	120(3)
C(9)-O(2)-H(2B)	114(3)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$). The anisotropic displacement factor exponent takes the form: $-2p^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	26(1)	26(1)	17(1)	-1(1)	1(1)	2(1)
C(2)	38(2)	26(2)	38(2)	6(1)	10(1)	5(1)
C(3)	39(2)	45(2)	48(2)	8(2)	12(2)	18(2)
C(4)	26(2)	64(2)	29(2)	2(2)	6(1)	-1(2)
C(5)	43(2)	40(2)	44(2)	1(2)	13(2)	-16(2)
C(6)	40(2)	25(2)	39(2)	-2(1)	10(1)	3(1)
C(7)	24(1)	32(2)	19(1)	-2(1)	0(1)	1(1)
C(8)	16(1)	19(1)	20(1)	-1(1)	0(1)	2(1)
C(9)	18(1)	19(1)	21(1)	-2(1)	-2(1)	1(1)
C(10)	17(1)	22(1)	23(1)	1(1)	4(1)	-2(1)
C(11)	28(1)	17(1)	19(1)	-1(1)	-2(1)	1(1)
C(12)	27(1)	23(1)	33(1)	3(1)	-2(1)	2(1)
C(13)	46(2)	24(2)	37(2)	4(1)	-17(2)	0(2)
C(14)	74(3)	22(1)	26(1)	5(1)	-10(2)	-9(2)
C(15)	60(2)	27(2)	20(1)	-1(1)	7(1)	-14(2)
C(16)	33(2)	24(1)	23(1)	-2(1)	3(1)	-3(1)
C(17)	66(2)	30(2)	21(1)	-5(1)	7(2)	-8(2)
C(18)	55(2)	18(1)	25(1)	-4(1)	-5(1)	-9(1)
C(19)	35(2)	19(1)	24(1)	2(1)	-2(1)	-3(1)
C(20)	54(2)	27(2)	33(2)	2(1)	-18(2)	-8(2)
C(21)	19(1)	21(1)	27(1)	0(1)	1(1)	-2(1)
C(22)	21(1)	21(1)	42(2)	-1(1)	-2(1)	-3(1)
C(23)	55(2)	31(2)	42(2)	-7(1)	-14(2)	-7(2)
N(1)	19(1)	16(1)	23(1)	-2(1)	0(1)	2(1)
N(2)	30(1)	19(1)	19(1)	0(1)	-1(1)	5(1)
O(1)	14(1)	25(1)	49(1)	-6(1)	-2(1)	2(1)
O(2)	14(1)	24(1)	32(1)	1(1)	-1(1)	1(1)
O(3)	34(1)	19(1)	29(1)	2(1)	3(1)	-1(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3).

	x	y	z	U(eq)
H(2A)	1429	4118	4111	41
H(3)	-1481	4072	4477	53
H(4)	-2741	2200	4748	48
H(5)	-1105	375	4622	51
H(6)	1809	435	4263	41
H(7A)	4457	1573	3990	30
H(7B)	4439	3049	4024	30
H(8)	2887	3124	3205	22
H(9)	6237	3231	3275	23
H(12A)	3355	3764	1649	33
H(12B)	2278	2476	1690	33
H(13)	1715	3394	869	43
H(14A)	4220	3668	289	49
H(14B)	4547	4490	793	49
H(15)	7282	3406	582	43
H(16A)	7817	2489	1404	32
H(16B)	6745	3771	1475	32
H(17A)	5867	1681	173	47
H(17B)	7282	1203	599	47
H(18)	4486	99	680	39
H(19A)	3984	464	1574	31
H(19B)	6137	482	1457	31
H(20A)	1774	1178	906	46
H(20B)	2489	1640	357	46
H(22A)	-616	-311	2905	34
H(22B)	1450	-624	2746	34
H(23A)	-971	811	2123	64
H(23B)	-486	-607	2014	64
H(23C)	1065	429	1963	64
H(1)	3550(40)	660(30)	3007(11)	14(7)
H(2)	5680(60)	1420(40)	2292(14)	38(10)

H(2B)	7570(60)	1520(30)	3101(14)	33(10)

Table 6. Torsion angles [°].

C(6)-C(1)-C(2)-C(3)	-1.3(5)
C(7)-C(1)-C(2)-C(3)	179.0(3)
C(1)-C(2)-C(3)-C(4)	1.0(5)
C(2)-C(3)-C(4)-C(5)	-0.9(5)
C(3)-C(4)-C(5)-C(6)	1.2(5)
C(2)-C(1)-C(6)-C(5)	1.5(5)
C(7)-C(1)-C(6)-C(5)	-178.7(3)
C(4)-C(5)-C(6)-C(1)	-1.5(5)
C(2)-C(1)-C(7)-C(8)	-80.7(3)
C(6)-C(1)-C(7)-C(8)	99.5(3)
C(1)-C(7)-C(8)-N(1)	-65.2(3)
C(1)-C(7)-C(8)-C(9)	174.0(2)
N(1)-C(8)-C(9)-O(2)	-49.9(3)
C(7)-C(8)-C(9)-O(2)	72.6(3)
N(1)-C(8)-C(9)-C(10)	73.2(3)
C(7)-C(8)-C(9)-C(10)	-164.3(2)
O(2)-C(9)-C(10)-O(3)	-156.2(3)
C(8)-C(9)-C(10)-O(3)	84.2(3)
O(2)-C(9)-C(10)-N(2)	24.6(3)
C(8)-C(9)-C(10)-N(2)	-95.0(3)
N(2)-C(11)-C(12)-C(13)	-176.7(2)
C(16)-C(11)-C(12)-C(13)	60.1(3)
C(19)-C(11)-C(12)-C(13)	-59.4(3)
C(11)-C(12)-C(13)-C(14)	-59.5(3)
C(11)-C(12)-C(13)-C(20)	59.9(4)
C(12)-C(13)-C(14)-C(15)	59.7(3)
C(20)-C(13)-C(14)-C(15)	-59.5(3)
C(13)-C(14)-C(15)-C(17)	60.4(3)
C(13)-C(14)-C(15)-C(16)	-59.9(3)
N(2)-C(11)-C(16)-C(15)	176.7(2)
C(12)-C(11)-C(16)-C(15)	-59.9(3)
C(19)-C(11)-C(16)-C(15)	59.8(3)
C(14)-C(15)-C(16)-C(11)	59.8(3)
C(17)-C(15)-C(16)-C(11)	-59.5(4)

C(14)-C(15)-C(17)-C(18)	-60.6(3)
C(16)-C(15)-C(17)-C(18)	59.3(4)
C(15)-C(17)-C(18)-C(20)	60.3(3)
C(15)-C(17)-C(18)-C(19)	-59.2(4)
N(2)-C(11)-C(19)-C(18)	179.5(3)
C(16)-C(11)-C(19)-C(18)	-60.7(3)
C(12)-C(11)-C(19)-C(18)	59.2(3)
C(20)-C(18)-C(19)-C(11)	-59.8(3)
C(17)-C(18)-C(19)-C(11)	60.2(3)
C(17)-C(18)-C(20)-C(13)	-59.0(3)
C(19)-C(18)-C(20)-C(13)	60.5(3)
C(12)-C(13)-C(20)-C(18)	-60.8(4)
C(14)-C(13)-C(20)-C(18)	58.5(3)
O(1)-C(21)-C(22)-C(23)	67.4(4)
N(1)-C(21)-C(22)-C(23)	-111.8(3)
O(1)-C(21)-N(1)-C(8)	-0.5(4)
C(22)-C(21)-N(1)-C(8)	178.7(2)
C(9)-C(8)-N(1)-C(21)	-140.4(2)
C(7)-C(8)-N(1)-C(21)	97.6(3)
O(3)-C(10)-N(2)-C(11)	-7.3(5)
C(9)-C(10)-N(2)-C(11)	171.9(2)
C(16)-C(11)-N(2)-C(10)	73.0(3)
C(12)-C(11)-N(2)-C(10)	-49.5(4)
C(19)-C(11)-N(2)-C(10)	-168.5(3)

Symmetry transformations used to generate equivalent atoms:

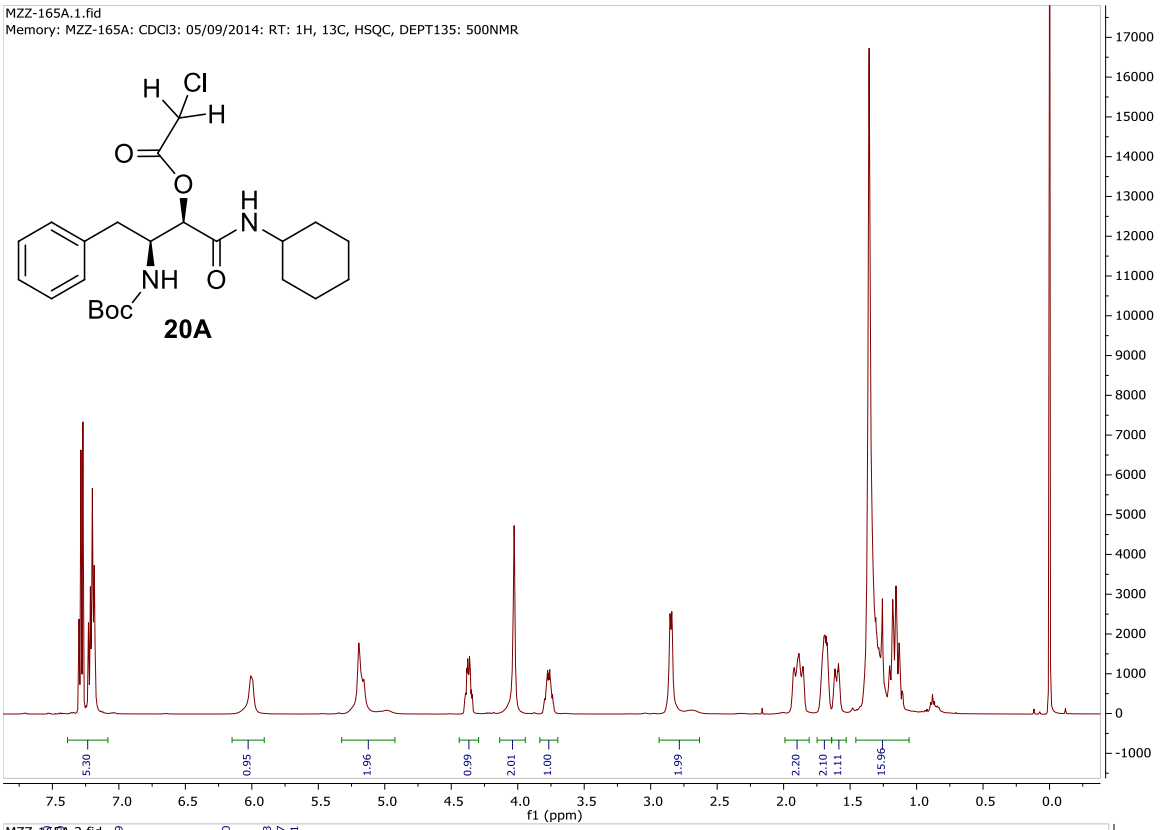
Table 7. Hydrogen bonds [Å and °].

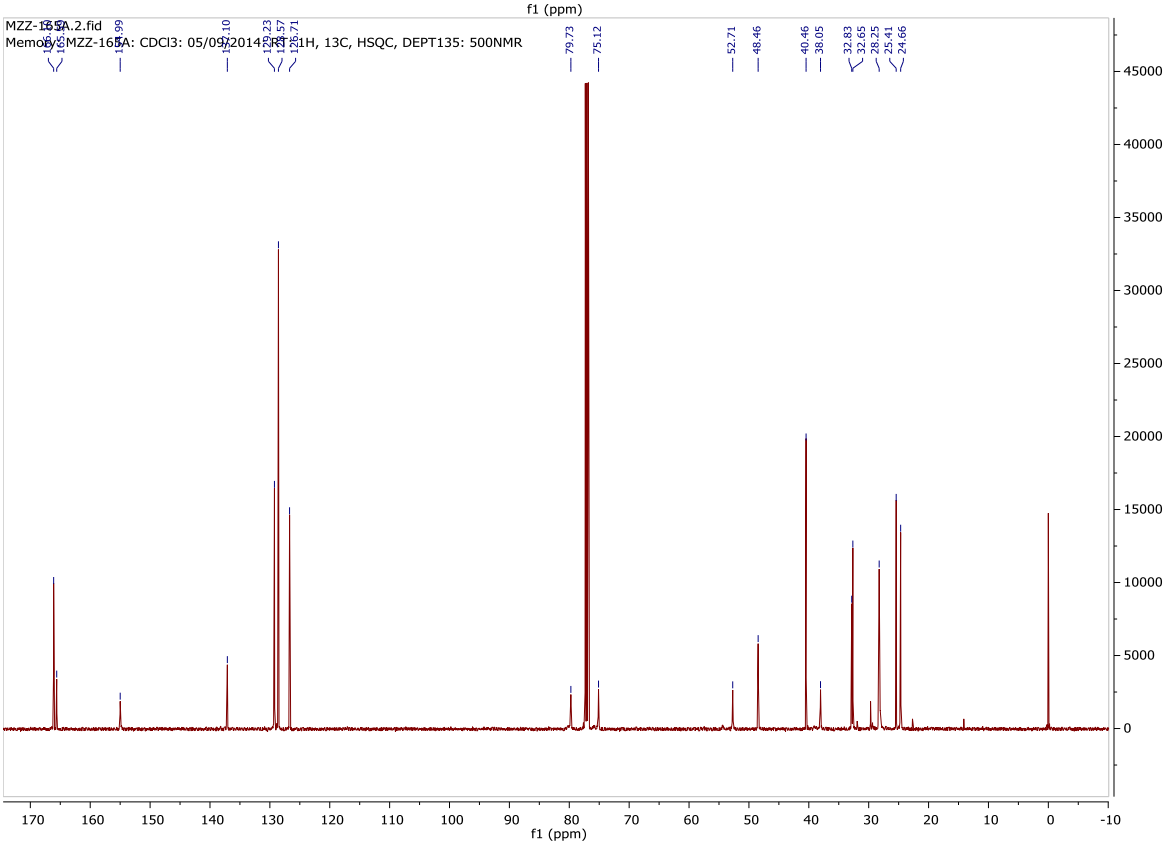
D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2B)...O(1)#1	0.77(4)	1.91(4)	2.667(3)	167(4)
N(1)-H(1)...O(3)#2	0.86(3)	2.22(3)	3.052(3)	162(3)
N(2)-H(2)...O(2)	0.84(4)	2.25(4)	2.710(3)	115(3)

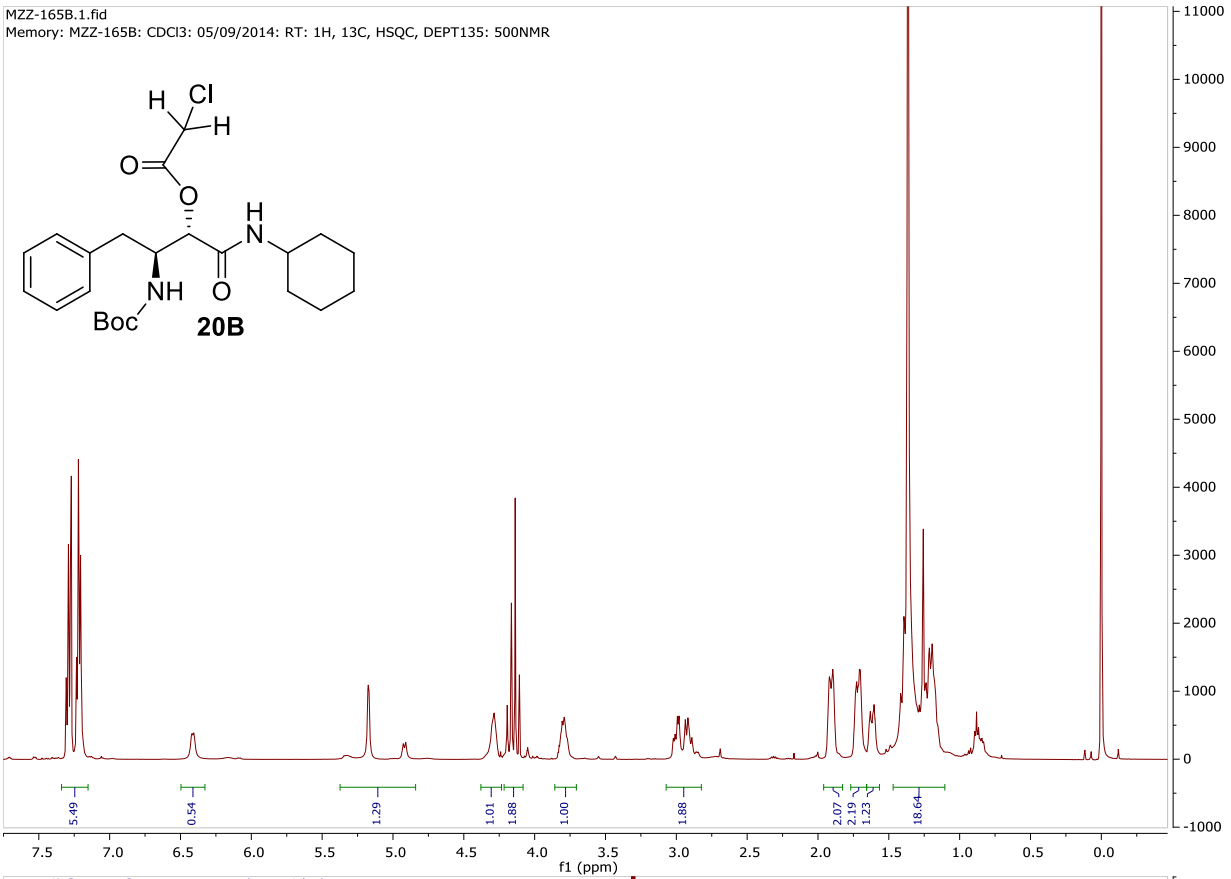
Symmetry transformations used to generate equivalent atoms:

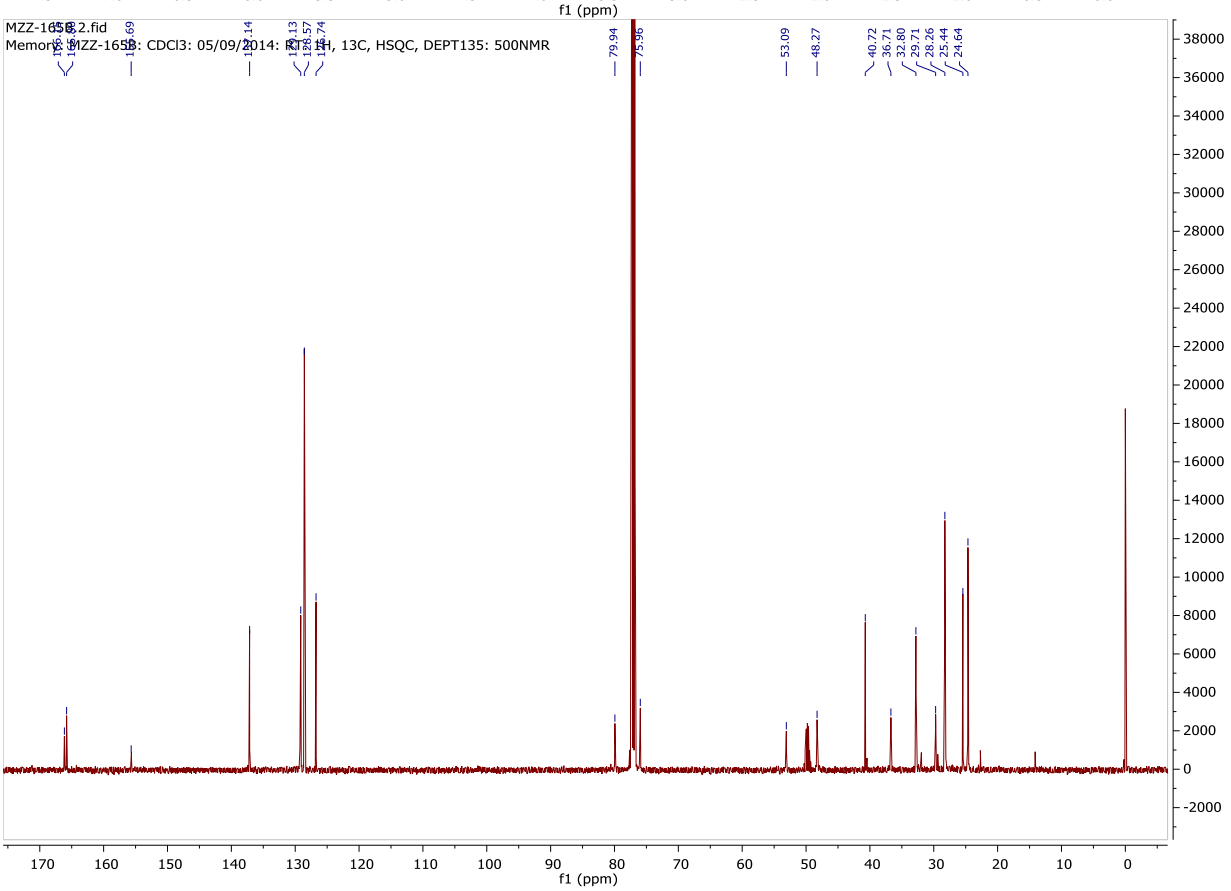
#1 x+1,y,z #2 -x+1,y-1/2,-z+1/2

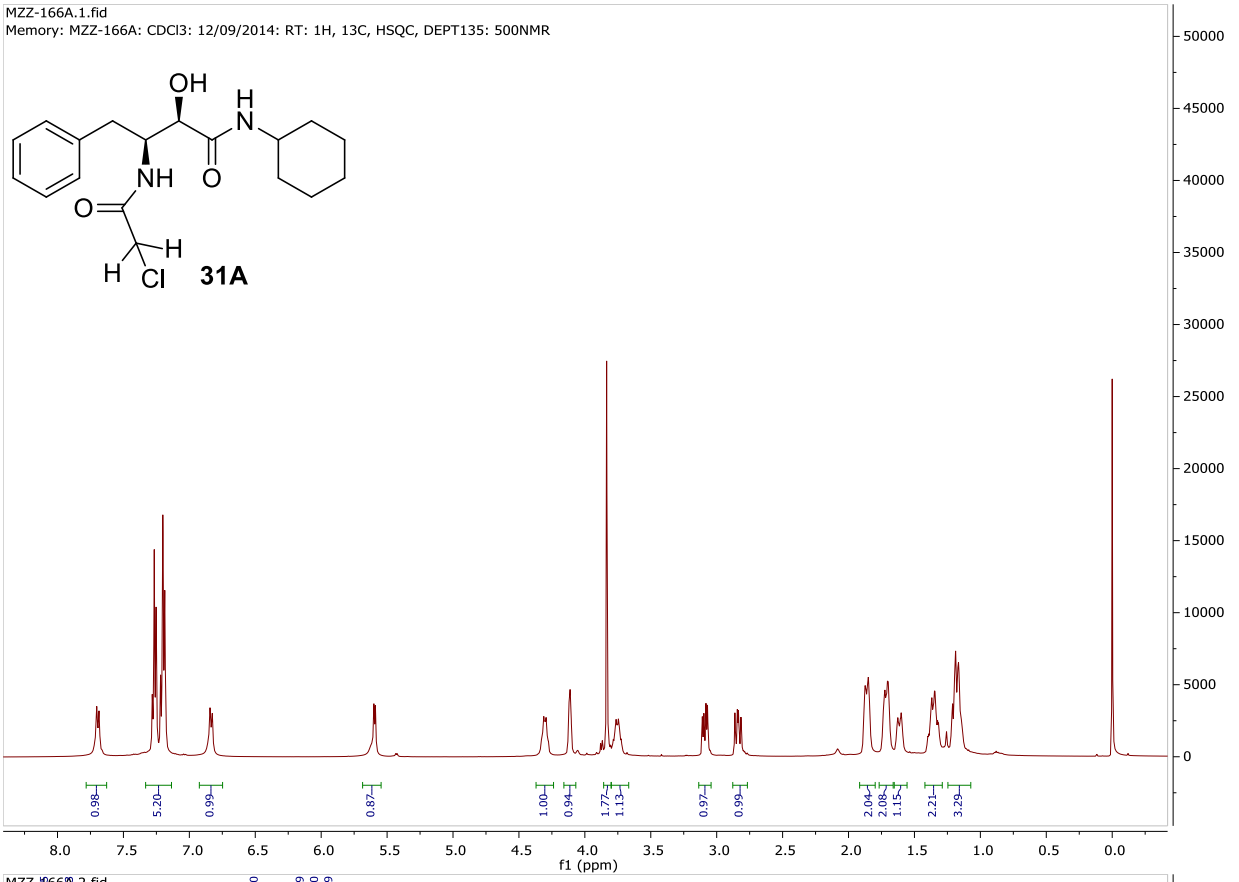
APPENDIX II: ^{1}H & ^{13}C NMR SPECTRA

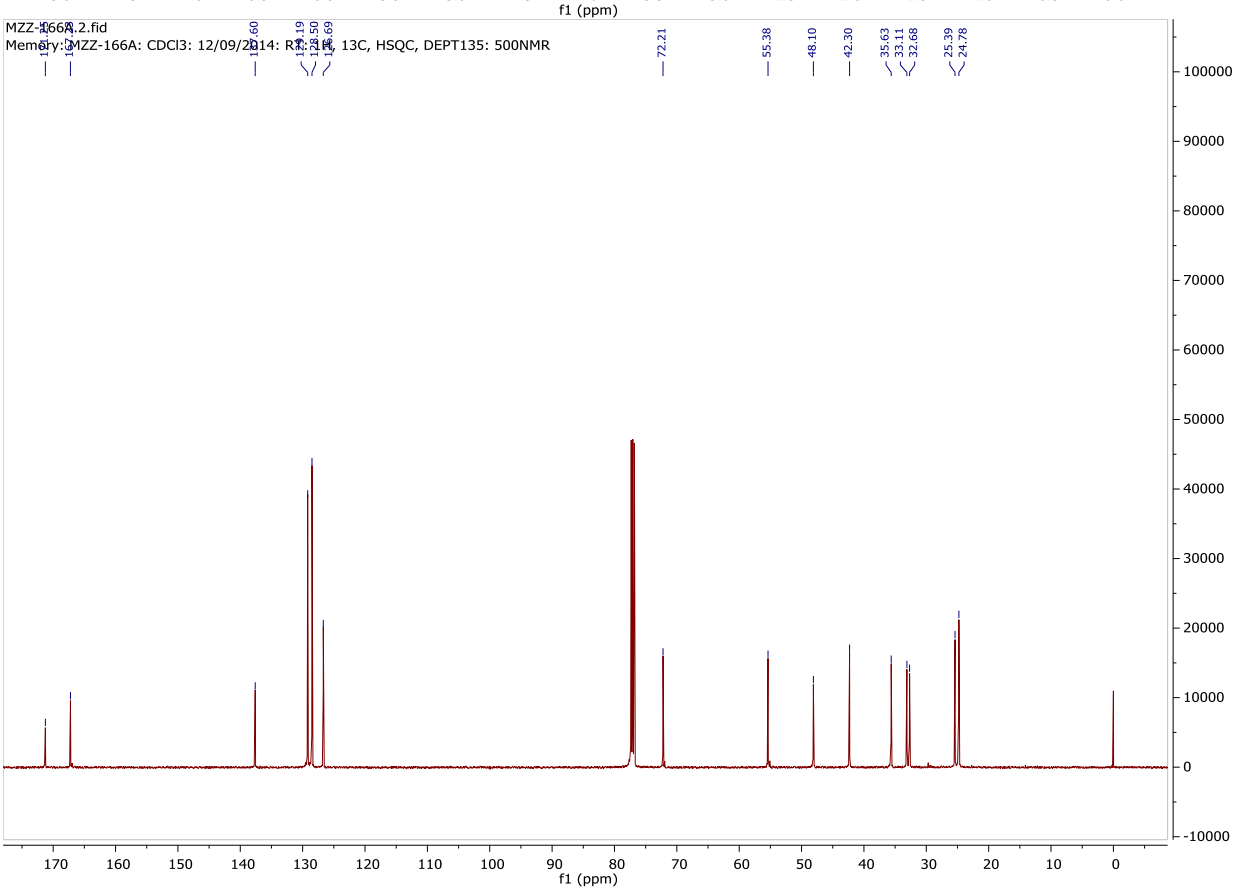


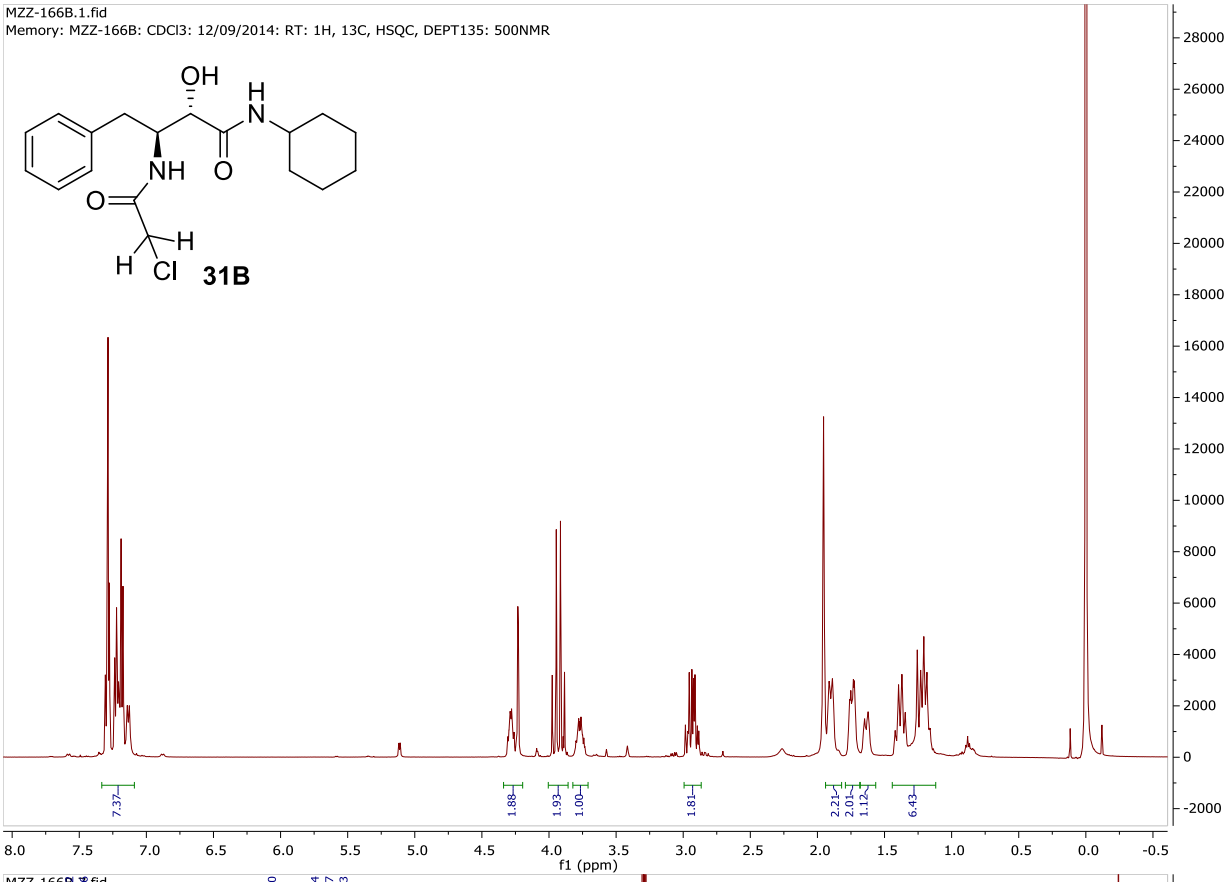


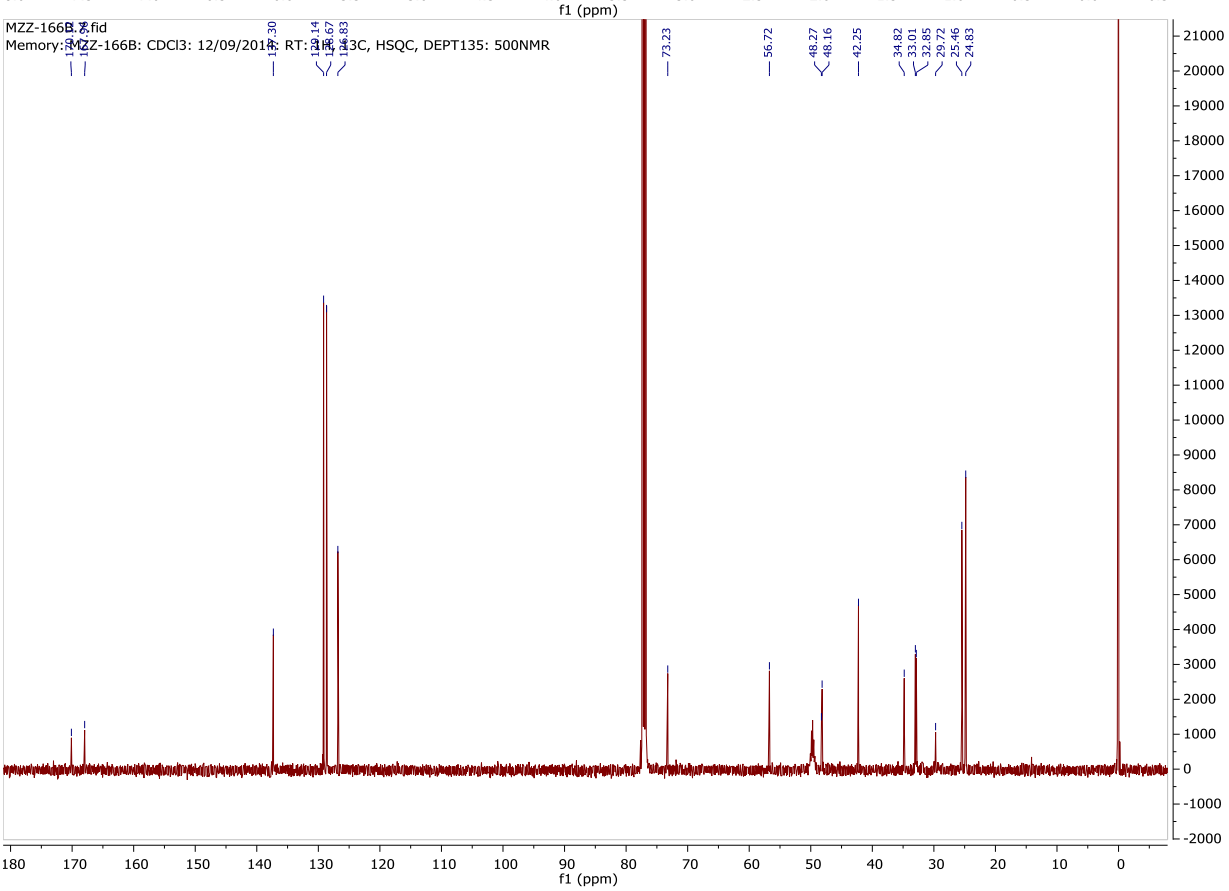

MZZ-165B.1.fid
Memory: MZZ-165B: CDCl3: 05/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
20B
5.49
0.54
1.29
1.01
1.88
1.00
1.88
2.07
2.19
1.23
18.64
f1 (ppm)

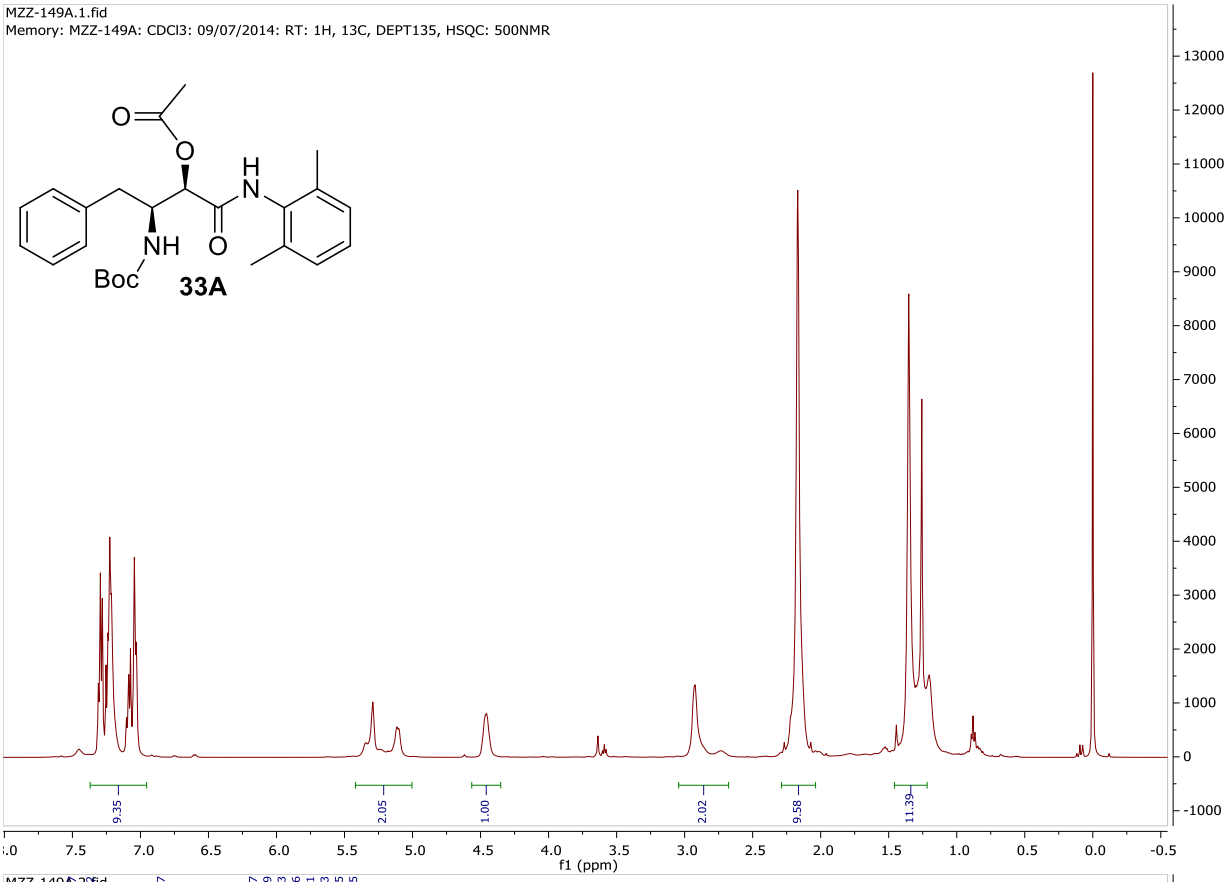

MZZ-165B.2.fid
Memory: MZZ-165B: CDCl3: 05/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
79.94
75.96
53.09
48.27
40.72
36.71
32.80
29.71
28.26
25.44
24.64
f1 (ppm)

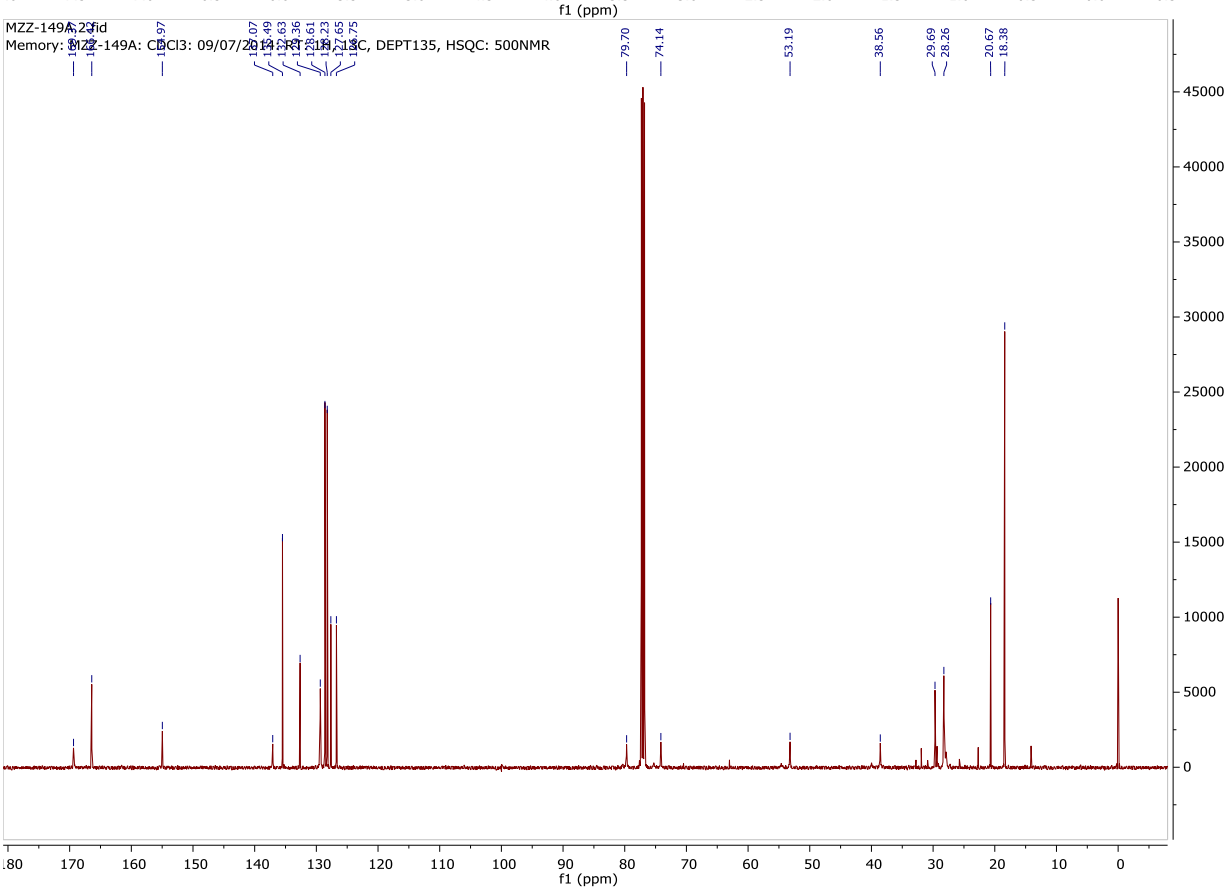

MZZ-166A.1.fid
Memory: MZZ-166A: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
OH
H
N
NH
O
O
H
H
Cl
31A
0.98
5.20
0.99
0.87
1.00
0.94
1.77
1.13
0.97
0.99
2.04
2.08
1.15
2.21
3.29
f1 (ppm)

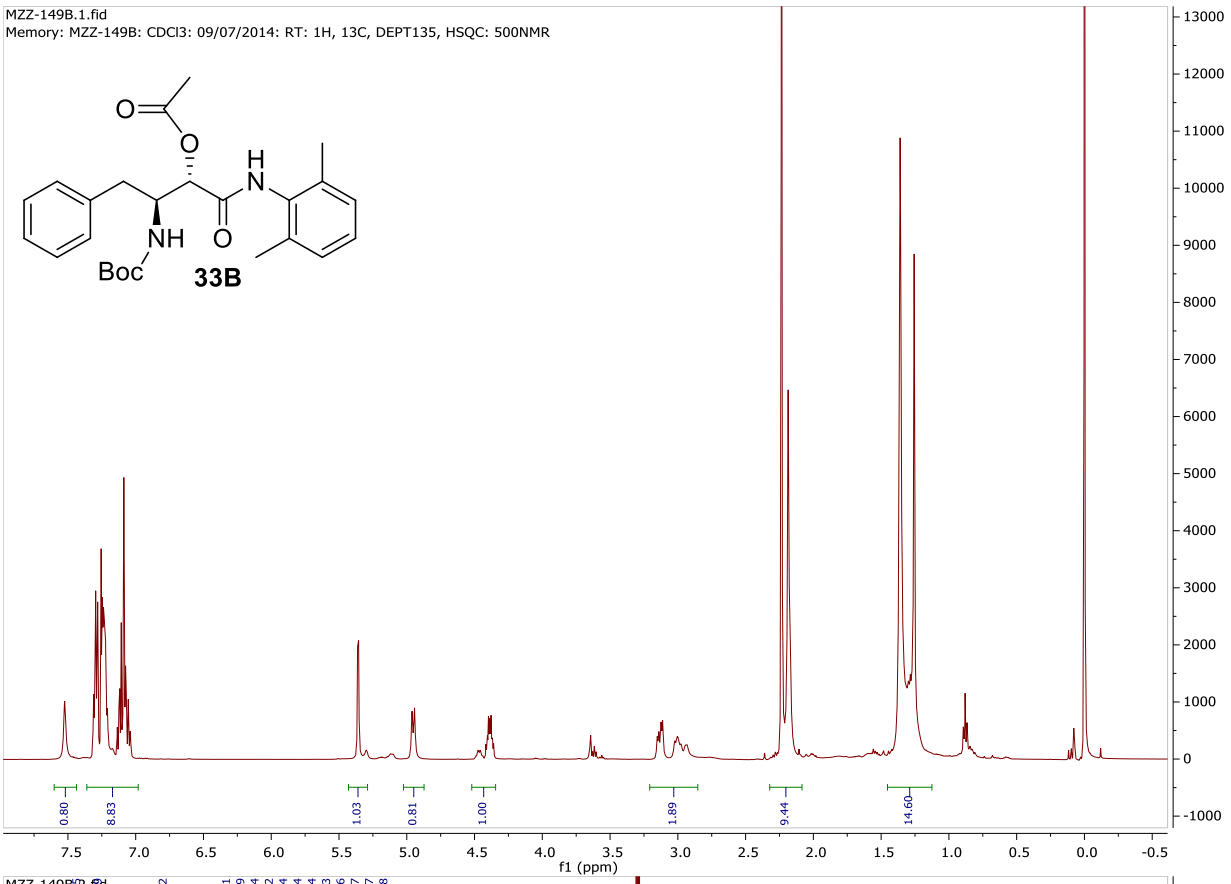

MZZ-166A.2.fid
Memory: MZZ-166A: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
172.60
129.19
128.50
126.69
72.21
55.38
48.10
42.30
35.63
33.11
32.68
25.39
24.78
f1 (ppm)

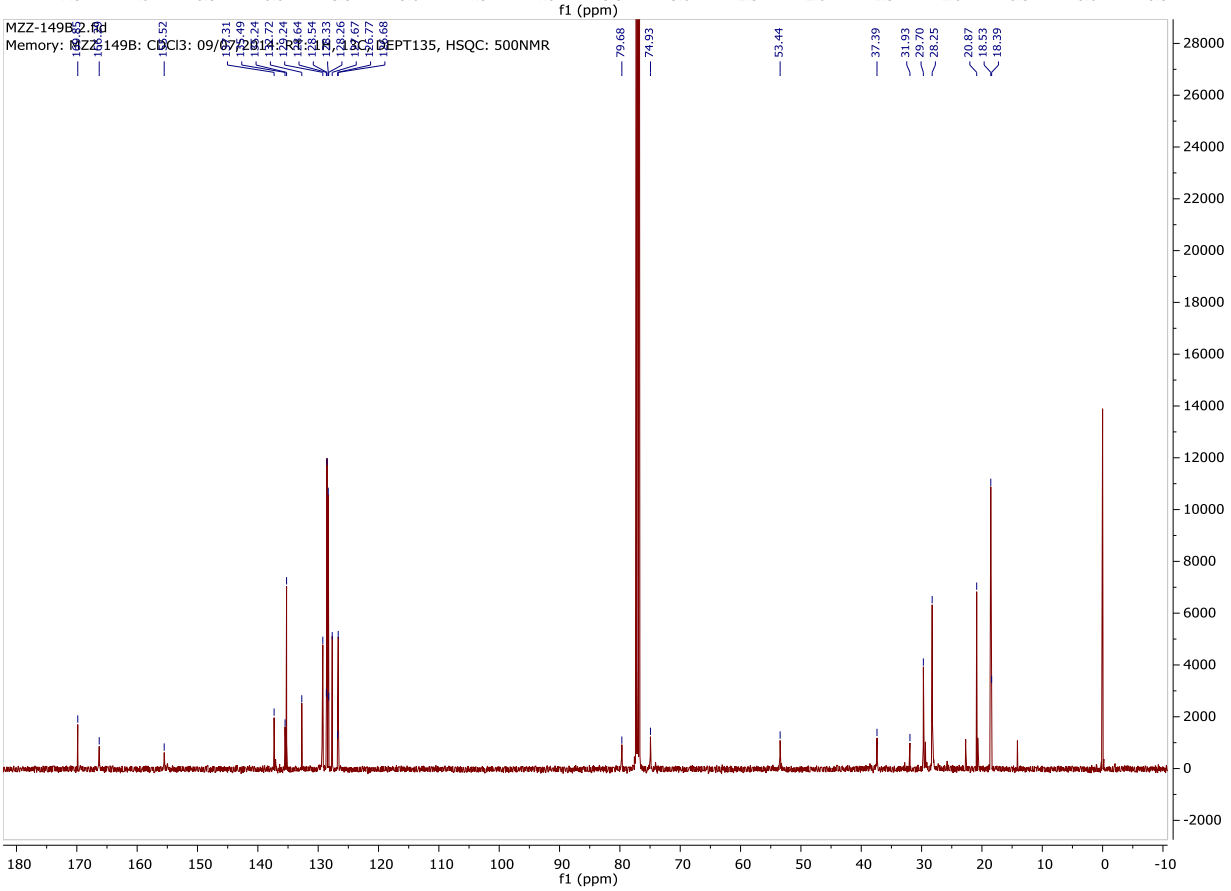

MZZ-166B.1.fid
Memory: MZZ-166B: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
OH
H
N
NH
O
O
H
H
Cl
31B
7.37
1.88
1.93
1.00
1.81
2.21
2.01
1.12
6.43
f1 (ppm)

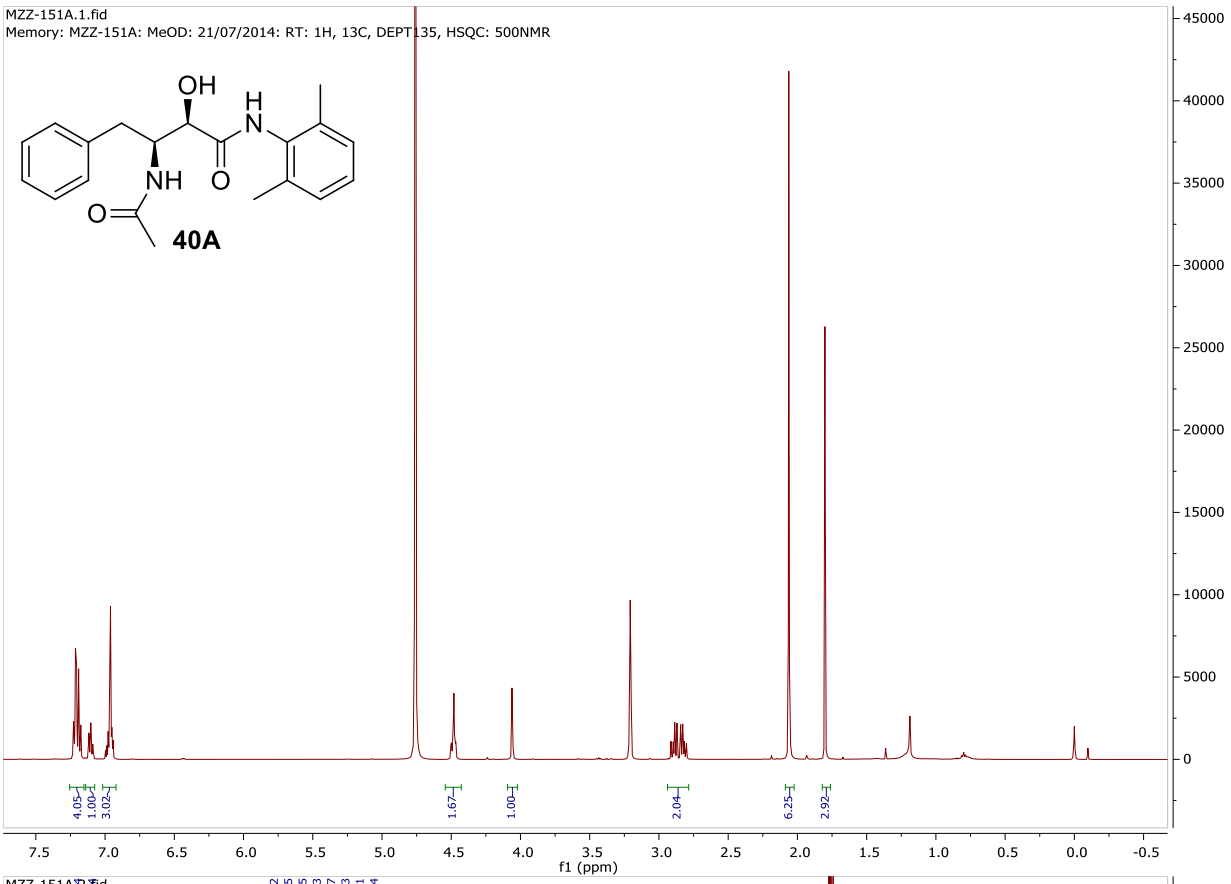

MZZ-166B.1.fid
Memory: MZZ-166B: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
73.23
56.72
48.27
48.16
42.25
34.82
33.01
32.85
29.72
25.46
24.83
f1 (ppm)

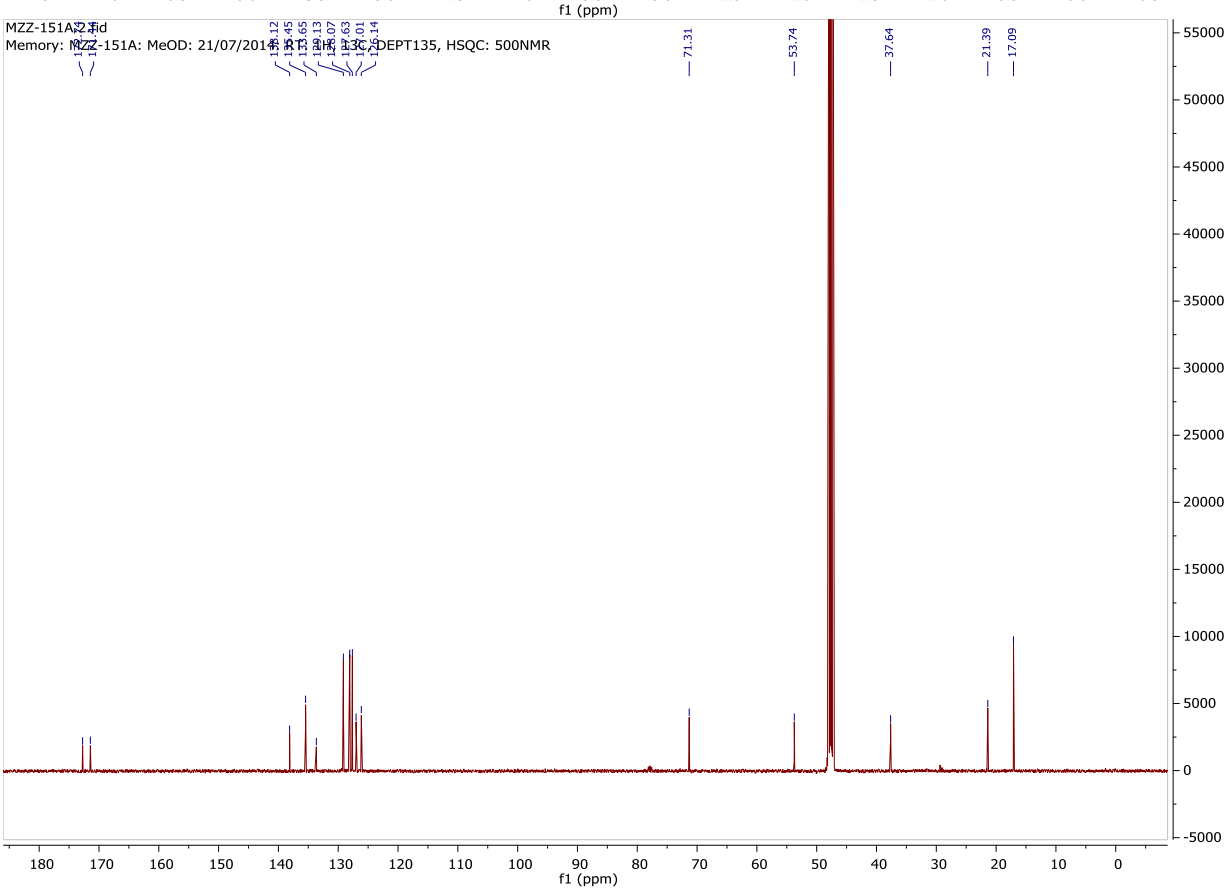

MZZ-149A.1.fid
Memory: MZZ-149A: CDCl3: 09/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
O
O
H
N
NH
O
Boc
33A
9.35
2.05
1.00
2.02
9.58
11.39
f1 (ppm)

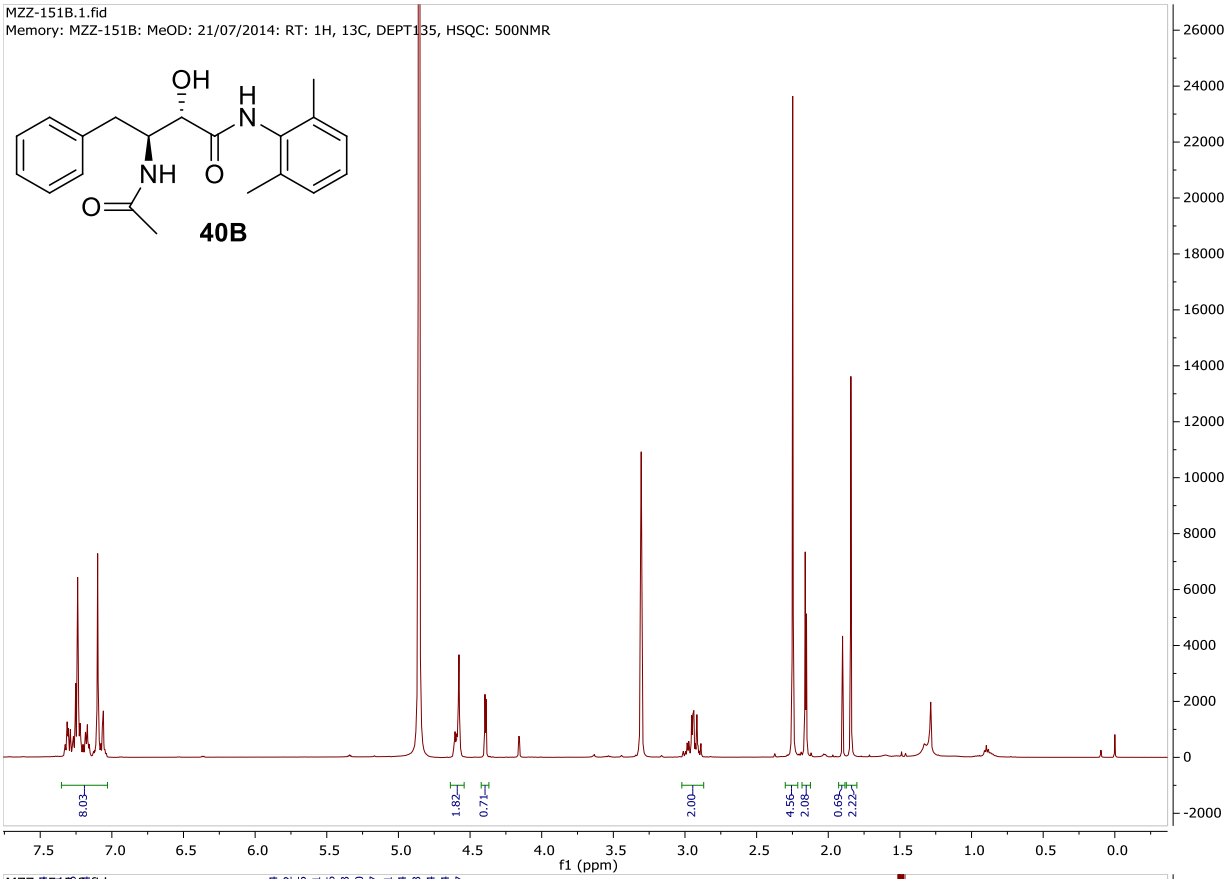

MZZ-149A.2.fid
Memory: MZZ-149A: CDCl3: 09/07/2014: RT: 13C, DEPT135, HSQC: 500NMR
79.70
74.14
53.19
38.56
29.69
28.26
20.67
18.38
f1 (ppm)

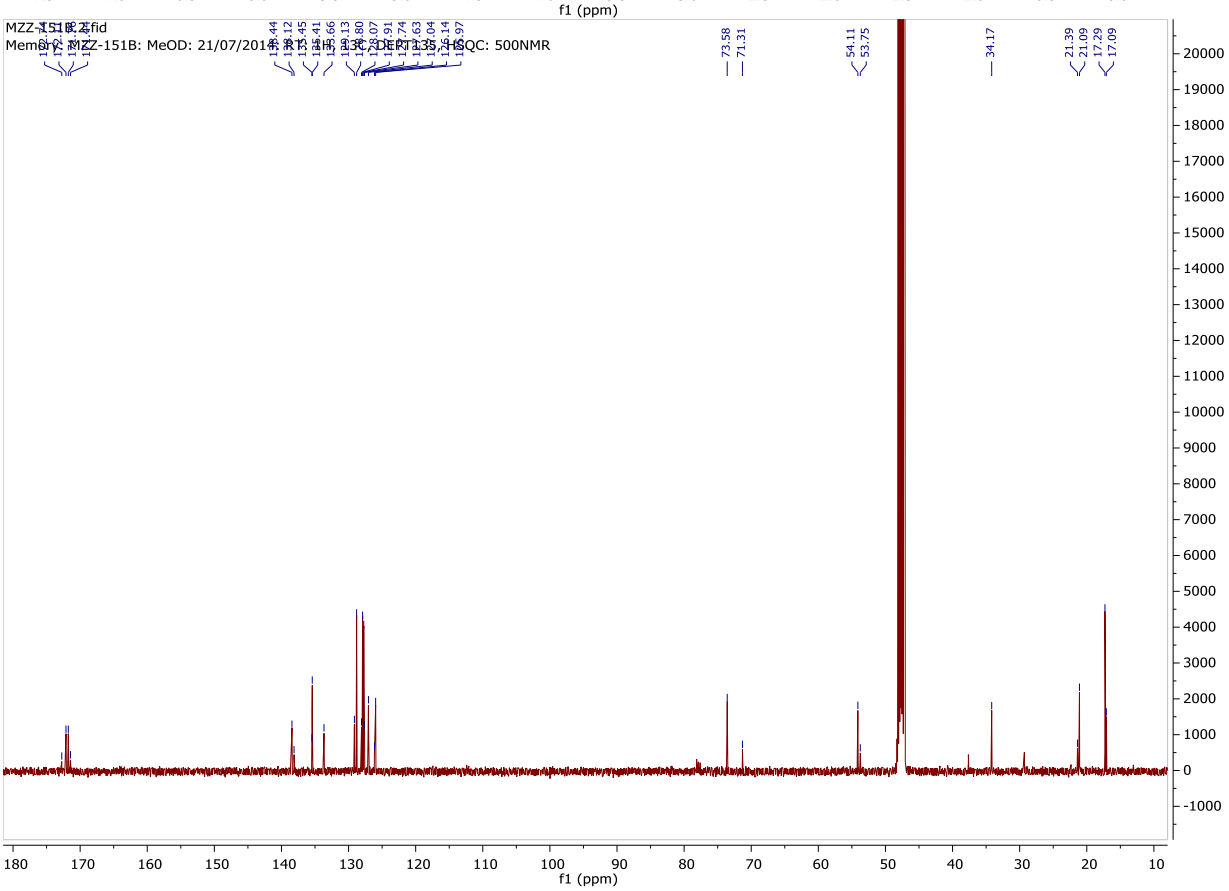

MZZ-149B.1.fid
Memory: MZZ-149B: CDCl3: 09/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
O
O
H
N
NH
O
Boc
33B
0.80
8.83
1.03
0.81
1.00
1.89
9.44
14.60
f1 (ppm)

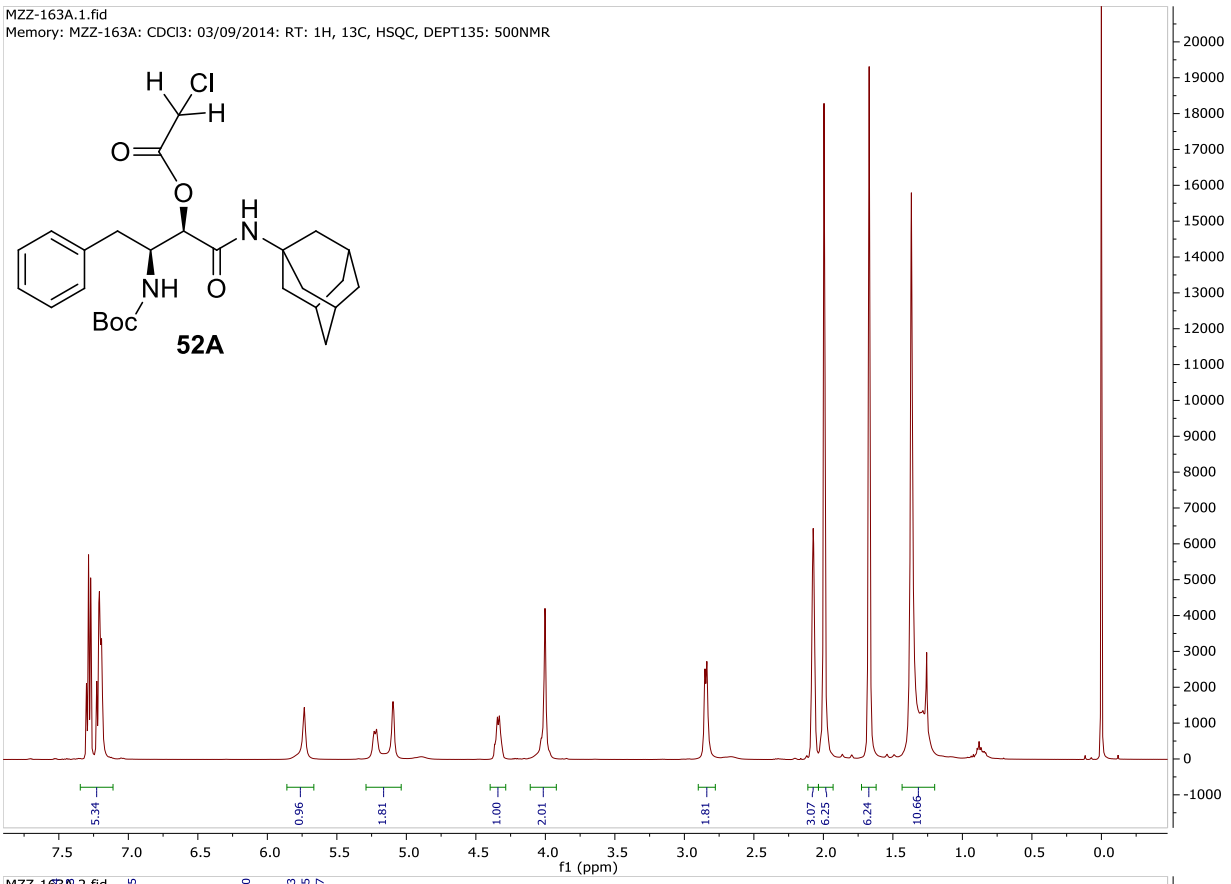

MZZ-149B2.fid
Memory: MZZ-149B: CDCl3: 09/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
79.68
74.93
53.44
37.39
31.93
29.70
28.25
20.87
18.53
18.39
f1 (ppm)

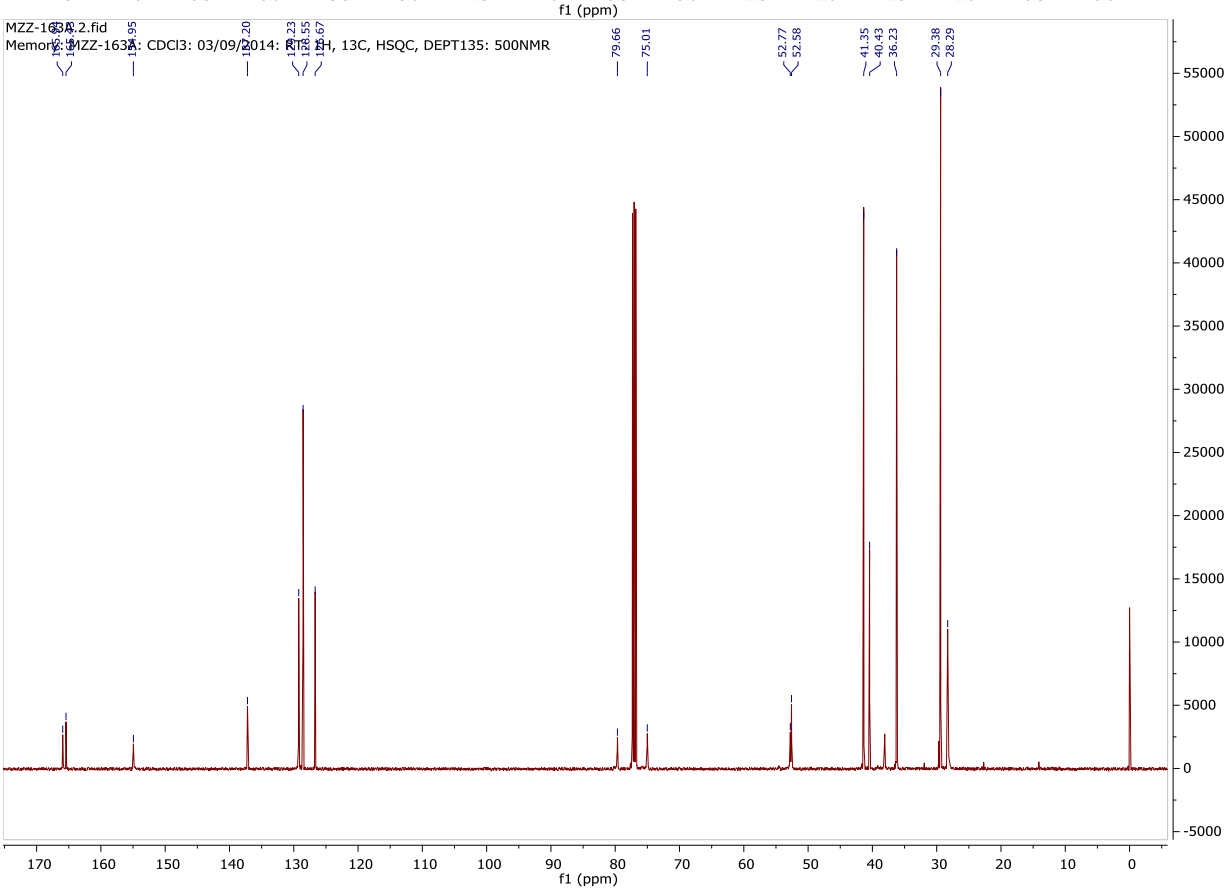

MZZ-151A.1.fid
Memory: MZZ-151A: MeOD: 21/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
OH
H
N
NH
O
O
40A
4.05
1.00
3.02
1.67
1.00
2.04
6.25
2.92
f1 (ppm)

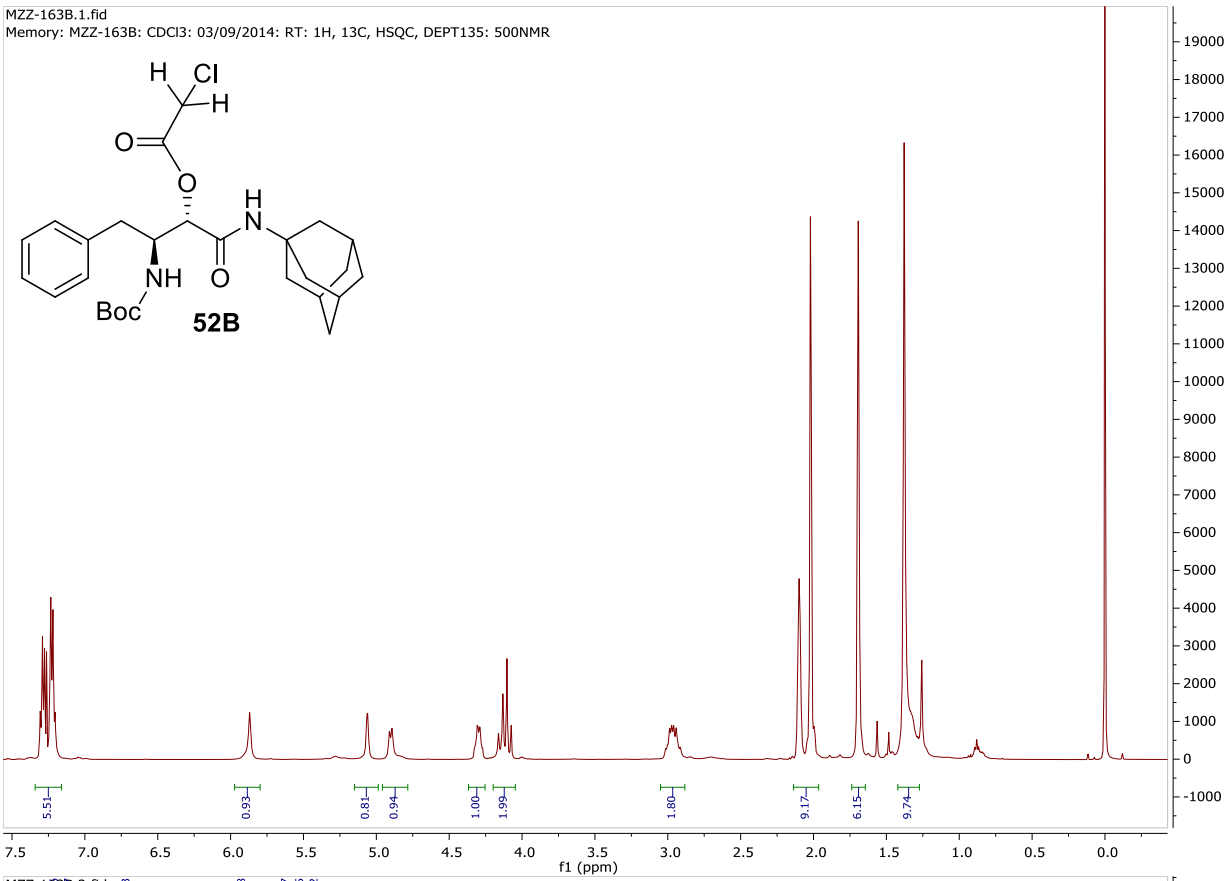

MZZ-151A.2.fid
Memory: MZZ-151A: MeOD: 21/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
71.31
53.74
37.64
21.39
17.09
f1 (ppm)

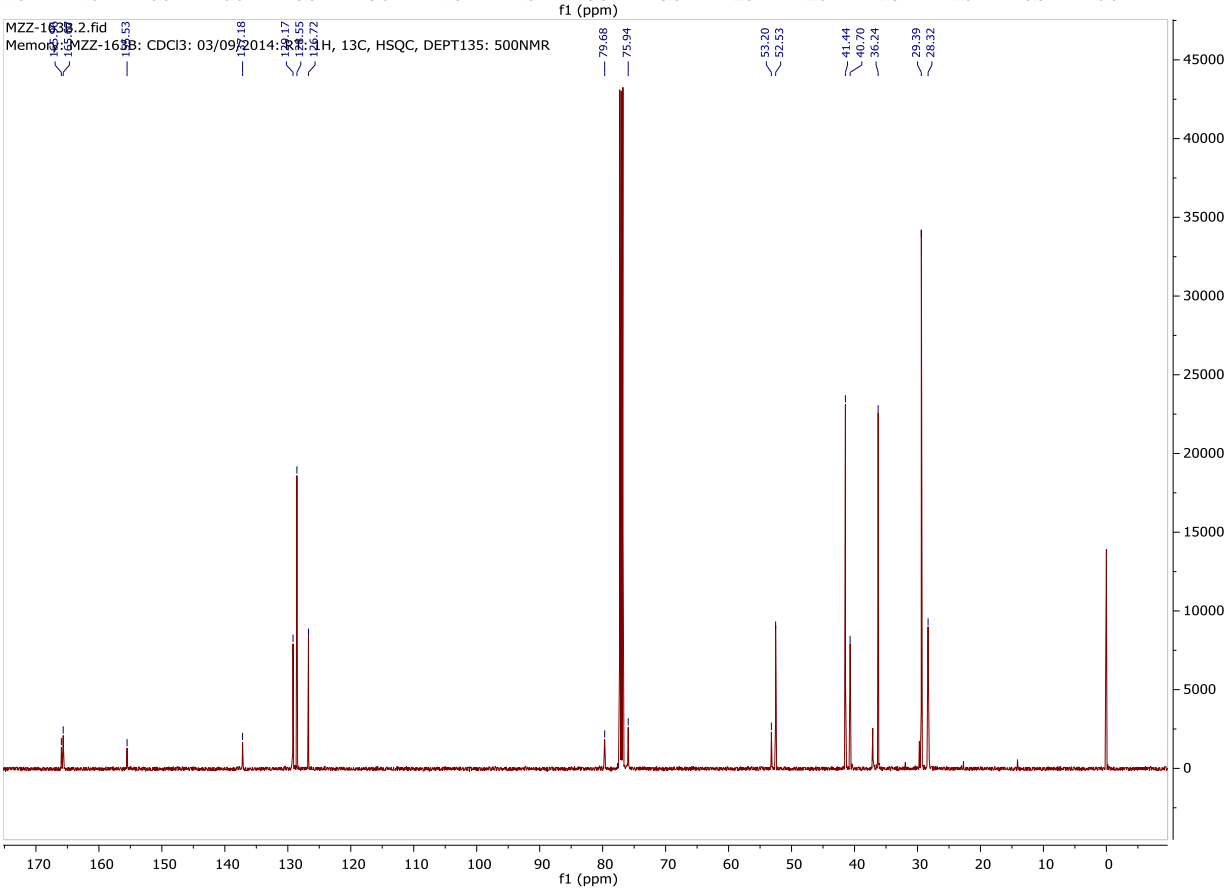

MZZ-151B.1.fid
Memory: MZZ-151B: MeOD: 21/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
OH
H
N
NH
O
O
40B
8.03
1.82
0.71
2.00
4.56
2.08
0.69
2.22
f1 (ppm)

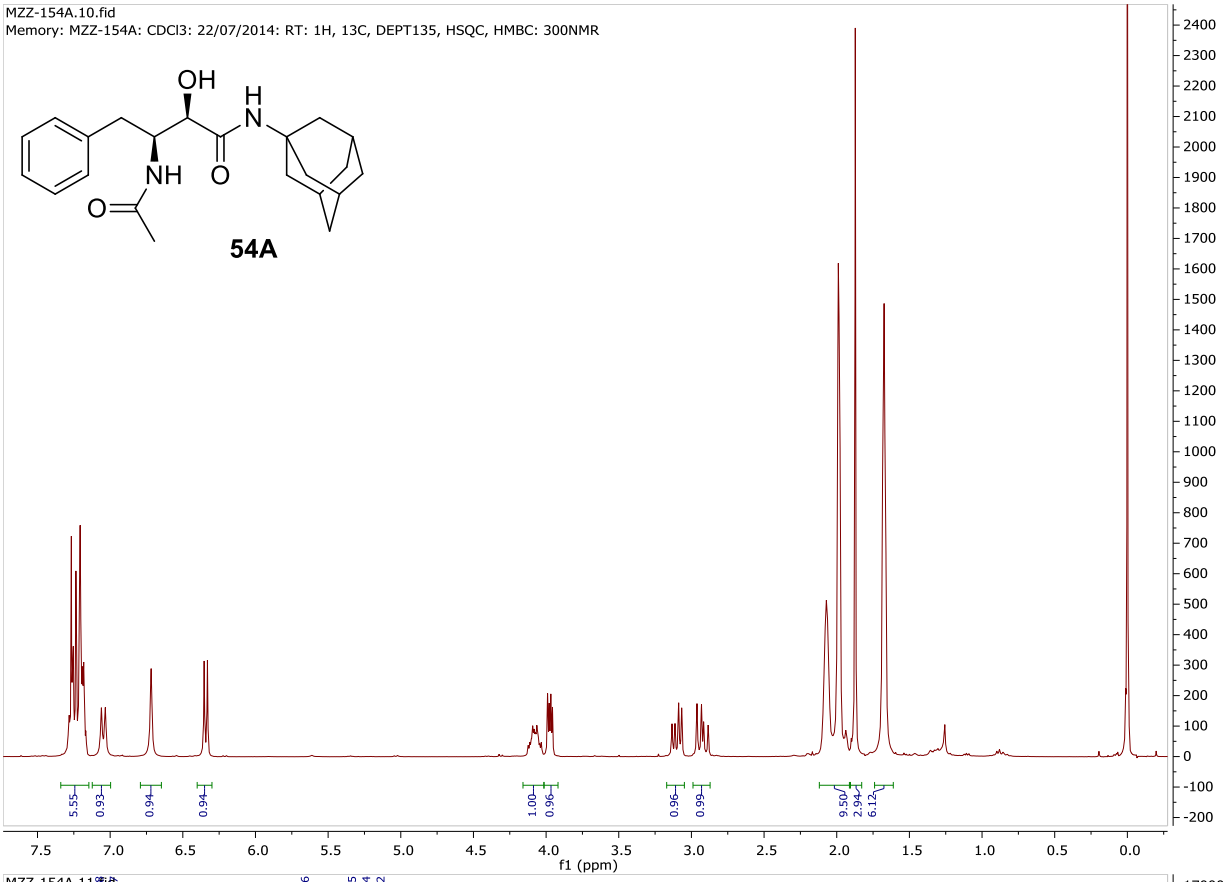

MZZ-151B.2.fid
Memory: MZZ-151B: MeOD: 21/07/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
73.58
71.31
54.11
53.75
34.17
21.39
21.09
17.29
17.09
f1 (ppm)

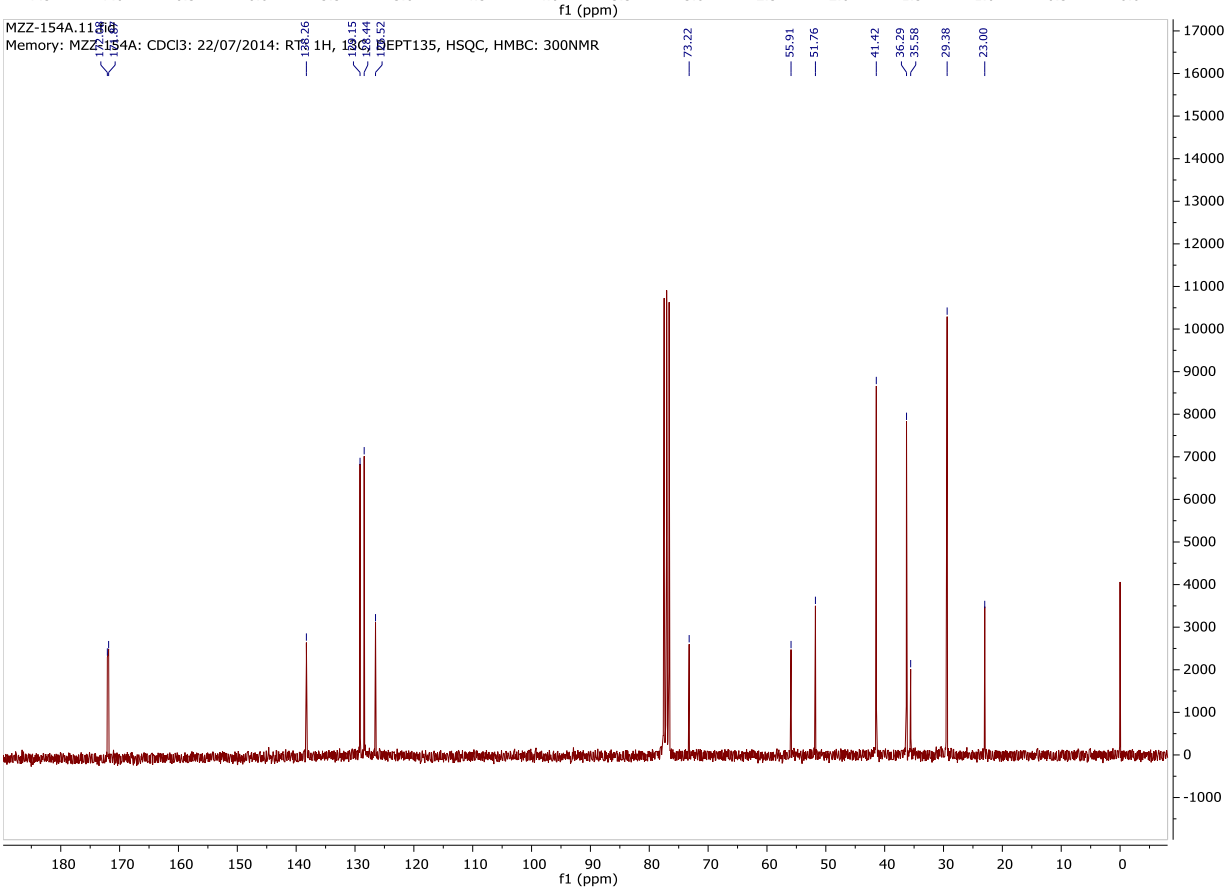

MZZ-163A.1.fid
Memory: MZZ-163A: CDCl3: 03/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
H
Cl
H
O
O
H
N
NH
O
Boc
52A
5.34
0.96
1.81
1.00
2.01
1.81
3.07
6.25
6.24
10.66
f1 (ppm)

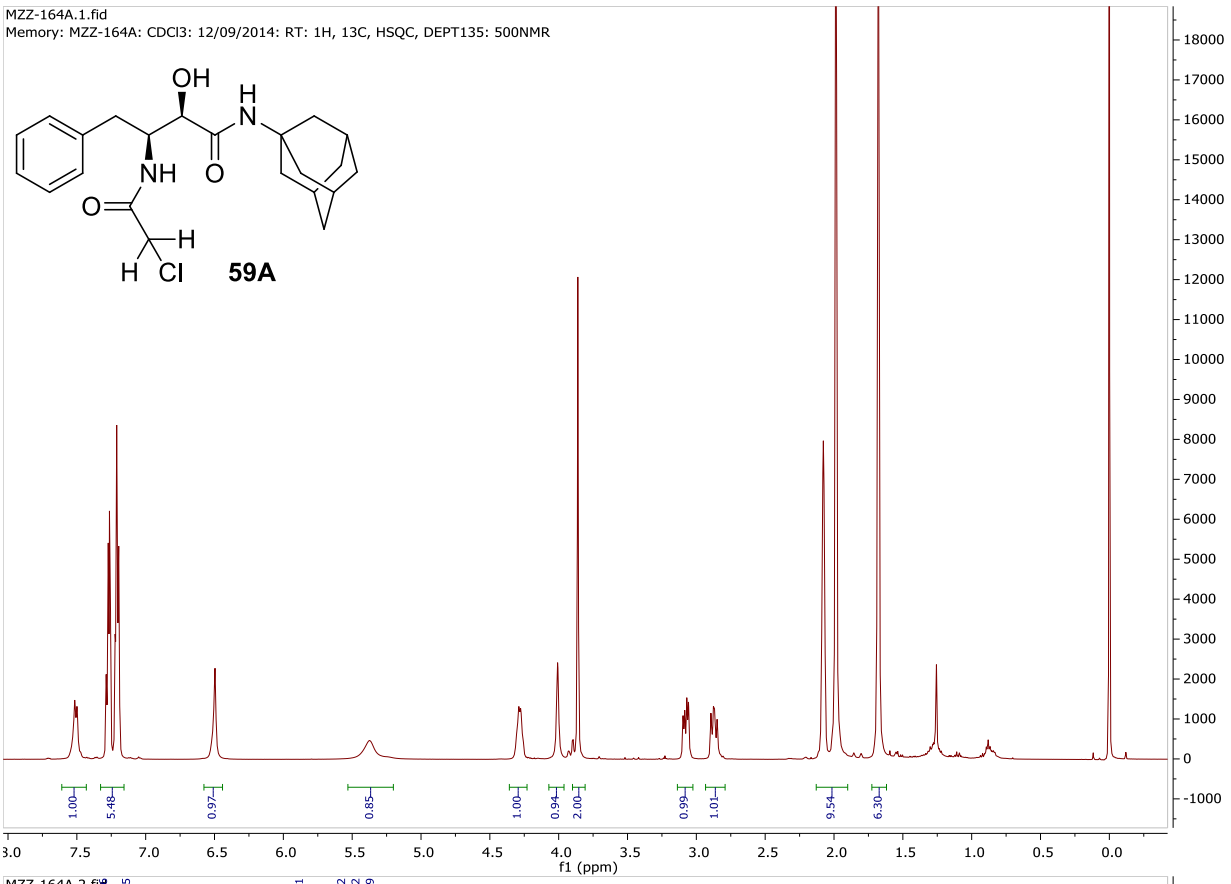

MZZ-163A.2.fid
Memory: MZZ-163A: CDCl3: 03/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
79.66
75.01
52.77
52.58
41.35
40.43
36.23
29.38
28.29
f1 (ppm)


MZZ-163B.1.fid
Memory: MZZ-163B: CDCl3: 03/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
H
Cl
H
O
O
H
N
NH
O
Boc
52B
5.51
0.93
0.81
0.94
1.00
1.99
1.80
9.17
6.15
9.74
f1 (ppm)


MZZ-163B.2.fid
Memory: MZZ-163B: CDCl3: 03/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
79.68
75.94
53.20
52.53
41.44
40.70
36.24
29.39
28.32
f1 (ppm)


MZZ-154A.10.fid
Memory: MZZ-154A: CDCl3: 22/07/2014: RT: 1H, 13C, DEPT135, HSQC, HMBC: 300NMR
OH
H
N
NH
O
O
54A
5.55
0.93
0.94
0.94
1.00
0.96
0.96
0.99
9.50
2.94
6.12
f1 (ppm)


MZZ-154A.11.fid
Memory: MZZ-154A: CDCl3: 22/07/2014: RT: 1H, 13C, DEPT135, HSQC, HMBC: 300NMR
138.26
130.15
129.44
125.52
73.22
55.91
51.76
41.42
36.29
35.58
29.38
23.00
f1 (ppm)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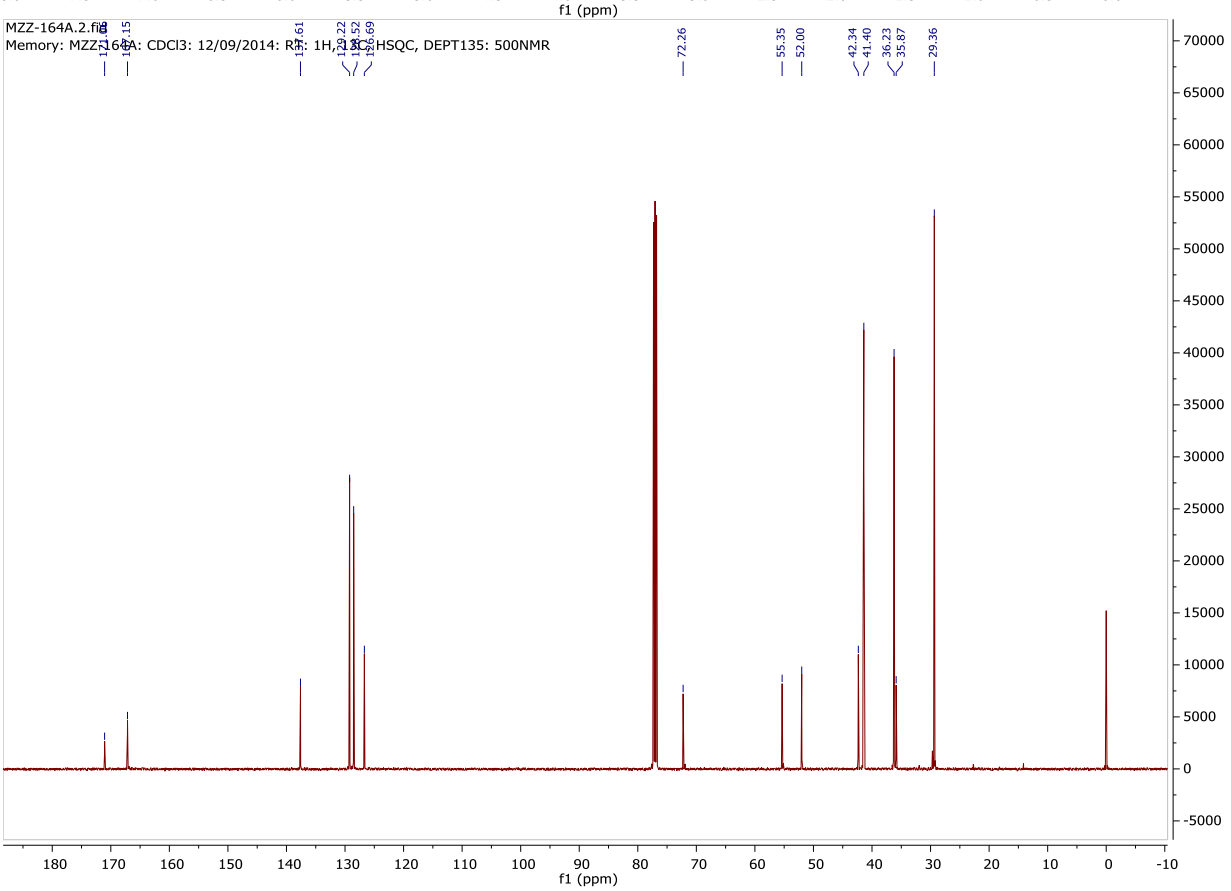
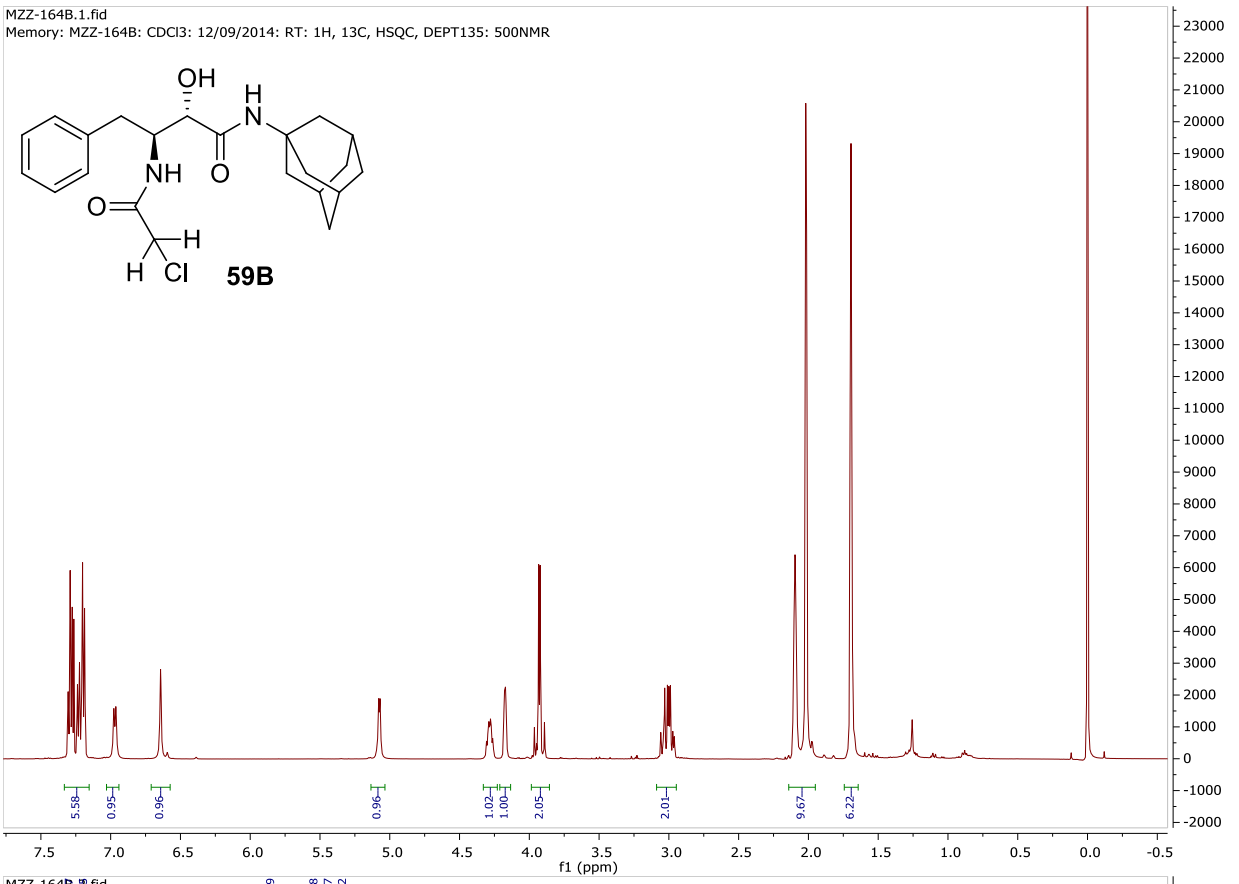
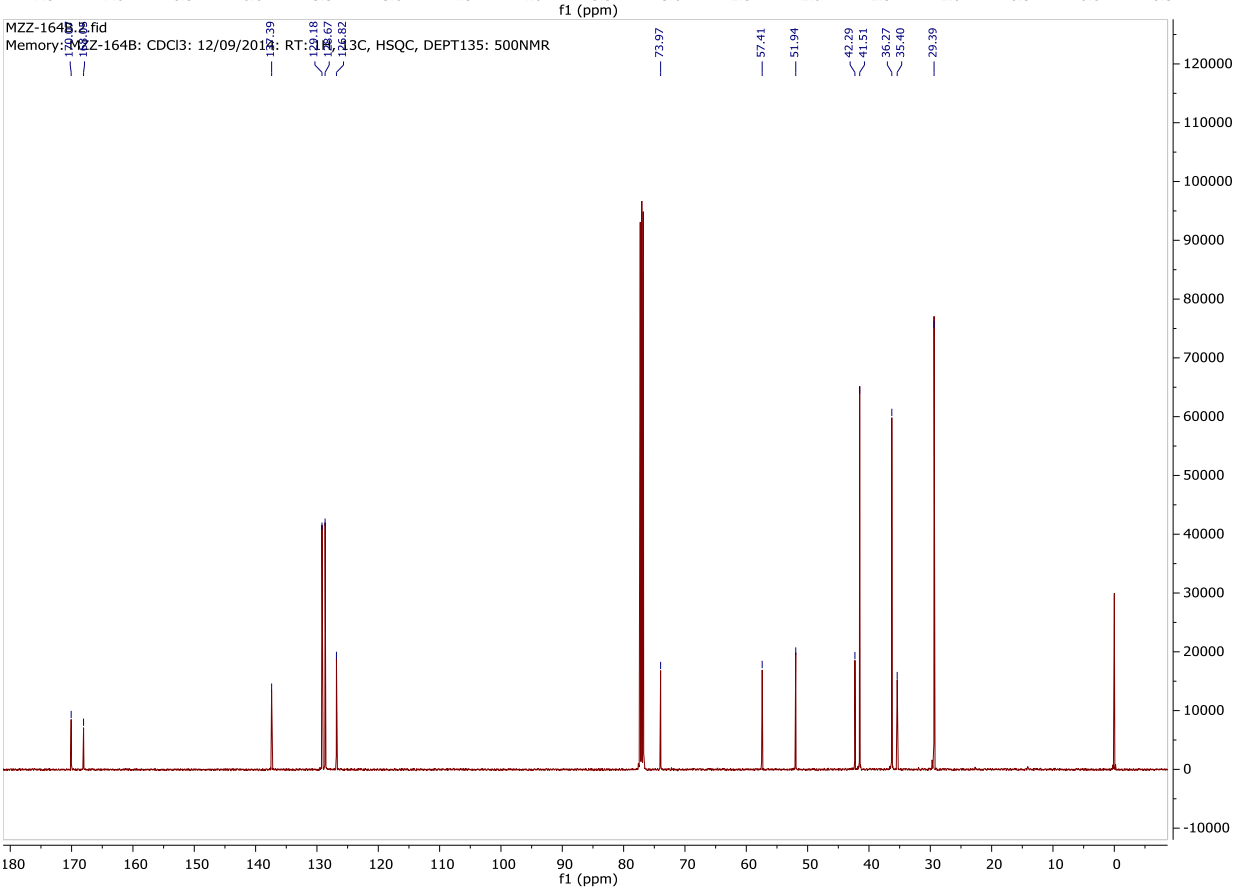
Memory: MZZ-164A: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
OH
H
N
NH
O
O
H
H
Cl
59A
1.00
5.48
0.97
0.85
1.00
0.94
2.00
0.99
1.01
9.54
6.30
f1 (ppm)


MZZ-164A.2.fid
Memory: MZZ-164A: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
72.26
55.35
52.00
42.34
41.40
36.23
35.87
29.36
f1 (ppm)


MZZ-164B.1.fid
Memory: MZZ-164B: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
OH
H
N
NH
O
O
H
H
Cl
59B
5.58
0.95
0.96
0.96
1.02
1.00
2.05
2.01
9.67
6.22
f1 (ppm)


MZZ-164B.1.fid
Memory: MZZ-164B: CDCl3: 12/09/2014: RT: 1H, 13C, HSQC, DEPT135: 500NMR
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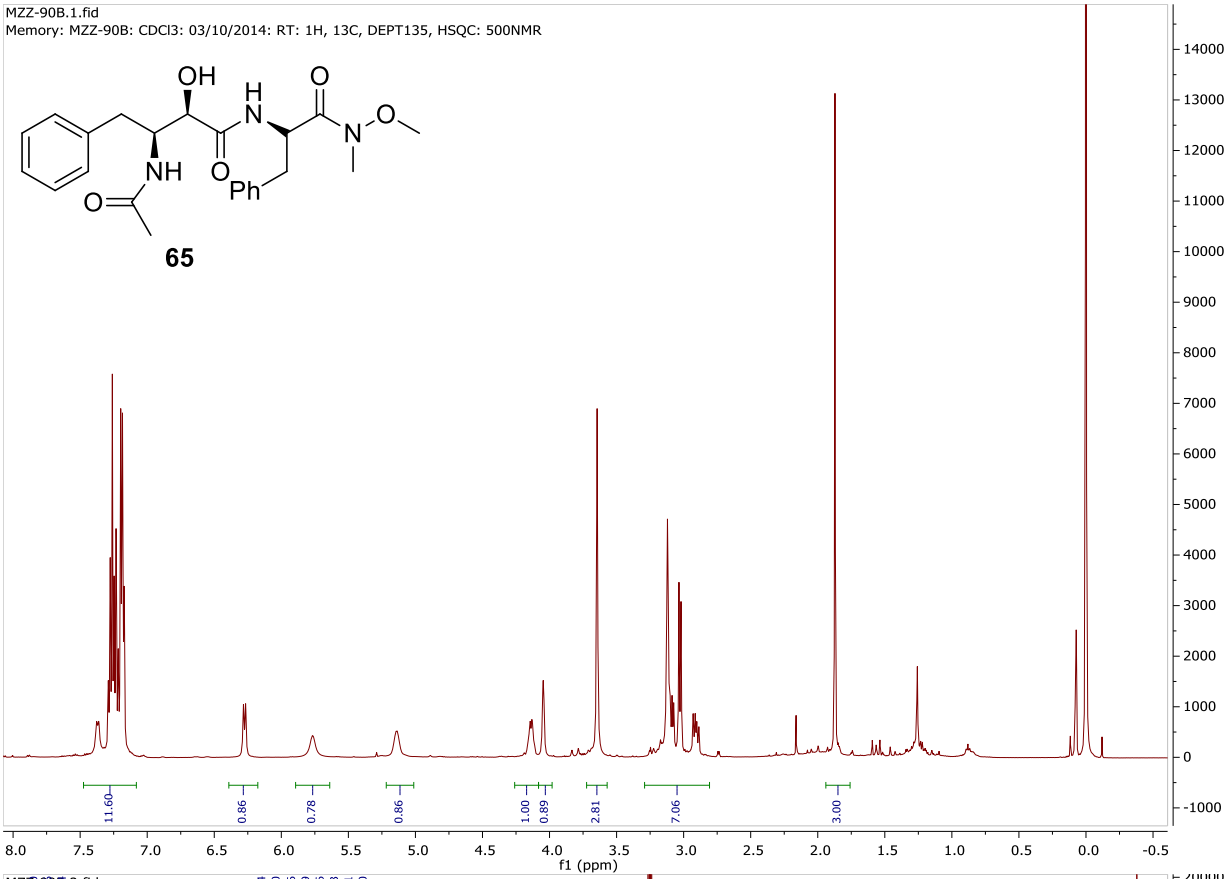
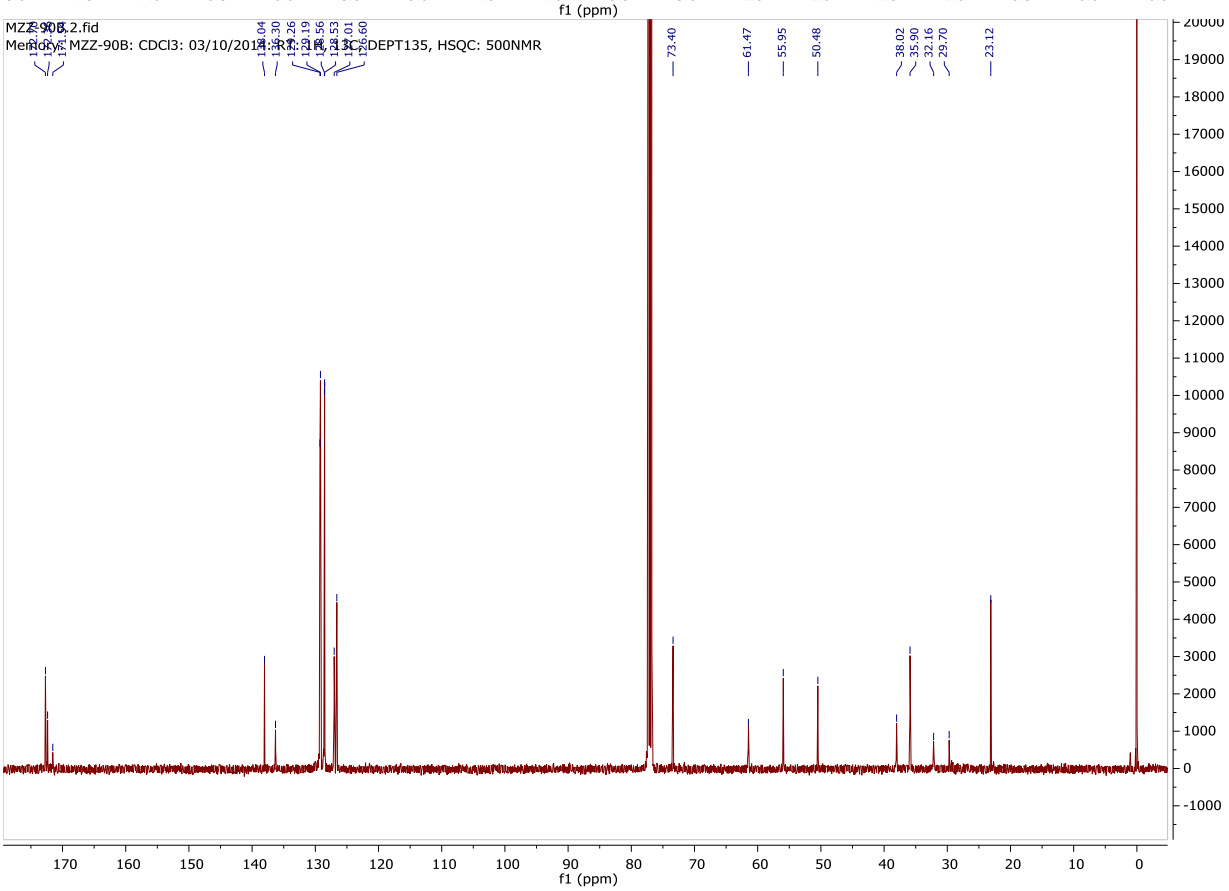
57.41
51.94
42.29
41.51
36.27
35.40
29.39
f1 (ppm)


MZZ-90B.1.fid
Memory: MZZ-90B: CDCl3: 03/10/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
OH
H
N
O
NH
O
Ph
N
O
O
65
11.60
0.86
0.78
0.86
1.00
0.89
2.81
7.06
3.00
f1 (ppm)


MZZ-90B.2.fid
Memory: MZZ-90B: CDCl3: 03/10/2014: RT: 1H, 13C, DEPT135, HSQC: 500NMR
73.40
61.47
55.95
50.48
38.02
35.90
32.16
29.70
23.12
f1 (ppm)

APPENDIX III

1. The *in vitro* single-cycle phenotypic assay for HIV-1

The HIV Virology Section at the National Institute for Communicable Diseases (NICD) employs a vector-based HIV-1 phenotypic assay for drug screening and resistance testing. For the assay, three vectors (plasmids) are used to create virus-like particles by transfection of mammalian cells (293T cells). Transient expression of the vectors in the cells produce the RNA of firefly luciferase that is packaged into a virus-like particle that contains the HIV-1 structural components (gag) and the three HIV-1 enzymes (reverse transcriptase, protease and integrase). Vesicular stomatitis virus G proteins are incorporated into the envelope surrounding the virus-like particle and allows for viral entry into the host cell.

Upon viral entry, the luciferase RNA is reverse transcribed by HIV-1 reverse transcriptase and the resulting cDNA integrated into the cell's genomic DNA by HIV-1 integrase. The assay allows for only a single integration event per virus-like particle. Once integrated, the luciferase gene is expressed and produces functional firefly luciferase that can be detected by the addition of a substrate. The luminescence that is generated during conversion of the substrate can be quantified on a luminometer, and is directly proportional to the amount of virus-like particles that infected the cell. If inhibitors are present that target either the reverse transcriptase or integrase, a reduced luminescence will be observed. Since HIV-1 protease is involved in the maturation of virions, protease inhibitors need to be incorporated into the virus-like particle during transfection and before infection. The inhibition of protease will result in a decreased luminescence after infection.

Procedure for screening the toxicity of compounds:

Before determining the antiviral activity of compounds, it is important to assess whether they are toxic to the cells that are used in the assay. Cell death, brought on by toxic compounds, would lead to a reduced luminescence in the drug screen assay and could be mistaken for inhibition. Such "false inhibitions" can be prevented by testing over non-toxic concentrations. To perform a toxicity screen, serial dilutions of a compound is prepared in the wells of a 96-well culture plate. After addition of 293T cells, the plate is incubated for 48 hours at 37°C under 5%

CO_2 and a humidified atmosphere. A medium control, which contains no compound, is included to represent full (100%) cell viability. Cell viability is then assessed in the all wells using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega) reagent. After approximately 1.5 – 2 hours of incubation at 37°C under 5% CO_2 and a humidified atmosphere, the absorbance of the wells are measured on a 96-well plate reader at 490 nm. The viability of the cells in each well is calculated relative to medium control. Activity screens are performed at concentrations with ≥ 80% cell viability.

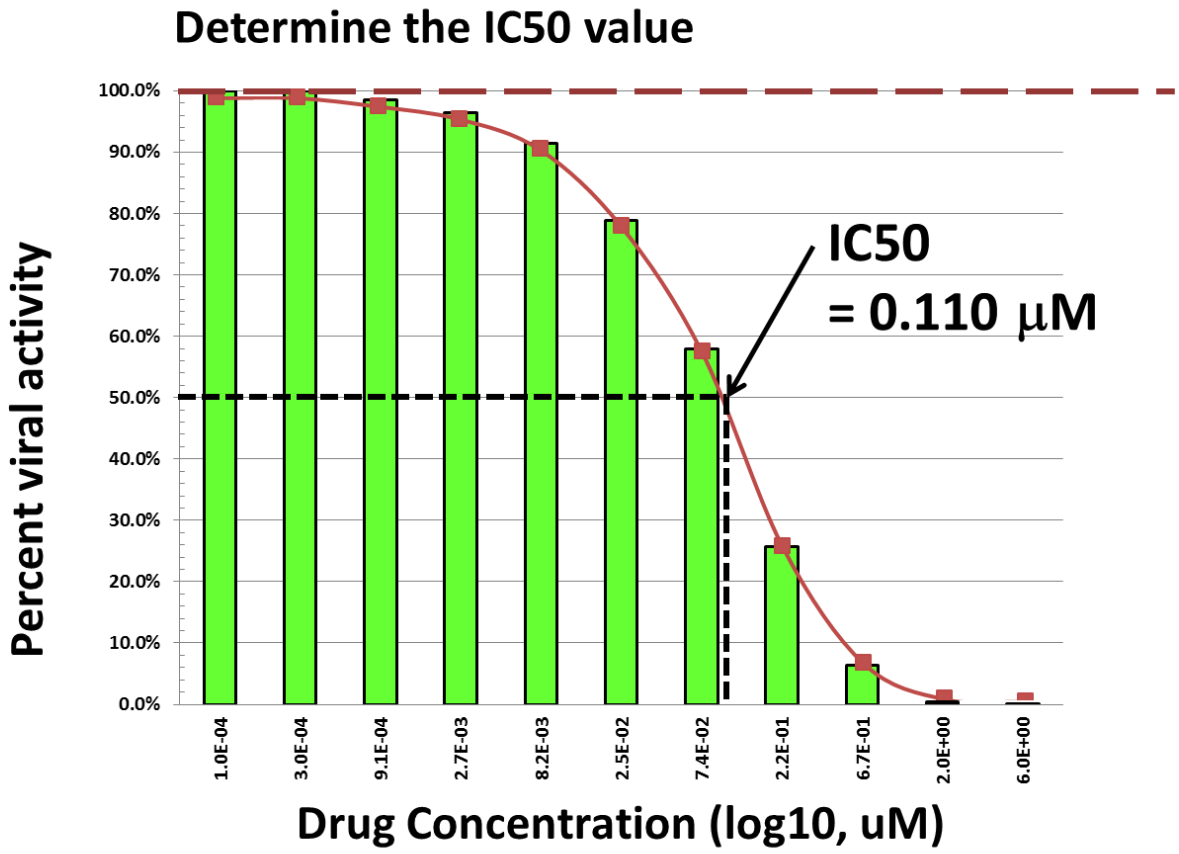
Procedure for screening potential protease inhibitors:

The transfection of 293T cells is performed 24 hours prior to the start of the assay. On the day of the assay, serial dilutions of potential protease inhibitors are prepared in the wells of a 96-well culture plate (i.e the drug plate). The transfected 293T cells are harvested and added to the drug plate. A medium control, which contains no inhibitor, is included to represent full (100%) viral activity. The plate is incubated for 24 hours at 37°C under 5% CO_2 and a humidified atmosphere. Supernatant is then transferred from the drug plate to a new 96-well culture plate (i.e. an indicator plate) that contains 293T cells in medium only. The plate is incubated for 48 hours at 37°C under 5% CO_2 and a humidified atmosphere. After incubation, the luciferase substrate (Bright-Glo™ Luciferase Assay System, Promega) is added to the wells of the plate and incubated at room temperature for 2 minutes. The contents of the wells are mixed by pipetting and transferred to a black 96-well plate. Luminescence is then measured and the amount of inhibition assessed.

Assay for inhibition:

During each assay, a medium control is included that will indicate the luciferase signal of a fully active virus. This is used to calculate the viral activity in the wells that contain the potential inhibitor. A decrease in luciferase activity (luminescence signal), relative to that of the medium control, indicates viral inhibition. A lack of inhibition is observed when the activities are similar. To quantify the amount of inhibition, the concentration needed to inhibit 50% of the luciferase activity (IC_{50}) is determined for the medium control and potential inhibitors. The fold change (FC) in difference between the IC_{50} of the medium control and potential inhibitors is then calculated. A FC=1 indicates similar IC_{50}'s and no inhibition, while FC>1 shows inhibition. Due to

variation in the assay, a FC≤3 does not indicate a significant amount of inhibition and such compounds can be considered inactive.



2. SYBR Green antimalarial drug sensitivity assay

The *P. falciparum* (FCR-3) strain was cultured *in vitro* at 37°C in 3% O_2, 5% CO_2, 92% N_2 and adjusted to a 0.625% parasitaemia/ 1.25% haematocrit before being incubated along with the compounds for 72 hours. The plates were then frozen overnight and once thawed, they were incubated in the dark, for 1 hour at room temperature with buffered SYBR green I. The fluorescence was read in a microplate reader with excitation and emission wavelength bands centered at 485 and 528 nm, respectively. The percentage inhibition was calculated taking the untreated and dihydroartemisinin control into account. Dihydroartemisinin was used as the positive control. At least three independent experiments were conducted.