

**SYNTHESIS OF PEPTIDOMIMETIC COMPOUNDS AS HIV-1
PROTEASE INHIBITORS**

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By

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Abstract

This research project has involved the design, synthesis and evaluation of novel peptidomimetics compounds as HIV-1 protease inhibitors. Here is presented one-step, two-step and three-step syntheses and the *in vitro* bio-assay studies of a series of fully characterized peptidomimetics as HIV-1 protease inhibitors candidate using the shortest and most cost effective synthetic routes.

The first series of compounds were accessed via a synthetic elaboration of Morita-Baylis-Hillman adducts by a Michael addition with benzylamine, proline or glycine esters to afford a series of β -amino- β' -hydroxycarboxylate esters in moderate to good yields. Base-catalyzed cyclization of non-benzylated aza-Michael adducts afforded a series of coumarin-3-hydroxy-2-methylenepropanoate esters in moderate yields.

The uncatalyzed direct amidation of diethyl tartrate/tartaric acid and tartaric acid osazone with selected amines/amino acids afforded a series of C_2 -symmetrical and unsymmetrical 1,2-dihydroxycarboxylates in moderate to very high yields. All the synthesized compounds were fully characterized using spectroscopic techniques. These conjugates, designed as potential HIV-1 inhibitors, were tested against the HIV-1 protease enzyme. A number of these ligands have exhibited inhibition levels and IC_{50} values comparable to ritonavir, permitting, therefore, their identification as lead compounds for the development of novel inhibitors.

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List of Abbreviations

AIDS	: Acquired Immunodeficiency Syndrome
AA	: Amino acid
Bn	: Benzyl
CHN	: Elemental analysis
DABCO	: 1,4-diazabicyclo[2.2.2]octane
DBU	: 1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	: Dichloromethane
DMAP	: 4-dimethyl aminopyridine
DMSO	: Dimethylsulfoxide
2D	: Two dimensional
DOPA	: 3,4-dihydroxyphenyl alanine
EWG	: Electron-withdrawing group
FT-IR	: Fourier-transform infrared spectroscopy
3HQ	: 3-quinuclidinol
5-HT3	: 5-Hydroxytryptamine receptor 3 receptor
5-HT4	: 5-Hydroxytryptamine receptor 4 receptor
HTS	: High-throughput screening
HAART	: Highly active antiretroviral therapy
HIV	: Human Immunodeficiency Virus
HR-MS	: High Resolution Mass spectrometry
MBH	: Morita-Baylis-Hillman
NMR	: Nuclear magnetic resonance
m.p.	: Melting point
MS	: Mass spectrometry
M.V.K.	: Methyl vinyl ketone
NCI	: National Cancer Institute
OMe	: Methyl ester
OEt	: Ethyl ester
PR	: Protease
rt	: Room temperature
SD	: Standard deviation
STD	: Saturation Transfer Difference

TLC : Thin Layer Chromatography
Prep-TLC : Preparative Thin Layer Chromatography
WHO : World Health Organization

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Chapter 1: Introduction

1.1. Background on the Human immunodeficiency virus

1.1.1. Origins

The origins and the probable causes of the virus currently known as the human immunodeficiency virus (HIV), the main causative agent of AIDS, has been controversial since the beginning of the 1980's meanwhile the number of deaths has been growing. Some researchers have believed the HIV to originate from “hunting and/or eating” of chimpanzees with Simian Immunodeficiency Virus (SIV) from the Democratic Republic of Congo (DRC) or from other West African countries (Figure 1).¹



Figure 1: Speculated origin country of the HIV (used with permission from <https://thenypost.files.wordpress.com/2015/02/22-1n019.jpg?quality=90&strip=all>) (accessed on 07/07/2017).¹

The increased occurrence of the *Pneumocystis carinii* pneumonia and Kaposi's cancer, in conjunction with other opportunistic infections, was observed in the U.S.A. in 1981 among previously healthy homosexual individuals and some drug abusers.²

It was found that immunosuppression was the main cause of these unusual cases of diseases and it was therefore identified as Acquired Immune Deficiency Syndrome (AIDS).³

The causative agent of AIDS was later identified as a human retrovirus in 1983 when it was isolated for the first time in France from a patient having several lymphadenopathies (which had been directly related to AIDS).⁴ Another virus, termed HIV type-2, was isolated from some individuals from West Africa and was found to have similarities with HIV type-1.⁵ It has been established that both HIV subtypes can cause AIDS. However, HIV type-2 has a longer pathogenic path.

Research has established the existence of similarities between the HIV and the SIV diagnosed from chimpanzees, through a comparative study of the phylogenetic tree analysis of HIV⁶ and other similar viruses isolated from nonhuman primates.⁷ HIV type-1 has been found to be directly related to the strain of the SIV from chimpanzees whereas HIV type-2 has been found to be directly related to the strain of SIV isolated from sooty mangabey monkey (*Cercocebus atys*).^{6,7} The genome homology of both HIV-1 and HIV-2 are roughly 40%.⁸ However, it has been speculated that both viruses came from African primates: the African chimpanzee (*Pan Troglodyte troglodytes*) for HIV-1⁹ and the sooty mangabey (*Cercocebus atys*) for HIV-2 respectively. Nevertheless, Gao, F. and co-workers speculated that the origin of HIV-1 remained uncertain.¹⁰

Both HIV-1 and HIV-2 have been proven to cause the same syndrome, which was then termed in late 1986 as the Acquired Immune Deficiency Syndrome (AIDS).¹¹ Different terms have been used to identify this virus such as "human retrovirus" (1983-1984).¹¹ Other terms include "Human T lymphotropic virus III (HTLVIII)"⁶ "lymphadenopathy-associated virus (LAV)" as well as the "AIDS-associated retrovirus (ARV)."¹¹

1.1.2. Infection and transmission

Individuals infected by HIV can infect others if treatment with antiretrovirals (ARVs) is not started as soon as possible to slow down its replication. HIV can be mainly transmitted among individuals by unprotected sexual intercourse or through blood contact from an infected person.¹² Upon entry, the HIV virus tends to infect and

deplete the helper T lymphocytes (known to regulate the system's immune response), resulting in a weakened immune system.¹³ Once the immune system is weakened, the patient becomes susceptible to opportunistic infections, leading to the development of AIDS.¹⁴ Owing to the key role played by the helper T-lymphocytes, onocytes and the viral infections within the macrophages, they have been both identified as targets for the HIV-1 amongst others.¹⁵

It has been observed that the helper T-lymphocyte surface marker (also called CD4+), acts as the receptor for the HIV viral surface glycoprotein gp120.¹⁶ Several tissues within the patient can also be infected, including in the central nervous system where a reservoir of the virus can be formed.¹⁷

It has been shown that the CD4+ count (concentration) in the blood is crucial in the determination of the progression of the infection. The normal concentration of CD4+ in the blood has been established to be 800-1200 cells/mm³; however, in the final phase of the disease, it is lower than 200 cells/mm³ of blood.¹⁸

At this very low concentration, the infection rapidly progresses toward the last stage, referred to as "AIDS" where the chance of survival becomes slight, as shown in Figure 2 below, which illustrates the different stages of HIV progression towards AIDS and the consequent failure of the immune system).¹⁸

In most cases, once the infection establishes itself (first infection phase) within the patient (or host), symptoms including headache, fever, lymphadenopathy, pharyngitis, rash, heart burn and gastrointestinal disturbance, can be manifested.¹⁹ This is followed by a subsequent asymptomatic phase, which can take about ten years before reaching the final/vulnerable stage where by the patient develop muscular wastage and many other related AIDS issues.²⁰

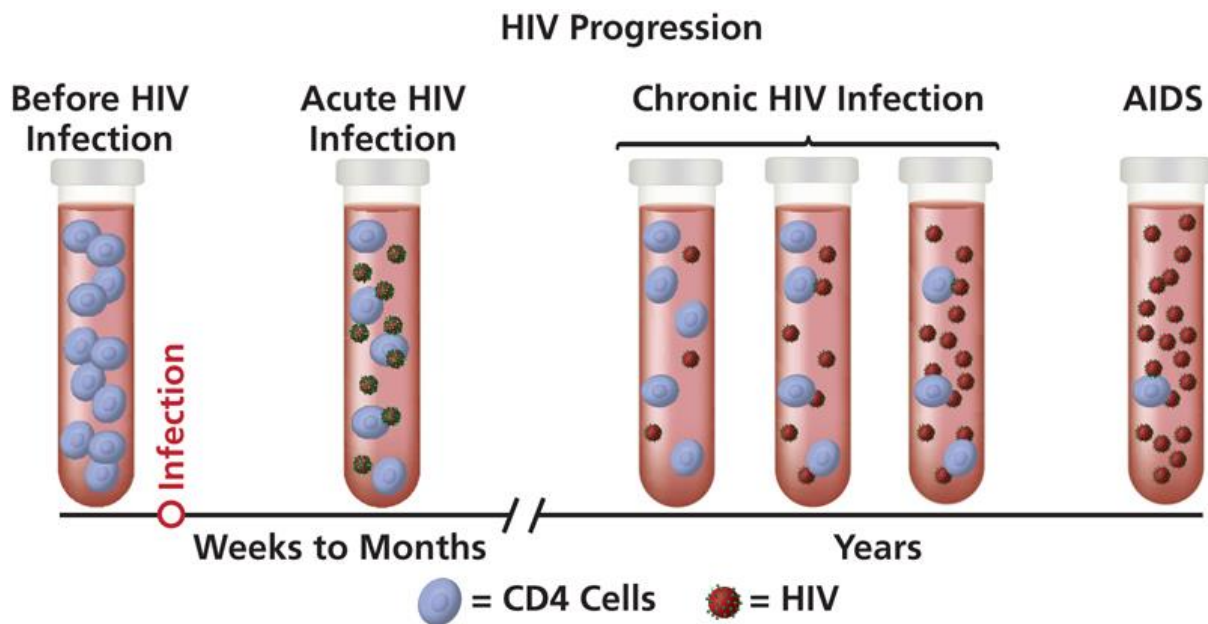


Figure 2: Different stages of the progression of HIV¹⁸ (used with permission from the cited reference).

1.1.3. Mortality and societal effects

Despite so many efforts and positive progress towards finding an effective cure or vaccine to eradicate HIV globally, up to now there is not yet a specific cure for this deadly virus that has been killing millions of people globally.²¹

According to the WHO report (2016), more than 76.1 million individuals have been infected globally with HIV-1 (since the start of the HIV epidemic), with an alarming 35 million reportedly dead already (Figure 3).²² The same source reported also that in 2016, about 0.8% (0.7-0.95) of all mature people aged between 15 and 49 years old were reported infected and/or living with HIV-1 globally. Sub-Saharan countries have been reported to be the most affected as shown in Figure 4 below.

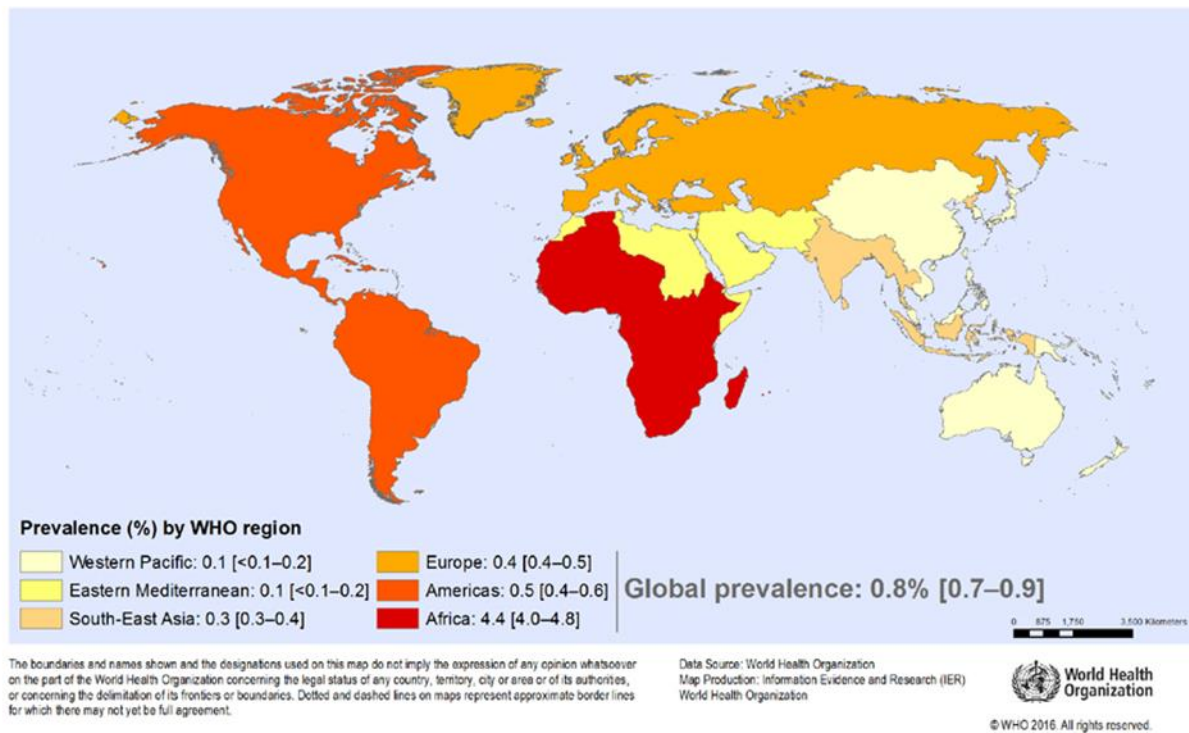


Figure 3: Adult HIV prevalence (15-49 years old) in 2015 by WHO region (adapted from http://www.who.int/gho/hiv/epidemic_status/prevalence/en/ (06/03/2017)).²²

UNAIDS Gap and the Global AIDS Response Progress Reports indicated that of all African countries, despite having the largest ART program worldwide, South Africa has been found to be the most infected country in 2015 with an estimation of 7 million people reportedly living with HIV-1.²³ Additionally, it was confirmed by the same source that during the same year, about 380 000 new infections were reported and 180 000 people died of AIDS.

The HIV-1 has affected and negatively affected mostly the poorest countries. Since the virus affects the whole immune system, infected individuals with HIV-1 becomes susceptible to many other diseases, increasing therefore their morbidity and mortality percentage.²⁴

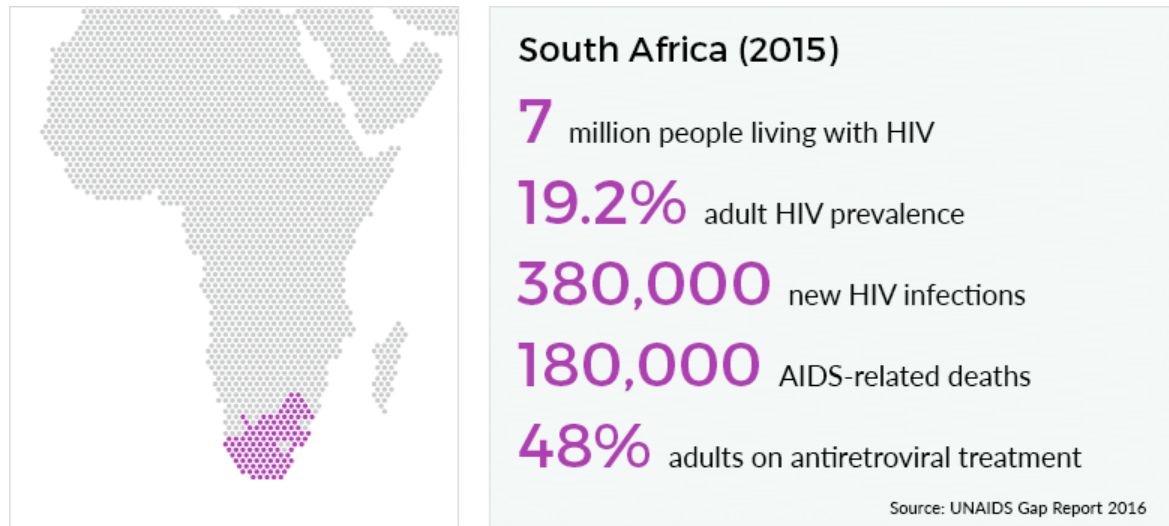


Figure 4: Reported HIV and AIDS in South Africa in 2015.²³

1.1.4. The lifecycle and structure of the HIV

As shown in Figure 5 below, the replication of HIV in a host occurs in seven steps. As soon as the virus enters the host, HIV viral proteins bind or attach themselves to receptors on the surface of the CD4+ cells.²⁵ This is followed by the entry (fusion) of the HIV virus into the CD4+ cells whereafter the virus releases the reverse transcriptase enzyme, allowing it to transform its RNA (its genetic content) into DNA and therefore facilitate its entry into the CD4+ cell's nucleus where it combines with the cell's DNA. Upon the entry into the nucleus of the CD4+ cell, the viral DNA encodes the long chains of the HIV polyproteins, which are used for replication. Newly synthesized polyproteins (Gag and Gag-Pol), relocate to the surface of the cell host where they assemble into immature HIV virions.²⁵

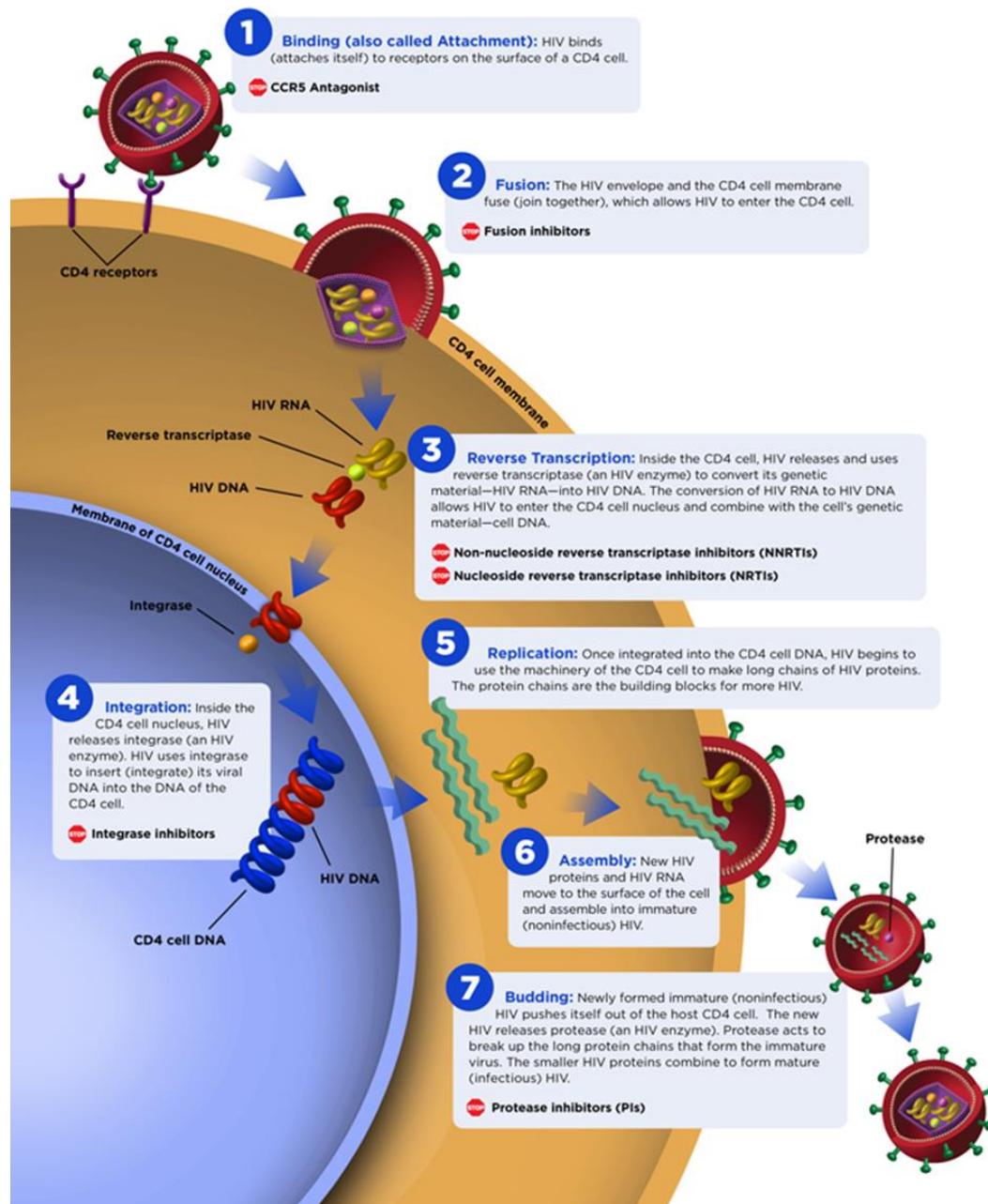


Figure 5: The HIV life cycle (adapted from <https://aidsinfo.nih.gov/images/factsheet/HIV-Lifecycle-> (accessed on 06/03/2017).²⁵

In the last step (also called Budding step), these immature virions come out of the host CD4+ cell. The viral protease then cleaves the polyproteins into the various viral proteins required, thus generating mature infectious HIV virions.²⁶ Once matured, HIV virions can then replicate and infect new cells and so on.

Research has established that HIV-1 and HIV-2 are RNA viruses, belonging to the *Retroviridae* family of retroviruses whose genome comprises duplicated copies of positive single-stranded RNA. Catalyzed by viral enzyme reverse transcriptase, this

RNA in return is converted into the DNA as soon as the cells become infected by the retroviruses. These retroviruses have been classified into seven genera, whereby the genus *Lentivirus* is generally characterized by a slow progress of disease after the infection phase. Therefore, HIV has been termed a “lentivirus” owing to the fact that its latency period can generally last a long time.²⁷

1.1.5. Different targets for anti-HIV chemotherapy

Within the replicative cycle, there is no “specific stage” as such to be considered as the most important because all of them are to be considered as major targets in the search for effective anti-HIV chemotherapy.²⁸ However, the reverse transcriptase and the protease phases have attracted much research attention in the development of potential anti-HIV therapies.²⁹

1.1.6. HIV-1 protease enzyme: structure and function

The HIV-1 (PR) has been identified as an aspartyl PR, which is crucial for the life cycle of HIV. As proposed for the first time by Mariusz Jaskolski *et al.*³⁰ PR enzyme catalyze the cleavage polyproteins such as the Gag and Gag-Pol around their scissile peptide bonds to generate matured infectious HIV virions. The interaction of a substrate with the PR has been shown to reduce the activation energy of the peptide bond cleavage reaction, by stabilizing the transition state.³¹ It has been established that the half-life of a peptidic bond at room temperature and neutral pH conditions has been estimated to be about 500 years.³² The PR enzyme’s catalytic ability allows the activation of the amide bond to facilitate cleavage reactions to occur rapidly at room temperature.

In 1985, it was suggested by Toh *et al.*, referencing the characteristic Asp-Thr-Gly sequence, that the PR of HIV belongs to the family of aspartic proteases.³³ This hypothesis was later on confirmed through the inhibition by pepstatine A (a selective aspartic protease inhibitor),³⁴ and also by the mutagenesis of the active site Asp 25, which led to elimination of the catalytic activity.³⁵ Aspartic proteases have been identified and/or found in fungi, in plants, and also in vertebrates. The aspartic

PR include: i) pepsin, ii) cathepsin D, iii) renin, iv) chymosin, v) penicillopepsin, and vi) Rhizopus pepsin. It has been shown that these different classes are “two-domain enzymes” and contain more than 300 residues, with the Asp-Thr-Gly sequence within one of the domains forming the active site.³⁶

X-ray crystallographic data obtained for the first time by Navia *et al.*³⁷ from Merck laboratories, and the more accurate HIV PR enzyme structure reported by Kent and coworkers³⁸ have presented HIV-1 PR as a homodimer, made up of two identical subunits of 99 amino acids, with one active site which is C_2 -symmetrical in the free form without a substrate. The active site is characterized by two Asp-Thr-Gly sequences formed by Asp25, Thr26 and Gly27 of each monomer respectively.

Within the homodimer, each monomer has an extended β -sheet region known to be a glycine-rich loop or flap which facilitates the binding of the substrate into the active site of the PR enzyme via each of the aspartyl residues, Asp-25 and Asp-25' located at the bottom of the cavity.³⁸ Therefore, the inhibitor or the substrate binds onto the aspartyl residues in its extended conformation, in which it's the interactions with the different amino acid side chains determines the enzyme's specificity.³⁹

Figure 6 below illustrates the standard nomenclature of a PR enzyme based on the conventional nomenclature of Schechter and Burger.⁴⁰ From the central aspartate core units within the active site of the PR enzyme, S1, S2, and S3 as well as S1', S2' and S3' correspond to different subsites. Using standard nomenclature, the S1 and S'1 (S2 and S'2, etc.) subsites are structurally equivalent. Subsites S1 and S1' and S3 and S3' are hydrophobic, except for Asp-29, Asp-29', Asp-30 and Asp-30'. The S3 and S3' are directly exposed to water molecule site.⁴¹ However, subsites S2 and S2' can possibly accommodate both polar and hydrophobic side chains. According to the same convention, P1, P2 and P3 and P1', P2' and P3' correspond to the side-chains of the PR substrate or the inhibitor. These side-chains are designated P1, P2 etc., outward from the scissile bond going towards the amino end of the enzyme, and P1' to P3' from the scissile bond going towards the carbonyl end.⁴⁰

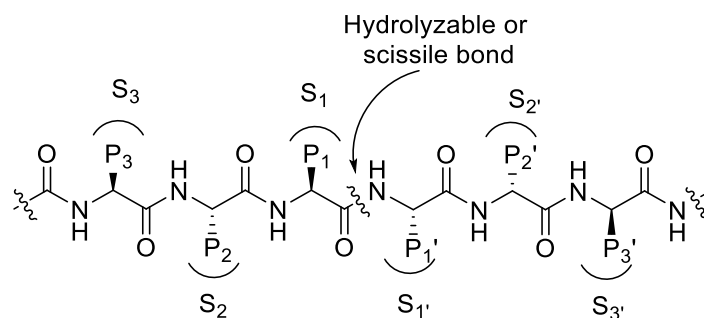


Figure 6: The standard nomenclature of subsites in the PR enzyme according to Schechter and Burger: P1---Pn', P1'---Pn' designates amino acid residues of peptide substrates. Their corresponding binding sites on the PR are referred to as S1---Sn', S1'---Sn' subsites.⁴⁰

More than 140 structures of HIV-1 PR, including its mutants and complexes with various inhibitors have been reported in the literature.⁴² The structure of the HIV PR enzyme homodimer complexed with inhibitor TL-3 (PDB ID: 3TLH),⁴³ is illustrated in Figure 7. In the presence of a substrate or inhibitor, the two molecular flaps of the PR enzyme move up to 7 Å and close over the substrate within the active site.

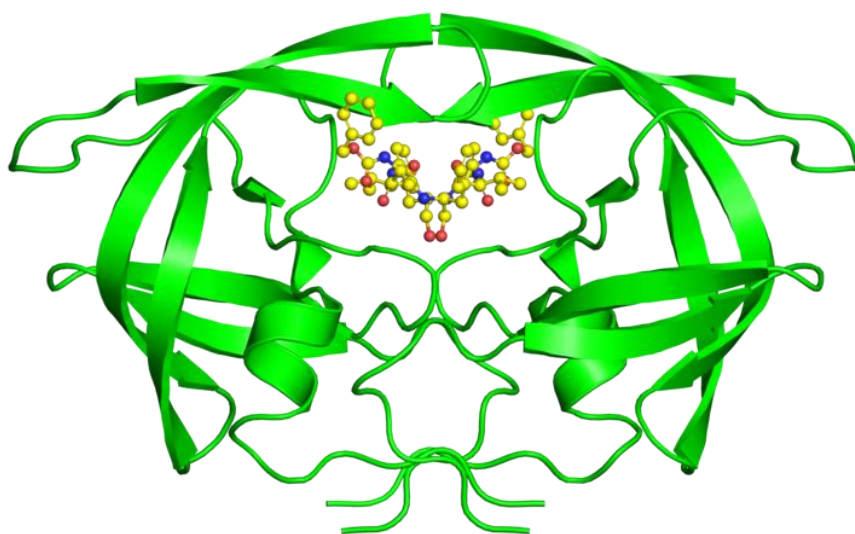


Figure 7: A schematic structure of HIV PR when complexed with a substrate TL-3 (PDB: 3TLH).⁴³

1.1.7. Mechanism of action of HIV protease

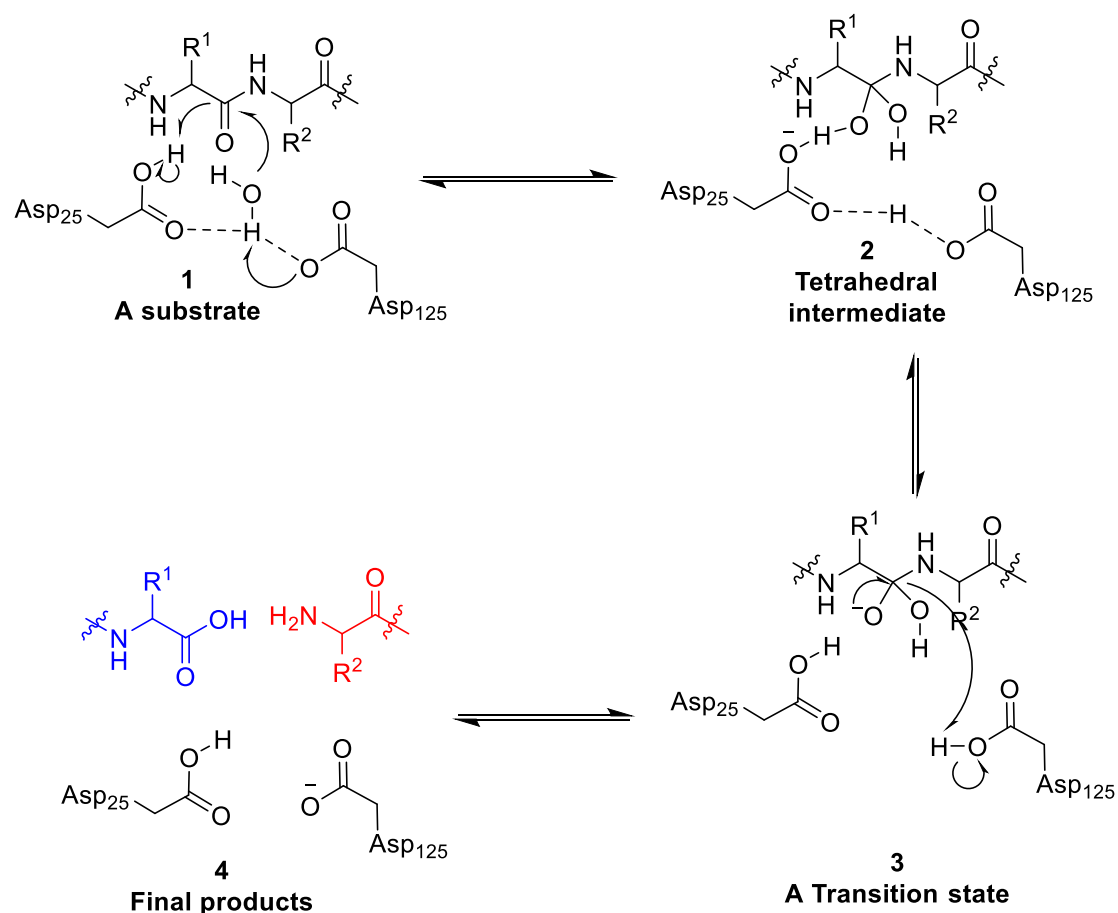
Protease enzymes are crucial in many biological processes, where they selectively catalyze the hydrolysis of peptide bonds through two different mechanisms, allowing their mechanistic classification into two broad classes.⁴⁴ The first class of PR

enzymes uses an activated water molecule in such a way to attack the scissile amide bond of the substrate. A zinc cation (the zinc metalloproteinases) or the two aspartyl β -carboxy groups situated at the active site (the aspartate proteases) have been proposed to promote the water molecule activation.

The second class is that to which HIV-1 PR belongs. These PR enzymes catalyse the selective hydrolysis of the peptide's amide bonds using a nucleophilic atom of an amino acid side chain to commence the hydrolysis. Through the second mechanism, the nucleophilic atom, (which can be a hydroxy group or a thiol), is firstly activated by another amino acid side chain. The resulting activated nucleophile in return attacks also the carbonyl of the scissile amide bond, forming an ester or a thioester acyl intermediate, which is then hydrolyzed by a water molecule, generating hydrolysis products.

The HIV-1 protease cleaves the Gag and Gag-Pol polyproteins proteolytically at specific sites of polyproteins and several cleavage mechanisms have been proposed. Many hypotheses have been made by researchers concerning the PR's mechanism of action. A schematic representation of the most generally accepted mechanism is presented in Scheme 1 below⁴⁵ based on the *Rhizopys chinensis* aspartic PR's crystallographic structure.

In this mechanism, the HIV PR hydrolyses the substrate **1** at its amide bond through the hydration process, which uses a "nucleophilic water molecule" between the two side-chains of the aspartic acid residues 25 and 125, which acts in conjunction with an aspartic acid, hydrolysing the peptide's scissile bond. This process results in a tetrahedral intermediate **2**, which gives the transition state **3** before generating the final products **4** through specific rearrangement.



Scheme 1: Proposed mechanism for HIV PR hydrolysis of a peptide substrate.⁴⁵

As illustrated in the study by conducted by Suguna *et al.*,⁴⁶ (Figure 8), when the PR enzyme was complexed with a reduced peptide inhibitor, the enzyme structure was modified as was its conformation, especially around the two flaps. They folded themselves over (closed position) this inhibitor, creating a “tunnel -like structure.” This movement is favoured by the hydrogen bonding formed between the flap residues Ile 50/50' and the water molecule, which is also H-bonded to two carbonyls within the inhibitor.

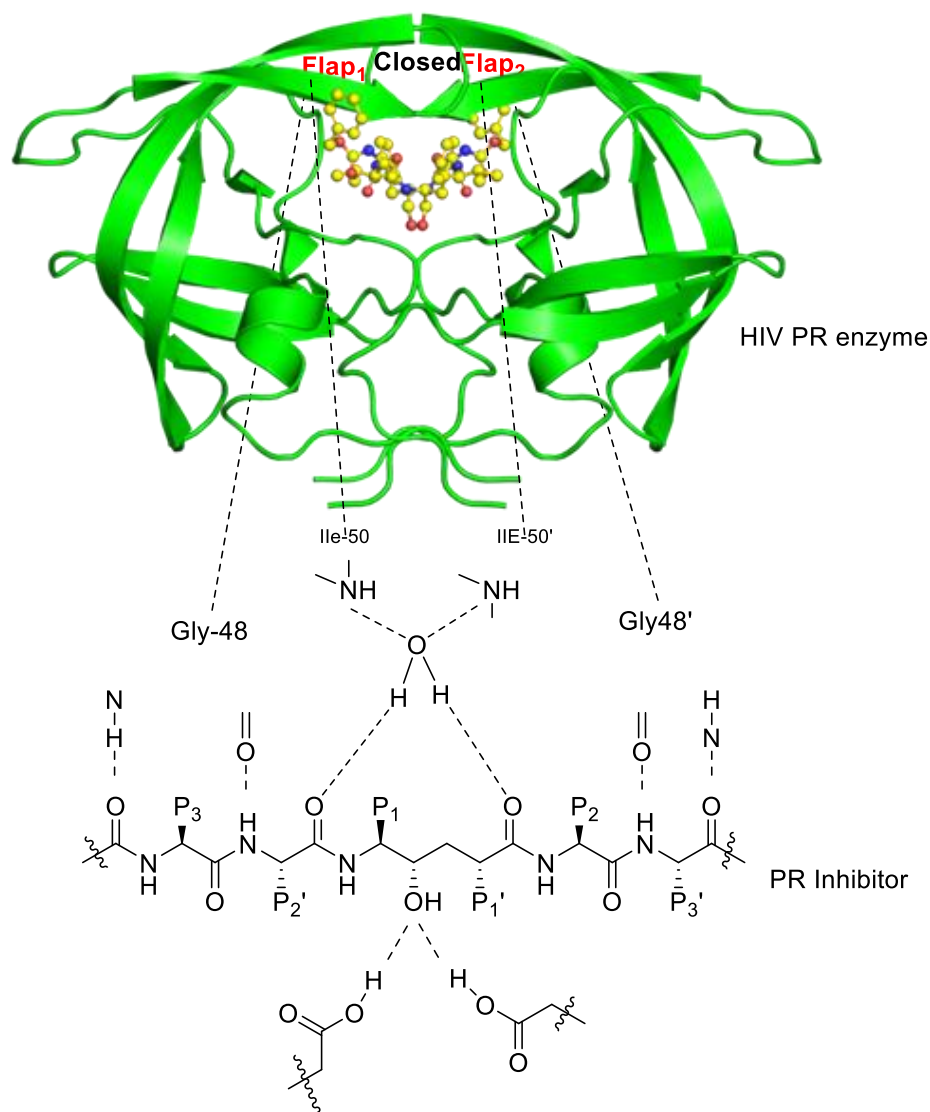


Figure 8: The two flap regions of HIV PR and their hydrogen bonding.⁴⁶

1.2. Rational Design of PR inhibitors

1.2.1. Current state of HIV-1 PR inhibitors

Techniques including the nuclear magnetic resonance (NMR), protein crystallography, and computational methods, have been intensively used in the structure-assisted rational drug design and discovery.⁴⁷ Their use has resulted in a better understanding of the PR enzyme's structure and mechanism of action, which have been used for the past decade in the rationalization of the design and new drugs development, based on peptide isosteres.⁴⁸ Some of these compounds

include statin and its analogs or cyclic urea incorporating the C₂-symmetrical scaffold.^{47,48} A few of the resulting compounds have been approved as anti-HIV/AIDS therapies by the FDA, and are further discussed in the following sections.

Studies have shown that, due to its key role during the replication cycle, any mutation and/or inhibition of HIV-1 PR's active site would result in a total blockage or disruption of the virus's ability to replicate and infect more cells. This makes HIV-1 PR one of the most significant targets for the development of anti-HIV/AIDS's chemotherapy as suggested for the first time by Kramer *et al.*⁴⁹ These authors observed that mutation of the pol-gene in the PR region prevented cleavage of the Gag polyprotein a necessary step for the maturation of HIV virion.

HIV-1 PR has many similarities³³ with other aspartic PRs, including mammalian enzymes such as pepsin, renin, and chymosin.³⁶ Pepstatin, known to be an inhibitor of mammalian aspartic PRs,⁵⁰ has been identified as an inhibitor of HIV-1 PR and other retroviral PRs, although it can be inactivated by active-site mutation.⁵⁰

It has been established that inhibition of the HIV-1 PR would lead to the formation of immature non-infectious virions and this has attracted great attention from the pharmaceutical industry, resulting therefore in several peptidomimetic and non-peptide PR inhibitors being developed by researchers.⁵¹ An exhaustive and more detailed list of all currently approved (up to date) peptidomimetics inhibitors is shown in Figure 9 below, with the latest ones recently approved in 2018.⁵¹

FDA Approval of HIV Medicines

'80- '84	1981 First AIDS cases reported in the United States				
'85- '89	1987 Zidovudine (NRTI)				
'90- '94	1991 Didanosine (NRTI)	1992 Zalcitabine (NRTI)	1994 Stavudine (NRTI)		
'95- '99	1995 Lamivudine (NRTI) Saquinavir (PI)	1996 Indinavir (PI) Nevirapine (NNRTI) Ritonavir (PI)	1997 Combivir (FDC) Delavirdine (NNRTI) Nelfinavir (PI)	1998 Abacavir (NRTI) Efavirenz (NNRTI)	1999 Amprenavir (PI)
'00- '04	2000 Didanosine EC (NRTI) Kaletra (FDC) Trizivir (FDC)	2001 Tenofovir DF (NRTI)	2003 Atazanavir (PI) Emtricitabine (NRTI) Enfuvirtide (FI) Fosamprenavir (PI)	2004 Epzicom (FDC) Truvada (FDC)	
'05- '09	2005 Tipranavir (PI)	2006 Atripla (FDC) Darunavir (PI)	2007 Maraviroc (CA) Raltegravir (INSTI)	2008 Etravirine (NNRTI)	
'10- '14	2011 Complera (FDC) Nevirapine XR (NNRTI) Rilpivirine (NNRTI)	2012 Stribild (FDC)	2013 Dolutegravir (INSTI)	2014 Cobicistat (PE) Elvitegravir (INSTI) Triumeq (FDC)	
'15- '18	2015 Evotaz (FDC) Genvoya (FDC) Prezcobix (FDC)	2016 Descovy (FDC) Odefsey (FDC)	2017 Juluca (FDC)	2018 Biktarvy (FDC) Cimduo (FDC) Ibalizumab (PAI) Symfi (FDC) Symfi Lo (FDC) Symtuza (FDC)	

Drug Class Abbreviations:

CA: CCR5 Antagonist; FDC: Fixed-Dose Combination; FI: Fusion Inhibitor; INSTI: Integrase Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; NRTI: Nucleoside Reverse Transcriptase Inhibitor; PE: Pharmacokinetic Enhancer; PI: Protease Inhibitor; PAI: Post-Attachment Inhibitor

Note: Drugs in gray are not available in the United States and/or are no longer recommended for use in the United States by the HHS HIV/AIDS medical practice guidelines. These drugs may still be used in fixed-dose combination formulations.



Figure 9: An updated list of all currently approved FDA peptidomimetics inhibitors of HIV-1 enzyme.⁵¹ (permission requested)

However, Figure 10 below shows chemical structures of some of peptidomimetics approved by the U.S.A. Food and Drug Administration (FDA) for the treatment of HIV infection. These include Norvir[®] (Ritonavir (**5**); approved in 1996),⁵² Invirase[®] (Saquinavir (**6**) approved in 1995)⁵³, Reyataz[®] (Atazanavir (**7**) approved in 2003). Also Agenerase[®] (Amprenavir (**8**) approved in 1999),⁵⁴ Kaletra[®] (Lopinavir (**9**), marketed with ritonavir approved in 2000),⁵⁵ Lexiva[®] (Fosamprenavir (**10**); approved in 2003) Crixivan[®] (Indinavir (**11**) approved in 1996),⁵⁶ Prezista[®] (Darunavir (**12**); non-peptidic PR inhibitor approved in 2006), Viracept[®] (Nelfinavir (**13**) a non-peptidic PR inhibitor approved in 1997),⁵⁸ and Aptivus[®] (Tipranavir (**14**) non-peptidic PR inhibitor approved 2005).

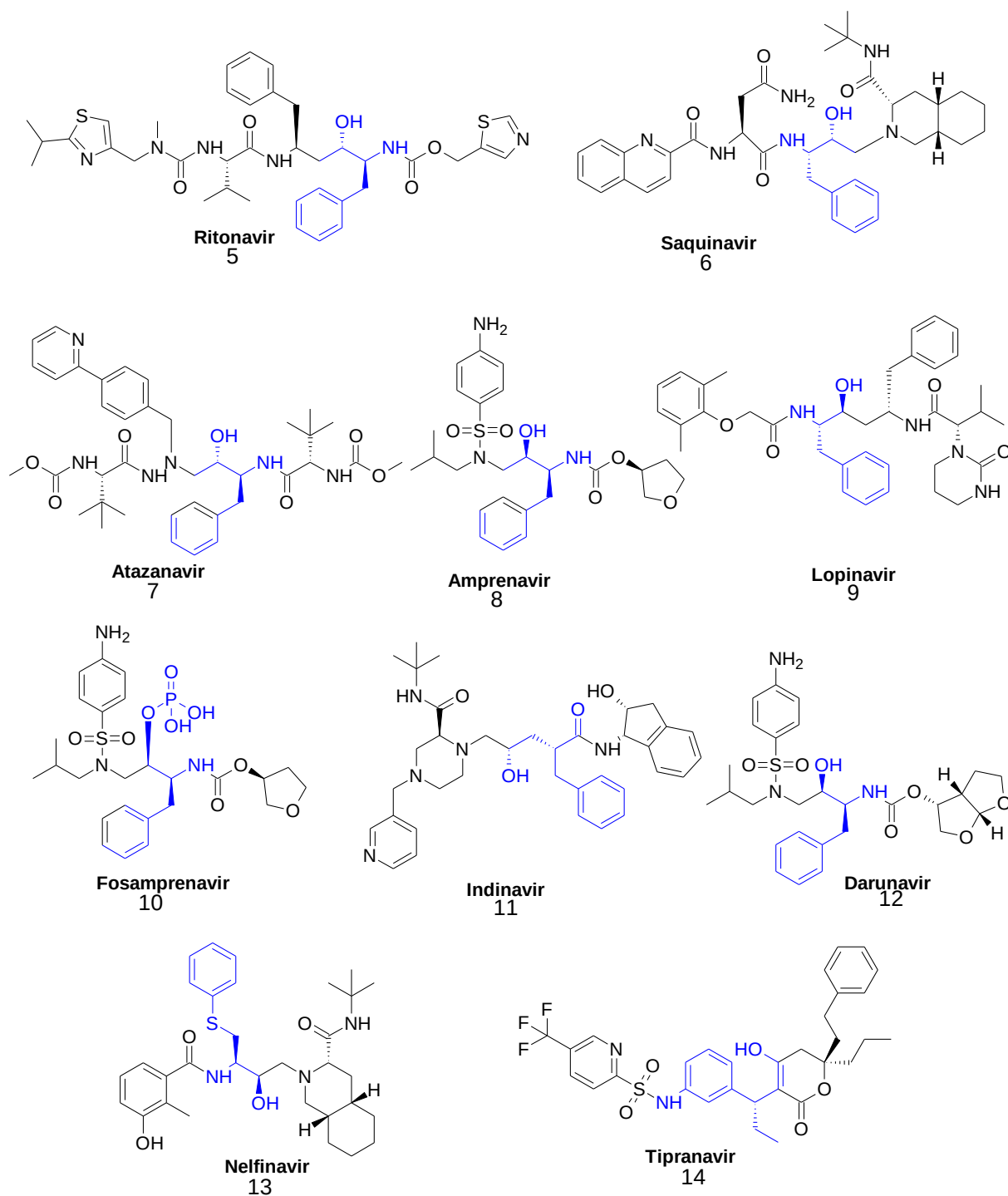


Figure 10: HIV-1 protease inhibitors currently approved by the U.S.A. Food and Drug Administration (FDA) for the treatment of HIV infection (see references 52-57).

Additionally, development and clinical testing of “therapeutic HIV vaccines” (which are able to not only prevent the diseases but also treat them too) are currently underway to assist HIV infected individuals.⁵¹ These therapeutic HIV vaccines are intended to (if effective) prevent the progression of the HIV to AIDS; to replace the

usage of long ARV therapies and therefore curing and/or removing totally the HIV from infected body. However, up-to-date, there is no FDA approved therapeutic vaccines.⁵²

These above-mentioned therapies, in use only for oral administration in the treatment of HIV, have been designed to block the replication of the virus thereby preventing progression of infection. However, their absorption within the gastrointestinal tract and their diffusion through anatomical barriers have been generally poor to moderate due to their extensive binding to plasma proteins, to cytochrome and to P-glycoprotein and the resulting efflux of the drug.⁵⁸ This results also in lowering the effective concentration of these therapies around the active sites (i.e. central nervous system)⁵⁹ or around genital tracts.⁶⁰ Therefore, to suppress the viral infection, the drug levels must be maintained above a suitable inhibitory concentration.

Lower drug concentrations in the target sites usually lead to an ongoing viral replication⁶¹, which results in the emergence of drug resistance. It has been recommended therefore that, with the exception of nelfinavir, these currently used therapies be co-administrated with a low dose of a potential inhibitor of cytochrome P450 (such as Ritonavir), in such a way to simplify the treatment regimens, thus improving the patient's adherence to therapy.⁶¹

Originally, Ritonavir was developed as “one of the most complex inhibitors inhibitor” of HIV protease. However, It has been not oftenly used nowadays due to its own antiviral activity, although still being used widely as a booster of other PR inhibitors. It was specifically designed to inhibit cytochrome P450-3A4 (CYP3A4), which is a particular liver enzyme, which metabolizes the PR inhibitors.⁶² Ritonavir's molecular structure specially inhibits CYP3A4 in such a way that even used in a very low dose, it can still able to enhance other PR inhibitors.⁶³

The rational design of cytochrome P450 inhibitors has recently attracted more attention.⁶⁴ This has resulted in several promising drugs being developed, with some currently in various clinical testing stages⁶⁵ viz Gilead Sciences' GS-9350⁶⁶, SPI-452 (developed by Sequoia Pharmaceuticals)⁶⁷, the TMC-41629 (developed by Tibotec)⁶⁸, the PF-03716539 (developed by Pfizer).⁶⁹

However, the co-administration of PR inhibitors with boosters has also been challenging due to side effects, which may be life threatening. Caution needs to be exercised for patients on HAART therapy involving pharmacokinetic boosters (such as hypolipidemics, antihistamines, or ergot alkaloids), and others therapies such as sildenafil, corticosteroids, or psychotropic agents.⁷⁰

Beside the prohibitive expense to those living in underdeveloped countries, tolerability (related to side effects), toxicity, and patients' adherence to these therapies have also been challenging. Research has shown that the side effects of the currently used inhibitors have often been more troubling than the symptoms of the HIV infection itself.⁷¹

Therefore, new approaches to the antiviral treatment, including novel HIV PR inhibitors, are needed to achieve a better control of HIV infection and/or replication, while sustaining therefore the patient's quality of life. These novel PR inhibitors must be developed with broad specificity (to overcome the drug-resistant HIV mutants) and must have better pharmacokinetic properties and lower toxicity.⁷²

Several factors have been determined to be crucial in the design of efficient protease inhibitors such as the incorporation of specific functional groups onto the scaffolds, which would interact with the hydrophobic and hydrophilic parts of the targeted molecule (TM).⁷³ This incorporation is done to improve both the pharmacokinetic as well as the pharmacodynamic properties of the new ligand or derivative. To improve absorption, the drug candidate should preferably be readily soluble in the aqueous medium which will facilitate its distribution within the host cells, but also sufficiently lipophilic to cross membranes.⁷⁴

Therefore, effective PR inhibitors should be designed in such a way to fit the size and shape of the enzyme's active site, with C₂-symmetrical structure since the PR enzyme itself is C₂-symmetric.^{37,38} This would cause the malfunction of the enzyme therefore blocking replication. Rational design of potent inhibitors necessitates information on molecular recognition indicators such as binding energy, molecular interactions and changes in conformation or stereochemistry. However, such properties can be obtained only through a structure-based drug design tools viz docking, structure-based virtual screening, and molecular dynamics studies. The

stereochemistry of a potential inhibitor ligand must complement the P1/P2 subsites of the protease enzyme.^{37,38}

1.2.2. Approaches used to rationally design PR inhibitors

1.2.2.1. Natural peptide modification approach

HIV-1 PR is an aspartic acid protease (AAP) and its mode of action is well known (**Section 1.1.8**), which also gives an idea of how it would interact with an inhibitor. This has inspired different strategies to design and synthesize several potential anti-HIV-1 PR inhibitors, including some of the currently approved by the FDA.

Strategically, a potential inhibitor of HIV-1 PR should have strong binding properties and also be stable to any proteolytic reaction. Finding such an inhibitor can include random and semi-random rational design strategies. The most obvious strategy, apart from random high throughput screening,⁷⁷ is to utilize the peptidic substrate as a template, with the scissile peptide bond substituted with a nonhydrolyzable hydroxyethylene or hydroxyethylamine core unit.

The incorporation of this isostere as the core unit is aimed to mimic the tetrahedral transition state of the PR catalysis or proteolytic reaction. However, the central hydroxyl group of the core unit of these inhibitors has been revealed by the crystal structures of HIV-1 PR enzyme, when complexed with these inhibitors, that is located between Asp-25 and Asp-25' and thus allowing therefore an electrostatic interaction of about 4 kcal mol⁻¹ between the two catalytic aspartates and the free hydroxy group (of the PR inhibitors).⁴¹

An example of how these strategies and/or modifications of the natural peptide have been used by researchers is given in Figure 11. The natural peptide (**15**), consisting of the C- and N-terminals, the backbone, and the side chain, each of which can be elaborated further in the discovery and development of potential peptidomimetics such as Ritonavir (**16**). The scissile bond of a natural peptide (highlighted in red) is replaced by a nonhydrolyzable hydroxyethylene or hydroxyethylamine moiety.

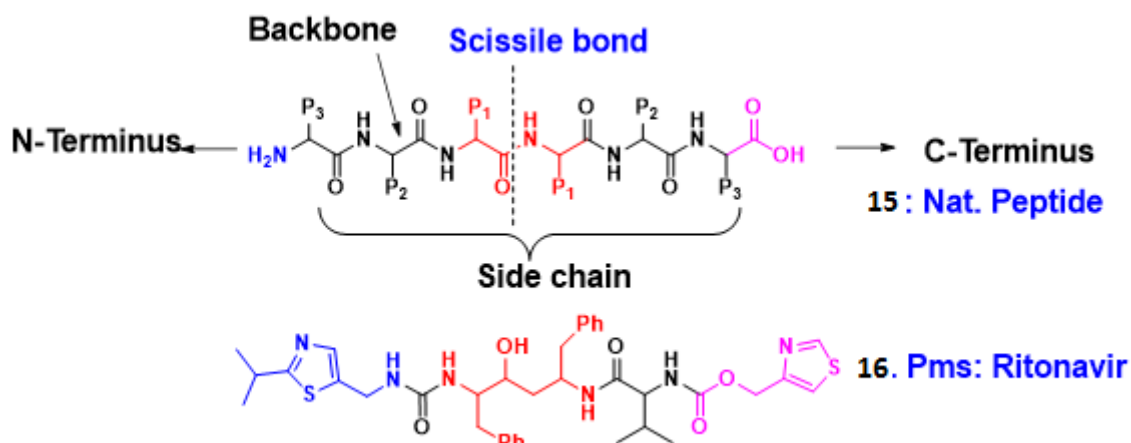
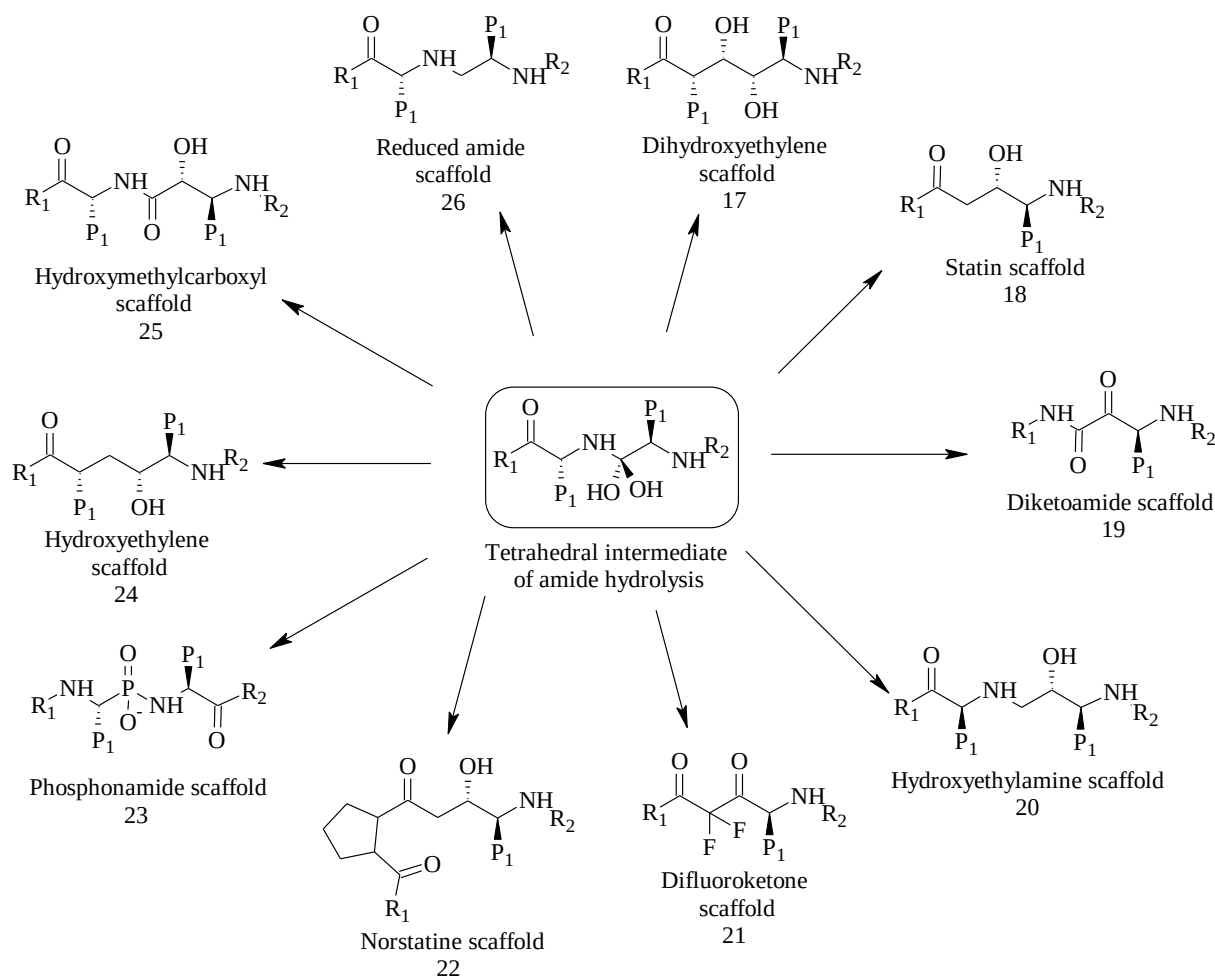


Figure 11: Schematic features of a natural peptide (15) for the synthesis of a peptidomimetic such as Ritonavir (16)

1.2.2.2. Transition state analogs

Research has shown that the transition-state of the substrate are known to possess higher affinity for the enzyme than the substrate.³¹ The knowledge of both HIV enzyme's cleavage mechanism as well as the closeness/resemblance of the tetrahedral intermediate to the transition-state, have facilitated the preparation of transition state analogs. This knowledge of transition-state analogs inhibitors was gained through many years of research of the human aspartic protease renin, which is involved in the regulation of high blood pressure.⁷⁸ In this regards, several noncleavable transition state isosteres have been used in the development and synthesis of potential HIV-1 PR inhibitors.

Some examples of transition state analogs are shown in Scheme 2 below. These include dihydroxyethylene (**17**), statin (**18**), diketoamidostatine⁷⁹ (**19**), hydroxyethylamine (**20**), difluoroketone (**21**), norstatine (**22**), phosphonamide (**23**)⁸⁰, hydroxyethylene (**24**), hydroxymethylcarboxyl (**25**), reduced amide (**26**), and more recently, silicon-based inhibitors.⁸¹



Scheme 2: Non-cleavable transition-state isosteres developed for the synthesis of HIV-1 PR inhibitors.⁴⁴

An example of the strategy of reduced amide's introduction into a peptide has been used in the development of the MVT-101, one of the earliest inhibitors of HIV PR⁸² (Figure 12).

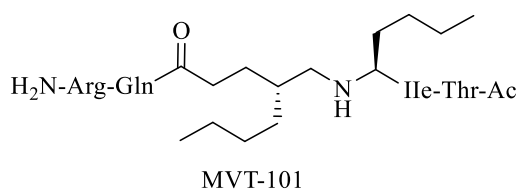


Figure 12: An example of a reduced amide inhibitor.⁸²

However, only moderate potency was achieved with most of the reduced amide compounds. Nevertheless, MVT-101 was the first inhibitor to be co-crystallized with the HIV-1 PR, giving valuable three-dimensional structural information of this enzyme (HIV PR) in its inhibited form.⁸³

The statine skeleton, **18** comprising five atoms, was employed as a dipeptide (six atoms) mimic and was incorporated in peptides to serve as inhibitors of HIV protease.⁸⁴ The mediocre potency that was achieved with this fragment was considered to be attributable to the lack of a P1' side-chain. However, inhibitors with the norstatine moiety, **22** which more closely resembles a single amino acid residue carrying an extra carbon, provided increased activity against HIV PR when incorporated into inhibitors.⁸⁵

Hydroxyethylene isosteres **24** constitute the basis for highly potent inhibitors and provided the first inhibitors to have activity against HIV in cell-based assays.⁸⁶ The improvement achieved with hydroxyethylene isosteres **24** encouraged investigation of dihydroxyethylene isosteres **17**, which had the potential for C₂ symmetry. It was found that the contribution from the second hydroxyl group was negligible, as suggested by similar binding affinities of hydroxyethylene and dihydroxyethylene based transition state analogs.⁸⁷ The hydroxyethylamine isosteres **20** are perhaps most successful amongst the previously known transition state analogs used in HIV protease inhibitors. This transition state analog was utilized in the first clinically approved HIV protease inhibitor, saquinavir⁵³ (Figure 10, structure **6**). Saquinavir (Ro 31-8959), which was discovered by Hoffmann-La Roche, is the first FDA approved drug (1995) for the treatment of AIDS. This potent inhibitor ($K_i = 0.12$ nM) has the hydroxyethylamine isostere replacement for the Phe-Pro cleavage site.

The structure of this inhibitor was quite interesting since it had the *R* stereochemistry at the carbon bearing the central hydroxy group, which was unique among aspartic protease inhibitors that were known at that time. Both Amprenavir ($K_i = 0.6$ nM), which was discovered by Vertex Pharmaceuticals⁸⁸ and approved by the FDA in 1999, and Nelfinavir ($K_i = 2$ nM), which was discovered by a collaborative effort between Lilly and Agouron⁸⁹ and approved by the FDA in 1997, have similar hydroxyethylamine isostere replacement to that found in Saquinavir.

The X-ray crystallographic structures of the HIV-1 PR complexed with some hydroxyethylene and dihydroxyethylene inhibitors have established that the central hydroxyl group of this core unit is situated between Asp-25 and Asp-25, making favorable electrostatic contacts. This interaction between the two catalytic aspartates

and the hydroxy group is worth more than 4 kcal mol⁻¹.⁴¹ A review that discusses in detail the various interactions of the different inhibitors with the binding site has been published.⁹⁰

When aiming to improving the pharmacological, safety and pharmacokinetic properties of current PR inhibitors, the synthesis of various acyl-substituted indinavir (and saquinavir) prodrugs, their in vitro stability with respect to hydrolysis and their anti-HIV activity and cytotoxicity in cells have to be seriously considered.⁹¹ It has been found that the hydrolysis of the ester bond and liberation of the active free drug have been so crucial in the inhibition of HIV PR enzyme. The more the hydrolysis is faster, the closer the anti-HIV activity is to that of the respective parent drug and this has been the case in most of the C-14-substituted indinavir and saquinavir derivatives (IC₅₀ from 10 to 360 nM for ester half-lives of 90 min to 40 h).⁹¹

In parallel, research has established that the level of HIV inhibition is very low for the prodrugs for which hydrolysis is very slow.⁹¹ This is the case with the myristoyl or oleyl saquinavir esters, owing to the stable masking of the hydroxyl that is part of the peptidomimetic non-cleavable transition state isostere responsible for the inhibitory potency of saquinavir (and indinavir). However, it has been found that the anti-HIV activity of the monosubstituted C-8 indinavir prodrugs does not correlate with their resistance to hydrolysis, since the C-8 hydroxyl of indinavir is not involved in the transition state isostere.⁹¹ Thiazole derivatives are the integral part of several biologically active molecules.⁹² In particular, 5-hydroxymethylthiazole⁹³ moiety has been one of the key intermediates of Ritonavir.⁹⁴

With regards to the above mentioned, The Merck group discovered a novel variation of the hydroxyethylene transition state analog in their drug, Indinavir ($K_i = 0.56$ nM),⁹⁵ which was approved by the FDA in 1996. Merck group rationalized that incorporating the cyclic urea core, to mimics the hydrogen bonding features of the key structural water molecule into an inhibitor, would replace the flap water molecule. Consequently this would lead to better binding energy due to the positive entropic effect that should be provided by the cyclic urea core. These inhibitors also contained a diol functionality (a dihydroxyethylene derivative) as a transition state mimic which would interact with the catalytic aspartates.⁹⁶ Therefore, several cyclic

urea inhibitors were developed and found to be potential candidates against HIV PR, although none of them have been commercially available to date.

1.2.2.3. N- and C-terminal duplicated strategies

While the above-mentioned strategies concern backbone and side chain modification of a natural template or peptide, another major strategy which has been used by researchers to design new HIV-1 PR inhibitors is to duplicate either the *N*- or *C*-terminals of a natural peptide (Figure 13). This strategy has been based on the C_2 -symmetric nature of the HIV-1 PR homodimer, which was revealed by the X-ray analysis of a native PR enzyme.

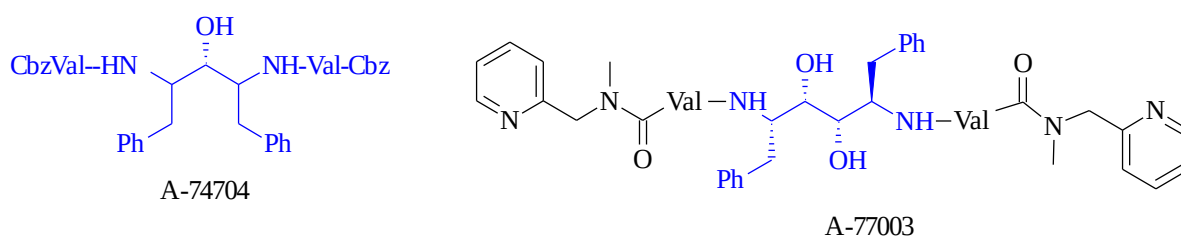


Figure 13: Examples of *N*-terminus duplicated inhibitors.

An example of these analogs was developed by Abbott, using an *N*-terminal duplication strategy to develop A-74704, as a pseudosymmetric diaminoalcohol⁹⁷, and the diaminodiol A-77003, which was used clinically as an intravenous agent.¹⁴² This strategy was further improved, resulting in the synthesis of the non-symmetric compound, the ritonavir (as a hydroxyethylene isostere), which has since been clinically approved.⁵²

Many other analogs have been developed by other research groups worldwide using *N*- and/or *C*-terminal duplication, although the *C*-terminal duplication strategy has been studied less extensively.⁹⁸ For example, Bone and co-workers reported a high-affinity inhibitor, L-700,417 for the first time using this concept⁹⁹ and a more peptide like inhibitor, which also exhibited a relatively high potency, was separately designed and synthesized by Babine and co-workers.¹⁰⁰

1.2.2.4. Two-fold symmetrical and pseudosymmetrical inhibitors

The design of two-fold symmetrical inhibitors, is based on the observation that HIV-1 PR enzyme itself is a symmetrical homodimer in the native state. Thus, the active site should exhibit two-fold symmetry.⁷⁵ The recognition of the binding subsite on either side of the active site by a symmetrical inhibitor should be advantageous. Figure 14 below shows the chemical structure of this type of inhibitor, a *1,4-diamido-dihydroxyethylene*-based scaffold, which constituted the major part of this current research project.

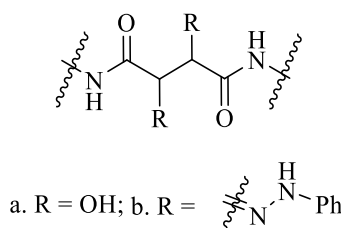
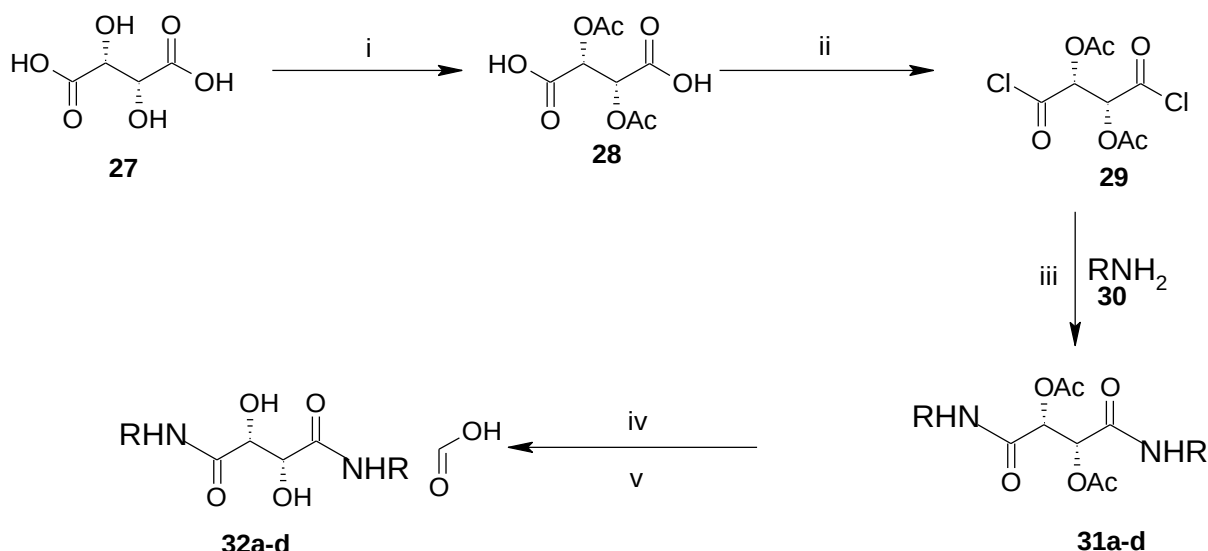


Figure 14: Typical chemical structure of a two-fold symmetrical or pseudosymmetrical inhibitor

Literature has described development of several two-fold C_2 -symmetrical and pseudosymmetrical inhibitors derived from (*R,R*)-tartaric acid as the chiral precursor of the central hydroxyethylene, dihydroxyethylene,⁹⁷ aminodiol,¹⁰¹ phosphinates¹⁰² and cyclic urea¹⁰³ cores, to which α -amino ester residues have been attached.¹⁰⁴ The synthetic approach used to access such compounds synthesized as potential HIV-1 PR inhibitors has been shown in Scheme 3 below.

The amino esters used were intended to interact with P1/P1' and P2/P2' subsites of the HIV-1 PR enzyme. José C. Barros *et al.*, reported that pseudopeptides inhibitors **31a-d** and **32a-d** were obtained in good yields without noticeable racemization (Scheme 3 below).¹⁰⁴ These authors also used a computational study on commercially available inhibitors to establish that inhibitors with C_2 symmetry show significant affinity for the HIV protease.¹⁰⁵ These results inspired them to prepare a series of pseudopeptides with C_2 -symmetry and a dihydroxyethylene core starting from tartaric acid. They demonstrated that these compounds **31a-d** and **32a-d** (Scheme 3) showed good inhibition with IC_{50} values in the range of Indinavir.¹⁰⁶



- a) R = Phenylglycine-OEt
 b) R = Asp-OEt
 c) R = Glu-OEt
 d) R = Gly-OEt

Scheme 3: Synthetic approach used in the synthesis of pseudopeptides derived from (*R,R*)-tartaric acid as potential inhibitors of HIV-PR enzyme. Reaction conditions: i) AcCl, reflux, 76%; ii) (COCl)₂, CH₂Cl₂, DMF; iii) Et₃N, CH₂Cl₂; iv) EtOH, H₂SO₄; v) LiOH, THF/H₂O.¹⁰⁴

In this approach, first protection of both hydroxyl groups of tartaric acid **27** was carried out, to give acetyl **28** ((*2R,3R*)-*O,O*-diacetyltartaric acid) in 76% yield. Product **28** was converted *in situ* to acid chloride **29** by reacting it with oxalyl chloride in dry DCM and catalytic amounts of DMF.¹⁰⁷ Coupling of **29** with selected amino esters **30a-d** was achieved in presence of triethylamine (Et₃N) in dry dichloromethane (CH₂Cl₂), resulting in pseudopeptides **31a-d** in good yields after recrystallization. Deprotection using EtOH/H₂SO₄ followed by an alkaline hydrolysis using LiOH in THF/H₂O¹⁰⁸ gave the diacid **32a-d** (Scheme 3).

Although this synthetic approach required no coupling agents (such as EDC¹⁰⁹ or DCC¹⁵³), protection and deprotection of the free hydroxyl groups of tartaric acid were necessary. Despite the fact that this approach of forming amide directly without the coupling agents was introduced as early as 1858,¹¹⁰ only a few cases have been reported in the literature.¹¹¹

In order to evaluate structure-activity relationships (SAR), of their novel compounds, they chose amino esters derived from α -amino acids having different lateral chains

viz glycine (no side chain), phenylglycine (an aromatic capable of π -stacking interactions), aspartic acid and glutamic acid (polar constituents). These derivatives were designed to interact with the target enzyme. They included a dihydroxyethylene core as an amide bond isostere for interactions with the Asp25–Thr26–Gly27 residues (active site) and a range of amino acid side chains for improved interaction with recognition sites of the enzyme. Due to their low molecular weight, it was assumed that these compounds should be effective in penetrating biological membranes and adhere to the basic Lipinski rules.^{112,113}

1.2.2.5. Cyclic urea-based protease HIV-1 PR inhibitors

Optimization of the P2/P2' and P3/P3' binding property of potential new drugs has been the most focus of many researchers. However, only a few studies have been addressing it, for an increased potency and translation by handling of the P1/P1' binding properties.¹¹⁴ Amongst these few studies, Urban and co-workers.¹¹⁵ were one of the first who have addressed the variations at P1/ P1' by illustrating several examples of an increased enzyme inhibition from just manipulations the P1/P1', although these compounds were still having peptide character.

After them, two more studies from Merck reported a different modification at one P1' side chain position in their indan-based inhibitor (L-685,434).¹¹⁶ It was shown replacing the P1' side chain's phenyl ring of their indan-based inhibitor with a 4-(3-hydroxypropyl) group or other tethered substituents increased its potency in binding affinity of about a 8-15fold. In 1993, Getman and co-workers implemented (hydroxyethyl)urea isostere-based as novel PR inhibitor, which tolerated a variation of the P1 substituent.¹¹⁷ However, the alkylation of the urea nitrogen projected a substituent into the S1 pocket, allowing incorporation of other groups and therefore several novel inhibitors with nanomolar binding constants have been developed and reported. These include the recent report by Budt and co-workers, who developed seven P1/P1' analogs from a series of a C₂-symmetric diol.¹¹⁸ These authors established that lipophilic P1/P1' substituents were ideal improved potency and binding activity.

Another novel route to HIV-1 PR inhibition based on a 7-membered cyclic urea scaffold has been reported by Lam and co-workers⁹⁶ which resulted in a series of orally bioavailable compounds, including DMP 323 and DMP 450 (see Figure 15 below), with nanomolar binding affinities. It was found by these authors that these inhibitors could bind symmetrically in the active site with the urea oxygen, which can accept two hydrogen bonds from the flap residues Ile 50/50'⁹⁶ whereas the p-hydroxymethyl substituent at P2 also could pick up an other hydrogen bond from the backbone NH of Asp 29/29' and Asp 30/30'.

Based on these above-mentioned observations from Lam and co-workers, it's evident that a diol oxygens is ideal for improved interaction with the catalytic aspartate region, hence, cyclic urea provides becomes a unbending scaffold for precise alignment of all the key structural components with their corresponding binding motifs. This precise stereochemical arrangement with the binding motifs/active sites has been a simple result from the unique *R,S,S,R*-configuration of the four adjacent chiral centers.⁹⁶

Nevertheless, this stereochemical arrangement has been critical for any optimal binding interactions between the inhibitor and the HIV-1 PR enzyme, which allows the inhibitors to create an effective hydrogen-bond-network between the aspartate residues and the flap region of the HIV-1 PR enzyme without intervention of any further water molecule as it is commonly the case with linear inhibitors.¹¹⁹

Although having good oral bioavailability in animals after the Phase I clinical trials, DMP 323 series has been withdrawn due to the high blood level variability in humans¹²⁰. A second candidate, DMP 450, has recently completed phase I trials and was shown to have an improved pharmacokinetic profile in humans.¹²¹

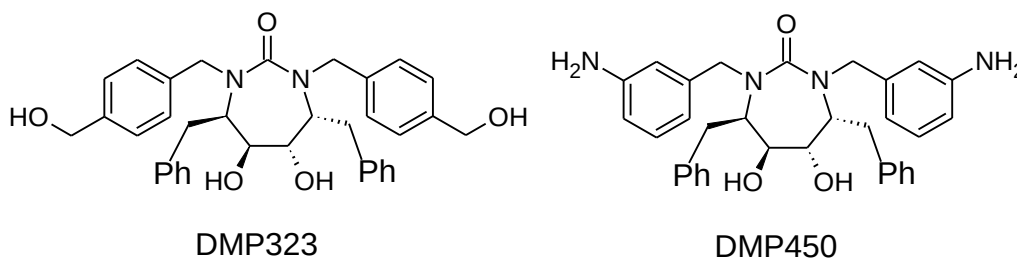
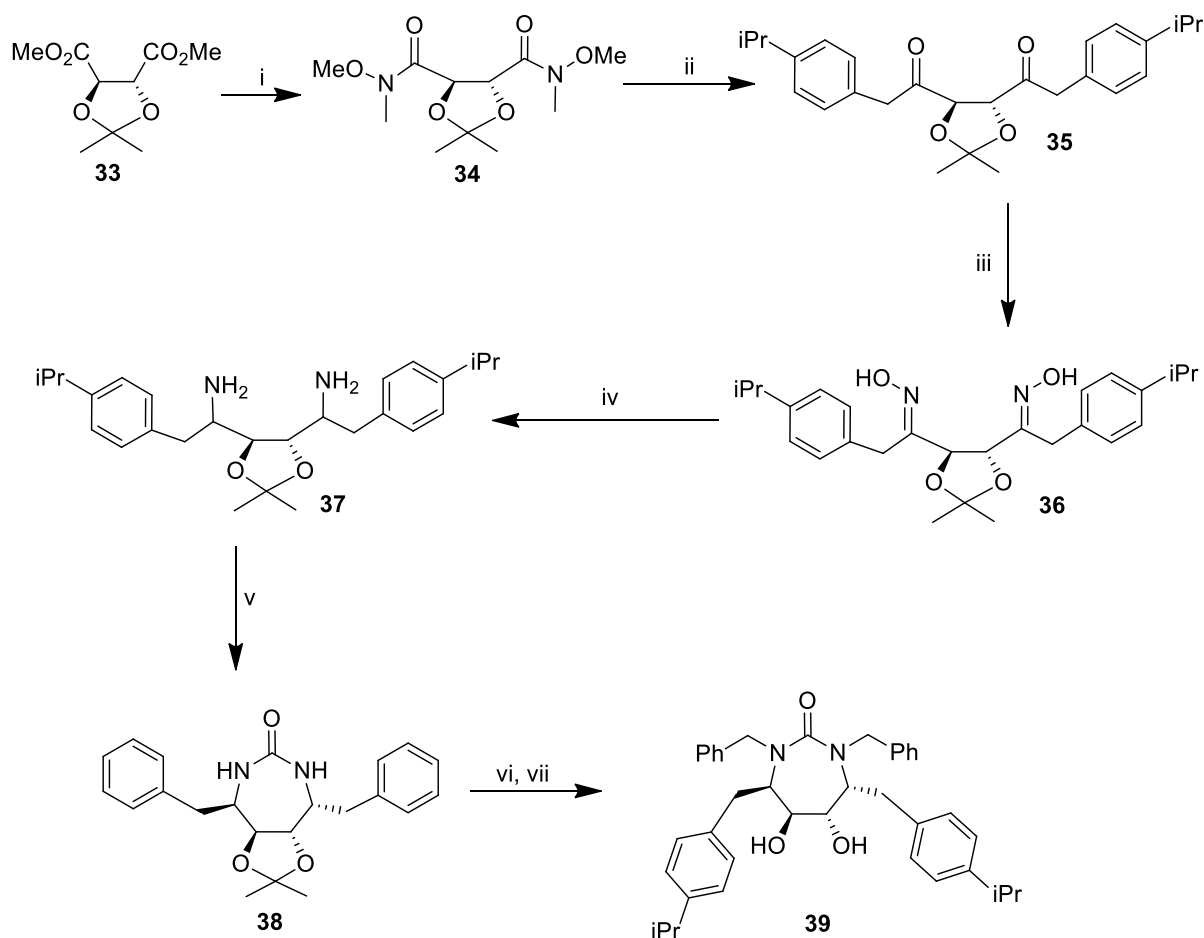


Figure 15: Typical HIV-1 PR inhibition based on a 7-membered cyclic urea scaffold.⁹⁶

Nugiel, D. A. and co-workers reported on a series of novel P1/P1'-substituted cyclic urea-based HIV-1 PR inhibitors using three different synthetic schemes.¹²² In their first approach, they used amino acid as starting materials to synthesize DMP 323 (Figure 15), whereas L-tartaric acid or L-mannitol starting material were used in the other two approaches. Using substrate control method, these authors succeeded to introduce the four adjacent *R,S,S,R* chiral centers of the cyclic urea scaffold and this depending on the desired P1/P1' substituent.¹²² It was found that several analogs had better binding affinity compared and that modulation of some physical properties of their prepared compounds resulted in a 10-fold improvement in translation, which consequently gave better overall antiviral activity.¹²² One of these approaches is summarized in Scheme 4 below.



Scheme 4: One of the Nugiel, D. A. and co-workers' proposed methods to access a series of novel P1/P1'-substituted cyclic urea-based HIV-1 PR inhibitors¹²²: Reagents and conditions: (i) HN(Me)OMe, HCl, AlMe₃, CH₂Cl₂, -10 °C, 83%; (ii) 4-i-Pr-BnMgCl, THF, 95%; (iii) H₂NOH, HCl, EtOH, H₂O, 82%; (iv) DIBAL, toluene, 58%; (v) CDI, tetrachloroethane, reflux, 62%; (vi) K+t-BuO⁻, BnBr, THF, 92%; (vii) HCl, MeOH, H₂O, 73%.

Using the approach described in Scheme 4 for the synthesis of cyclic urea **39**, L-tartaric acid was used as the chiral precursor to introduce the desired P1/P1' substituent using substituted benzyl organometallic reagents and this was achieved through a convergent exploitation of the key Weinreb amide **34**.¹²² Treatment of (-)-dimethyl 2,3-O-isopropylidene-L-tartrate **33** with N,O-dimethylhydroxylamine hydrochloride and trimethylaluminum¹²³ gave the bis-Weinreb amide **34**, which was also converted into the diketone **35** (in 95% yield) by treatment with an excess of p-isopropylbenzylmagnesium chloride in THF. The oxime isomer **36** was obtained as a mixture of E:E isomer (major) along with the E:Z isomer (unseparated minor isomer) after treatment of **35** with hydroxylamine hydrochloride. The oxime was later reduced selectively to the diamine **37** as single *R,S,S,R* stereoisomer¹²⁴ (in moderate yield),

after treatment with an excess of DIBAL using the reaction conditions as described in Scheme 4 above.

Rationally, Nugiel, D. A. and co-workers observed that using the THF (which is coordinating solvent), changed the selectivity and generated the *R,S,S,S* isomer of the diamide **37** as the major product. It was presumed therefore that the selectivity of DIBAL (in a noncoordinating solvent), would be a result of its complexation with the acetonide oxygens. This would generate a bulky adduct that undergoes a kind of sterically controlled attack from additional hydride to finally produce the desired *R,S,S,R* stereoisomer.¹²² This observation is in accord with the Williams' mechanism, involving the reduction of hydroxy oxime ethers diastereo-selectively.¹²⁵

Treatment of the diamine **37** with 1,1'-carbonyldiimidazole produced an imidazolide intermediate, which was cyclized in the presence of tetrachloroethane to form the cyclic urea **38** (in 62% yield), which was further alkylated with benzyl bromide to finally give the cyclick urea **39** (in 67% yield).

These two last approaches, including the two-fold symmetrical (**section 1.2.2.4**) and pseudosymmetrical inhibitors as well as the cyclic urea-based protease HIV-1 PR inhibitors (**section 1.2.2.5**), have greatly guided our choice of using tartaric acid and/or its derivatives such as diethyl tartrate and tartaric acid osazone as precursors in the design of new compounds using computational chemistry.

1.2.2.6. Structure- activity relationships of C₂- symmetric HIV-1 PR inhibitors

Many current therapies against HIV-1 PR have an hydroxyethylene core unit which contains the free hydroxyl group (-OH) required for hydrogen bonding between the PR inhibitor and the carboxylic acid (-COOH) on the Asp-25 and Asp-25' residues located in the PR enzyme's active site (**Figure 16**).¹²⁶

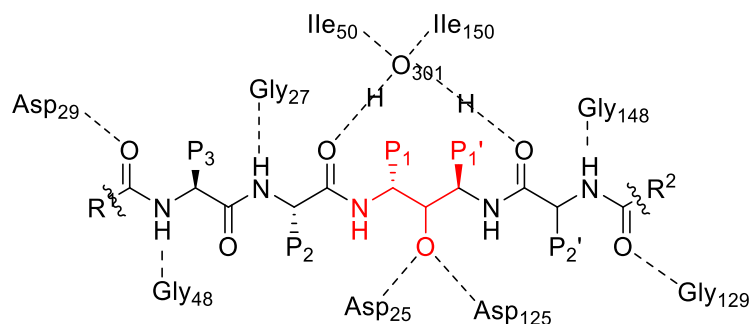


Figure 16: An image of a PR inhibitor attached to the HIV PR active site (adapted from ref.44).

As shown in Figure 16, the hydrogen bonds between the water molecule, the residues Ile50 and Ile50', and the carbonyl groups of the inhibitor play a key role, connecting it with the flap regions of the active site of the PR enzyme. However, for a non-peptide-like inhibitor, a proton acceptor may replace the tetracoordinated water molecule and therefore allow a direct interaction with the two isoleucine residues located on the flaps of the active site.⁴⁴

S1, S1', S2 and S2' subsites within the active site of HIV PR recognize the hydrophobic amino acids present in the natural substrates. Some residues in the enzyme active site can also form additional hydrogen bonds with hydrophilic groups on the PR inhibitor. The SAR's profound knowledge of the HIV PR has contributed so much in the elaboration and design of novel inhibitors with improved potency against the HIV-1 PR enzyme.

1.2.2.7. Molecular modelling and computer-aided drug design

While the SAR studies between HIV-1 PR and its inhibitors have been used in drug design, computer-aided drug discovery, also referred to as "macromolecular docking", studies have been intensively used to understand the binding mode in such a way to optimize inhibitor design. It has been established through different studies, which involved energy minimization using molecular mechanics¹²⁷ that calculated contribution of the main-chain atoms to the total interaction energy was between 56% and 68%, which gives a considerable contribution to the total binding energy.

Computer-aided drug discovery is a computer modelling of the “quaternary structure” of different protein–protein- and/or protein–nucleic acid complexes formed by the interactions of two or more biological macromolecules.¹²⁷

This method has been used to predict or calculate, through simulation, the free energy of binding of the ligand-receptor pair through analysis of the intramolecular recognition parameters.¹²⁷ This method, in conjunction with X-ray crystallography and NMR spectroscopy, has become a meaningful tool in the development, design and synthesis of new drugs with enhanced properties. It has also permitted the establishment of the structure-activity relationships (SAR) of newly synthesized ligands with the target molecule/protease enzyme. Thus, it has been used in exploring different conformations of ligands at the active site of the targeted PR enzyme.¹²⁷

For example, the molecular modelling and computer-aided drug design technic has been used by López-Rodríguez, M. L. and co-workers¹²⁸ in the design of novel selective 5-HT₄ ligands through a comparative study of the 5-Hydroxytryptamine receptor (5-HT₄) and 5-Hydroxytryptamine receptor (5-HT₃) pharmacophores. The resulting conformational analysis performed on the active and inactive 5-HT₃ and 5-HT₄ compounds permitted an estimation of a pharmacophore for these both ligands.

Cosequently, many other factors such as the regions of steric tolerance and intolerance, based on van der Waals surfaces of active and inactive 5-HT₃ and 5-HT₄ ligands for both receptors were also proposed. The proposed steric model for the 5-HT₄ receptor-binding site has been supported by experimental results from the synthesis of two new active and selective ligands: **40** (K_i (5-HT₃) = 3.7 nM; K_i (5-HT₄) > 1000 nM) and **41** (K_i (5-HT₃) > 10 000 nM; K_i (5-HT₄) =13.7 nM). The chemical structures of these two novel ligands **40** and **41** are shown in Figure 17 below.¹²⁸

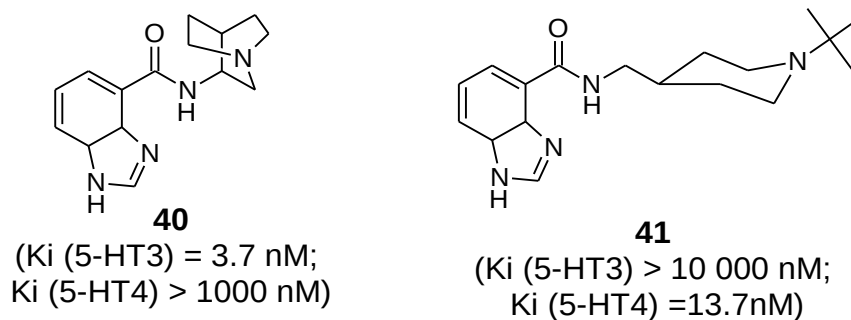


Figure 17: An example of two new active ligands resulting from the proposed steric model for the 5-HT4 receptor-binding site.¹²⁸

Another example is the design and discovery of an HIV-1 integrase inhibitor, the caffeic acid phenethyl ester (CAPE) **42** illustrated in Figure 18 below.¹²⁹ HIV-1 integrase (IN) has been one of the most important enzyme, which mediate the integration of HIV-1 DNA into host chromosomal targets. Therefore IN has been one of most attractive targets for the development of anti-AIDS drugs.

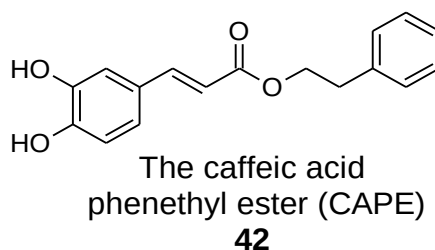


Figure 18: The chemical structure of the caffeic acid phenethyl ester (CAPE) **42**.¹²⁹

A 3D study has been conducted by the National Cancer Institute (NCI) database from a pharmacophore hypothesis based on this well-known CAPE **42**, about 267 structures have been found to be matching the CAPE **42** pharmacophore, of which, 60 were tested in an in vitro assay against HIV-1 IN. Results from this assay showed that 19 of those 60 inhibited both the 3'-processing and strand transfer.¹³⁰

Therefore, the relevance of the proposed pharmacophore was further tested using a small 3D validation database of known HIV-1 IN inhibitors (which had no overlap with the group of compounds found in the initial search). The result of this search confirmed the existence of the postulated pharmacophore by also suggesting a further existence of a possible second pharmacophore relevant in the binding to IN.¹³¹ A further screening using the second pharmacophore in a 3D search of the NCI database, revealed 10 novel structurally diverse HIV-1 IN inhibitors, of which a few were potential candidates with $IC_{50} < 30$ nM.¹²⁹

From the above mentioned, several studies have established that the 3D computer aided technology along with other traditional medicinal chemistry techniques, has been a powerful tool in the development of novel HIV-1 leads. Generally, the computer aided drug design technics have been used to increase the speed and efficiency in the drug discovery process. This techinc is not a direct route to new drugs however, it can provides more details, which rationalize the drug desing.

However, when the structure of the receptor analyzed is unknown, the drug design could be based on key features of a well-known active ligand and/or a pharmacophore model, from which some properties of the receptor site may be inferred. And this has been established as “ligand-based drug design”¹³² which involves the construction of compounds, which have same features with an active compound (ligand similarity approach) and the application of quantitative structure-activity relationship (QSAR) data.¹³³

Different tools have been employed in docking studies, including the search algorithms (example the genetic algorithm), Monte Carlo algorithms, fragment-based algorithms, and molecular dynamics algorithms. However, apart from these, there are also some tools such as GOLD, FlexX, DOCK, and ICM, which have been used for high throughput docking simulations. In addition, various kinds of molecular docking procedures involving either ligand/target flexible (or rigid based upon the objectives of docking simulations)^{133, 134} like flexible ligand docking (target as rigid molecule), rigid body docking (both the target and ligand as rigid molecules) and flexible docking (both interacting molecules as flexible) have been reported.

Therefore several docking studies¹³⁶ based on the crystal structures of the inhibitor complexes of HIV-1 PR have been reported where docking methods and algorithms were tested using the structural data and experimental characteristics by Monte Carlo docking¹³⁷ or by comparison with de novo constructed inhibitors using the fragment-based method.¹³⁸

Other studies examined empirical free energy as a target function in docking and design, showing the advantages of this approach over the calculation of interaction energy.¹³⁹

The combination of both computer-aided and laboratory experimental methods have played major roles in the discovery, design and development of new potent drugs, some of which became clinical candidates as described previously in this thesis.

1.3. The Morita-Baylis-Hillman reaction and its applications in the synthesis of HIV-1 PR inhibitors

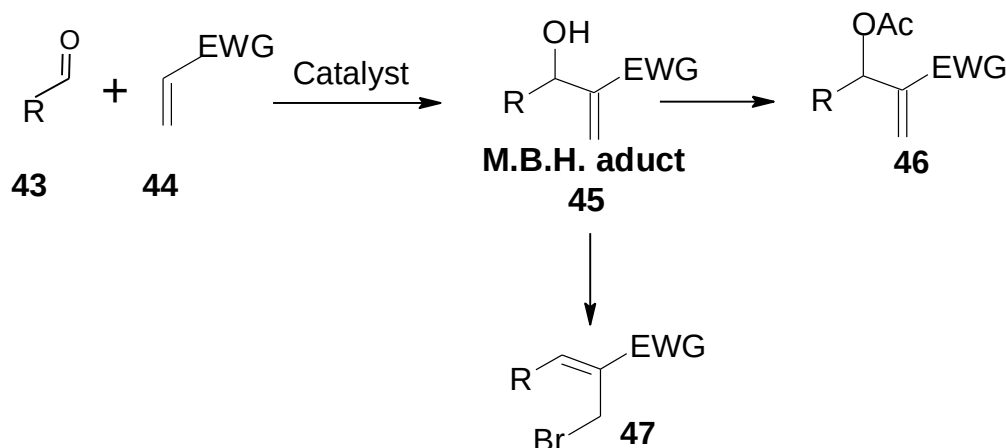
Wei and Shi have shown that the classical MBH reaction involves the formation of α -methylene- β -hydroxycarbonyl compounds **45** through combination aldehydes **43** and α,β -unsaturated carbonyl compounds **44** in the presence of a tertiary amine such as **48** or phosphine as a catalyst (Scheme 5).¹⁴⁰ These main three components features the MBH reaction while enabling a wide selection of substrates.

It has been described as an atom-economic, carbon-carbon bond-forming in a three-step reaction (combining successive Michael, aldol and elimination reactions in one pot), between activated alkenes at their α -position and carbon electrophiles in the presence of a catalyst (or catalytic system), to produce densely functionalized molecules referred to as MBH adducts.¹⁴¹

When using prochiral electrophiles, a stereogenic centre will be generated thus providing the asymmetric version of the reaction. The densely functionalised products (MBH adducts), which have three functional groups in close proximity to each other, are very useful synthons in the construction of various synthetic targets, including some natural products.

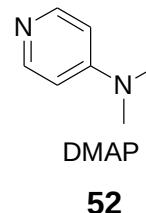
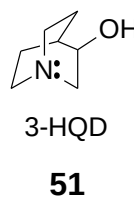
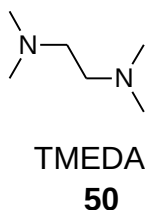
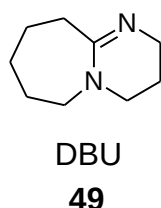
Intramolecular elaboration of the adducts can afford heterocyclic and carbocyclic compounds.¹⁴¹ Interest in the reaction initially grew slowly due to various challenges, but has grown exponentially in recent decades as its synthetic potential drew the attention of synthetic organic chemists. In the process, the breadth of the three essential elements used in the reaction, viz., the activated olefin, catalyst and electrophile has also grown significantly.¹⁴¹ The numerous publications on various aspects of the reaction are proofs of its phenomenal growth in recent years.¹⁴¹

As shown in Scheme 5, this reaction can accommodate both aliphatic and aromatic electrophiles. Together with its aza-analogss, the MBH reaction has been one of the most extensively used carbon-carbon bond forming reactions for many decades.

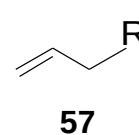
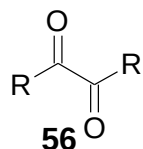
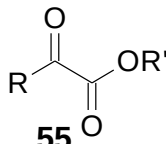
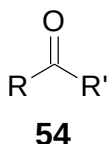
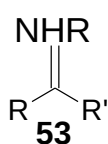


EWG : $PO(OEt)_2$, $CONH_2$, CO_2R , $SOPh$, CO_2Me , CHO , SO_2Ph , CN , SO_3Ph , etc.
 R : Alkyl, Ar or H.

Catalysts:



Other electrophiles used:



Scheme 5: The main MBH reaction: EWG, catalysts and electrophiles.¹⁴⁰⁻¹⁴¹

The activated alkenes used in MBH reaction have varied from cyclic and acyclic alkenes to alkynes and even allenes. Among the most commonly used organocatalysts for this reaction, 1,4-diazabicyclo[2.2.2]octane **48** (DABCO), 1,8-diazabicyclo[5.4.0]undec-7-ene **49** (DBU), tetramethylethylenediamine **50** (TMEDA) and 3-quinuclidinol **51** (3HQ) and *N,N*-dimethylaminopyridine (DMAP) **52**.¹⁴²⁻¹⁴⁴ Tertiary phosphines have also been found to catalyse this reaction.¹⁴⁵

Since the MBH reaction involves two components including the activated alkene and an electrophile, several combinations of reaction partners have been generated, especially, the aza-B.H. reaction, which has been is an important variant of MBH reaction using imines as electrophiles.

Although in most cases aldehydes, ketones, or imines are employed as prochiral electrophiles, a few reports on the use of allyl halides,¹⁴⁶ alkyl halides,¹⁴⁷ and epoxides¹⁴⁸ (**53-58**), have been documented, with one or more stereogenic centres, leading to asymmetric MBH versions.¹⁴⁹ The MBH adducts and their derivatives have been extensively utilized in the synthesis of several heterocycles and other cyclic frameworks.¹⁴³

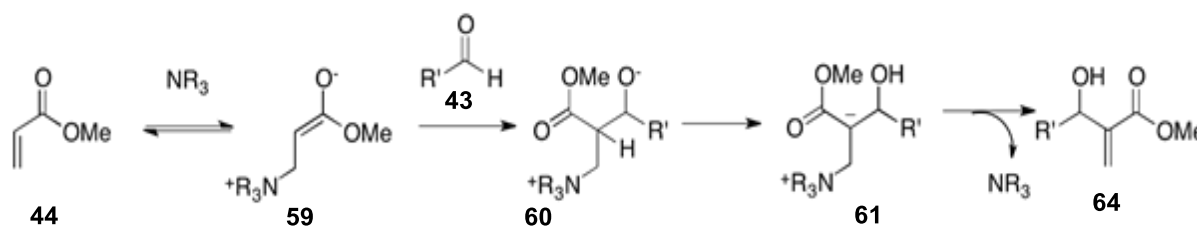
The use of cheap and readily available starting materials has been another advantage since the final product contains a chiral center from a pro-chiral starting material (electrophile) and more functionalities in the proximity, facilitating further modifications. Elaboration of different functionalities such as acetylation (**46**) or bromination (**47**) of MBH adduct as shown in Scheme 5, have given access to several complex scaffolds, which includes the syhthesis of coumarins and much more products.¹⁴³

1.3.1. Mechanism of the MBH reaction

The wide variety of reactants, catalysts and reaction conditions reported for the MBH reactions has been challenging in the elucidation of its mechanism, and therefore, the first proposed mechanism for this reaction was done by Hoffman.¹⁵⁰ This mechanism commences with the nucleophilic aza-michael addition of DABCO to MVK **44** giving a zwitterionic aza-enolate **59** which then reacts with benzaldehyde **43** in an aldol-type reaction to produce zwitterion **60** which can undergo E2 or E1_{CB} elimination (**61**), to release DABCO following proton migration, to provide the MBH adduct **64** as shown in Scheme 6 below.¹⁵⁰

The rate-determining step was thought to be the addition of the enolate to the aldehyde however, since MVK is highly reactive, a competition reaction may also occur in which a second molecule of MVK reacts with the zwitterionic enolate to

afford the competition product on release of the catalyst as shown in Scheme 7 below.^{140, 141}



Scheme 6: First proposed mechanism of the MBH reaction.¹⁵⁰

Later on, Hill and Isaacs conducted some kinetic experiments to investigate the mechanistic details¹⁵¹ of this reaction and established that the rate of reaction between acrylonitrile and acetaldehyde was found to be first order in concentrations of acrylonitrile, acetaldehyde, and DABCO. These authors proposed therefore that the aldol addition step, which involves all three reactants, would be the rate-determining step of the MBH reaction. They also claimed that they did not observe kinetic isotope effect using α -deuterated acrylonitrile.

The rate of MBH reaction was accelerated by the build-up of product (autocatalytic effect), which could not be rationalized by the mechanism. In addition, the formation of a significant amount of 'unusual' dioxanone byproduct in the MBH reaction of aryl aldehydes with acrylates was not expected. These have been some of critics raised around this first proposed mechanism.

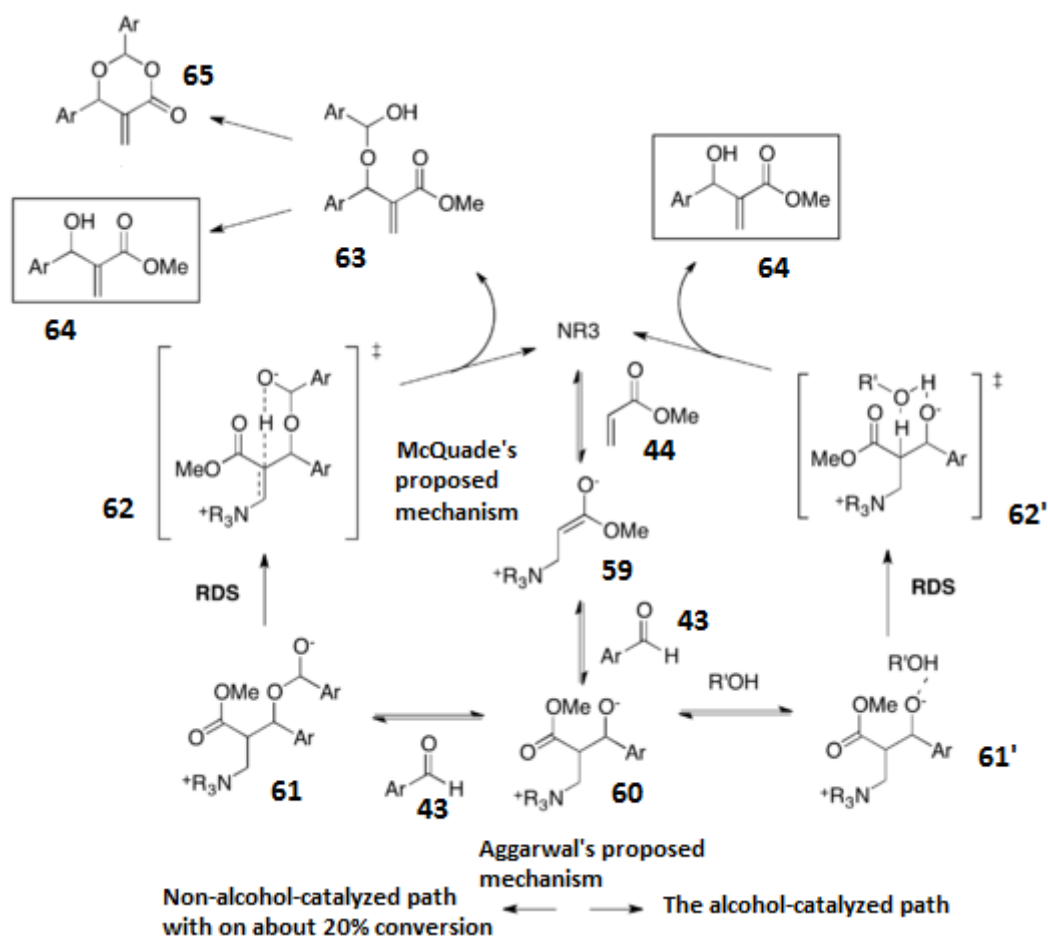
Therefore, further re-evaluation of the MBH reaction's mechanism was undertaken by McQuade and co-workers¹⁵² and Aggarwal and co-workers¹⁵³, using both kinetics and theoretical studies, focusing on the proton-transfer step. According to McQuade, the MBH reaction between methyl acrylate and p-nitrobenzaldehyde is second order relative to the aldehyde and shows a significant kinetic isotope effect at the α -position of the acrylate (5.2 in DMSO). Regardless of the solvents the KIE were found to be greater than two, indicating the relevance of proton abstraction in the rate-determining step.

Based on these new data, McQuade proposed a new mechanism, suggesting that the proton transfer step is the rate-determining step. First and second steps are not changed, but after the first aldol addition, the second addition of aldehyde occurs to form a hemiacetal alkoxide. Then the rate-determining proton transfer step via six-membered transition state releases the adduct **63** (as described in Scheme 7 below), which further reacts to produce MBH product **64** or dioxanone byproduct **65**. This mechanism accounts for the formation of dioxanone byproduct.

In another hand, Aggarwal focused on the autocatalytic effect and observed that the catalytic quantities of MBH product or methanol removed this effect. Thus he proposed that at early stage of the reaction non-alcohol catalyzed mechanism, equivalent to McQuade's proposal, operates, while after 20% conversion alcohol-catalyzed mechanism dominates (Scheme 7).

In this later stage, alcohol R'OH assists the rate-determining proton transfer step via six membered transition state. Aggarwal and Harvey modeled the two pathways using density functional theory calculations and showed that the computed energy profile matches well with the experimental kinetic isotope effect and observed rate of reaction.¹⁵³

In addition, Aggarwal and co-workers also demonstrated that the overall enthalpic barrier of the alcohol-catalyzed pathway is slightly smaller than that of the non-alcohol-catalyzed pathway, rationalizing that as the alcohol (MBH product) concentration increases the alcohol-catalyzed pathway starts to dominate, exhibiting the autocatalysis. Scheme 7 give combined mechanistic proposals of both McQuade and co-workers¹⁵² and Aggarwal and co-workers.¹⁵³



Scheme 7: A combined mechanistic proposals of both McQuade and co-workers¹⁵² and Aggarwal and co-workers.¹⁵³

Although arguments of both McQuade's and Aggarwal's studies have attracted much attention recently, there are a number of issues not resolved yet. First, McQuade's proposal for the role of the intermediate **63** is not clearly proven. Because **63** could be formed simply by addition of **64** to an aldehyde, formation of **63** and **65** could be happening outside of the MBH mechanism. McQuade asserts that the rate-determining step involves two molecules of aldehyde because the reaction rate is second order in aldehyde, but does not explain why Hill and Isaac observed first order for their substrates.

Indeed, the enormous variability of substrates for MBH reaction is a constraint for probing the general mechanism of MBH reaction in a unified manner. In addition, Aggarwal previously suggested that rate-determining step of the reaction changes from proton transfer to aldol addition over the course of the reaction, based on the fact that primary kinetic isotope effect disappears after 20% conversion,¹⁵² but the

subsequent computational studies concluded that the proton transfer step still has the highest barrier in the late stage of reaction. The discrepancy between kinetic and computational results implies that there still are mechanistic aspects of MBH reaction not understood well.

Recently, Coelho and co-workers¹⁵⁴ have used ESI-MS data to provide experimental data to support the dualistic nature of the reaction's proton transfer step, thus granting the first structural evidence for both McQuade's and Aggarwal's mechanistic propositions for this rate-determining step of the reaction.

Following up with the Aggarwal postulates, a computational investigation of the MBH reaction mechanism was also undertaken by Robiette and co-workers¹⁵⁵, which focused on an amine-catalysed coupling of benzaldehyde and methyl acrylate under protic solvent-free conditions. Robiette and co-workers looked at the mechanism under these reaction conditions and how the mechanism and the rate-determining step respond in the presence of alcohols.

Their predictions in the absence of protic solvent were that deprotonation of the α -position was the rate-determining step, which occurred via a cyclic transition state. In the presence of an alcohol (or water), a slightly lower energy pathway is provided, in which the alcohol operates as a shuttle in the transfer of the proton from carbon to oxygen.

1.3.2. Shortcomings of the MBH reaction

MBH reaction has been challenging. Mainly the time requirement for completion of the reaction has been one of the most encountered challenge. This reaction can take days or even weeks depending on the aldehyde (or electrophile) activated alkene and the catalyst in use.¹⁵⁶ However, many efforts at improving the reaction rate have involved the use of dual catalysts, high-pressure conditions, microwave irradiation, ultrasound, temperature variation, solid support, polar solvents, ionic liquids and additives.^{142, 157}

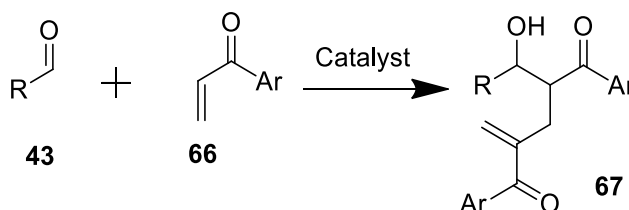
It has been found that the combination of dioxane, methanol, and water with organic solvents, in the presence of a stoichiometric amounts of catalyst or co-catalysts such

as the LiClO_4 and/or CaH_2 together with DABCO in combination with proline and imidazole (in some cases as additives) would result in improved yield some how depending on the substrates used.

In addition, since MBH reaction has a great extent of variability in reaction substrates, developing reaction conditions, which would be suitable for some combination of substrates become therefore challenging. For example, β -substituted activated olefins, vinyl sulfones, and vinyl sulfoxides exhibit low reactivities, slowing or preventing sometimes the reaction to occur. Competing reactions of substrate functionalities are also problematic. Acroleins are susceptible undergo oligomerization, and allenolates easily undergo cycloaddition reactions. The development of suitable conditions for using alkyl halides and epoxides as electrophiles has been also challenging.

Despite the broad scope, atom economy, and generality of the reaction, the slow rate of the MBH reaction (reaction times of 7 to 14 days or even longer are not uncommon, even with 25 to 100 mol % catalyst) for aliphatic aldehydes and electron-rich benzaldehydes often limits the synthetic utility of the process. Ketones are generally not reactive enough to take part in the reaction in a synthetically useful manner under ordinary conditions. However, due to the highly negative volume of activation, sluggish MBH reactions, including those using ketones as substrates, can be realized by conducting the reaction under high pressure (up to 20 kbar).¹⁵⁸

High reactivity of the activated alkene could also be a problem. The MBH reaction of an aryl vinyl ketone **66** with an aldehyde **43** is not straightforward, since the reactive aryl vinyl ketone readily adds first to another molecule of aryl vinyl ketone via Michael addition, then the adduct adds to the aldehyde to form a double MBH adduct **67** as shown in Scheme 8 below.¹⁵⁹



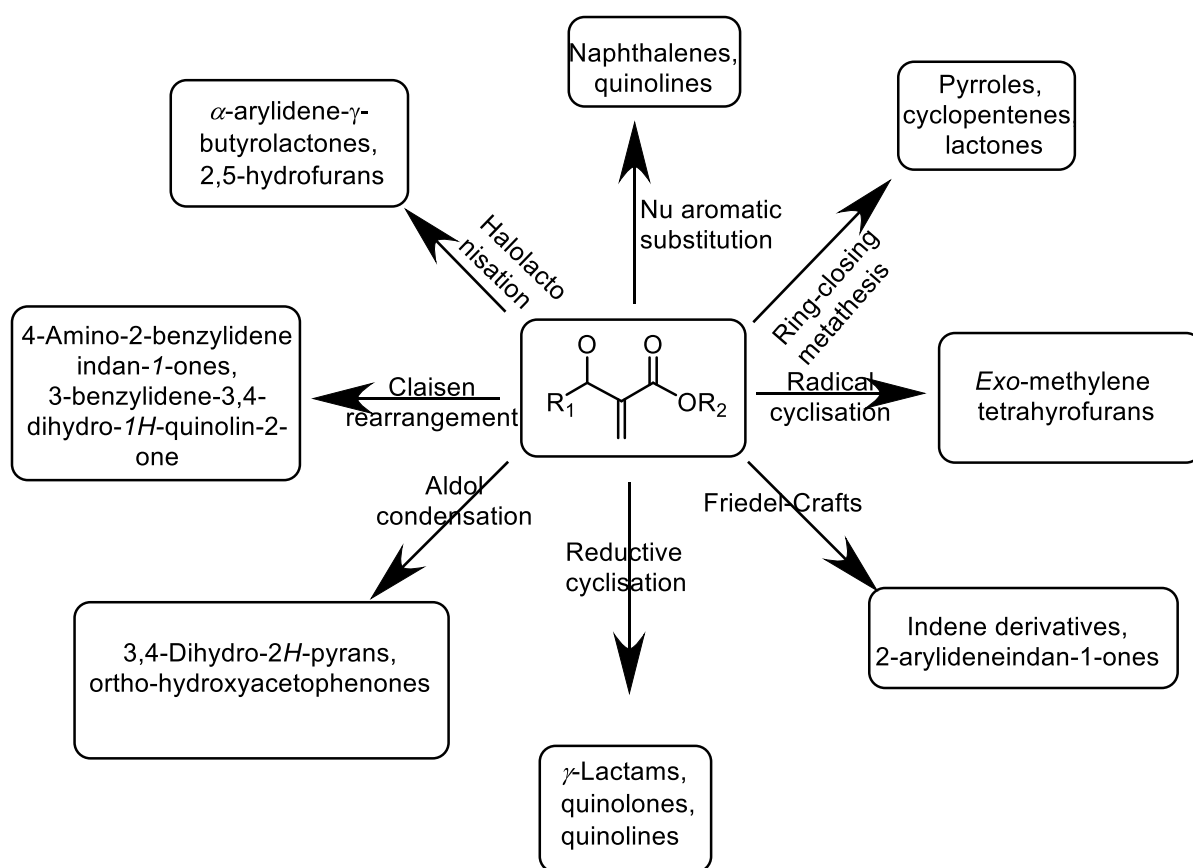
Scheme 8: A double MBH adduct formation from an aryl vinyl ketone with an aldehyde

A general solution to asymmetric MBH reaction of diverse substrates is still missing as well. Overall, MBH reaction is not yet at a mature stage, and there is still much room for development of powerful and general catalytic systems. Much more still needs to be done to circumvent these shortcomings of the MBH method.

1.3.3. Modifications of the MBH adduct

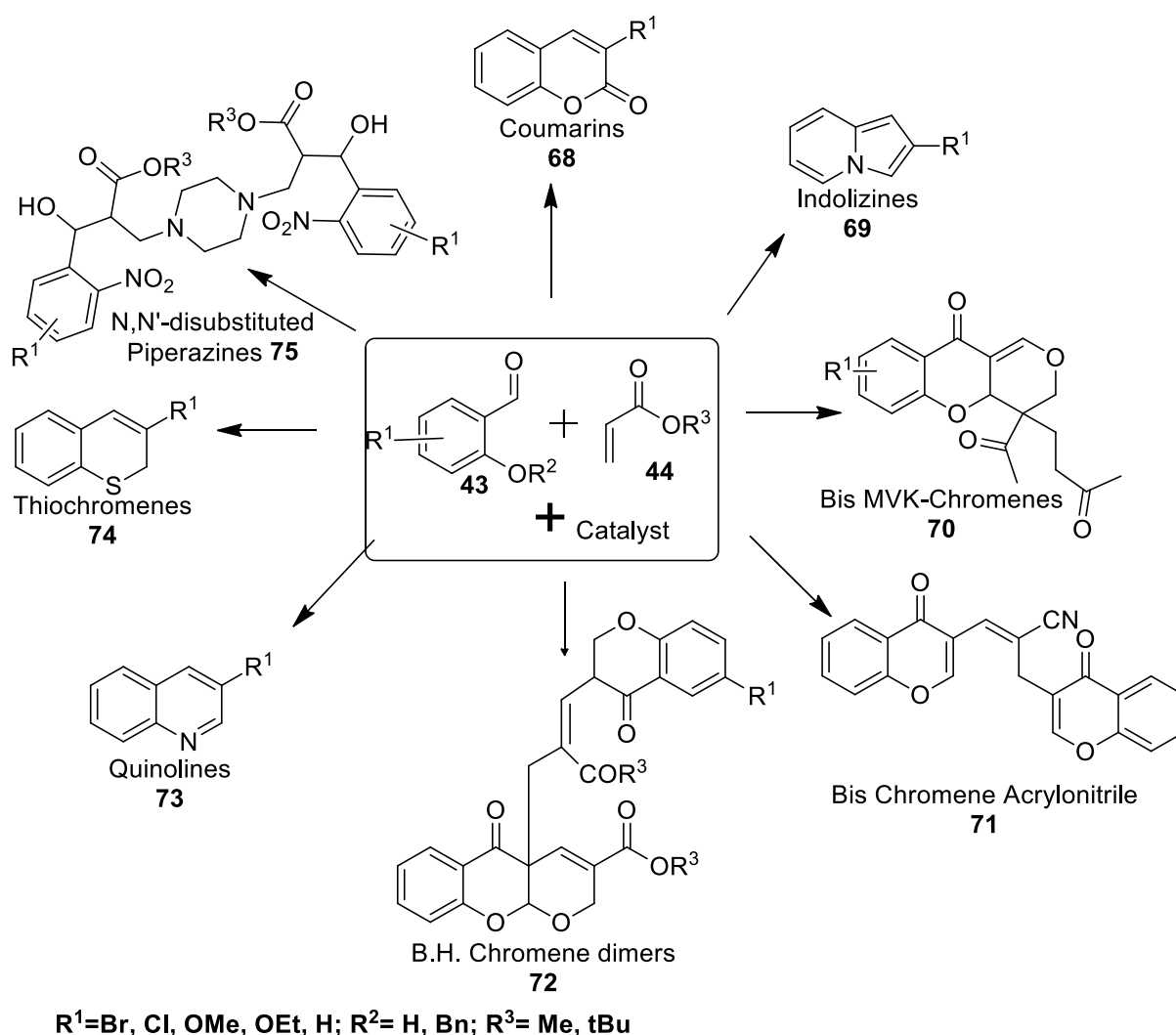
The MBH reaction affords adducts which contain the allylic alcohol and ketone functional groups which allows them to be used as precursors for further transformations or modifications to access derivatives used in drugs and natural products.¹⁶⁰

Based on many chemical transformations of MBH adducts that have been reported, Lee and co-workers¹⁶¹ have identified eight categories, each of which is characterised by a particular key reaction. These categories, their key reactions and examples of the corresponding products are shown in Scheme 9 below.



Scheme 9: Some of possible chemical reactions and transformations that can be made from the MBH adduct scaffolds.¹⁶¹

Amongst others, some of important modifications of the MBH adducts includes the formation of a series of compounds such as include indolizines,¹⁶² quinolines,¹⁶³ thiochromenes,¹⁶⁴ chromenes, coumarins^{165,166} and the complex polycyclic systems^{167,168} as shown in Scheme 10 below. However, since the synthesis of some of these MBH adducts' modifications have been previously undertaken previously in our research group; therefore we sought to discuss them with more details in the next **Section 1.3.4** below.



Scheme 10: Some of different compounds accessed via the Baylis-Hillman reaction in our group.

Coumarin **68** (or 2*H*-chromen-2-one, 2*H*-1-benzopyran-2-one, *o*-hydroxycinnamic acid lactone or 2-oxo-1-benzopyran) is a benzo- α -pyrone, which is a heterocyclic

compound in which a benzene ring is fused to a pyrone ring with an oxygen atom at the α -position.¹⁶⁹ In most cases, Coumarins have been formed *via* the shikimate-chorismate biosynthetic pathway.¹⁷⁰

Daphnin **76** (7- β -D-glucopyranosyloxy-8-hydroxycoumarin) was the first coumarin derivative to be isolated (in 1812 from *Daphne alpine*) and shortly afterwards (1820), coumarin itself was isolated from the tonka bean (*Dipteryx odorata* L.) by Vogel.^{169,171}

Coumarin and its derivatives have been found to occur naturally in a wide array of plants where they are known to regulate plant growth and absorb UV radiation.^{169,171}

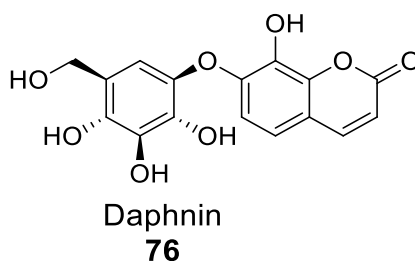


Figure 19: Chemical structure of Daphnin.^{169,171}

However, according to their structures, coumarins have been classified into eight subtypes viz: (i) simple coumarins, which includes hydroxylated, alkoxyated or alkylated compounds on the benzene ring, and their glycosides (e.g. umbelliferone **77**);^{171,172} (ii) furanocoumarins (or furocoumarins) – coumarin derivatives with a five-membered furan ring attached to the coumarin moiety, exemplified by the linear furanocoumarin psoralen **78** and the angular analogue angelicin **79**; ^{171,172} (iii) pyranocoumarins containing a six-membered ring attached to the coumarin moiety, as in the linear pyranocoumarin xanthyletin **80** and the angular analogue seselin **81**;¹⁷² (iv) pyrone-substituted coumarins, which bear substituents at positions 3 or 4, e.g., warfarin;¹⁷² (v) benzocoumarins, e.g., ellagic acid **82**; ¹⁷¹ (vi) coumestans (e.g., coumestrol **83**); ¹⁷¹ (vii) isocoumarins (e.g., hydrangenol **84**);¹⁷¹ and (viii) complex structures, which include a coumarin moiety (e.g., novobiocin **85**).¹⁷¹ Chemical structures of these subtypes of coumarins are shown in Figure 20 below.

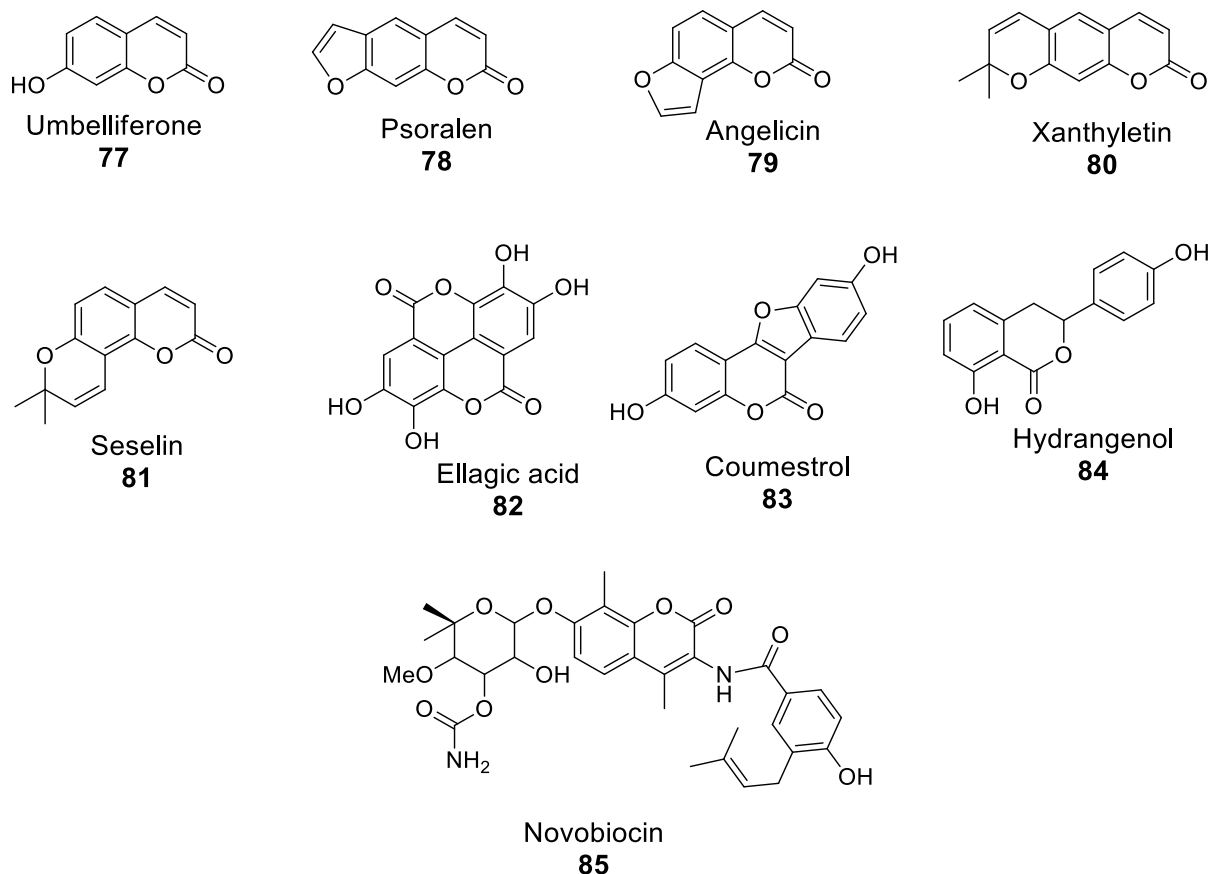


Figure 20: The eight subtypes of Coumarins according to their structures. ^{171,172}

Several naturally occurring coumarin derivatives have found application in various fields including pharmaceutical, perfumery and cosmetics, as well as alcoholic beverages.¹⁷³ Properties and biological activities of coumarins derivatives include anticoagulant (e.g., tromexan **86**),¹⁷⁴ antibiotic (e.g., novobiocin **85**), antipsychotic (e.g., compound **87**),¹⁷⁵ antitumor (e.g., umbelliferone **77**)¹⁷⁶ and anti-HIV properties (e.g., compounds **88** and **89**).¹⁷⁷⁻¹⁷⁹ Thus, coumarins derivatives continues to be attract many attention of researchers.

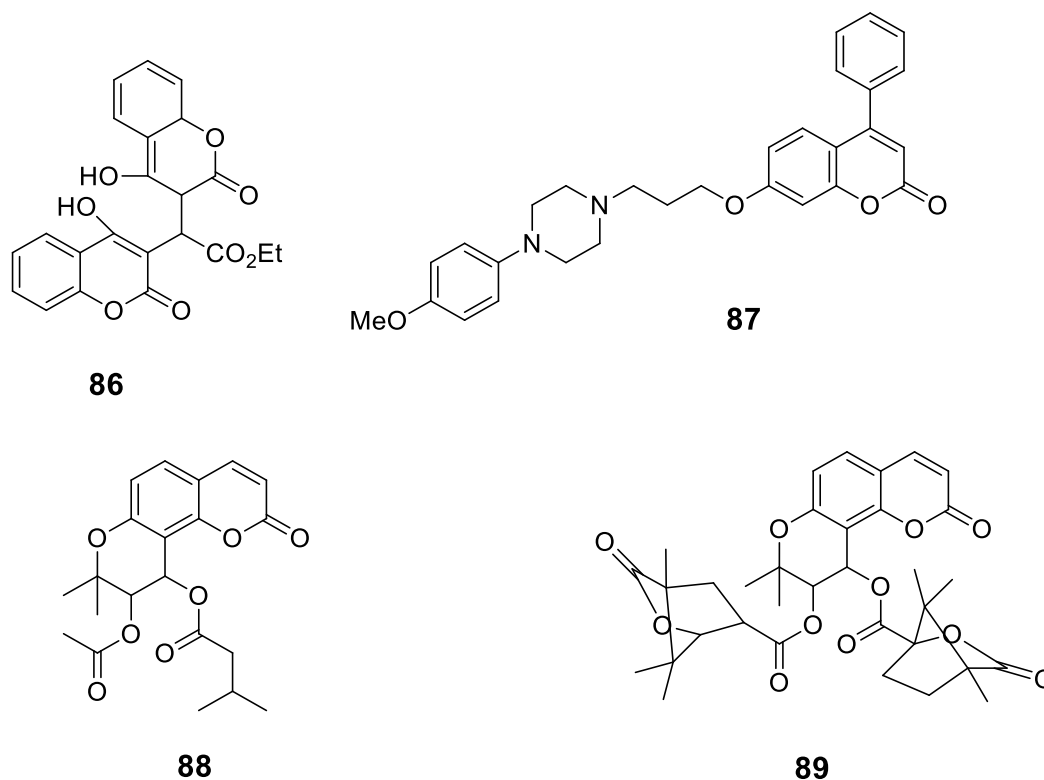
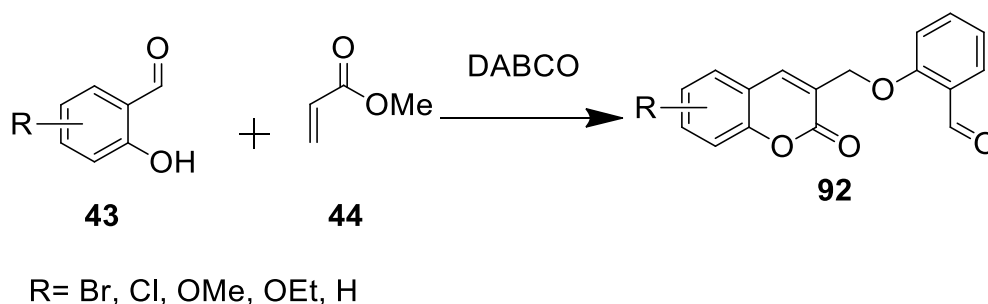


Figure 21: Examples of some biologically active coumarin derivatives as described from references 174 to 179.

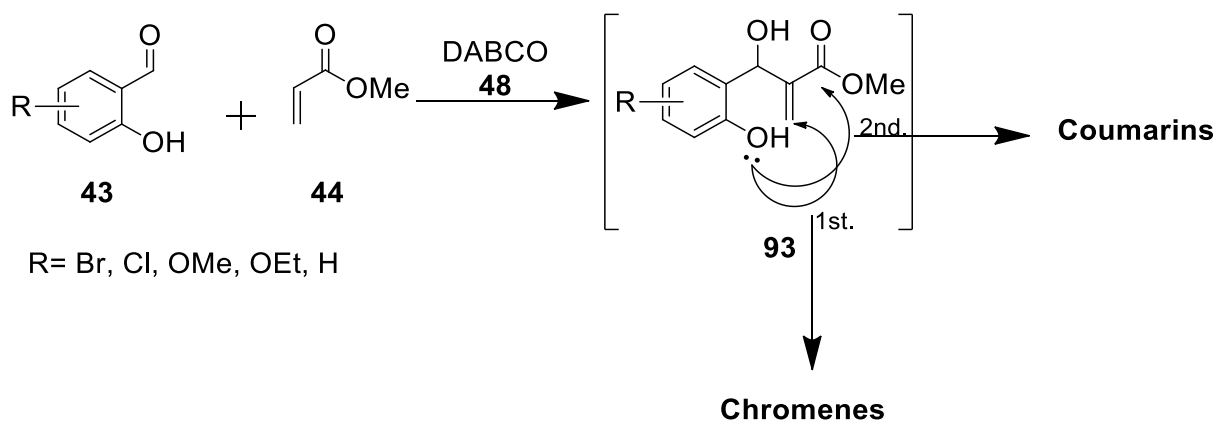
1.3.4. Previous work from our research group on MBH method

Within our research group, MBH reaction has been extensively applied in the construction of different benzannulated heterocycles compounds, with isolation of various unexpected products during recent years, and these have provided the platform for the present study. Compounds obtained in earlier studies have been presented in Scheme 10.

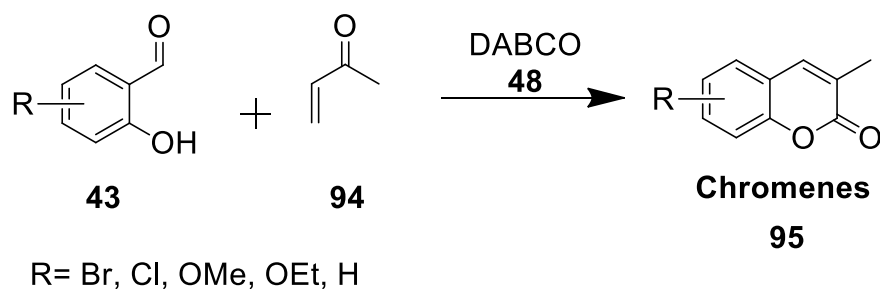
There has been extensive work on the reactions of salicylaldehydes with activated alkenes in the presence of DABCO catalyst and on exploring the mechanism of the MBH reaction.¹⁸⁰ For example, Bode established the route to indolizines^{162,181} and on reacting salicylaldehyde **43** with methyl acrylate **44**, observed that a coumarin derivative **91** had formed instead of the expected Baylis-Hillman adduct (Scheme 11).¹⁸²

Scheme 11: Reaction of salicylaldehyde with methyl acrylate.¹⁸²

Later on, it was established by Robinson that the MBH reaction shown in Scheme 11 actually give access to a cascade of chromene and coumarin derivatives as presented in Scheme 12 below.^{183,184} This has since been proposed to proceed via an intermediate Baylis-Hillman adduct **93**, which spontaneously cyclises via a conjugate addition following the 1st path, which or the acyl substitution following the 2nd path.¹⁶⁷

Scheme 12: Non-regioselective Baylis-Hillman cyclisation as proposed by Robinson.¹⁶⁷

However, using a variety of activated alkenes (such as **94**) to preclude acyl substitution (using also a CH_2Cl_2 - H_2O solvent system), Nocanda cyclized regioselectively the MBH adduct and afforded chromenes **95** as shown in Scheme 13 below.¹⁶⁸



Scheme 13: Regioselective Baylis-Hillman cyclisation to chromenes asproposed by Nocanda.¹⁶⁸

Another modification of the MBH adducts was undertaken by Musa, who synthesized coumarin derivatives using the *O*-benzylated MBH adducts **97** (route **A**, Scheme 14). This author was able to isolate 3-substituted coumarins **98** and **99** of different types depending on the haloacid used for the cyclisation procedure.

However, he also established that when using tert-butyl acrylate **102** as the activated alkene, it was possible to isolate the MBH adduct **103** without prior protection of the free hydroxyl group of the aldehyde **91**, thereby affording a convenient and chemoselective route to 3-substituted coumarins **99** (route **B**, Scheme 14).¹⁸⁵⁻¹⁸⁷

He also explored the susceptibility of the three electrophilic centres in 3-(halomethyl) coumarins to nucleophilic attack (Figure 22), and concluded that various N- and C-nucleophiles attacked exclusively at the exocyclic C-1' electrophilic centre.¹⁸⁸

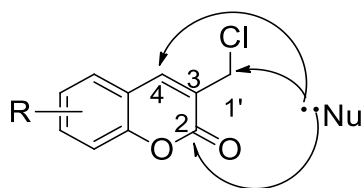
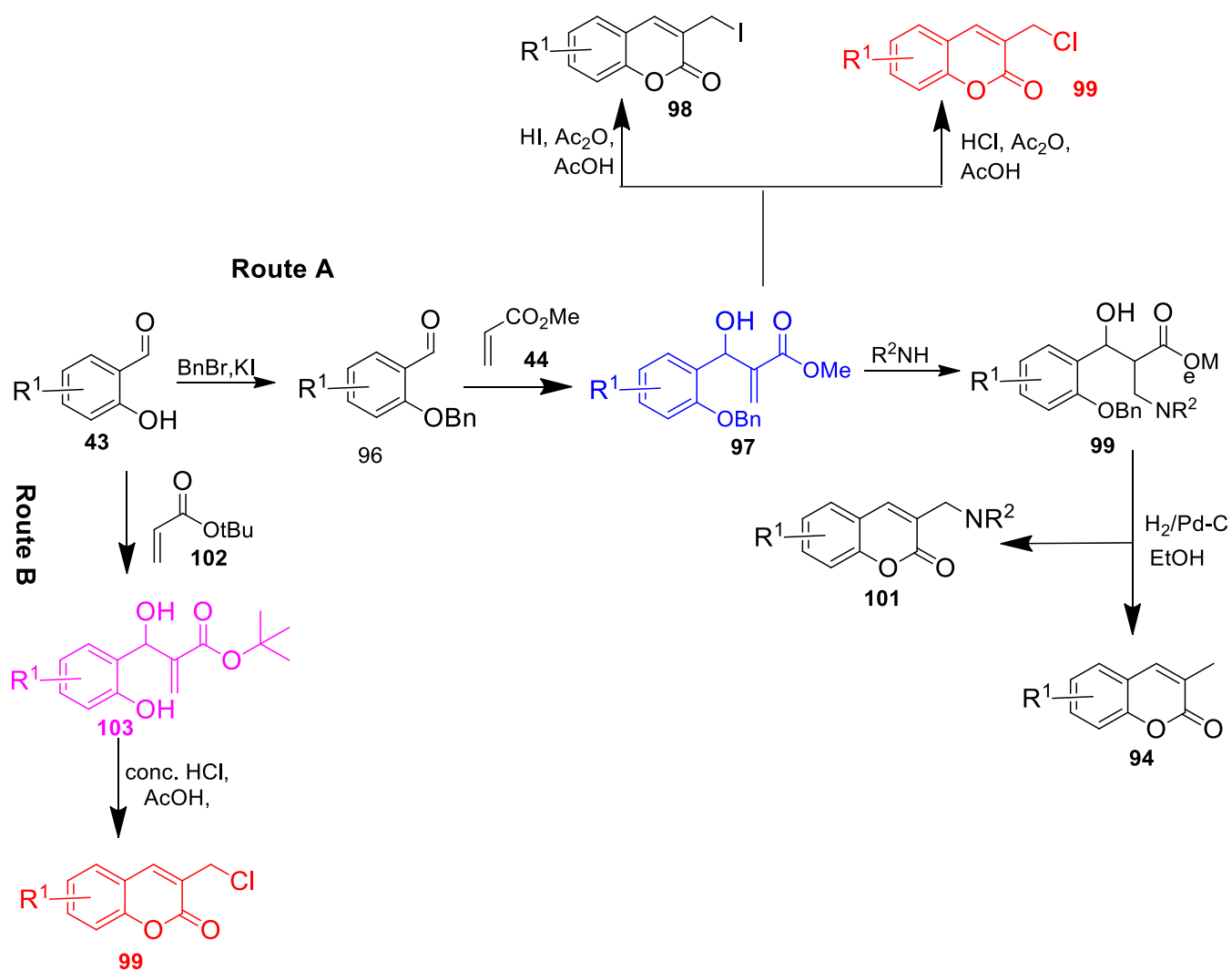
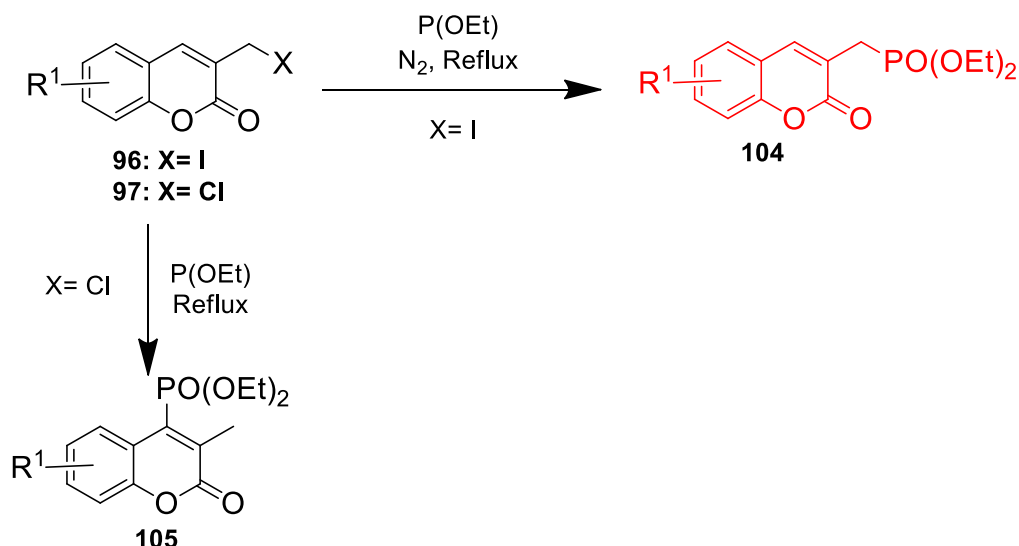


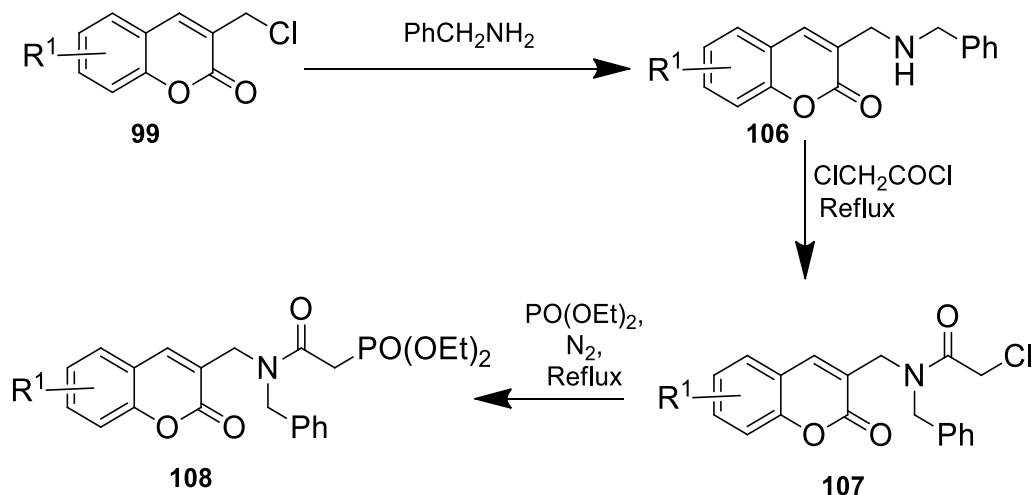
Figure 22: Possible modes of nucleophilic attack on 3-halomethyl coumarins.¹⁸⁸

Scheme 14: Cyclisation of unprotected Baylis-Hillman adducts to coumarins.¹⁸⁷

In the same way, using the Michaelis-Arbuzov reaction, Rashamuse obtained substitution by the para-nucleophile, triethylphosphite, at the 4-position on the coumarin nucleus. He also revealed the possibility of accessing to either the 1'-phosphonated (**104**) or the 4-phosphonated (**105**) coumarin analogues chemoselectively, depending on the halide group as described in Scheme 15.¹⁸⁹

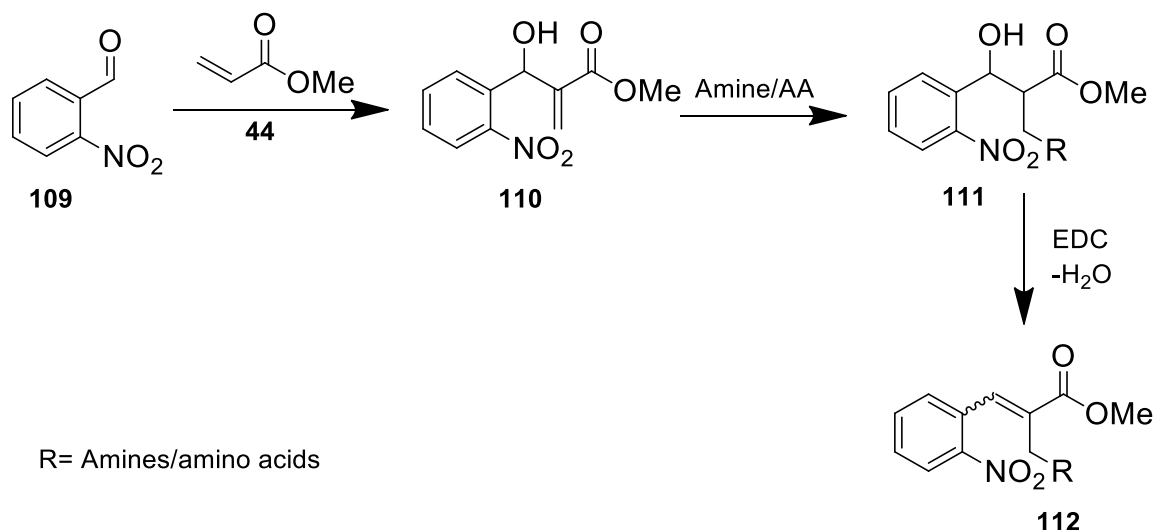
Scheme 15: Michaelis-Arbuzov reaction of 3-(halomethyl)coumarin analogues.¹⁸⁹

In addition, Rashamuse also prepared a series of phosphonated 3-(benzylaminomethyl)coumarin derivatives **108** by subjecting coumarin analogues which were accessed via MBH methodology to step-wise amination, chloroacetylation and Michaelis-Arbuzov phosphonation reactions as shown in Scheme 16).¹⁹⁰ Preliminary in silico docking studies of the hydrolysed analogues of these compounds as potential HIV-1 PR inhibitors, were also undertaken.¹⁹⁰

Scheme 16: Synthesis of phosphonated 3-(benzylaminomethyl)coumarin analogues as proposed by Rashamuse.¹⁹⁰

In another study undertaken in our group to access potential HIV-1 IN inhibitors, Lee succeeded to prepared some cinnamate esters **112** via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-mediated dehydration of the

hydrocinnamate derivatives **111** produced, in turn, by amination of MBH adducts (Scheme 17), the products were obtained as diastereomeric mixtures, with the (E)-isomer predominating.¹⁹¹



Scheme 17: Synthesis of cinnamate esters via Baylis-Hillman methodology.¹⁹¹

1.4. Importance of simplicity in synthesis of drug molecules

Apart from time-consuming issue related to the synthesis of current therapies, the use of expensive, non-green methods and the issue of the antiviral drug resistance mutation have been the most common shortcoming.

The fact that a drug binds to the enzyme's active site with measurable or high affinity does not automatically imply that it should be considered as a potential drug but it has to have several properties such as being selective or specific to avoid or minimize its side effects.^{192,193}

Additionally, its concentration within the cells of the host should remain at levels far above the inhibition constant (K_i) to ensure an effective treatment. It should also exhibit oral bioavailability. Another valuable property is that any potential drug should be able to treat a large set of viral genotypes and work in synergy (or at least be compatible) with complementary drugs⁶¹, in such a way to overcome any insurgence of drug resistance during the treatment.^{192,193}

Furthermore, treatment for an infection such as HIV needs to be taken for a lifetime, and this using combination of several drugs. Since HIV is particularly prevalent among the poor, a potential drug needs to be designed using very simple methodology (involving green chemistry), and readily available and inexpensive starting materials, so it can be cost effective and available to all categories of people, especially from the very poor background. The high price related to the current therapies is partially due to long processes/routes used to manufacture them.

However, these above-mentioned properties have been found, in most cases, to be challenging. Even for Ritonavir, for example, designed with C_2 -symmetry by Abbott's laboratories in 1994 as ABT-538, or A-84538, and an extensively used booster, is made in more than ten steps, using non-green methods and very expensive chemicals, employing several catalysts and generating numerous by-products. These by-products render the purification of the target molecule difficult, which results in poor yields of the desired product.

1.5. Aims of this research project

This research project is a part of an ongoing study, which is aimed at designing and synthesizing new truncated peptidomimetics as potential HIV-1 PR inhibitors. Initially, specific objectives of this study were to

- ❖ Synthesize, using the MBH reaction, both *O*-benzylated and non-benzylated MBH adducts from various salicylaldehyde substrates and different acrylates;
- ❖ Synthesize different aza-Michael adducts, via nucleophilic addition of various amino acids/amines onto the MBH adducts; to afford new truncated hydroxy- β -amino-methyl-ester derivatives;
- ❖ Base-catalyzed cyclization of the non-benzylated aza-Michael adducts to afford 3-(aminomethyl)coumarins analogs;
- ❖ Fully characterize all the synthesized ligands;
- ❖ Do the cytotoxicity test and PR inhibition bioassay using Ritonavir as standard.

In addition to these initial aims, which were embedded in the ongoing work of our research group, we wanted to explore the application of symmetrical and unsymmetrical diamides derivatives of tartaric acid. This chiral carbohydrate derivative is cheap and readily available, and can be readily transformed into a range of derivatives. However, utilization of condensing and activating agents in the amidation process has been progressively undesirable as environmentally benign alternatives to standard chemical transformations are required.¹⁹⁴

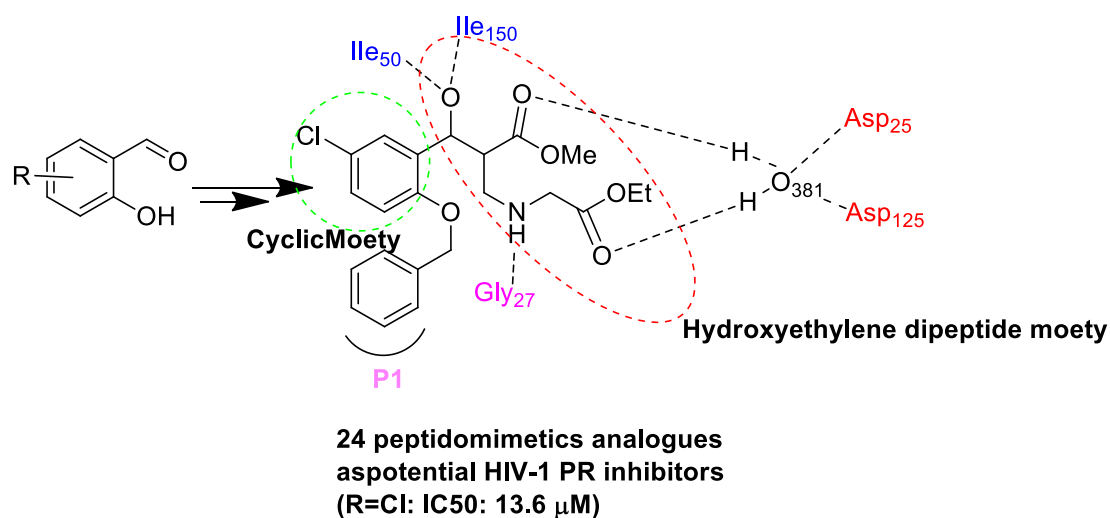
Additionally, catalytic solutions to several chemical reactions have been designed in such a way to maximize reactions yields while being atom efficient, and minimising the associated E-factor for the transformation.¹⁹⁵ For these reasons, uncatalyzed direct condensation of a carboxylic acid with an amine would be the most desirable way to build an amide bond. Therefore, the new aims were to:

- ❖ Explore the synthesis of both C₂-symmetrical and C₂-unsymmetrical 1,4-diamides using *uncatalyzed direct reaction* of diethyl tartrate or tartaric acid (in some cases) and tartaric osazone with corresponding amino acids/amines;
- ❖ Characterize all the synthesized ligands; and
- ❖ Perform Cytotoxicity and PR inhibition bioassay.

Chapter 2: Results and Discussions

2.1. Synthesis and evaluation of aza-Michael adducts of methyl 3-[(2-benzyloxy)phenyl]-3-hydroxy-2-methylenepropanoate esters as potential HIV-1 protease inhibitors

The MBH adduct features hydrophobic moieties, which help to bind the molecule appropriately in the active site. Potential binding interactions between some of the products and proximal amino acid residues in the HIV-1 protease receptor have been revealed, while in vitro bioassays have shown that many of the compounds exhibit a measure HIV-1 PR inhibition activity, three of them with IC₅₀ values of less than ca. 22 μ M. Scheme 18 below illustrates the above mentioned binding of the hydroxy group/carbonyl and the aryl/hydrophobic groups with the active site of the HIV-1 PR enzyme.



Scheme 18: Typical features of the aza-MBH adducts binding to the HIV-1 PR enzyme's active site

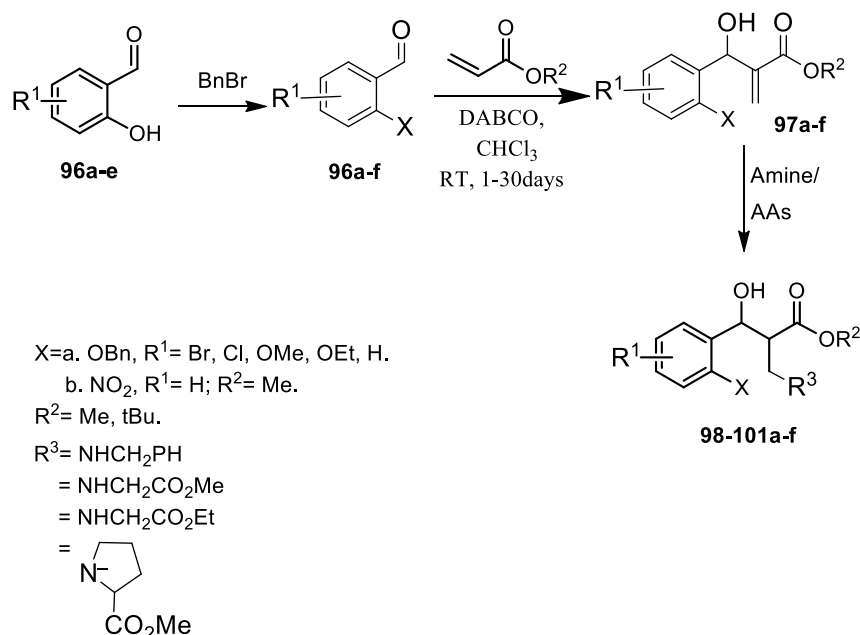
Many clinically approved HIV-1 PR inhibitors, such as ritonavir **16**,¹⁹⁶ contain a hydroxyethylamine or hydroxyethylene dipeptide isostere and would otherwise be the scissile site, and it has been noted that the configuration of the hydroxyl-bearing asymmetric centre is crucial to their inhibitory activity.^{197,198} In this research, the

development of potential HIV-1 PR inhibitors, hetero/cyclic derivatives, such as shown in Scheme 18¹⁹⁹ have been prepared which contain the hydroxyethylene dipeptide isostere present in ritonavir **16**, truncated analogues such as the indolizine derivative,²⁰⁰ as well as AZT-conjugates as potential dual-action inhibitors.²⁰¹⁻²⁰³

In this first section, the applications of the MBH methodology in the synthesis and evaluation of aza-Michael adducts of methyl 3-[(2-benzyloxy)phenyl]-3-hydroxy-2-methylenepropanoate esters **99a-e** as potential HIV-1 protease inhibitors is discussed.

The enzyme inhibition and cytotoxicity results of all the final compounds synthesized are also presented. The hydroxyl group, which is a feature of all MBH adducts, is important for optimising the binding of the substrate in the active site of the HIV-1 protease enzyme. This was one of the reasons why the MBH was chosen as one of the methods used in this work for the synthesis of potent inhibitors.

The pathway used in this approach involved several steps, viz., i) the benzylation of the 2-hydroxybenzaldehydes **43**, to afford the products **96a-e**; ii) a MBH reaction between each of the O-benzylated aldehydes and methyl acrylate to afford the B.H. adducts **97a-e**; and lastly iii) conjugate addition of different amino acids and/or amines to the MBH adducts **97a-e** to generate the target analogues **98-101a-e**. The overall reaction scheme of the planned synthesis is summarized in Scheme 19 below. Most of the aza-Michael adducts synthesized in this thesis (**98-101a-e**) have been previously prepared in our group. However, no bio-assay tests were not done so far, hence they were resynthesized here.



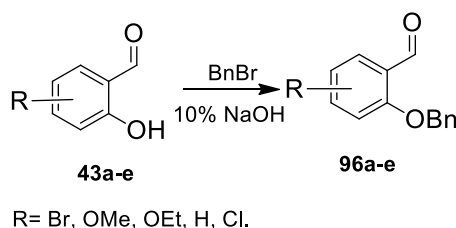
Scheme 19: *Plan for the synthesis of methyl 3-[(2-benzyloxy) phenyl]-3-hydroxy-2-methylenepropanoate esters 98-101a-f.*¹⁶⁵⁻¹⁶⁸

The same procedure was followed for the MBH reaction using 2-nitrobenzaldehyde as starting material with tert butyl acrylate to afford product **97f** followed by the aza-Michael addition reaction to afford a small library of products **98-101f** as shown in Scheme 19 above. These compounds have not yet been described in the literature to the best of our knowledge.

2.1.1. Synthesis of benzyl ethers ethers¹⁶⁵⁻¹⁶⁸

Morita-Baylis-Hillman reactions of 2-hydroxybenzaldehydes with methyl (or ethyl) acrylate are known to result in the spontaneous formation of complex mixtures of 2-chromene and coumarin derivatives as described by Bode *et al.*¹⁶² This can be circumvented by benzylation of the 2-hydroxy group of the salicylaldehyde derivatives, in such a way to allow the isolation of the MBH adducts and control of subsequent reactions.

As shown in Scheme 20 below, compounds **96a-e** were synthesized by the benzylation of substituted and unsubstituted 2-hydroxybenzaldehydes as described in the literature.¹⁶⁵⁻¹⁶⁸



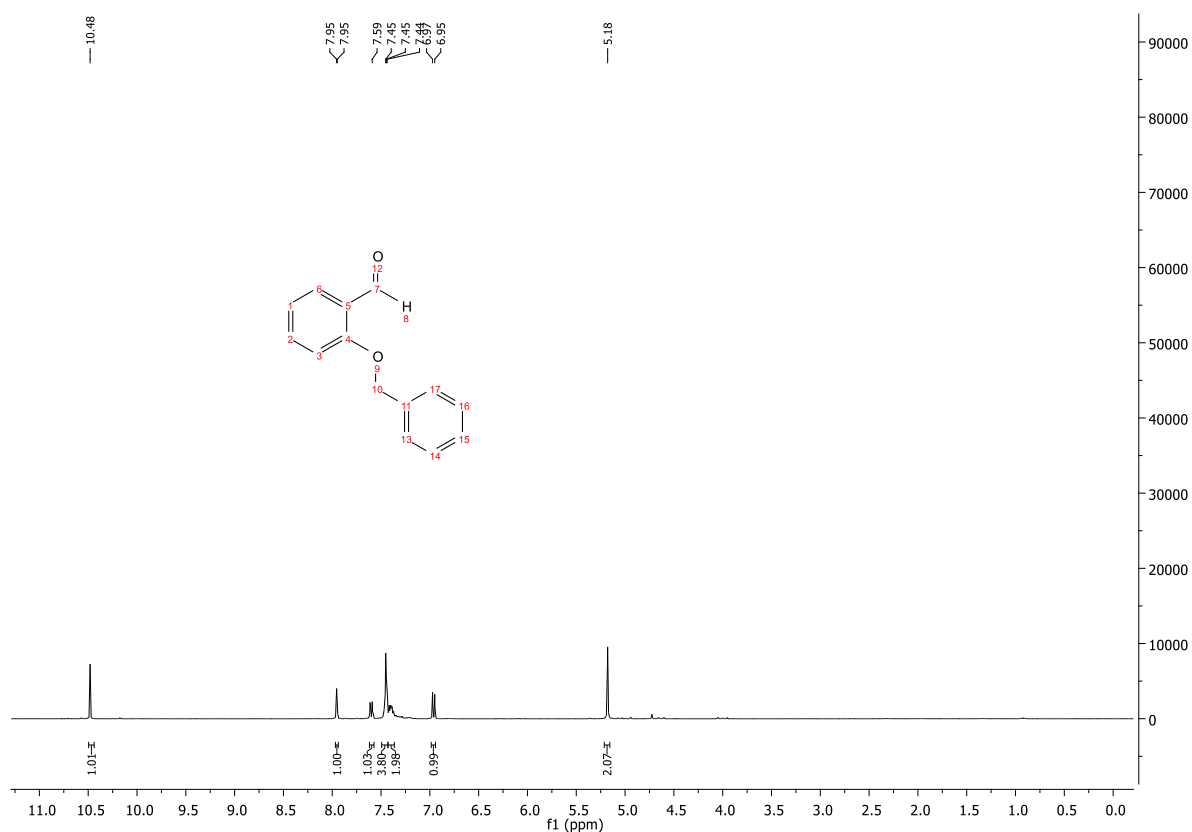
Scheme 20: O-benzoylation of salicylaldehyde. ¹⁶⁵⁻¹⁶⁸

This was achieved in moderate to high yields (70-96%) by refluxing 2-hydroxybenzaldehyde derivatives with benzyl bromide in the presence of 10% NaOH_(aq) for 30 minutes. The extraction of the product followed an acid-work-up to neutralize the base. The combined results are presented in **Table 1** below.

Table 1: The combined results of the benzylation of different 2-hydroxybenzaldehydes and their % isolated yields. ¹⁶⁵⁻¹⁶⁸

Compound	R ¹	% Isolated Yields
96a	H	70
96b	OMe	86
96c	OEt	80
96d	Br	98
96e	Cl	96

The appearance of a singlet at 5.18 ppm, integrating for 2H on the ¹H NMR spectrum of a representative compound **96a** (Figure 23) confirmed the formation of this product. This peak corresponds to the benzylic protons and appears as a singlet due to the absence of neighbouring protons around this methylene carbon. The singlet at 10.4 ppm corresponds to the aldehydic proton while the aromatic proton signals were observed between 6.96 and 7.95 ppm.



The product formation was also confirmed by ^{13}C NMR (Figure 24). The signals at 70.8 ppm and 188.4 ppm correspond to the benzylic and carbonyl carbons respectively, while the remaining signals correspond to the aromatic carbons.

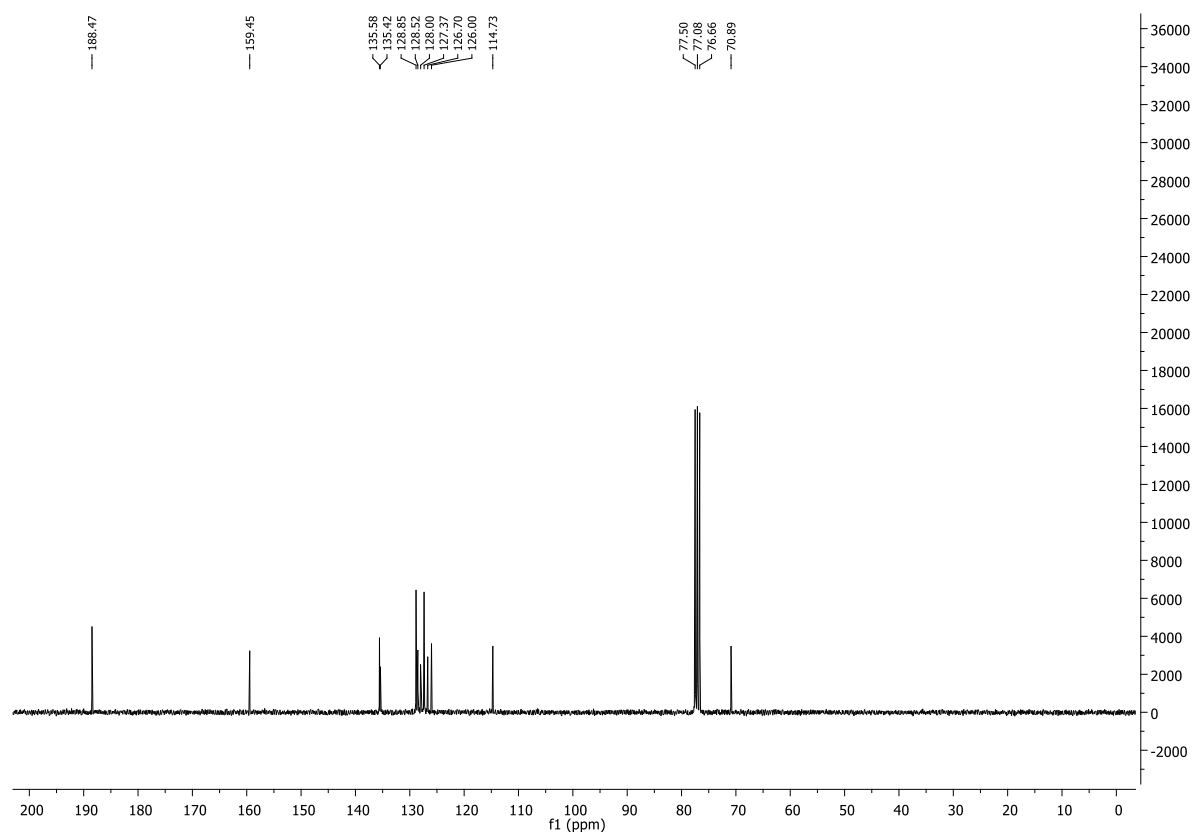
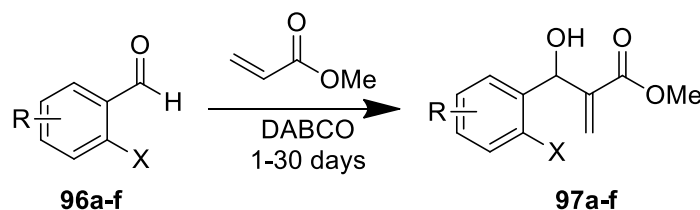


Figure 24: ^{13}C NMR (100 MHz) spectrum of the compound **96a** in CDCl_3 ²⁰¹⁻²⁰²

The peak at 70.9 ppm in the DEPT 135 NMR spectrum confirmed the presence of the benzylic carbon adjacent to the benzene group. These results correspond with the published data. ²⁰¹⁻²⁰²

2.1.2. Synthesis of the MBH adducts (**97a-f**) from the *O*-benzylated salicylaldehyde (**96a-e**) and 2-nitrobenzaldehyde with methyl acrylate

In order to access the MBH adduct, methyl acrylate was used as the activated alkene and this was the reason why the hydroxyl functional group of the benzaldehydes required protection (Scheme 21).



X= a. OBn; R= H, Br, Cl, OMe, OEt

b. NO₂; R= H only

Scheme 21: The MBH reaction of benzyl ethers and 2-nitrobenzaldehyde with methyl acrylate using DABCO as catalyst.
165-168

The solvent used for the MBH reaction (CHCl₃) was first passed through basic alumina to remove any contaminating HCl, thus preventing the acid-catalysed deprotection of the benzylated salicylaldehydes. As shown in Scheme 21, benzaldehyde derivatives (1 molar equivalent) were dissolved in a small volume of chloroform and then reacted with methyl acrylate (3 molar equivalents), at room temperature with stirring, in the presence of the DABCO catalyst.¹⁶⁵⁻¹⁶⁸ This reaction took between 7 and 30 days to run depending on the aldehyde substrate used. However, the reaction involving the nitrobenzaldehyde and the methyl acrylates took generally less time (0-5 days) to complete in high yields when compared to the benzylated salicylaldehydes, which took 7-30 days, or even more for the methoxy- and ethoxy-substituted compounds.

Work-up afforded the Baylis-Hillman adducts. The crude mixture was purified using both column chromatography and preparative thin layer chromatography (prep-TLC) to afford the MBH adducts **97a-f** in yields ranging from 26 to 61% as shown in Table 3 below. Apart from 97f, all the remaining compounds 97a-e have been described.¹⁶⁵⁻¹⁶⁸

Table 2: The results of the synthesis of MBH adducts and their % isolated yields.

Compound	R	% Yields isolated
97a	H	31
97b	OMe	26
97c	OEt	28
97d	Br	61
97e	Cl	57
97f	NO ₂ ¹	60

¹ Made from nitrobenzaldehyde, not bearing a benzyl ether

It was observed that the methoxy- and ethoxy-substituted and the unsubstituted 2-hydroxybenzaldehydes took much longer than the 4-bromo or 4-chloro-substituted ones, which gave better yields than the others did. This corresponds with earlier observations that the electron-deficient aldehydes are more reactive under these conditions. However, as mentioned before, the nitro compound having a strongly electron withdrawing reacted more quicker and this in terms of reaction time but gave moderate yields in the first trial, which improved later on after the reaction time was also increase in some attempt.

The synthesis of the MBH adduct **97d** was confirmed first by the disappearance of the singlet corresponding to the aldehyde proton at 10.4 ppm as shown in Figure 25 below.

The second strong evidence was the appearance of the two vinylic proton signals at 5.58 ppm and 6.22 ppm integrating for 1H each. These two peaks are characteristic of the formation of a MBH adduct and correspond to the new alkene functional group which forms from the attachment of the methyl acrylate. The doublet at 5.82 ppm, integrating for 1H, corresponds to one proton on the methine carbon bearing the hydroxyl functional group (-CH(OH)).¹⁶⁵⁻¹⁶⁸

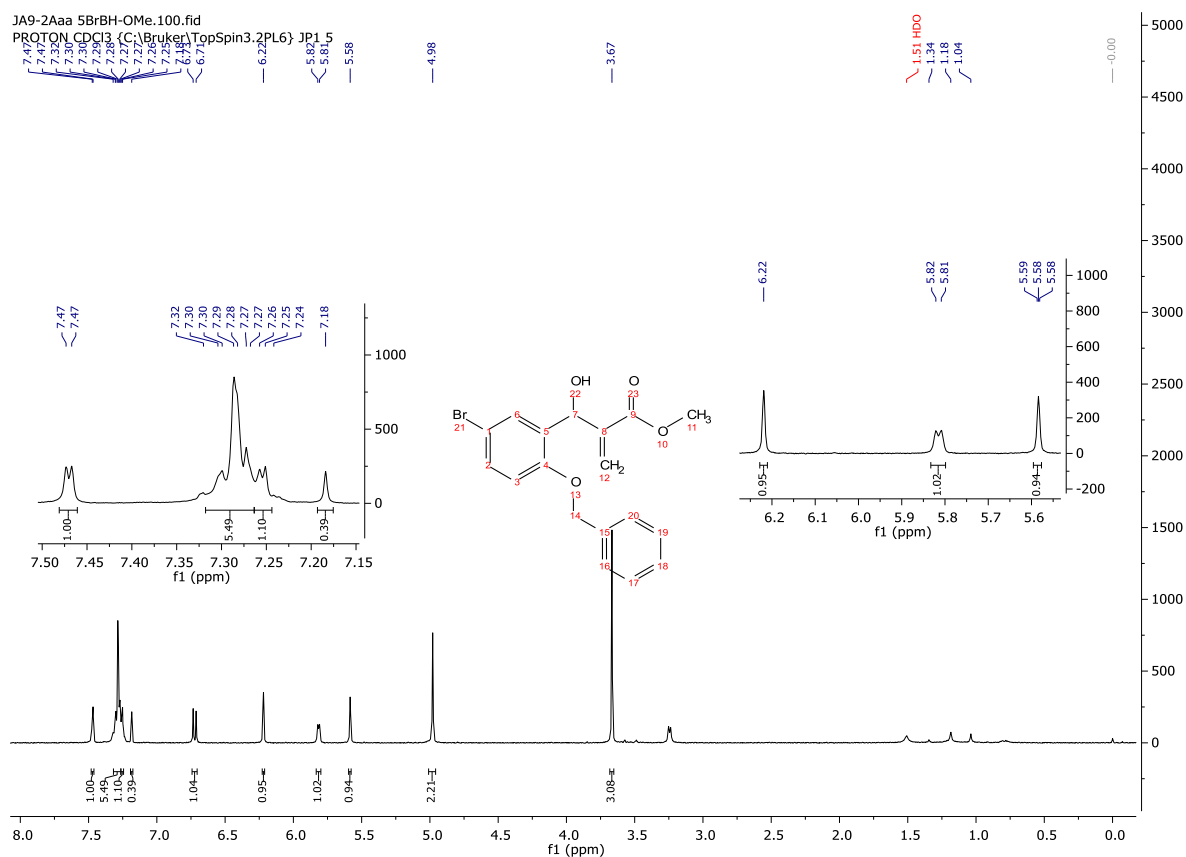


Figure 25: The ^1H NMR (400 MHz) spectrum of compound **97d** in CDCl_3 .¹⁶⁵⁻¹⁶⁸

The singlet at 4.98 ppm integrating for 2H corresponds to the two benzylic protons, while the singlet at 3.67 ppm integrating for 3H corresponds to the methyl group from the methyl acrylate. Other peaks between 6.72 ppm and 7.47 ppm correspond to the 8 aromatic protons (8H). The disappearance of the aldehyde signal at 190.3 ppm as observed using the ^{13}C NMR (Figure 26), further confirmed the synthesis of this compound **97d**.¹⁶⁵⁻¹⁶⁸

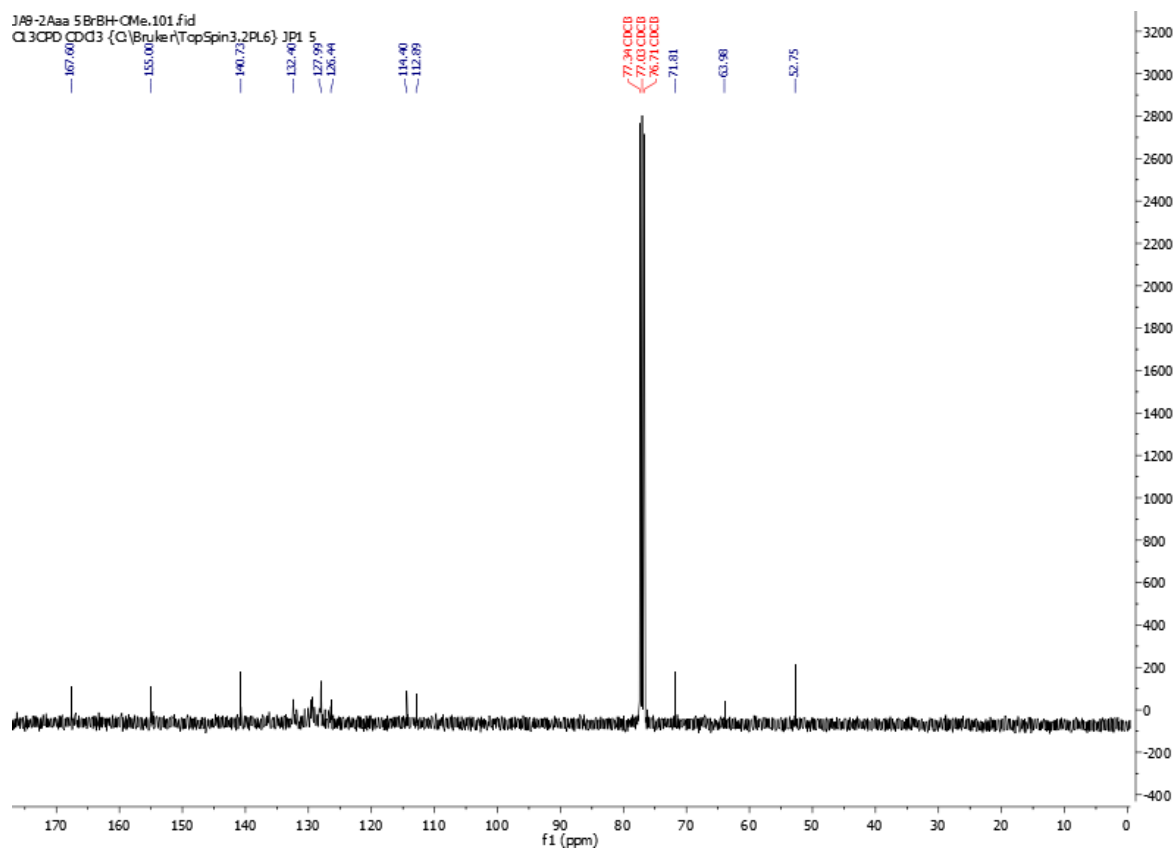


Figure 26: The ^{13}C NMR (100 MHz) spectrum of **adduct 97d** in CDCl_3 .¹⁶⁵⁻¹⁶⁸

Analysis of the DEPT 135 NMR spectrum (Figure 27) confirmed the methyl, 3-methine and benzylic carbons at 52.0, 67.7 and 70.4 ppm respectively. The methylene signal at 126.5 ppm confirmed attachment of the two vinylic protons on the same carbon and all these results and the remaining observed signals agreed with the literature.¹⁶⁵⁻¹⁶⁸

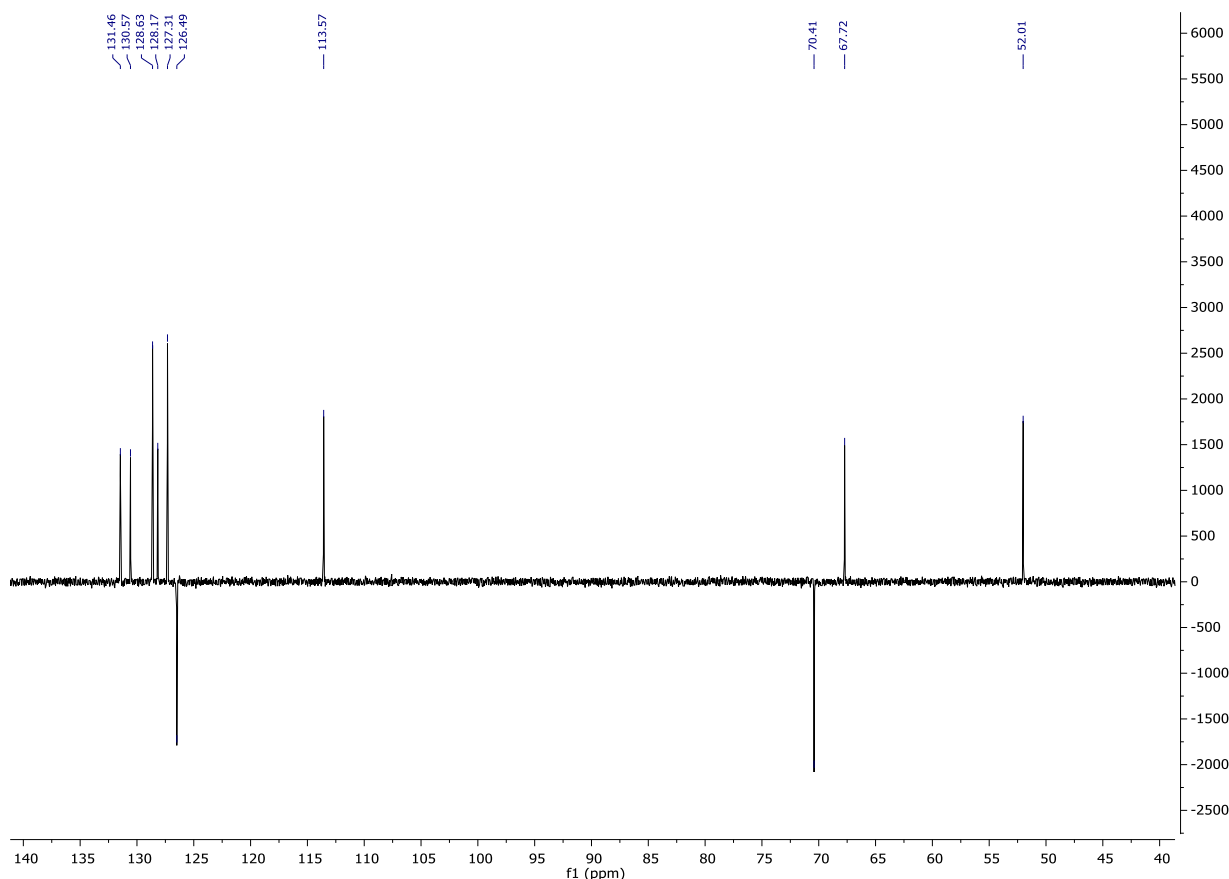


Figure 27: The DEPT 135 (400 MHz) NMR spectrum of adduct 97d in CDCl_3

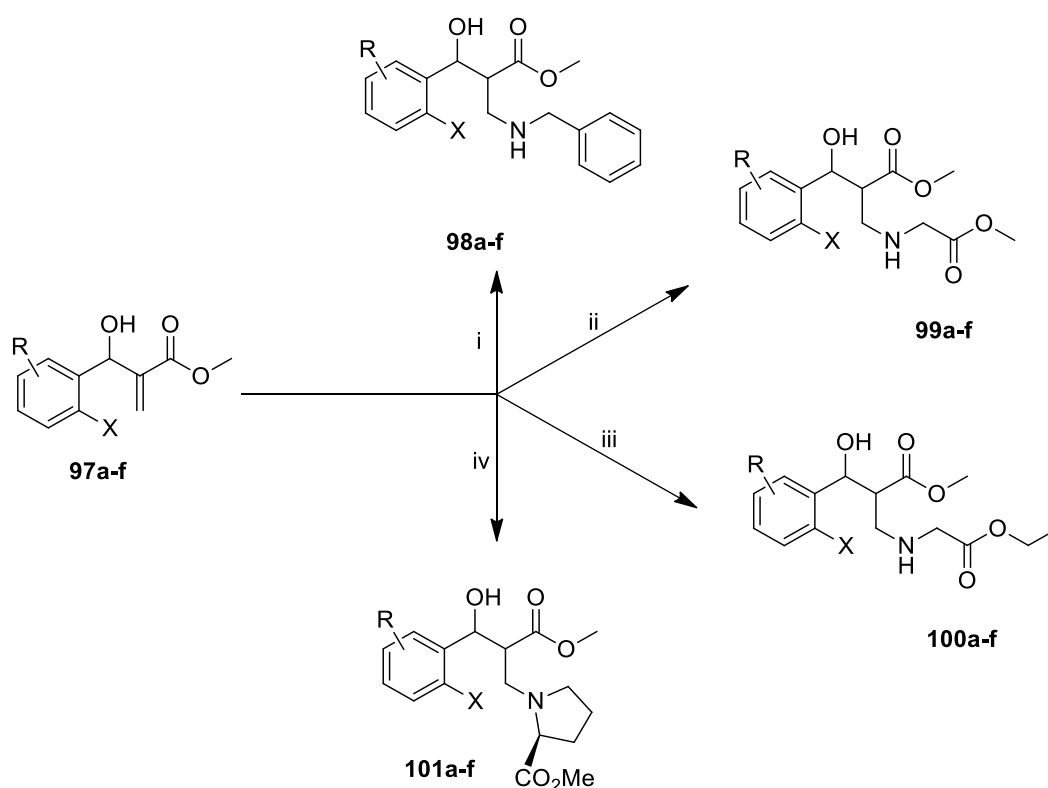
2.1.3. Synthesis of the aza-Michael adducts.²⁰¹⁻²⁰²

It is well known that the MBH reactions typically afford α,β -unsaturated carbonyl products which can serve as Michael acceptors – a property which we have used successfully in the synthesis of various benzannulated heterocyclic systems.¹⁶⁵⁻¹⁶⁸

The conjugate addition of a nitrogen-containing nucleophile to α,β -unsaturated carbonyl compounds gives access to β -amino carbonyl compounds, also known as aza-Michael addition adducts. Different methods, including the Mannich reaction, have been used to generate β -amino-carbonyl containing compounds and several catalysts, both bases and acids as well as Lewis acids have been used for this reaction.

MBH adducts are α,β -unsaturated carbonyl compounds which can be further elaborated in the development of β -hydroxycinnamate analogues by reacting them

with primary or secondary amines or amino acids through aza-Michael-type addition as described in Scheme 22 below.



X= a. R= H, 3-OMe, 3-OEt, 5-Br, and 5-Cl; X= OCH₂Ph
b. R= H, X= NO₂

Scheme 22: Synthetic route for aza-Michael adducts of methyl 3-[(2-benzyloxy)phenyl]-3-hydroxy-2-methylenepropanoate esters 97-101a-f. Reagents and conditions: i) benzylamine, MeOH, rt, 10-12 days; ii) methyl glycine.HCl, tetrabutylammonium bromide (TBAB), Sodium acetate (NaOAc), MeOH, rt, 10-12 days; iii) ethyl glycine.HCl, TBAB, NaOAc, MeOH, rt, 10-12 days; iv) (S)-proline.HCl, TBAB, NaOAc, MeOH, rt, 10-12 days.

As illustrated in Table 3 below, the MBH adducts **97a-f** were reacted with Glycine methyl ester, glycine ethyl ester and proline methyl ester (racemic mixture), by stirring in dry THF for up to 10 days at room temperature (Scheme 22) to access the 3-[(2-benzyloxy)phenyl]-3-hydroxy-2-methylenepropanoate esters **98-101a-f**. The aza-Michael reactions of the racemic MBH products **98-101a-f** has introduced a new stereogenic centre and are expected to afford diastereomeric adducts. Based on previous results from our group, it was found that ¹H NMR analysis of the crude benzylamino derivatives **98a-d** revealed the presence of a pair of diastereomers in each case and permitted estimation of the corresponding diastereomeric excess (% d.e. = 30-37%^{165c}; Table 3). Purification using preparative layer chromatography

(PTLC) permitted the isolation of the diastereomers, albeit in ratios, which differed somewhat from those estimated by NMR analysis of the crude mixtures.

In this study, TLC analysis of the crude products from these derivatives revealed several spots, which possibly correspond to the different diastereomers previously isolated by Olasupo, I. and co-workers^{165c} in the synthesis of **98-101a-e**. For this project, only the major products were isolated by flash column chromatography followed by preparative TLC to afford single isomers of the conjugate addition products **98-101a-f** in 39-95% yields.

Neutralisation of the hydrochloride salts of the amino acids was effected *in situ* by addition of the phase transfer catalyst, tetrabutylammonium bromide (TBAB), and sodium acetate. The Michael addition involving use of the hydrochloride salts of methyl and ethyl glycine appeared to be diastereoselective. Though glycine is not chiral and thus making it very difficult to resolve the diastereomers, in some cases single diastereomers have been isolated following chromatography and the TLC-MS.

Similar purification of the aza-Michael adducts **99a-d**, **109a-d** and **101a-d** using methyl glycine, ethyl glycine and L-proline as the respective nucleophiles afforded single diastereomers in yields ranging from very low to high yields (39-95%; Table 3), whereas the 3-methoxyphenyl and 3-ethoxyphenyl analogues **101b** and **101c**, respectively, were not resolved under these conditions.^{165c}

Table 3: Reaction yields and diastereomeric ratios of the aza-Michael adducts **98-101**.^{165c;201-202}

Product	R ¹	R ² (Amine/AA)	Yield (%) ^a	Yield (%) ^b	Resolved diastereomers (%) ^c	Resolved diastereomers (%) ^d
98a	H	Benzylamine	71	74.8	49 & 19	30 ^d
98b	OMe	Benzylamine	50	76.0 (50)	53 & 18	35 ^d
98c	OEt	Benzylamine	53	83.1 (53.5)	55 & 18	37 ^d
98d	Br	Benzylamine	49	72.7 (49)	49 & 15	34 ^d
98e	Cl	Benzylamine	53	-	- ^e	
98f	NO ₂	Benzylamine	67	-	- ^e	

99a	H	Glycine-OMe	95	56.1 (94.8)	19 ^f
99b	OMe	Glycine-OMe	89	68.5 (89)	61 ^f
99c	OEt	Glycine-OMe	82	49.9 (81.6)	50 ^f
99d	Br	Glycine-OMe	83	52.2 (83)	13 ^f
99e	Cl	Glycine-OMe	95	-	- ^e
99f	NO ₂	Glycine-OMe	78	-	- ^e
100a	H	Glycine-OEt	92	50.3 (92)	6 ^f
100b	OMe	Glycine-OEt	85	58.1 (85)	14 ^f
100c	OEt	Glycine-OEt	88	56.4 (83)	11 ^f
100d	Br	Glycine-OEt	79	56 (79)	21 ^f
100e	Cl	Glycine-OEt	93	-	- ^e
100f	NO ₂	Glycine-OEt	70	-	- ^e
101a	H	Proline-OMe	46	39.3 (46)	6 ^f
101b	OMe	Proline-OMe	55	59.1 (55)	- ^e
101c	OEt	Proline-OMe	40	51.7 (40.5)	- ^e
101d	Br	Proline-OMe	51	52.3 (51.2)	37 ^f
101e	Cl	Proline-OMe	53	-	- ^e
101f	NO ₂	Proline-OMe	53	-	- ^e

^a Isolated yield in this current project. ^b Isolated yield before resolution in our group.^{165c;201-202} ^c Isolated yields of the resolved diastereomers in our group. ^d Diastereomeric excess (%) values obtained from the crude ¹H NMR spectra. ^e Not resolved. ^f Single diastereomer obtained after purification in our group.^{165c;201-202}

The above Table 4 illustrates different results from what we found in comparison with what has been described in the literature from our research group.²⁰¹⁻²⁰² The general low yields observed in most cases with these compounds **98-101a-f** may be due to many different causes. These including their poor solubility, low nucleophilicity, and more importantly the generation of many by-products (including diastereomeric excesses in some cases, which were resolved previously in our group as mentioned before) as well as the use of “co-catalysts” and/or phase transfer agents, constituted the major challenge during the purification step, and therefore only major products were isolated and analysed.

All of the aza-Michael adducts were fully characterised using elemental analysis (CHN), and NMR (1-D ^1H and ^{13}C and, where appropriate 2-D) methods in combination with available literature.²⁰¹⁻²⁰² Assignment of peaks in Figure 28 was made possible by also using the NMR data of the starting materials. The ^1H NMR analysis of the compound **98a** confirmed the disappearance of the characteristic of MBH adduct vinylic proton signals between 5-6.5 ppm. The singlet at 2.02 ppm integrating for 1H corresponds to the proton directly attached to the nitrogen as shown in Figure 28 below.

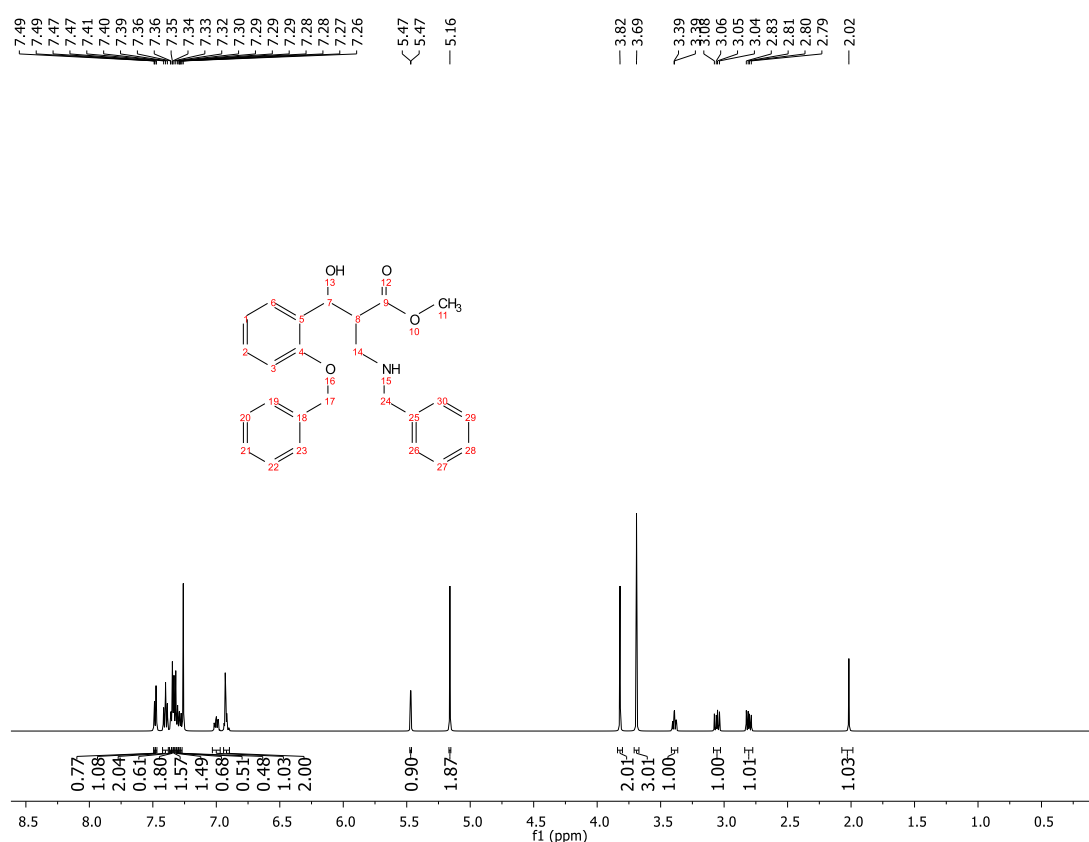


Figure 28: The ^1H NMR (300 MHz) spectrum of compound **98a** in CDCl_3 .^{191,193-194, 206}

The two doublets of doublets (dd) at 2.81 ppm and 3.06 ppm and integrating for 1H each correspond to the two diastereotopic protons of the methylene group between the α -H and the nitrogen atom. The triplet of a doublet (td) at 3.39 ppm, which integrates for 1H, corresponds to the α -H of the carbon adjacent to the carbonyl carbon.

This proton is flanked by two protons on one side (from the methylene group) which splits it into triplet and the proton on the carbon bearing the hydroxyl group, which splits it further into a doublet of triplets. The three protons of the methyl ester functional group resonate at 3.69 ppm as a singlet due to the absence of any neighbouring protons.

The singlet at 3.82 ppm, which integrates for 2H confirmed the formation of the product **98a**. It corresponds to the two protons on the methyl group between the nitrogen atom and the benzene group of the amine. The doublet at 5.47 ppm, which integrates for 1H, corresponds to the only proton adjacent to the hydroxyl functional group and the α -H, which is responsible for its appearance as doublet. The other remaining fourteen peaks between 7.05 ppm and 7.48 ppm correspond to the aromatic protons.

This compound was also confirmed by the ^{13}C NMR spectrum analysis (Figure 29), which showed the presence of the benzylic carbon peaks related to the methylene groups both adjacent to benzene groups, as well as all the other expected peaks.

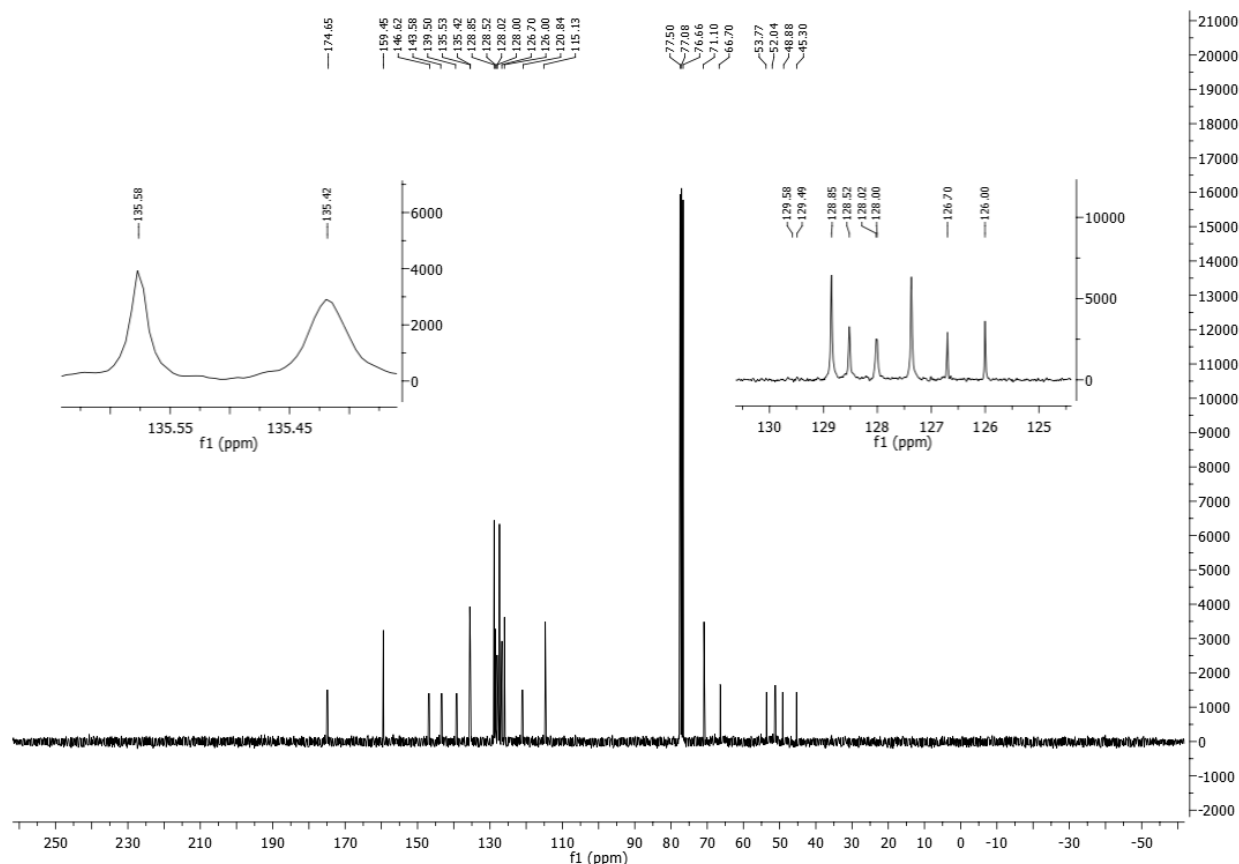


Figure 29: The ^{13}C NMR (75 MHz) spectrum of compound **98a** in CDCl_3 .

2.1.3.1. HIV Protease assay

The reaction mixture consisted of 0.6 $\mu\text{g/mL}$ HIV-1 subtype C protease, the buffer (1 M NaCl, 0.1 M sodium acetate, 1 mM EDTA, 1 mM dithiothreitol, 0.1% bovine serum albumin, 5% DMSO, pH 4.7) containing 8 μM fluorogenic substrate peptide (HIV protease substrate 1, Sigma-Aldrich). Test compounds were diluted in reaction buffer and reactions initiated by the sequential addition of the protease and substrate peptide to yield final compound concentrations of 20 μM . Reactions were carried out in a total volume of 200 μL in 96-well black plates for 20 minutes at 37 $^{\circ}\text{C}$ and fluorescence measured in a Spectramax M3 plate reader (excitation 340 nm, emission 485 nm) (Molecular Devices). Percentage enzyme activity relative to control wells (reactions without inhibitors) was calculated after subtracting background fluorescence readings (reactions without inhibitors or enzyme). Dose-response analysis to determine the IC_{50} values of selected compounds was carried out as described above, except that compounds were included in the reactions as 10-fold serial dilutions starting at 1 μM . Non-linear regression analysis of % enzyme

activity vs. log[compound] was carried out using GraphPad Prism (v. 5.02). Ritonavir was included as a standard reference inhibitor in all assays and results are presented in Figure 30 below

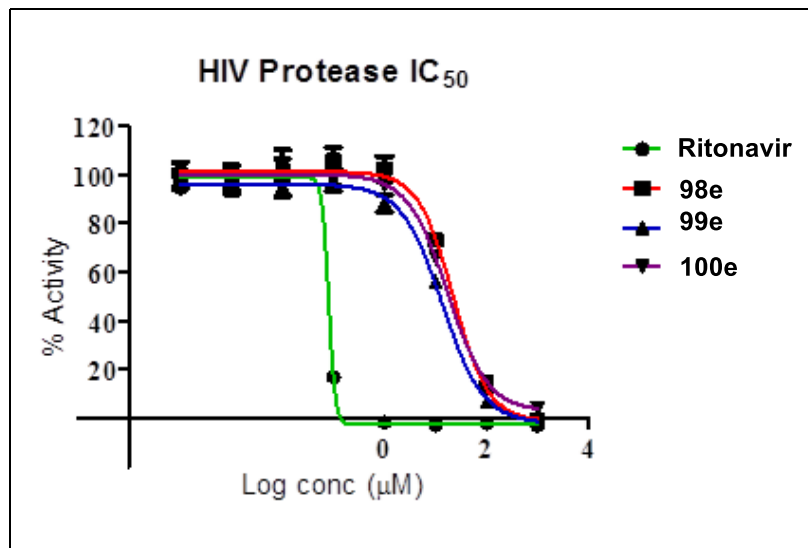


Figure 30: Plots of % activity of HIV-1 PR against concentration of ritonavir and ligands **98e**, **99e** and **100e**.

2.1.3.2. HeLa cell toxicity assay

HeLa cells (Cellonex) were plated at a density of 1.5×10^4 cells per well in 96-well plates and cultured overnight in DMEM with 5 mM L-glutamine (Lonza) supplemented with 10% (v/v) fetal bovine serum and antibiotics (penicillin, streptomycin, amphotericin B) in a 5% CO₂ incubator at 37°C. Test compounds were added to a final concentration of 20 μM and incubation continued for a further 48 hours. The concentration of viable cells in the wells was determined by adding a resazurin-based reagent (*in vitro* toxicology assay kit, Sigma-Aldrich) according to the manufacturer's instructions and monitoring fluorescence (excitation 560 nm, emission 590 nm) in a plate reader. Percentage cell viability relative to control wells (cells without compound) was calculated after subtracting background fluorescence readings (wells without cells). Results are presented in Figure 31 below.

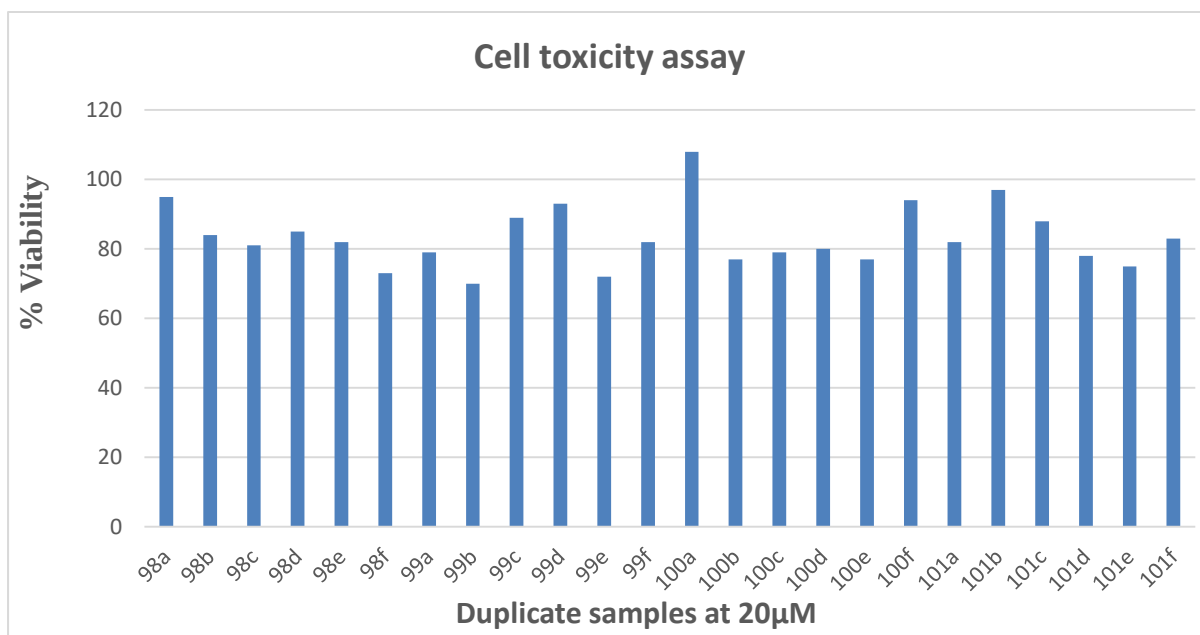


Figure 31: The bar graph of the percentage HeLa cell viability $\pm$ SD obtained for compounds **98-101a-f**.

Bioassay and docking data for the series of ligands (**98-101a-f**) showing the overlay of the *RR*, *RS*, *SR* and *SS* diastereomers of the aza-Michael adduct **98e** docked in the active-site of HIV-PR (2R5P), using Autodock (Figure 32); the hydrogen-bonding interactions between the docked *RR* diastereomer of the aza-Michael adduct **98e** (Figure 32) and proximate amino acid residues in the active-site in of HIV-PR (2R5P) using Autodock and the overlay of the *RR*-diastereomer of the aza-Michael adduct **98e** (Figure 33) and the control ligand docked in the active-site of HIV-PR (2R5P) using Autodock as well as the mean Autodock energies for ligands in the PDB HIV-1 PR crystal structures, 1HXB, 1HXW, 5T2Z and 2R5P results of the assays are described below. All these results are tabulated in Table 4 below.

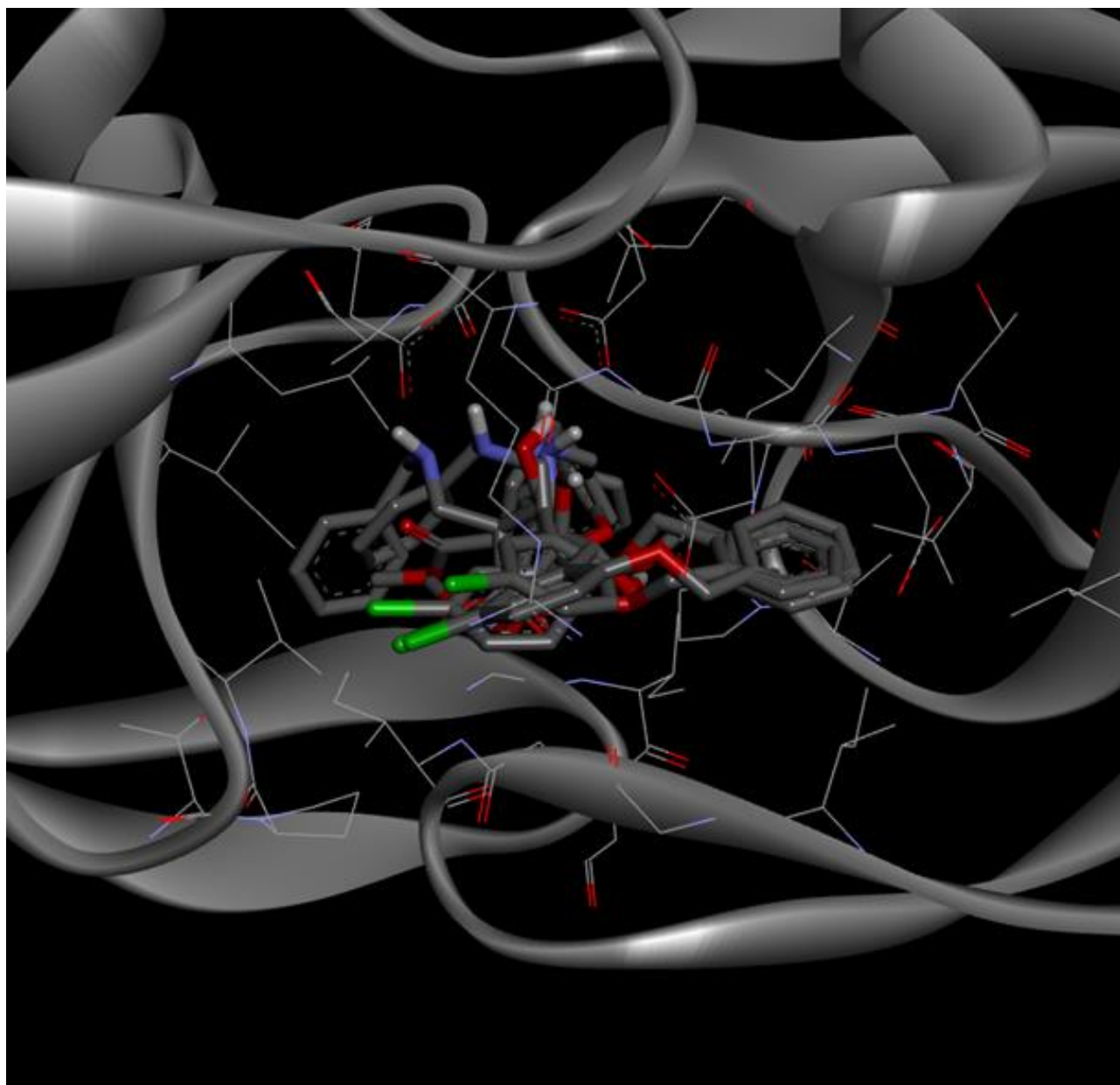


Figure 32: Overlay of the RR, RS, SR and SS diastereomers of the aza-Michael adduct 98e docked in the active site of HIV-PR (2R5P) using Autodock. Protein residues in the active site are shown in wireframe, coloured by atom type, the ligands as sticks, coloured by atom type.

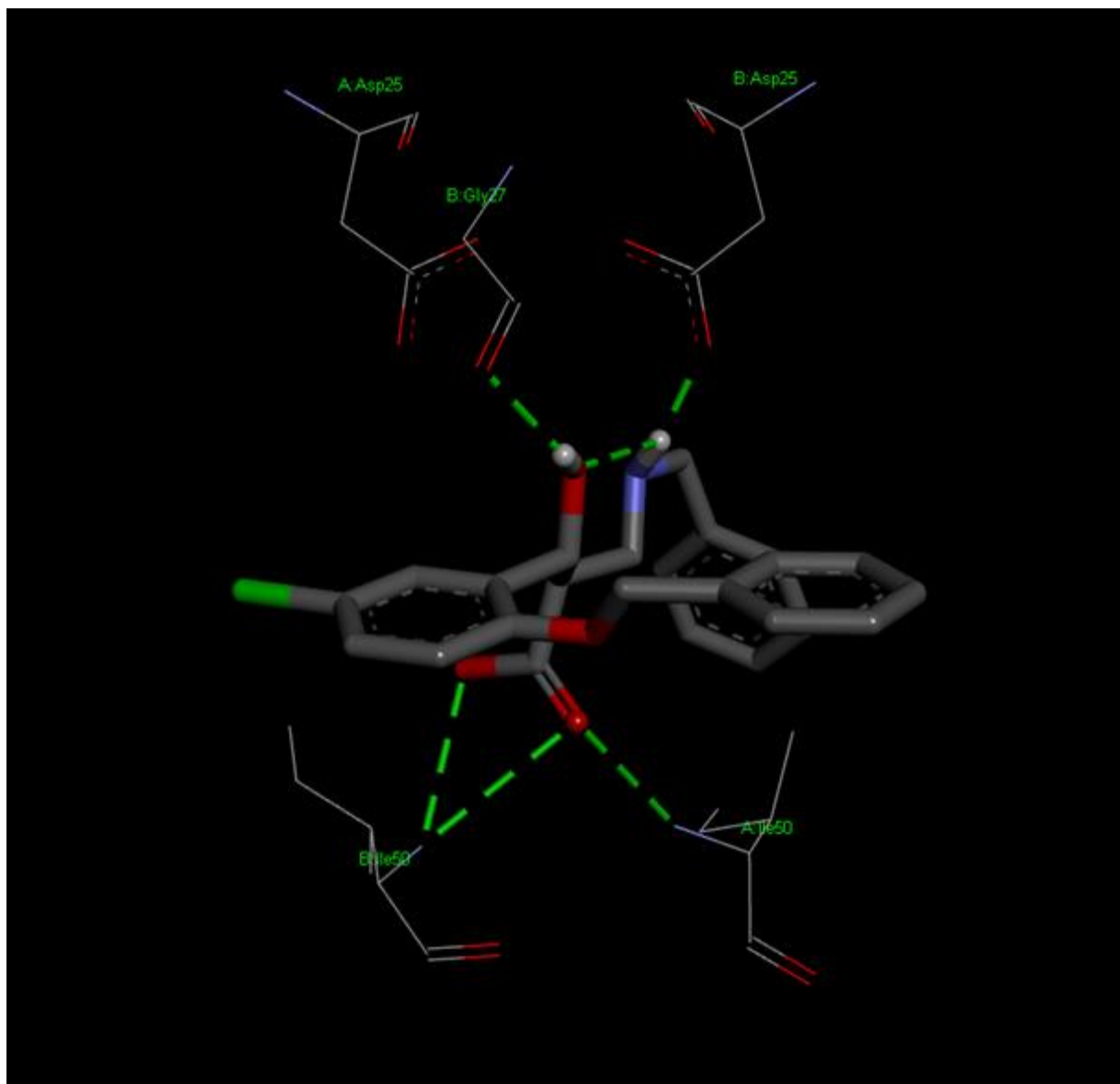


Figure 33: Hydrogen-bonding interactions between the docked RR diastereomer of the aza-Michael adduct 98e and proximate amino acid residues in the active site in of HIV-PR (2R5P) using Autodock. Protein residues in the active site are shown in wireframe, coloured by atom type, the ligand as sticks, coloured by atom type, and the potential hydrogen-bonds as green dashes.

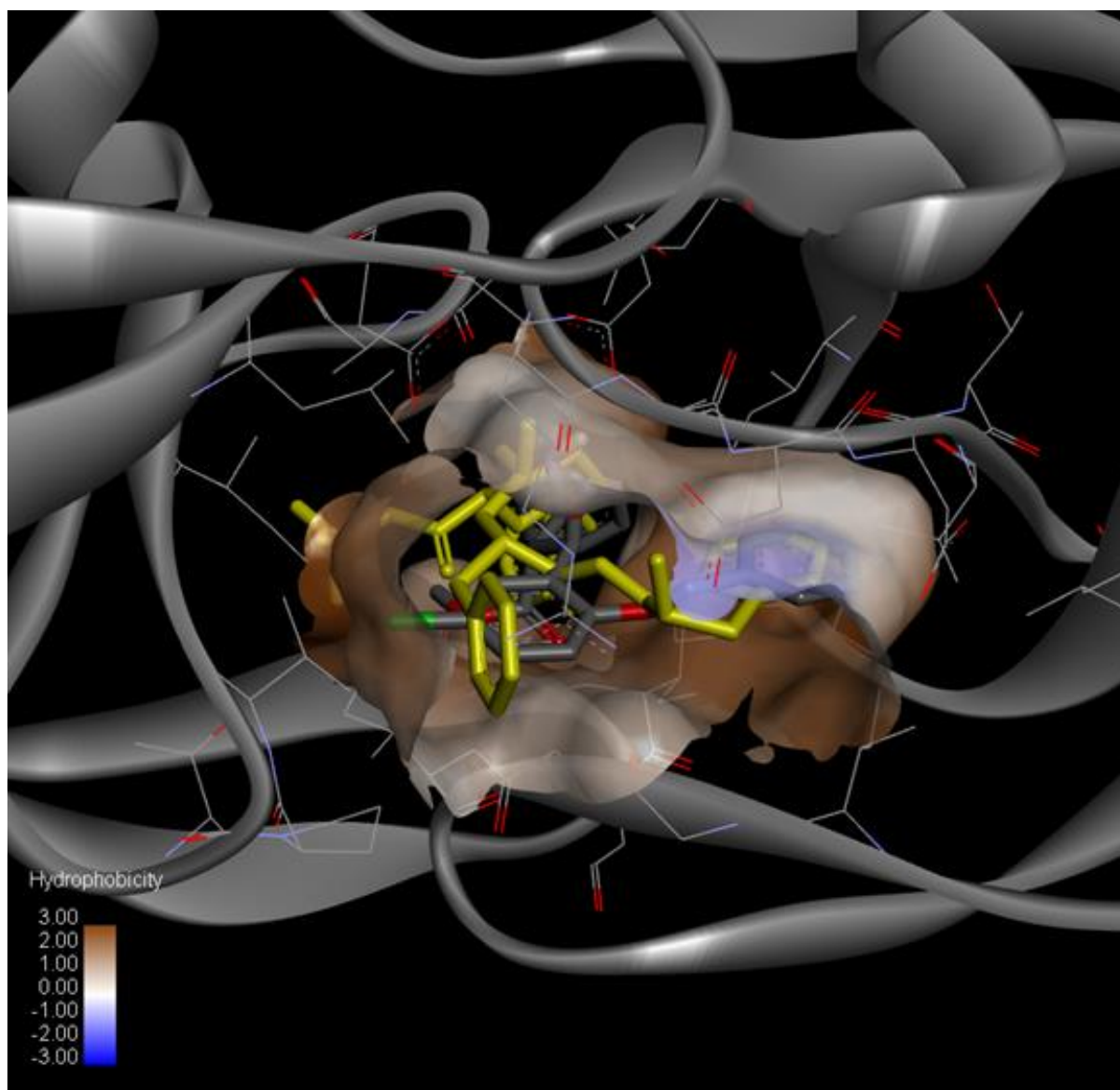


Figure 34: Overlay of the *RR*-diastereomer of the aza-Michael adduct **98e** and the control ligand docked in the active site of HIV-PR (2R5P) using Autodock. Protein residues in the active site are shown in wireframe, coloured by atom type, the ligand as sticks, coloured by atom type, the control ligand coloured yellow, and the receptor surface shaded to reflect hydrophobicity levels.

The activity of the HIV-1 PR enzyme in the presence of each of the ligands at a concentration of 20 μM , the corresponding percentage inhibition at this concentration and the percentage viability of HeLa cells at 20 μM have been presented in Figure 30, and Table 4 below. With a few exceptions, the synthetic ligands exhibited some inhibition of HIV-1 PR although lower than Ritonavir. Variation of the amino moiety appeared to have relatively little effect on inhibition of HIV-1 PR, since ligands representative of three of the sets of aza-Michael adducts reflect similar inhibition of HIV-1 PR at 20 μM (**98c**, 58.4%; **98e**, 62.4%; **99e**, 62.1%; **100d**, 51.1%; and **100e**, 78.5%). The 5-chloro derivative in three of the four sets (**98e**, **99e** and **100e**)

exhibited encouraging HIV-1 PR inhibition. Since most of compounds showed IC₅₀ values less than 50%, IC₅₀ values were determined only for four of the ligands exhibiting greater than 50% HIV-1 PR inhibition highlighted in green in Table 4.

These IC₅₀ were low ($\leq 22.2 \mu\text{M}$), the most active being the 5-chlorophenyl ethyl glycine derivative **100e** (IC₅₀ = 13.6 μM). None of the ligands showed significant toxicity against HeLa cells at 20 μM as compared to the Ritonavir although being more active.

Docking of the *O*-benzylated aza-Micheal conjugate **98e** into the HIV-1 PR receptor cavity was explored. The X-ray crystal structure of the receptor cavity of the HIV-1 PR enzyme (PDB 1HXW) was used,⁵² and the structures of the derivative **98e** and the protein were prepared using Discovery Studio Visualizer.²⁰⁵ The ligand occupying the receptor cavity and all water molecules were removed from the original PDB file, hydrogen atoms were added and each atom assigned an Autodock Type using AutoDock Tools (ADT). The Autodock 4.2 programme²⁰⁶ was used to explore the binding mode of compound **98e**, when docked in the HIV-1 PR active site using a flexible dock approach.

The protein active site and surrounding residues were mapped using the AutoGrid 4.2 algorithm, and a generic algorithm was used to perform the conformation search. Atom maps were generated for all potential interactions between the ligand and the active site residues, while the Arg8 catalytic residues were kept flexible. For docking calculations, Gasteiger partial charges²⁰⁷ were assigned to compound **98e** and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. Docked conformations were then viewed and explored using Discovery Studio Visualizer.²⁰⁵

Since the benzyl group was introduced to fit into the S1 hydrophobic pocket of the HIV-1 PR active site, the docking studies revealed several potential hydrogen-bonding interactions between proximate protein residues (Arg8, Gly27, Gly27', Asp25, Asp25', Ile50 and Ile50') and our aza-Micheal ligand **98e** (and some others) (Figure 33 and Figure 34). It is apparent that compound **98e** fits well in the active site

of HIV-1 PR and this is supported by the relatively close correspondence between the docked conformation of compound **98e** and the X-ray crystal structure of the known inhibitor, Ritonavir, in the active site.

This perhaps explains why some of these compounds exhibit good HIV-1 PR inhibition when compared with Ritonavir. The proximity of **98c** nucleus to residues Gly49 and Ile50 also explains why the presence of hydrogen-bonding donor groups, such as ethoxy, results in some binding and consequently some inhibition as evidenced in the bioassay results (Table 4).

Table 4 below represents the overall bioassay and docking data for the series of ligands (**98-101a-f**). In this table, the percentage activity at 20 μ M of HIV-1 PR, the corresponding percentage HIV-1 PR inhibition and the toxicity (as % viability of HeLa cells at 20 μ M) as well as the mean Autodock energies for ligands in the PDB HIV-1 PR crystal structures, 1HXB, 1HXW, 5T2Z and 2R5P are presented. The highlighted ligands are the ones, which have shown some inhibition activity against the HIV-1 PR enzyme.

Table 4: Bioassay and docking data for the series of ligands (**98-101a-f**) showing: the % activity at 20 μ M of HIV-1 PR, IC50 values, the corresponding % HIV-1 PR inhibition and the toxicity (as % viability of HeLa cells at 20 μ M) and mean Autodock energies for ligands in the PDB HIV-1 PR crystal structures, 1HXB, 1HXW, 5T2Z and 2R5P.

Compd.	HIV-1 PR % Inhibition	HeLa ^b % Viability	IC50 Val	Docked energy (Kcal/mol)							
				1HXB		1HXW		5T2Z		2R5P	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
98a	13.3	73.7	nc	-9.0	0.2	-9.0	0.2	-9.8	0.2	-9.9	0.2
98b	36.2	84.4	nc	-9.0	0.4	-9.0	0.4	-9.8	0.1	-10.0	0.4
98c	58.4	89.7	15.4	-8.8	0.2	-8.8	0.2	-10.0	0.1	-9.8	0.3
98d	-11.6	85	nc	-9.6	0.2	-9.6	0.2	-10.4	0.1	-10.2	0.3
98e	62.4	77.2	17.3	-9.3	0.4	-9.3	0.4	-10.3	0.3	-10.2	0.2
98f	8.055	73.7	nc	-7.9	0.3	-7.9	0.3	-9.4	0.1	-9.1	0.4
99a	-2.78	79.1	nc	-7.6	0.2	-7.6	0.2	-8.5	0.2	-8.5	0.2
99b	43.1	70.5	nc	-7.5	0.4	-7.5	0.4	-8.5	0.2	-8.3	0.2
99c	1.89	81.1	nc	-7.5	0.4	-7.5	0.4	-8.6	0.2	-8.3	0.5

99d	16.2	93.7	nc	-7.8	0.4	-7.8	0.4	-9.1	0.3	-8.9	0.2
99e	62.1	94.9	22.2	-7.9	0.2	-7.9	0.2	-9.0	0.2	-8.8	0.4
99f	7.02	80.5	nc	-7.0	0.3	-7.0	0.3	-8.0	0.2	-7.6	0.4
100a	-8.31	109	nc	-7.6	0.4	-7.6	0.4	-8.6	0.2	-8.3	0.4
100b	7.84	82.4	nc	-7.4	0.3	-7.4	0.3	-8.4	0.1	-8.8	0.4
100c	6	79.5	nc	-7.4	0.3	-7.4	0.3	-8.5	0.3	-8.2	0.1
100d	51.1	82.4	nc	-8.0	0.1	-8.0	0.1	-9.1	0.4	-8.6	0.5
100e	78.5	82.7	13.6	-8.0	0.2	-8.0	0.2	-9.1	0.1	-8.9	0.1
100f	21.7	94.7	nc	-7.0	0.2	-7.0	0.2	-8.1	0.3	-7.9	0.2
101a	8.65	82.7	nc	-7.8	0.1	-7.8	0.1	-8.6	0.2	-8.6	0.2
101b	13.01	97.6	nc	-7.4	0.3	-7.4	0.3	-8.4	0.3	-8.6	0.2
101c	18.8	88.2	nc	-7.4	0.2	-7.4	0.2	-8.5	0.2	-8.3	0.3
101d	-2.73	78.2	nc	-7.9	0.4	-7.9	0.4	-8.9	0.2	-9.0	0.3
101e	7.16	75.6	nc	-7.8	0.3	-7.8	0.3	-8.7	0.4	-8.8	0.3
101f	-41.52	83	nc	-7.1	0.3	-7.1	0.3	-8.1	0.0	-8.0	0.2

^a At 20 μ M; ritonavir as positive control with 100% inhibition. ^b At 20 μ M; emetine, as positive control, IC₅₀ 0.04-0.1 μ M. ^{nc}

Non calculated IC₅₀.

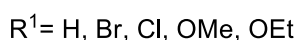
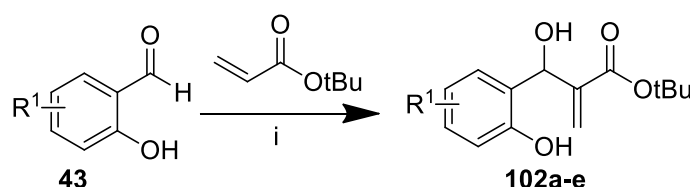
The percentage inhibition values as shown in Table 4 confirm that some of our ligands exhibit low to moderate percentage inhibition of the HIV-1 PR enzyme when compared to the inhibition value of the Ritonavir, which was used as a standard for the experiment. The fact that the ligands exhibit some inhibition could be perhaps, sufficient proof to warrant further synthetic modification in the hope of increasing the inhibition levels.

In summary, a series of 24 β -amino- β' -hydroxy esters have been successfully synthesized by subjecting a range of *O*-benzylated salicylaldehyde-derived MBH adducts to aza-Michael reactions with benzylamine, glycine esters or proline esters. The resulting adducts exhibited some *in vitro* activity against HIV-1 PR at 20 μ M, six of them with greater than 50% inhibition; low micromolar (13.6 – 22.2 μ M) IC₅₀ values were obtained for three of these ligands.

A tentative *In silico* docking studies clearly indicated the capacity of the HIV-1 PR receptor to accommodate the ligands, and revealed significant correlations between ligand-binding affinities and *in vitro* PR inhibition data.

2.1.4. Synthesis of *tert*-butyl 3-[(2-hydroxy)phenyl]-3-hydroxy-2-methylene propanoate esters.

As mentioned before, the use of *tert*-butyl acrylate as an activated alkene allows isolation of salicylaldehyde-derived MBH adducts and their regioselective cyclisation to coumarin derivatives.¹⁶⁵⁻¹⁶⁸



Scheme 23: Synthetic plan for *aza*-Michael adducts of *tert*-butyl 3-[(2-hydroxy)phenyl]-3-hydroxy-2-methylenepropanoate esters. Reagents and conditions: i) *tert*-butyl acrylate, 20% TEA(aq), rt., 6 hours; ii) Amines/amino acids, MeOH, rt, 3-6 hours.^{140, 165-168}

2.1.4.1. Synthesis of the MBH adducts (102a-e) with *tert*-butyl acrylate.

Some of the limitations of the MBH reaction are the low yields and slow reaction rate among others, providing ample scope for improvement. To accelerate the normally sluggish MBH reaction, the differences between conventional stirring and microwave-assisted irradiation procedures have been explored previously in our research group. In an attempt to improve the yield of the MBH reaction, adducts **102a-e** were synthesized by reacting a range of salicylaldehyde derivatives with *tert*-butyl acrylate in the presence of aqueous triethylamine (TEA) (very cheap and commercially available) as catalyst instead of DABCO (Scheme 23) as described by Basavaiah, D. *et al.*, (2000 and 2003)^{140, 209} and Nakagawa *et al.*, (1995).²⁰⁸

The use of water or methanol as a co-solvent has been shown to dramatically enhance both yield and the reaction time from 7-30 days with very low yields to only 3-6 hours with a very high yields. Also, the use of water as a co-solvent with 1,4-dioxane, tetrahydrofuran (THF), acetone, etc., has been described in the literature, although the mechanism is still not well understood.^{140,208,209}

As mentioned before, a careful attention to the MBH mechanism has revealed a sort of cascade of some “charged transition states as well as intermediates” which is

thought to be stabilized by some polar solvent or mixture, including water or methanol and this through “intermolecular charge-dipole interactions as well as hydrogen-bonding interactions.” Researchers have postulated that these key steps are believed to be responsible of the dramatic improvement in the aqueous Triethylamine (TEA) catalyzed MBH reaction.²⁰⁹

However, these hypotheses have to do with the zwitterionic intermediates, which define the MBH reaction mechanism. These zwitterionic intermediates are not be affected by the substrate, but the extent to which solvent polarity and steric bulk affects the reaction is substrate dependent.

The MBH adducts **102a-e** were prepared by stirring a mixture of one molar equivalent of salicylaldehyde, 3 molar equivalents of the *tert*-butyl acrylate (^tBu) and aqueous TEA at room temperature for a period of 3-6 hours (Scheme 23). TEA and DABCO are very similar structurally (with the same number of carbons and that all that is missing from TEA is the second nitrogen). However, it was amazingly observed that the yields and the running time of the MBH reaction were dramatically improved, up to 77-97% yields of the corresponding MBH adduct(s), when using TAE as catalyst and this after purification as shown in Table 5 below, when compared to the previous DABCO mediated approach. Nevertheless, the mechanism by which TEA gives better results using the *t*Bu and non-benzylated benzaldehydes still to be well understood.

The results as described in Table 5 are even far better than previously described in the literature by T. O. Olomola *et al.* (2010 and 2013).²⁰¹⁻²⁰²

Table 5: Combined results of the improved MBH reaction to access adducts 102a-e

Compound	R ¹	% Yield isolated
102a	H	77
102b	OMe	84
102c	OEt	81
102d	Br	97
102e	Cl	95

Compounds **102a-e** have been described in the literature and therefore, the assignment of peaks of this compound **102d** was facilitated by a simple compareason with previously described published data from our group.²⁰¹⁻²⁰³ Compound **102d** was confirmed by ^1H NMR analysis that showed the presence of the diagnostic vinylic signals at 5.63 ppm and 6.05 ppm, integrating for 1H each (Figure 35). The doublet at 5.65 ppm, which integrates for 1H, corresponds to the methine proton adjacent to the hydroxyl functional group. This methine proton in some cases couples with the hydrogen of the OH group to split as doublet.

The peak at 1.54 ppm (in CDCl_3) and 1.26 ppm (in DMSO-d_6) which integrates for 9H corresponds to the *tert*-butyl ester group while the remaining three signals from 6.73 ppm to 7.20 ppm in the aromatic region (3H) integrating for 1H each correspond to the substituted benzene group.

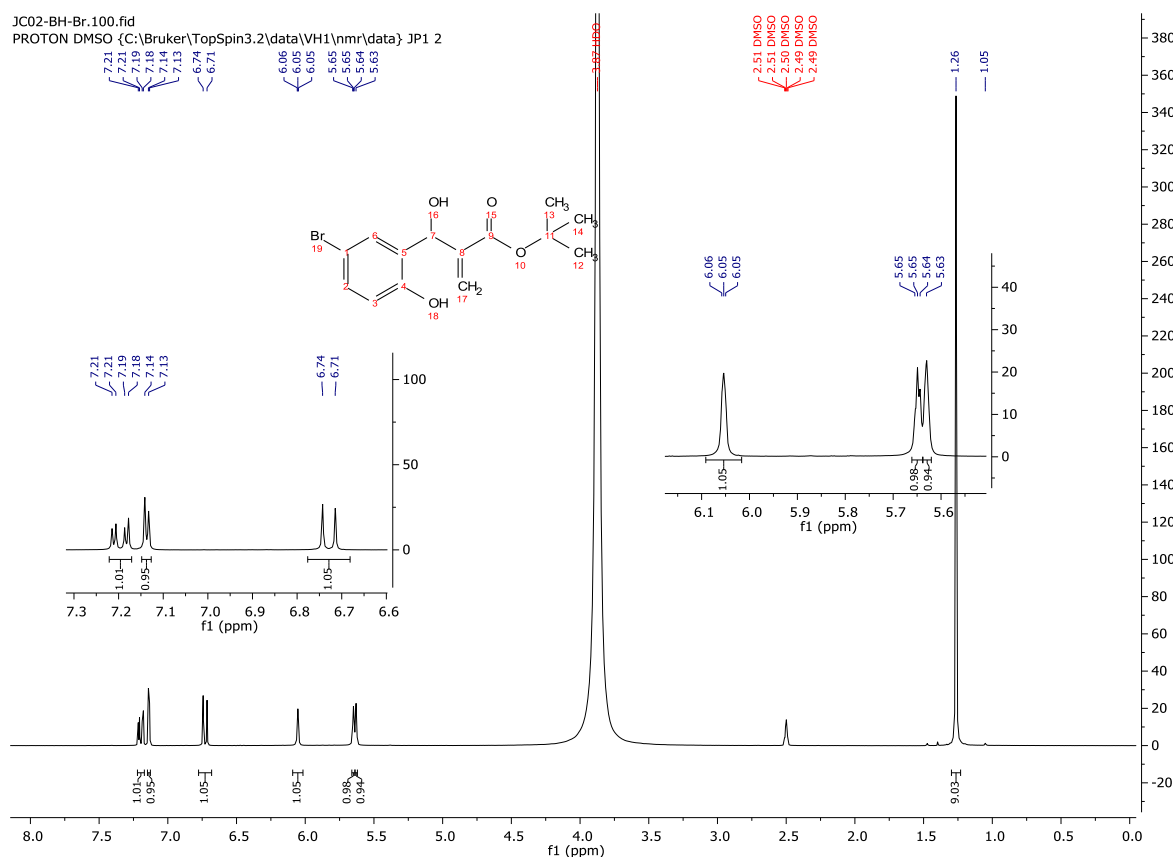


Figure 35: ^1H NMR (400 MHz) spectrum of the compound **102d** in DMSO-d_6 .

The ^{13}C NMR spectrum also confirmed this compound by the presence of the vinylic carbon at 123.8 ppm (Figure 36), which was observed as a negative signal in the DEPT-135 spectrum (Figure 37). The signal of the three carbons of the *tert*-butyl group appears at 27.8 ppm. All the other expected peaks have been identified. The structural assignments of the NMR signals of compound **102d** were confirmed finally by HSQC spectrum as shown in Figure 38 below.

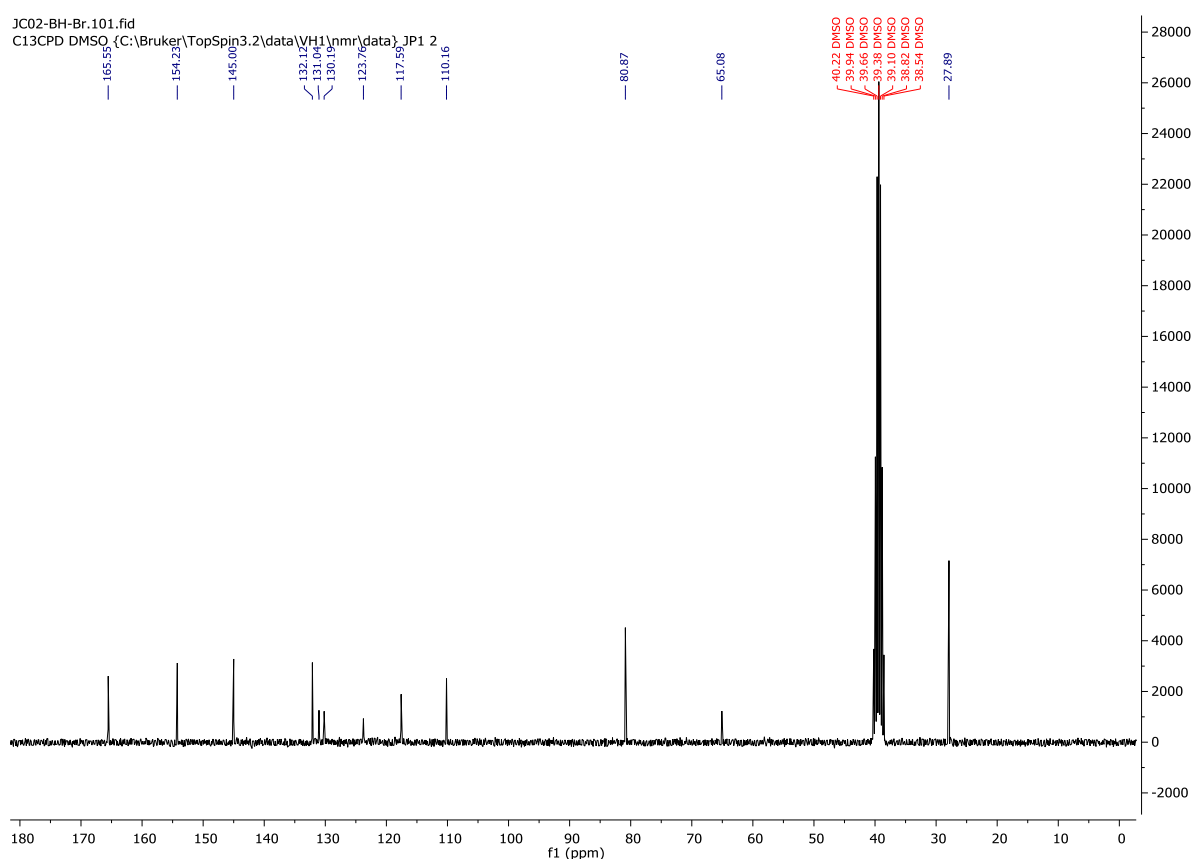


Figure 36: The ^{13}C NMR (100 MHz) spectrum of the compound **102d** in $\text{DMSO}-d_6$.

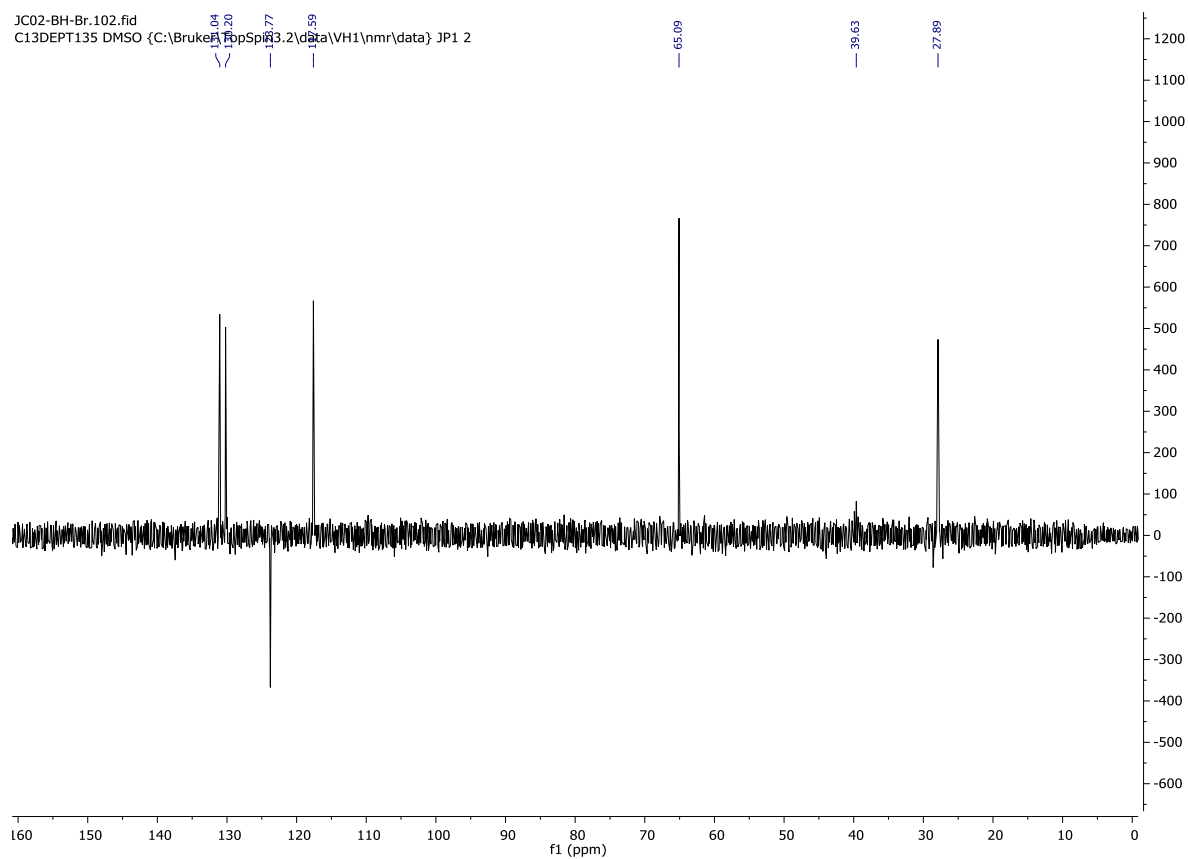


Figure 37: The 400 MHz DEPT 135 NMR spectrum of 102d in DMSO- d_6 .

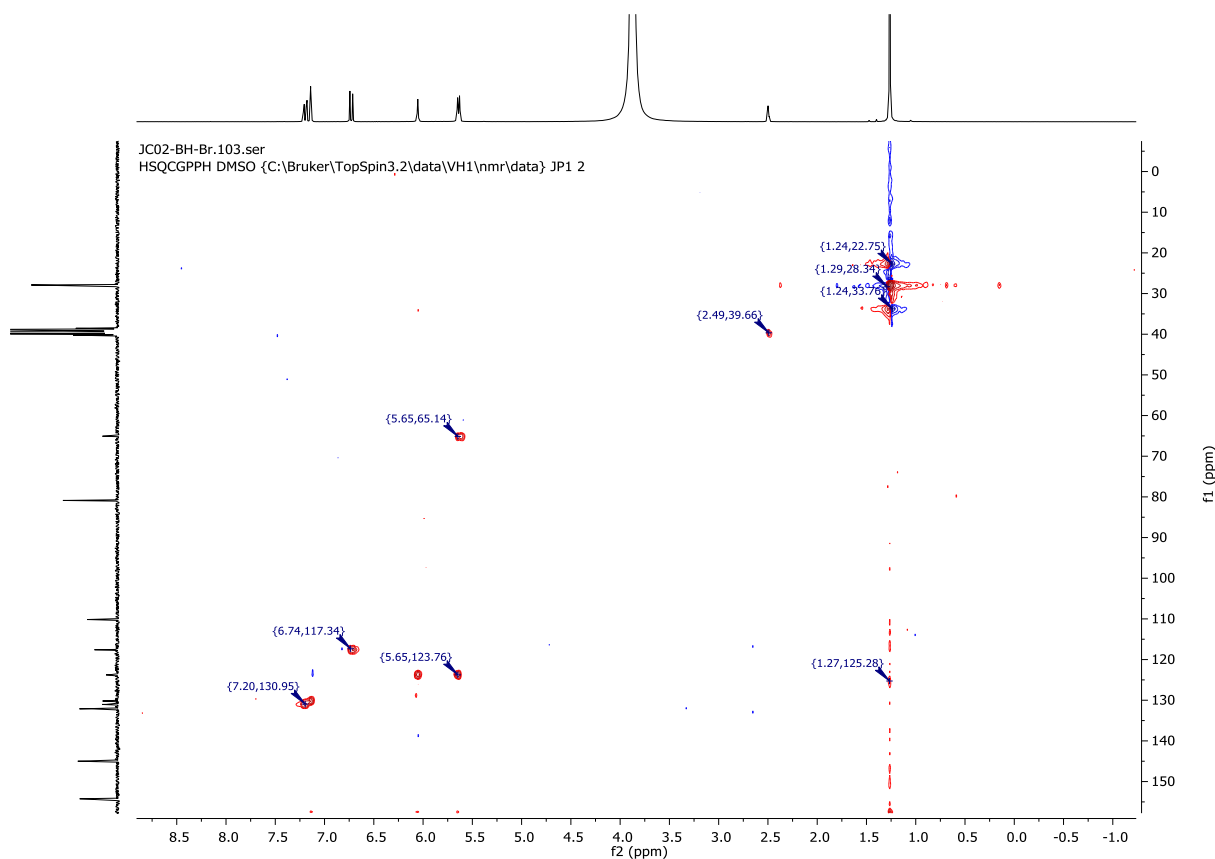
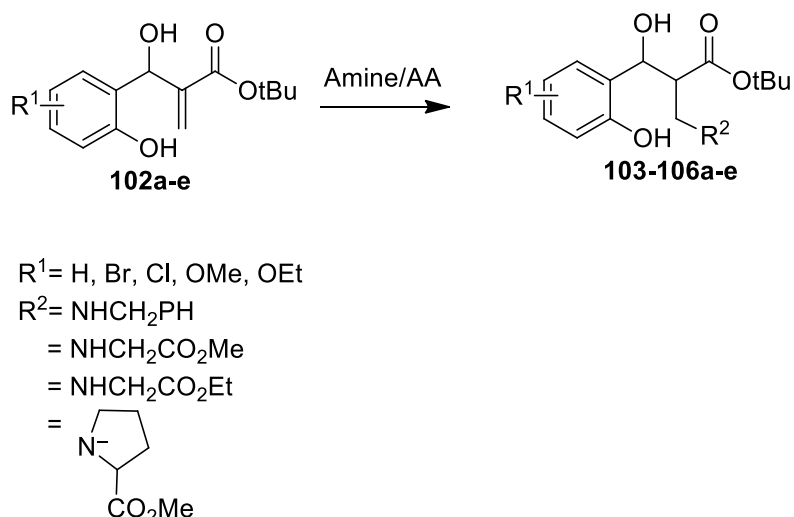


Figure 38: The 400 MHz HSQC spectrum of 102d in DMSO- d_6 .

All these results as well as the identified signals were in agreement with the literature.^{165-168,201-203}

2.1.4.2. Synthesis of the aza-Michael adducts 103-106a-e.

As illustrated in Scheme 17 below, the analogues **103-106a-e** were synthesized with slight modifications of the previously used method. Here, except where both reagents were solids, the neat reaction mixtures were stirred at room temperature for about six hours. Solids were dissolved in a minimum of dry THF.



Scheme 24: Synthetic plan for the synthesis of aza-Michael adducts **103-106a-e**: Reaction conditions: i) Benzylamine, 0.5-6 hrs; ii) Glycine-OMe, 0.5-6 hrs; iii) Glycine-OEt, 0.5-6 hrs; and iv) Proline-OMe, dry THF, 0.5-6 hrs

The mixture was stirred for 0.5 - 6 hours in a stoppered flask monitoring with TLC. When TLC showed no starting materials, the reaction mixture was then subject to short column chromatography over silica gel followed by a prep-TLC and the desired pure major product isolated in 81-99% and fully characterized. The results are presented in Table 6 below. These yields are far better when compared to those obtained previously and combined in Table 3.²⁰¹⁻²⁰²

Table 6: Combined results of the aza-Michael adducts **103-106a-e**.

Compound	R ¹	R ² (Amine)	Time (h)	% Yields
103a	H	Benzylamine	5-6	87
103b	OMe	Benzylamine	5-6	85
103c	OEt	Benzylamine	5-6	79
103d	Br	Benzylamine	2-4	97
103e	Cl	Benzylamine	2-4	99
104a	H	Glycine-OMe	5-6	85
104b	OMe	Glycine-OMe	5-6	88
104c	OEt	Glycine-OMe	4-6	83
104d	Br	Glycine-OMe	2-4	98
104e	Cl	Glycine-OMe	2-3	98

105a	H	Glycine-OEt	5	80
105b	OMe	Glycine-OEt	5-6	89
105c	OEt	Glycine-OEt	4-6	85
105d	Br	Glycine-OEt	2-4	97
105e	Cl	Glycine-OEt	3-4	98
106a	H	Proline-OMe	5-6	90
106b	OMe	Proline-OMe	4-6	81
106c	OEt	Proline-OMe	5-6	80
106d	Br	Proline-OMe	3-4	99
106e	Cl	Proline-OMe	3-4	98

Nucleophilic addition onto the alkene methylene of the MBH adduct introduced two stereocenters and/or two chiral centers in the aza-Michael scaffolds at the alpha-position (carbon 8) and the beta position (carbon 7), which resulted in the formation of four stereoisomers.

However, in each case, only major products were isolated, characterized and used for further study studies (bioassay, and cytotoxicity), and for further reaction (such as their cyclization reaction to 3-(aminomethyl)coumarin derivatives, as detailed in the next section). The signal assignment of the NMR spectra of **103d** was facilitated by comparison with the precursor and this was found to be in agreement with the literature.^{165-168, 201-202}

The ¹H NMR spectrum confirmed the formation of the aza-Michael adduct **103d** (Figure 39). The disappearance of the vinylic signals and the appearance of the multiplet at 3.06 ppm integrating for two overlapping protons, which are the alpha-proton as well as the second proton of the methylene carbon 17, located between the alpha-carbon and the nitrogen atom, was the first evidence of the new product formation. The doublet at 5.21 ppm and integrating for 1H corresponds to the methine proton adjacent to the hydroxyl functional group. In the precursor, this signal appeared as a singlet, because of the absence of neighboring proton(s), whereas in the aza-Michael adduct, it appears as a doublet. The doublet at 3.93 ppm integrating

for 2H correspond to the two methylene protons on the carbon 19, which is between the nitrogen atom and the aromatic ring of the benzylamine moiety.

The ^1H NMR spectrum (Figure 39) revealed also the presence of another multiplet at 2.94 ppm, which integrates for 2H. They correspond to the methylene group between the α -proton and nitrogen atom of the benzylamine (whose three aromatic protons appeared between 7.43 ppm and 7.48 ppm). However, despite being in a same environment, it was observed that the two protons on carbon 17, adjacent to the α -carbon, coupled with the α -proton and with the proton (1H) on the nitrogen atom independently. The proton on the nitrogen however did not couple to anything, but appeared as a singlet at 1.89 ppm. The last singlet at 1.50 ppm integrating for 9H (3x 3H) corresponds to the protons of the *t*-butyl ester.

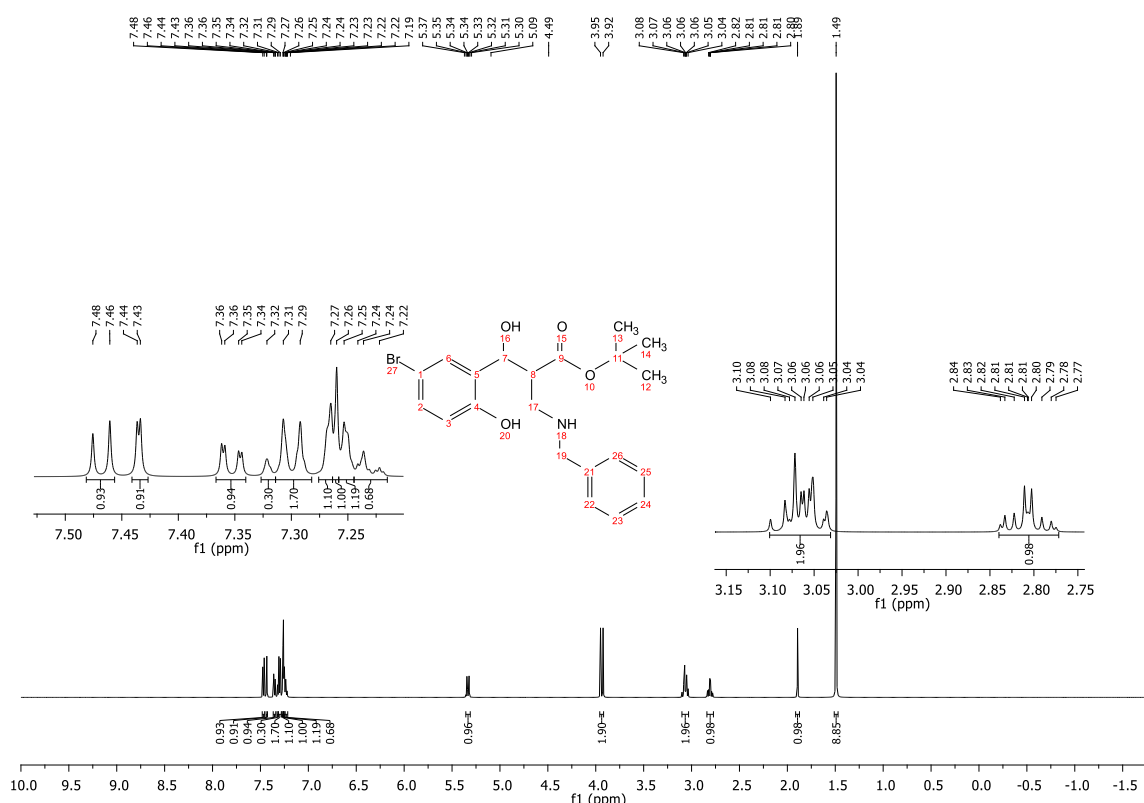


Figure 39: The ^1H NMR (300 MHz) spectrum of **103d** in CDCl_3 .

The ^{13}C NMR and the 2D NMR confirmed this compound. As shown in Figure 40 below, the three carbons of the *tert*-butyl group appear at 29.9 ppm on the ^{13}C NMR spectrum, while the methine carbon signal appears at 70.8 ppm. The α -carbon signal

appears at 51.9 ppm whereas the two methylene carbons both next to the nitrogen atom appear at 44.4 ppm and 55.1 ppm respectively.

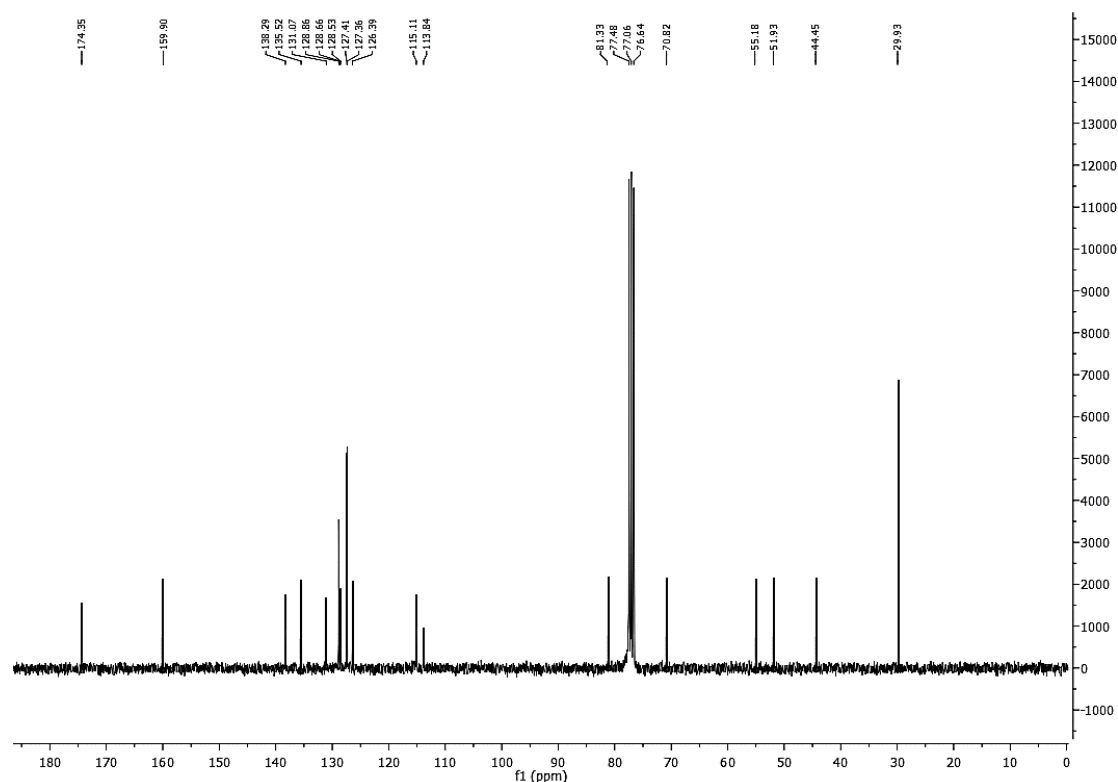


Figure 400: The 75 MHz ^{13}C NMR spectrum of 52d in CDCl_3 .

2.1.4.3. *In vitro* biological evaluation

The synthetic ligands **103-106a-e** were all examined for inhibition of HIV-1 protease and for cell toxicity, as determined by the viability of HeLa cells at a concentration of 20 μM . The % activity, % inhibition, % HeLa cell viability and the $\pm\text{SD}$ obtained for compounds **103-106a-e** at 20 μM are presented in Figure 41 and Table 7 below.

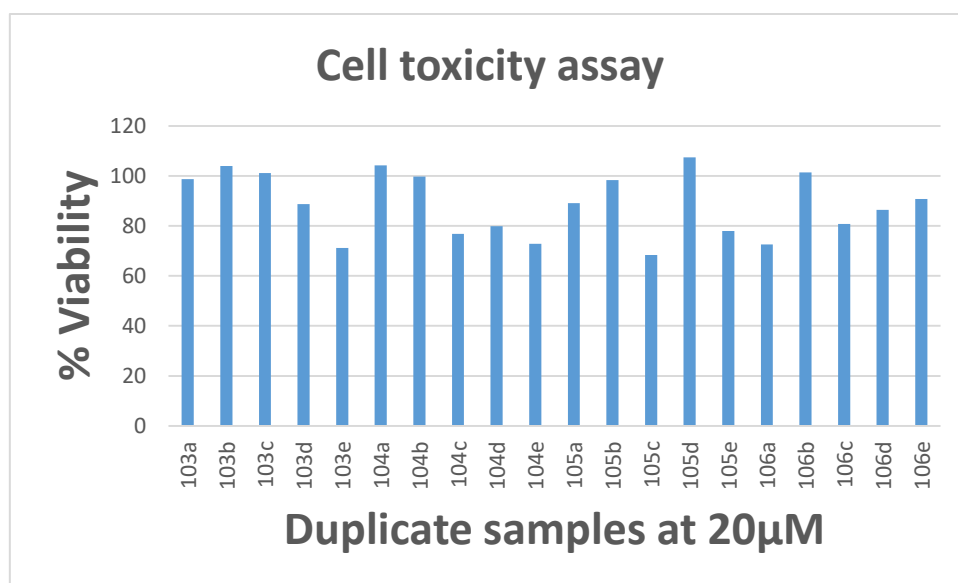


Figure 41: The bar graph of the % HeLa cell viability obtained for compounds **103-106a-e**.

Table 7: The % activity, % inhibition % HeLa cell viability and the $\pm$ SD obtained for compounds **103-106a-e** 20µM.

Compound	% Activity	% Inhibition at 20µM	Standard deviation	Percentage viability
103a	87.59491	12.40509	595.3839	98
103b	76.55445	23.44555	672.4585	103
103c	95.32541	4.674593	704.9855	101
103d	67.63643	32.36357	1264.307	88
103e	102.7365	-2.73652	3232.892	71
104a	98.07645	1.923552	178.1909	104
104b	86.98953	13.01047	422.1427	99.
104c	98.15775	1.842254	398.8082	76
104d	81.16516	18.83484	5494.927	79
104e	100.8362	-0.8362	963.7865	72
105a	91.34184	8.658159	296.2777	89
105b	93.99126	6.008739	1056.418	98
105c	92.83713	7.16287	799.7378	68
105d	141.5269	-41.5269	20081.83	107
105e	64.28291	35.71709	10156.88	77
106a	71.99164	28.00836	295.5706	72
106b	89.23247	10.76753	878.2266	101
106c	71.99744	28.00256	1122.886	80
106d	108.1675	-8.16747	2847.519	86
106e	86.21431	13.78569	1308.855	90

Unfortunately, although none of them was found toxic none of the synthetic ligands exhibited sufficient inhibition of HIV-1 PR to warrant further investigation, and therefore no IC₅₀ values were determined. It was observed that the absence of the benzyl group on these non-benzylated scaffolds **103-106a-e** has dramatically affected the activity of these compounds, in terms of both inhibition as well as the molecular weight of our ligands, which dropped significantly.

As mentioned in before, due to the hydrophobic character of the S1 and S1' subsite in HIV-1 PR, enzyme active site,⁴⁰ most of the present inhibitors have hydrophobic moieties, in many cases a benzyl group, at the P1 and P1' position.²¹⁰ It was found that the benzyl group on previous analogues **98-101a-f**, could occupy such hydrophobic pocket within the HIV-1 PR enzyme, and thus enhancing thereby the binding of such ligands. Therefore, based on to the substrate/protein nomenclature by Schechter and Berger,⁴⁰ it is not surprising that the absence of the benzyl group on analogues **103-106a-e** decreases the effectiveness of their inhibition (Table 7), since it was intended likely to fill up the S1 and S1' hydrophobic pocket of the HIV-1 PR enzyme. This potentially resulted in a poor fit of these **103-106a-e** ligands in the active site of the HIV-1 PR enzyme and therefore poor inhibition of the HIV-1 PR enzyme as presented in Table 7 above. Based on these observed results, no IC₅₀ value was calculated therefore.

These results were disappointing, but it motivated a further step to improve our ligands by cyclization of the non-benzylated aza-Michael adducts into coumarin derivatives since it has been well documented (also from our research group),²⁰¹⁻²⁰² that coumarin containing scaffolds exhibit some inhibition properties towards the HIV-1 PR.

2.1.5. Synthesis of coumarin-3-hydroxy-2-methylenepropanoate esters 107-110a-e.

The aim of this cyclization was to access a series of substituted 3-(aminomethyl)coumarins through MBH methodology. These were prepared previously by Nagesam *et al.*,²¹¹ who pioneered access to some antibacterial 4,6-dimethyl-3-(aminomethyl) coumarins from 3-(chloromethyl)-4,6-dimethylcoumarin,

and by Savel'ev *et al.*²¹², who used the Mannich reaction to prepare substituted pyrrolidinomethyl- and piperidinomethyl-coumarins.

As mentioned before, it has been established previously from our group by Musa *et al.*,^{165,166} that the acid (HCl) catalysed cyclization of the MBH adducts made from *tert*-butyl acrylate and non-benzylated 2-hydroxy salicylaldehydes afforded 3-(chloromethyl)coumarins and α -(chloromethyl)cinnamic acids.

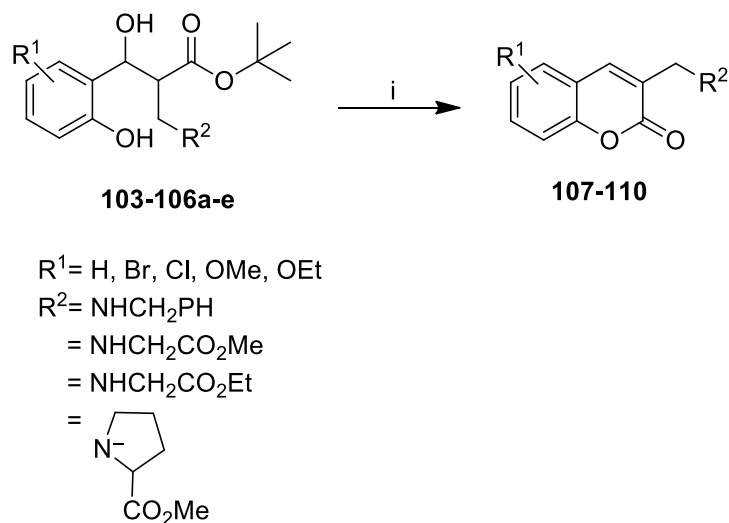
Later on, Faridon and co-workers²¹³ from our group demonstrated that cyclisation of the same MBH adducts under different conditions such as the base-catalysed reaction (KOH solution refluxing in acetonitrile for 5 hours), afforded chromene and chroman-4-ol derivatives instead of the expected coumarin derivatives. They concluded that the acid-catalysed cyclisation of the MBH reaction proceeds through acyl substitution, whereas the cyclization appears to proceed via conjugate addition when using basic conditions.

Since in all these previously cited cases the cyclization were done before doing the nucleophilic substitution onto the MBH adducts, it was therefore decided for this current research project, to investigate a new approach. Here, the aza-Michael adducts were first prepared from MBH adducts, accessed from the treatment of the non-benzylated 2-hydroxy salicylaldehydes and *tert*-butyl acrylate using the aqueous TEA as catalyst, to access compounds **103-106a-e** and then secondly the base-catalysed cyclization on these aza-Michael adducts was done to access the 3-(aminomethyl)coumarin derivatives **107-110a-e** as illustrated in Scheme 25 below.

2.1.5.1. Synthesis of ligands

The 3-(methylamino)coumarin analogues were synthesized in moderate to high yields (53-80% yields) as shown in Scheme 25 below, through the base-catalysed cyclization of the precursors **103-106a-e** in the presence of ethanolic potassium hydroxide by stirring at room temperature for about 8 hours or by refluxing for 5 hours as described previously by Faridoon *et al.* (2015).²¹³ However as mentioned before, the base-catalysed cyclization in our research group was done prior derivatization of the MBH adducts with the appropriate amines/amino acids.^{201-202, 213} In this research, a novel route has been evaluated, in which the MBH adducts were

first converted to the aza-Michael analogs **103-106a-e**, followed by the cyclization to afford analogs **107-110a-e** (as described in Scheme 25 below), in good yields.



Scheme 25: The synthetic plan for the synthesis of aza-Michael adducts of coumarin-3-hydroxy-2-methylenepropanoate esters **107-110** derivatives: Reaction conditions: 15% ethanolic KOH, reflux, 5 h.²¹³

Due to the lack of a chiral centre within the scaffold, only single products were isolated after column chromatography followed by a prep-TLC. Results are described in Table 8 below.

Table 8: Combined results and percentage yields for the cyclization reaction, which afforded compounds **107-110a-e**.

Compound	Isolated % yields
107a	66
107b	60
107c	58
107d	78
107e	75
108a	60
108b	53
108c	55
108d	80
108e	70
109a	62
109b	58
109c	55
109d	68

109e	74
110a	63
110b	60
110c	61
110d	80
110e	77

The compound **107d** was prepared as described above in 78% yields and confirmed by the NMR analysis and the purity was confirmed by the combustion analysis. Although prepared using a new method, which has not been previously described, some of these products have been prepared before. In addition, the NMR spectra of the precursors were used as guidance in the assignment of the new compounds and these agreed with the literature.²⁰¹⁻²⁰² Only one product was observed in each case when following the reaction with TLC.

As shown in Figure 42 below, the singlet at 7.94 ppm integrating for 1H (corresponding to the signal at 138.7 ppm on the ¹³C NMR spectrum, Figure 43), confirms that the compound formed is the coumarin derivative **107d** instead of the chromene. It was also observed that the hydroxyl group on the precursor (the MBH adduct) was lost which suggested that the product formed was a 4-H 3(aminomethyl)coumarin derivative as indicated by the ¹H NMR spectrum (Figure 42).

Further evidence confirming this hypothesis is the *absence* of a multiplet integrating for 1H on carbon 7 corresponding to a methine group. In case the hydroxyl group was there, this would have introduced a stereogenic centre within the molecule.

The other signals appearing between 7.84 ppm and 7.09 ppm correspond to the 8H from the aromatic rings as expected whereas the singlets at 3.94 ppm and 3.33 ppm, integrating for 2H each, correspond to the methylene protons on carbon 9 and 12 respectively. The singlet at 2.81 ppm, integrating for 1H, corresponds to the proton attached to the nitrogen atom. In some cases, it was observed that this proton caused the two methylenic protons to split as a doublet, or doublet of doublets, depending on the solvent and the frequency of the NMR instrument.

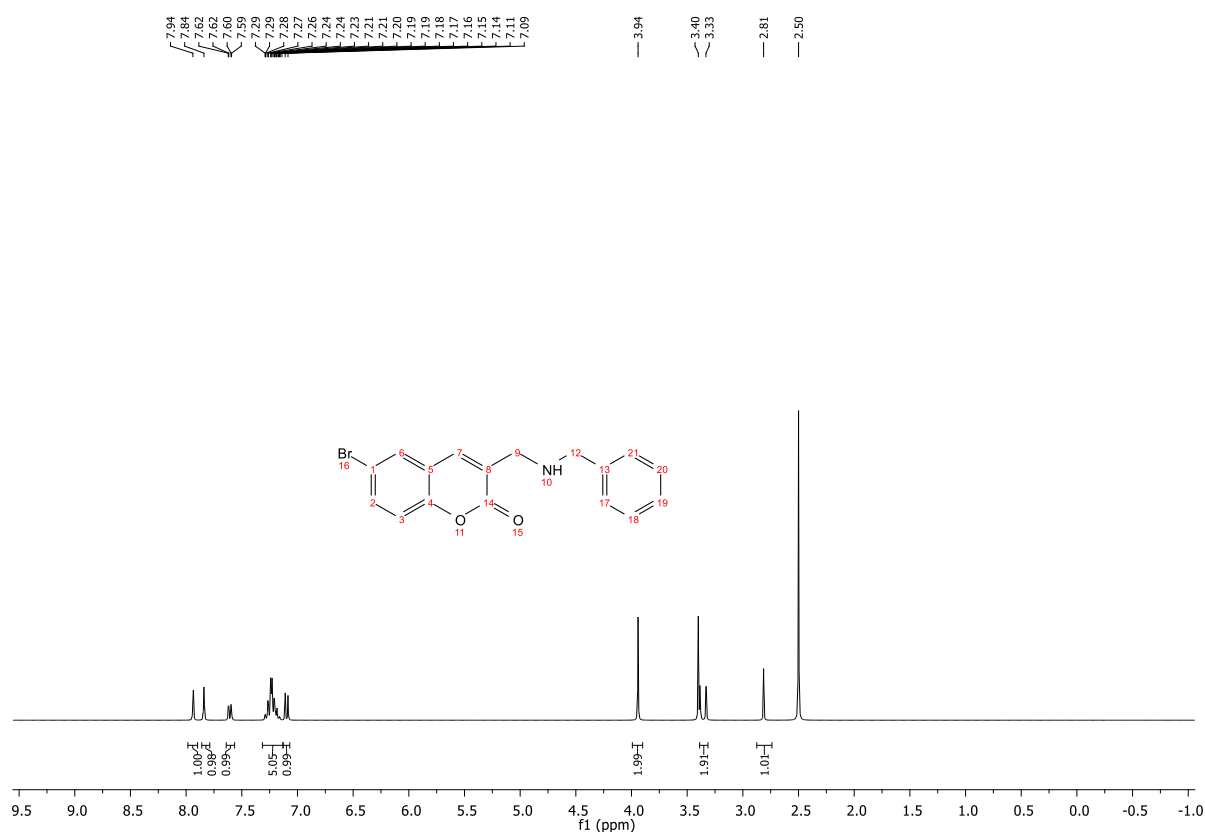


Figure 42: ^1H NMR (300 MHz) spectrum of **107d** in $\text{DMSO}-d_6$.

All the expected 17 carbon signals were confirmed by the ^{13}C NMR as shown below.

The two methylene groups were confirmed by the presence of the peaks at 52.4 ppm and 53.6 ppm in the ^{13}C NMR as shown in Figure 44. These signals were also confirmed as the only negative signals on the DEPT 135 NMR spectrum (Figure 44) and these assignments were in agreement with the literature.²⁰¹⁻²⁰² Except from compounds **107-110b-e**, all the remaining compounds (**107-110a**) have been described from our group. Here they are repeated using the new method as described above (in page 94 of this thesis).

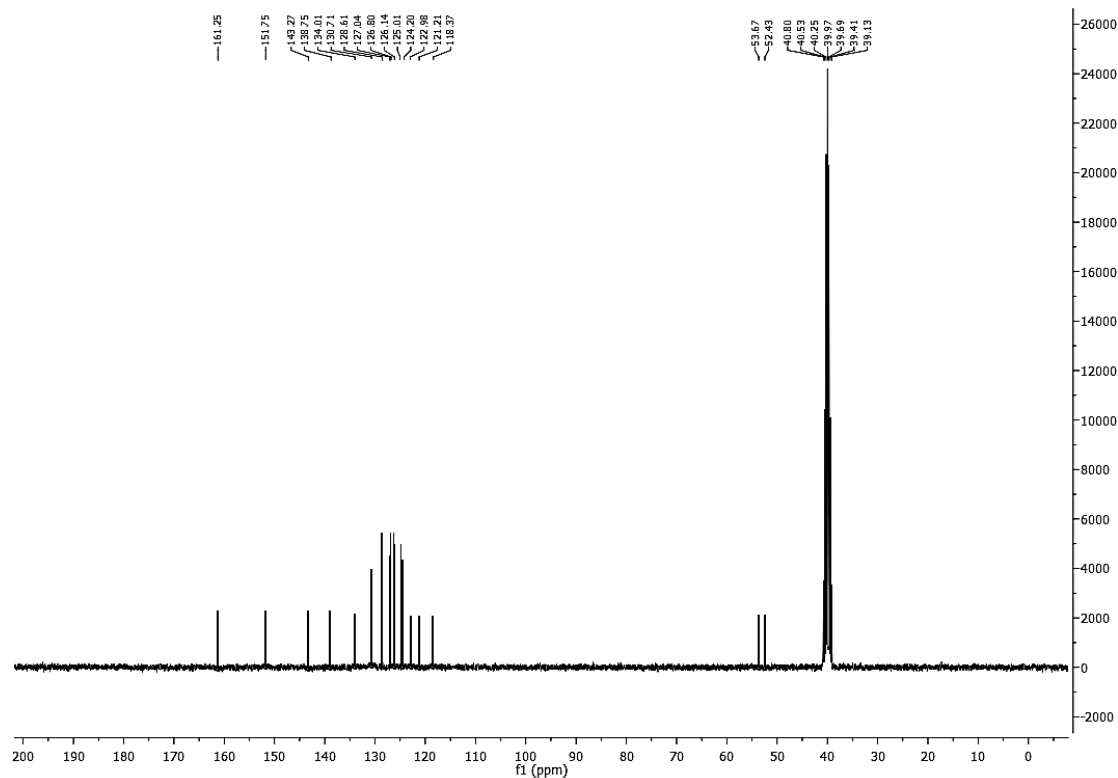


Figure 43: The ^{13}C NMR (75 MHz) spectrum of **107d** in $\text{DMSO } d_6$.

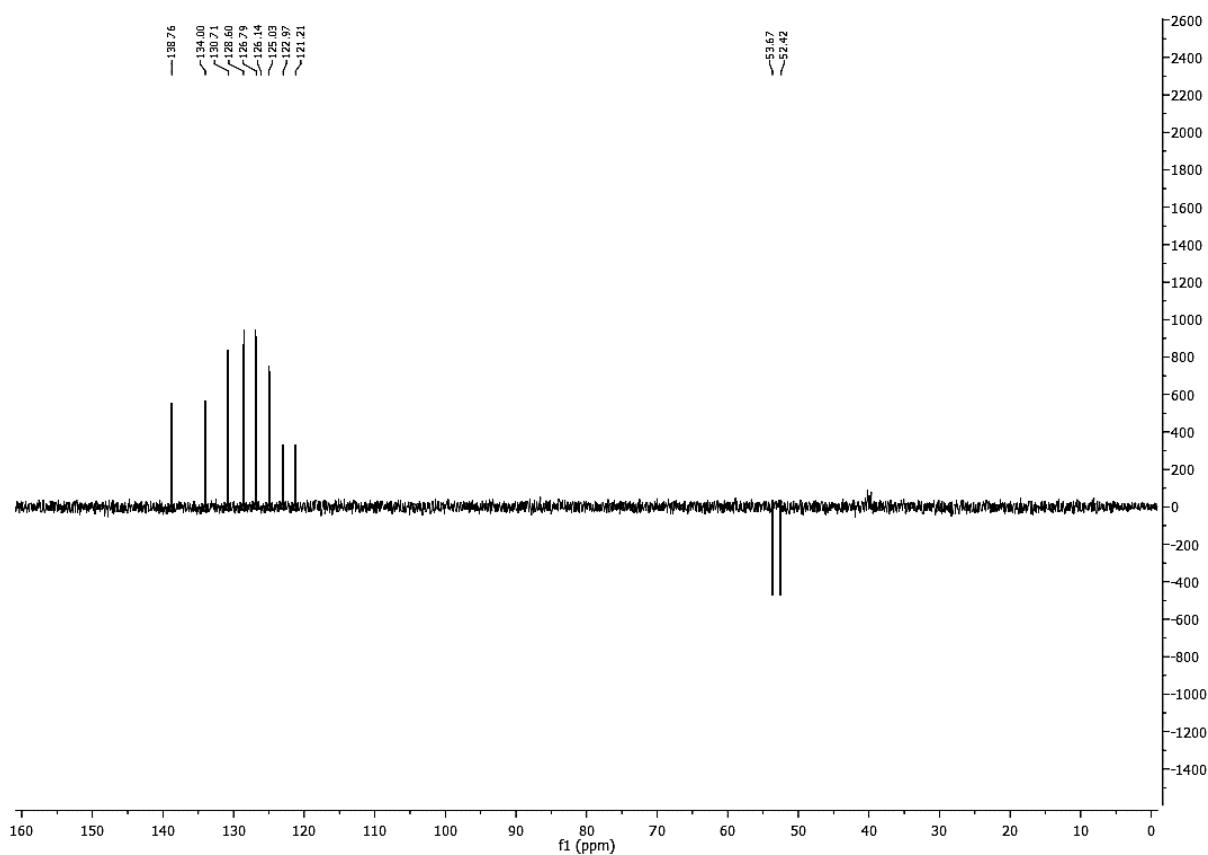


Figure 44: The DEPT 135 NMR spectrum of **107d** in $\text{DMSO } d_6$.

2.1.5.2. *In vitro* biological evaluation of the 3-(aminomethy) coumarin derivatives 107-110a-e

All the synthetic ligands **107-110a-e** were examined for inhibition of HIV-1 protease and for cell toxicity, as determined by the viability of HeLa cells at a concentration of 20 μ M. Results are presented in Figure 46 showing the bar graph of HeLa cell % viability whereas the % activity, the % inhibition and the HeLa % viability of these compounds are presented in Table 9 below.

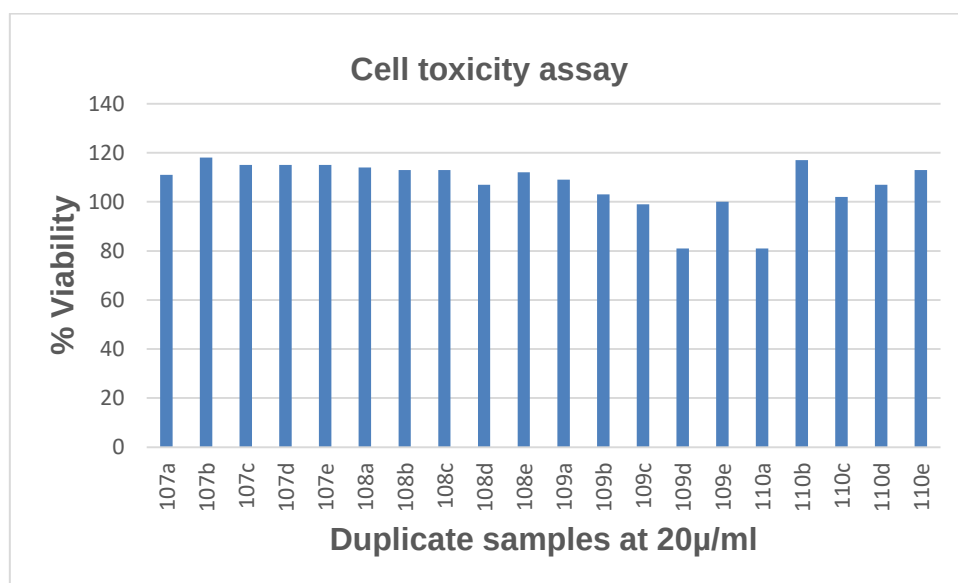


Figure 45: The bar graph of the % HeLa cell viability for compounds 107-110a-e.

Table 9: The % activity, % inhibition and % HeLa cell viability obtained for compounds 107-110a-e

Compound	% Activity	% Inhibition at 20 μ M	% Viability	IC50 (%)
Ritonavir	0.878276063	99.12172	-	99.9548
107a	0.08386721	38.91613	111.	nc*
107b	-0.163075131	33.1631	118	nc
107c	13.38613861	36.61386	115	nc
107d	3.857891672	34.14211	115	nc
107e	21.84740827	45.15259	115	45
108a	14.02679091	38.97321	114	nc

108b	6.755969715	30.24403	113	nc
108c	1.733255679	29.26674	113	nc
108d	20.72684916	35.27315	107	nc
108e	-1.155503786	42.1555	112	42
109a	1.684333139	26.31567	109	nc
109b	-0.062900408	38.06290	103	nc
109c	20.42399534	32.5760	99	nc
109d	6.015142691	36.98486	81	nc
109e	2.839836925	41.16016	100	41
110a	5.283634246	34.71637	81	nc
110b	13.09726267	28.90274	117	nc
110c	-2.485730926	21.34857	102	nc
110d	2.935352359	39.06465	107	nc
110e	0.56144438	48.43856	113	48

nc= not calculated IC50 value

Based on these results, the ligands synthesised exhibited less than 50% inhibition of HIV-1 PR and therefore no IC₅₀ values were determined. The loss of the free hydroxyl functional group on the scaffolds was thought to be one of the reasons for the low activity observed on the coumarin scaffolds since it is necessary for the H-bonding of other analogues onto the active site of the HIV-1 protease.¹²⁶ However, these results are much better than the aza-Michael precursors. It was noted that the set of 5-chloro-containing analogues were still showing relatively higher activity when compared to the others. Example of **107e** (45%), **108e** (42%), **109e** (41%) and **110e** (48%).

2.2. Synthesis and evaluation of the C₂-symmetrically and C₂-unsymmetrically substituted hydroxyethylene isosteres.

2.2.1. Synthesis and evaluation of the C₂-symmetrically substituted hydroxyethylene isosteres.

The 1,4-bis-amide moiety incorporated with a dihydroxyethylene skeleton has been found to lead to potential inhibitors.^{96,122} The work of Abbot Laboratories⁹² and of other research groups, suggested the possibility of developing a diamide diol skeleton using simple chemistry from readily available building blocks.^{96,122,214} Thus, a one-step access to a series of new diamide hydroxyethylene peptidomimetic inhibitors has been developed, using the uncatalyzed direct amidation reaction developed by Charville *et al.*²¹⁵

This was achieved using tartaric acid derivatives: tartaric acid (2,3-dihydroxysuccinic acid), diethyl tartrate (diethyl 2,3-dihydroxysuccinate) and tartaric acid osazone. Tartaric acid and its derivatives are inexpensive chiral pool compounds, which are commercially available and have been used in the construction of numerous chiral molecules. For example, in 2011 Gonzalez, S.V. *et al.*²¹⁶ designed a series of optically active, tartrate based amphiphiles, with interesting supramolecular properties. In this work both symmetric and unsymmetrically substituted tartaric diamides were made.

Also, Nugiel *et al.*,¹²² reported a series of novel S₁/S₁'-substituted cyclic urea-based HIV-1 protease inhibitors which were accessed using three different approaches, two of which used L-tartaric acid or L-mannitol as the starting material. Włostowski and co-workers²¹⁷ who have done significant work on tartaric acid and its O-acyl derivatives. They also elaborated more on the synthesis and applications of tartramides, tartrimides and O-acyltartramides and imides for more review on the use tartaric acid and its derivatives.

Although free tartaric acid was used in a few trial cases, the diester gave better synthetic results due to improved solubility. We anticipated that the stereochemistry of these compounds (tartaric acid or its derivatives) as well as their functional groups such as hydroxyl groups (on tartaric acid or diethyl tartrate), or Schiff base (on tartaric acid osazone), would facilitate interactions through hydrogen bonding with the structural water which is attached to the flap residues Ile 50/150 at the active site of the HIV PR enzyme.

The aim at using specifically tartic derivatives was to design inhibitors capable to bind symmetrically in the active site of the HIV-1 PR enzyme via the readily available two free oxygens atoms. These two oxygens atoms can accept two hydrogen bonds

from the flap residues Ile 50/50'. The diol oxygens are intended to interact with the catalytic aspartate region. In this regards, tartaric acid and its derivatives (as well as cyclic urea) can be therefore strong building blocks for potential inhibitors with an accurate alignment of all the key structural elements with their corresponding binding motifs. This alignment key feature comes from the unique R,S,S,R configuration of the two contiguous chiral centers of tartaric acid/diethyl tartrate. Anticipately, tartrates-based scaffolds would generate an efficient hydrogen bond network between the aspartate residues and the flap region of the HIV-1 PR enzyme without the intervention of a water molecule (commonly found in linear inhibitors).⁸⁰ This feature (stereochemical arrangement of tartaric acid/diethyl tartrate) has been critical for optimal binding interactions of our scaffolds to the HIV-1 PR enzyme.¹²²

Therefore, a potential inhibitor should contain some free hydroxyl group, which would form the hydrogen bonds. In addition to the hydrogen-bond network, the main-chain amide nitrogen atoms of the inhibitor can also be hydrogen-bonded to the carboxylate oxygens Asp25 and Asp125, respectively. The choice of tartaric acid or diethyl tartrate as the main core unit was to develop a series of compounds, which would interact tightly, through the hydrogen-bonding of the free hydroxyl group, with the catalytic residues. This close positioning of such inhibitor's hydroxyl to the carboxylate oxygens is not only caused by the attraction between these groups, but also by the interactions between the inhibitor's side chains and the S1-S3 site residues.

As discussed in the first chapter, the HIV-1 protease cleaves the precursor protein at nine positions. In seven of these, the P1 or P1' amino acid is a phenylalanine or tyrosine. Therefore, by homology, the use of benzyl groups as P1/P1' substituents on the C2/C5 carbons is expected to improve inhibitor binding, as is the case in a large number of inhibitors. The use of the osazone was partly inspired by the introduction of hydrophobic bulk at S1/S1', and partly due to its serendipitous availability in the laboratory.

2.2.1.1. Diamide tartrates synthesis

Peptides have been widely synthesized firstly in several steps, via the carbodiimide (CDI) mediated reaction between a carboxylic acid and an amine or amino acid (AA), or the use of acid chlorides and anhydrides as activated acid derivatives.²¹⁸⁻²¹⁹

These approaches are not green due to the production of undesirable and harmful by-products. Uncatalyzed direct formation of amides from acids and amines, is an economically attractive alternative reaction for amide bond formation and was therefore the method of choice in this study.

Tartaric acids have been used in the preparation of tartramides by many methods, including microwave irradiation in the presence of the appropriate amines.²²⁰⁻²²¹

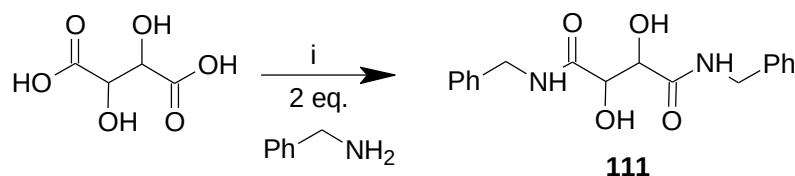
The Nugiel, D. A., and co-workers example has been described in Scheme 4, chapter 1, where they used, in one of their approaches, L-tartaric acid as the chiral precursor for preparing P1/P1'-substituted cyclic ureas.¹²²

Another recent example has been reported by Gonzalez, et al.²²² using the thermolysis or microwave irradiation of mixtures of L-tartaric acid and hexylamine, to form *N,N'*-dihexyltartramide in good yield, with partial racemization.

2.2.1.2. Synthesis of the C₂-symmetrical ligands **111-122(ab)**.

Symmetrically substituted 2,3-dihydroxysuccinamides **111-122(ab)** were accessed via a direct uncatalyzed amidation reaction as first described by Charville, H. et al.²¹⁵, in 2011 and subsequently by others.

Initially, one molar equivalent of acid (tartaric acid for the first trial while optimizing the reaction conditions) was heated under reflux to allow for acid dimerization, which is believed to be the activation step.²¹⁵ Thereafter two molar equivalents of benzylamine were added (Scheme 26). The reaction vessel was equipped with either a Dean-Stark or molecular sieves in to remove any resulting water from this reaction. After cooling to room temperature, the desired product precipitated and was purified by flash column chromatography, followed by preparative-TLC to afford symmetrically substituted 2,3-dihydroxysuccinamide **111a** very high yield (96%).



Scheme 26: Reaction of tartaric acid with benzylamine to afford the product **111a**

The product **111a** was confirmed after a full characterization, with the help of 2D NMR, FT-IR, MS, melting point, and high-resolution mass spectrometry. The data were found to be in agreement with published assignments.²²³

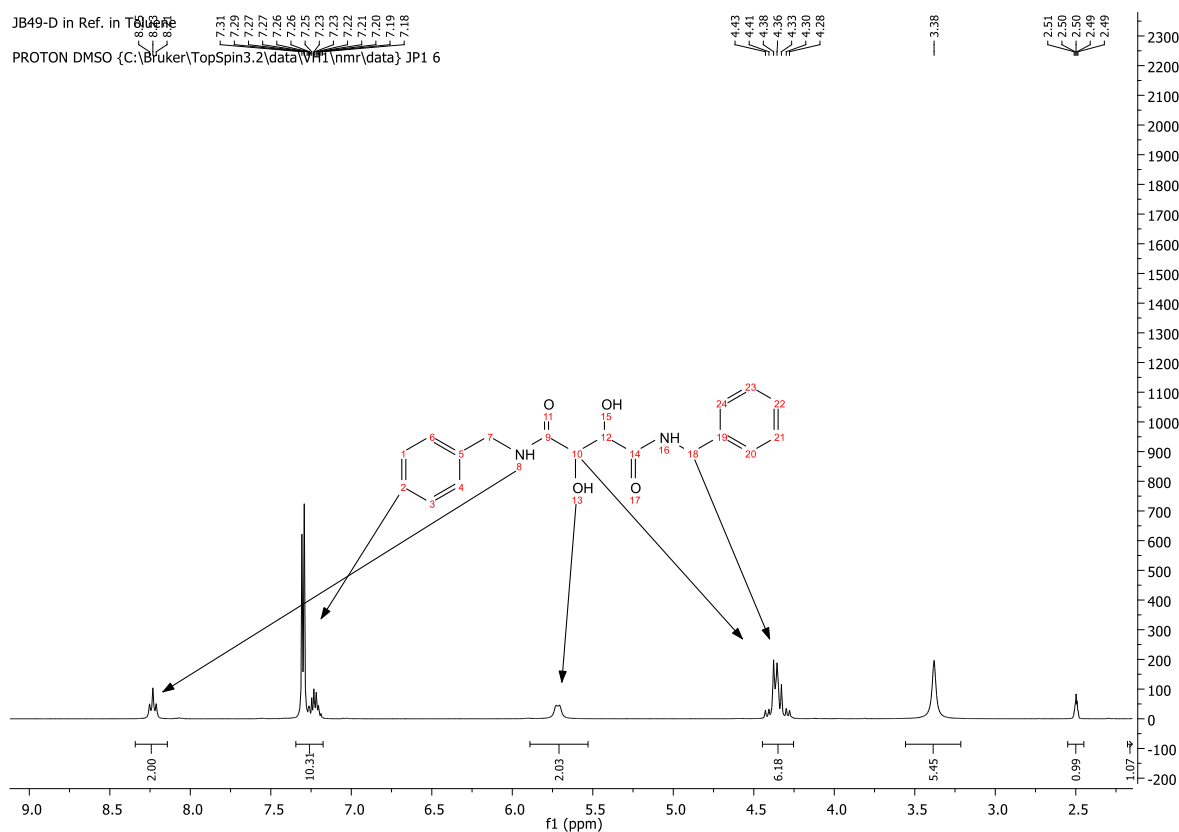


Figure 46: ^1H NMR (100 MHz) spectrum of **111a** in DMSO-d_6 .

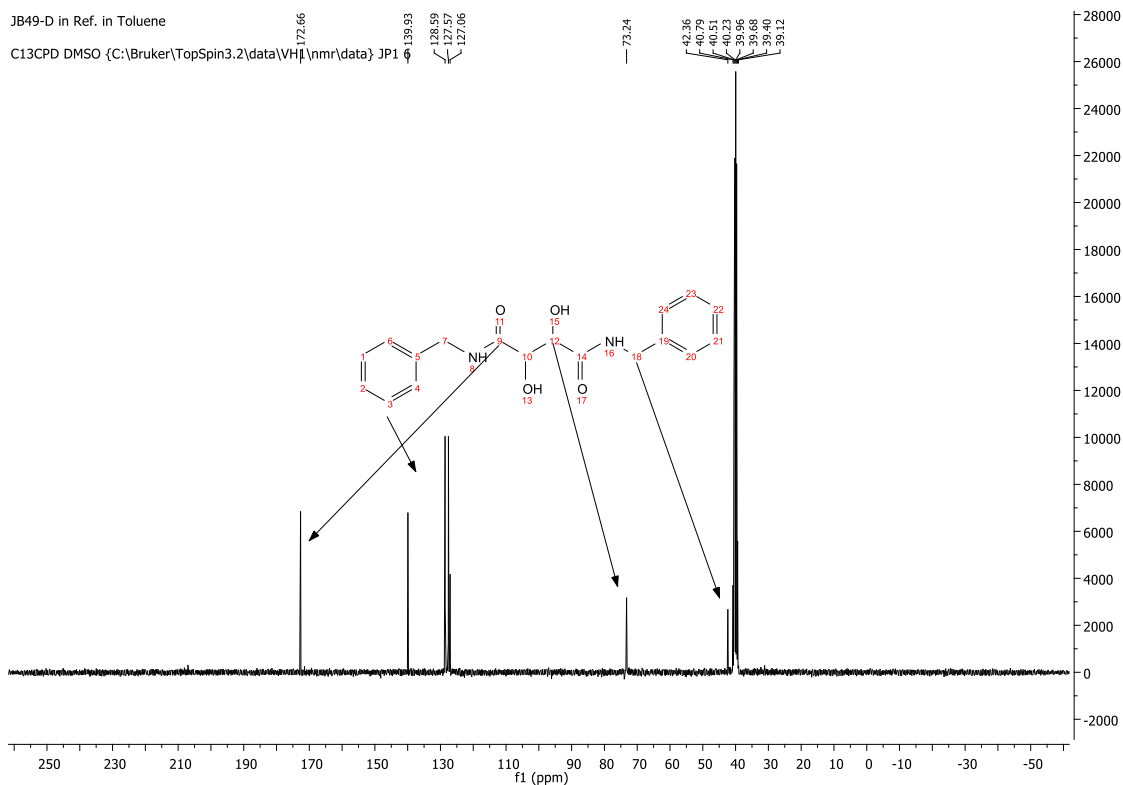


Figure 47: ^{13}C NMR NMR (75 MHz) spectrum of the 111a in DMSO-d_6 .

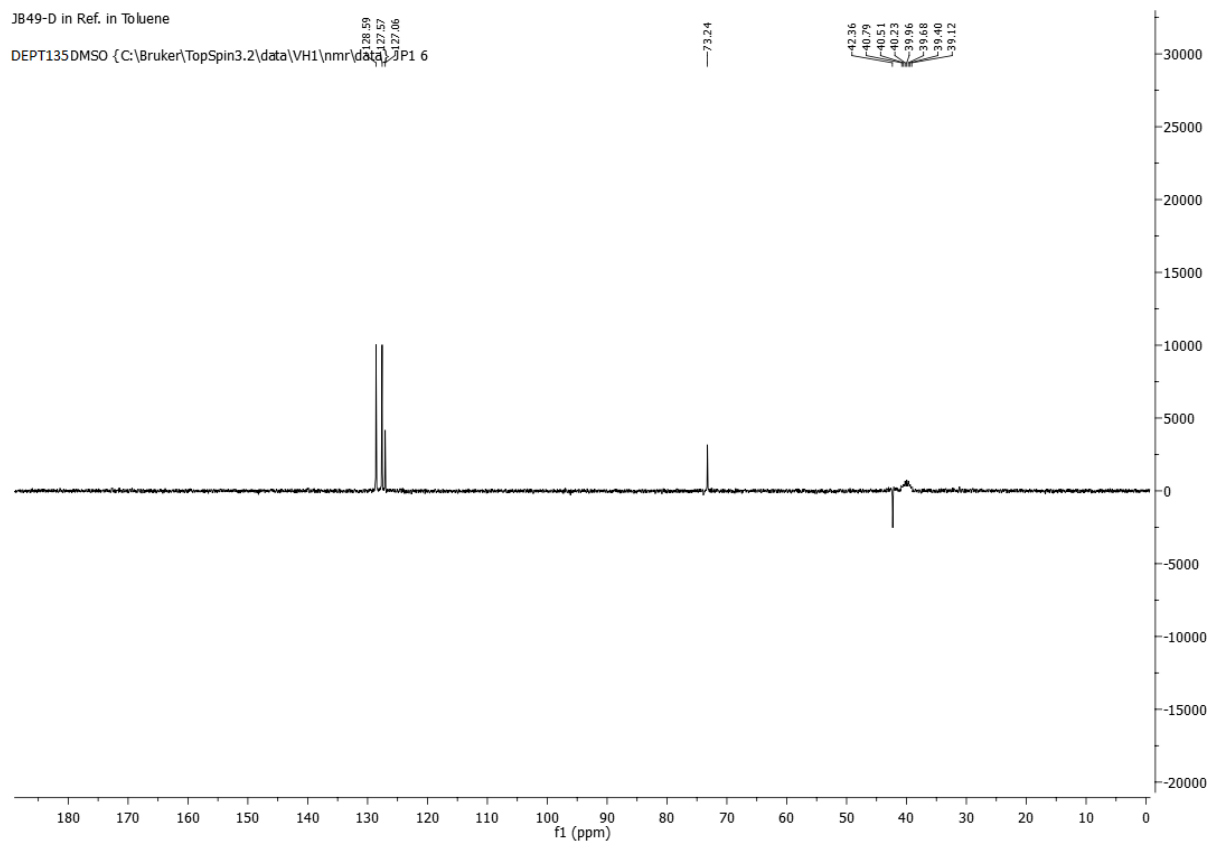


Figure 48: DEPT 135 (300 MHz) spectrum of 111a in DMSO-d_6 .

One of the most characteristic signals, which confirmed the amide bond formation, was the triplet at 8.23 ppm, integrating for 2H. This corresponds to the two amide protons. This evidence was revealed by both ^1H and HMBC spectra analysis. HMBC spectrum (Figure 50) revealed that this proton has a direct correlation with both the amide carbonyl carbon 9 and carbon 14 (whose signal is at 172.6 ppm) as well as the neighbouring benzylic carbon 7 and 18 at 42.1 ppm. The two benzylic protons are the responsible for coupling to this one proton attached to nitrogen.

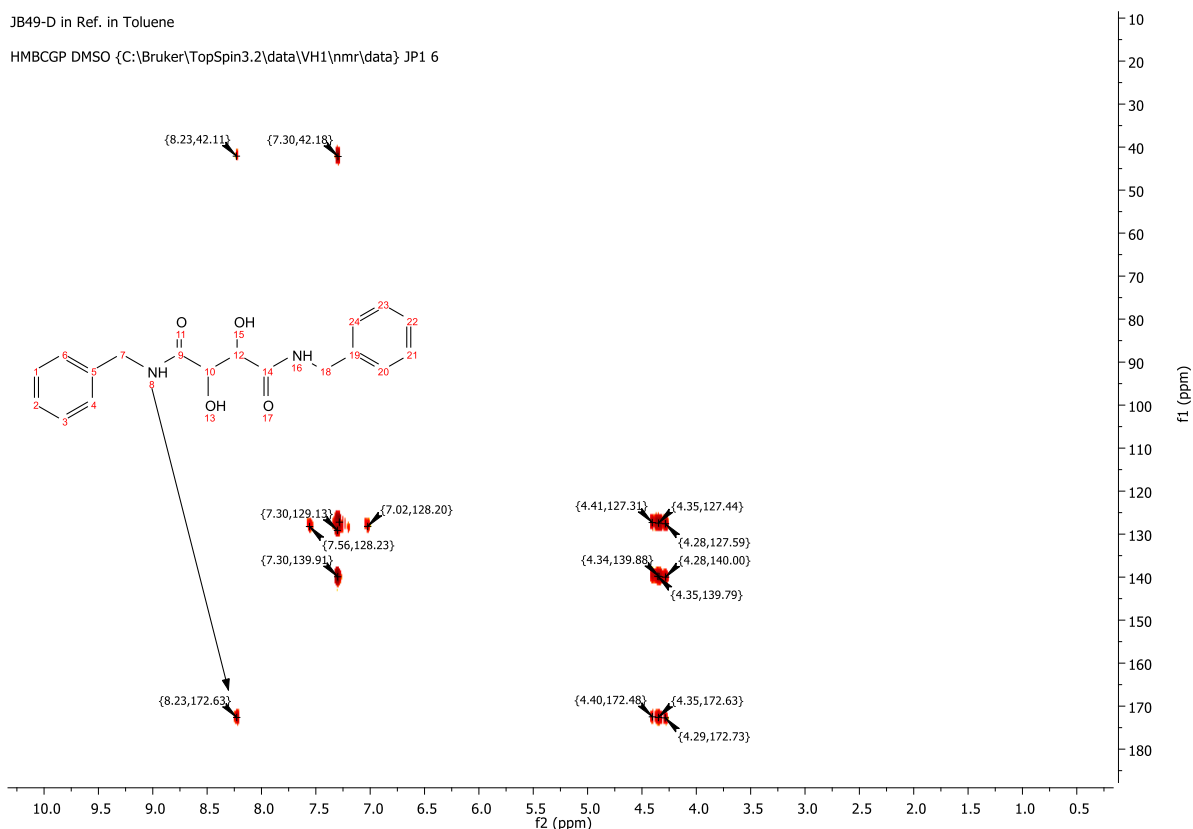


Figure 49: HMBC NMR (300 MHz) spectrum of **111a** in DMSO- d_6 .

The dibenzyl tartrate diamide **111a** was also characterized and confirmed by the electrospray mass spectrometry (ES-MS) analysis (Figure 51). The calculated mass of this compound is 328.1492. ES-MS spectroscopy revealed ions of m/z 329.36, 351.1312 and 367.1051 which corresponds to the mass of **111a** using $(\text{M}+\text{H})^+$, $(\text{M}+\text{Na})^+$ and $(\text{M}+\text{K})^+$ adducts. Thus, the sum of analytical data (NMR, infrared and mass spectrometric confirmed that the symmetrical diamide had been successfully synthesised.

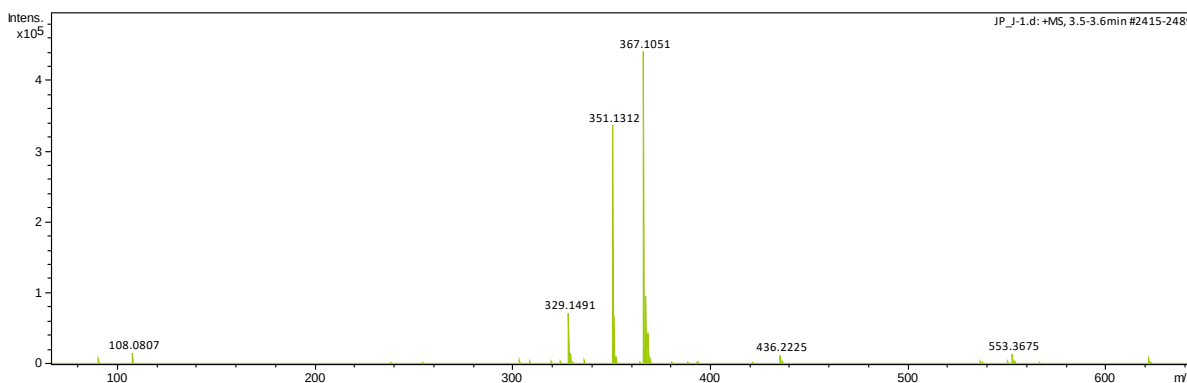
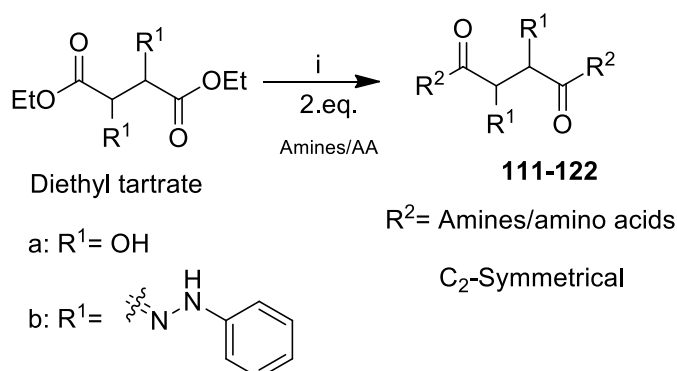


Figure 50: High-resolution mass spectrum of **111a** in methanol.

Although compound **111a** has been described and prepared by Sigma Aldrich,²²³ using different catalysts, there is no literature describing its synthesis using a direct uncatalyzed amidation reaction so far. The synthesis of compound **111a** was repeated using diethyl tartrate instead of the free tartaric acid. The same results were observed, except that in the second instance the reaction took less time.

After having successfully prepared the first symmetrically substituted diamide tartrate **111a** from a free dicarboxylic acid in a one-step synthesis as described above, we sought to explore the method further; by using the diethyl tartrate and tartaric acid osazone (these two derivatives of tartaric acid are also cheap and commercially available). These diacids were then derivatized with selected amines/amino acids (which would enhance the substrate-enzyme's interactions and/or recognize the S1-S3 sites residues of the enzyme), in a one-step reaction, using the same reaction conditions as shown in Scheme 27 below and results are combined in Table 10 below.



Scheme 26: Synthetic route of the 2,3-dihydroxysuccinamide ligands **111-122(ab)** with C_2 -symmetrical stereochemistry. Reaction conditions: i) Solvent, reflux, 2 h; amine/amino acid, reflux, 2-6 h.

Table 10: Combined results of the synthesis of the 2,3-dihydroxysuccinamide ligand 111-122ab.

ID	R ¹	R ²	%yield ^a
111	a	Benzyl amine	96
112	a	DOPA	91
113	a	Tyrosine-OMe	90
114	a	Sulfaguanidine	77
115	a	Sulfadiazine	88
116	a	Tryptophan	43
117	b	Tyrosine	89
118	b	Sulfamethoxazole	40
119	b	Sulfamethizole	97
120	b	Glycine-OEt	63
121	b	Proline-OMe	79
122	b	Iso-leucine	75

^aYields (%) given here refer to the compounds isolated after column chromatography followed by a preparative-TLC while the purity was confirmed by ¹H NMR spectra.

The novel and symmetrically substituted compound **113a** was synthesized using diethyl tartrate and tyrosine methyl ester as starting materials and the full structural confirmation was made possible by the application of one and two dimensional (2D) NMR, whereas the purity was confirmed using the elemental analysis. In most cases, the purification of the target compounds was not challenging since they precipitated as soon as the reaction was cooled to room temperature. However, with the Sulfamethoxazole and Sulfamethoxazole containing scaffolds the presence of two extra free amine groups at the other end. This could be associated with— leading to poor recovery from chromatographic purification.

The structure of the symmetrically substituted *bis*-tyrosine-OMe tartrate amide **113a** was also confirmed by the FT-IR spectrum. The presence of the amide functional

groups, a necessary characteristic of a successful synthesis of the desired scaffold, was confirmed by the appearance of both amide and ester carbonyl stretching bands in the IR spectrum at 1654 and 1744 cm^{-1} respectively, corresponding to the tartrate-tyrosine and tyrosine methyl ester linkages respectively. This was supported also by the appearance of signals at 173.6 ppm and 157.1 ppm in the ^{13}C NMR spectrum (Figure 53).

The remaining signals in the proton and carbon NMR spectra were assigned using HMBC (Figure 54) and DETP 135 (Figure 55) spectra. The $-\text{CH}$ signal of the tyrosine moiety was readily identified in the proton NMR spectrum as the only triplet (4.06 ppm), as a result of it coupling to the benzylic methylene protons (CH_2) of the tyrosine (Figure 52). This facilitated the ready assignment of the corresponding carbon signal at 53.9 ppm using the HMBC correlation (Figure 54) as well as the benzylic protons at 3.07 ppm using the COSY spectrum. The two sets of multiplets at 7.00 and 6.71 ppm, integrating in total to 8H (2 x 4H) correspond to the aromatic protons from the tyrosine methyl ester (Figure 52). Their integrals are rationalized by considering the symmetry within both the aromatic ring as well as the whole molecule. The HMBC spectrum (Figure 54) revealed that the doublet integrating for 1H at 7.00 ppm, which was attached to the carbon atom 31 (according to HSQC correlations), correlated to carbon 25. The carbon bearing this proton appears in the ^{13}C NMR (Figure 53), at 131.0 ppm as well as the DEPT 135 spectrum (Figure 55). Due to the presence of a neighboring electron donating or releasing group, the doublet at 6.70 ppm, integrating for 1 x 2H (due to the plan of symmetry within the molecule), with a $J = 3$ Hz, (on the carbon 32) correspond also to the aromatic ring. This proton is shielded by the OH group.

Due to the plane of the symmetry within the molecule, the overlapping singlets at 4.31 ppm, which integrate for 6H corresponds to the two methine protons (2H) on carbon 8 and carbon 9 respectively, both adjacent to hydroxyl functional groups, whose proton appears at the same position as well as the 2H attached to the two nitrogen atoms as shown in Figure 51. This was demonstrated by examination of the HMBC correlations. The HMBC spectrum revealed another that this signal at 4.31 ppm, which is related to a direct correlation from the carbonyl carbon 13 to the CH,

NH and OH all at the same position. These three correlations were interpreted as evidence of the direct link between tyrosine and tartrate, confirming formation of the diamidediol **113a**.

The amide carbon appears on the ^{13}C NMR spectrum at 173.5 ppm (amide region) while the carbonyl carbon 26 of the methyl ester appears at 169.9 ppm on the ^{13}C NMR (Figure 52). The proton on the nitrogen atom appears at 4.15 ppm (overlapping) which correlates with the carbonyl carbon at 173.5 ppm of the amide (Figure 53). The signal at 35.5 ppm (on ^{13}C NMR) correspond to the two $-\text{CH}_2$ of the phenyl groups. The signal at 157.1 ppm (^{13}C NMR) correspond to the two methines bearing the free hydroxyl groups. Others signals are presented in the experimental section.

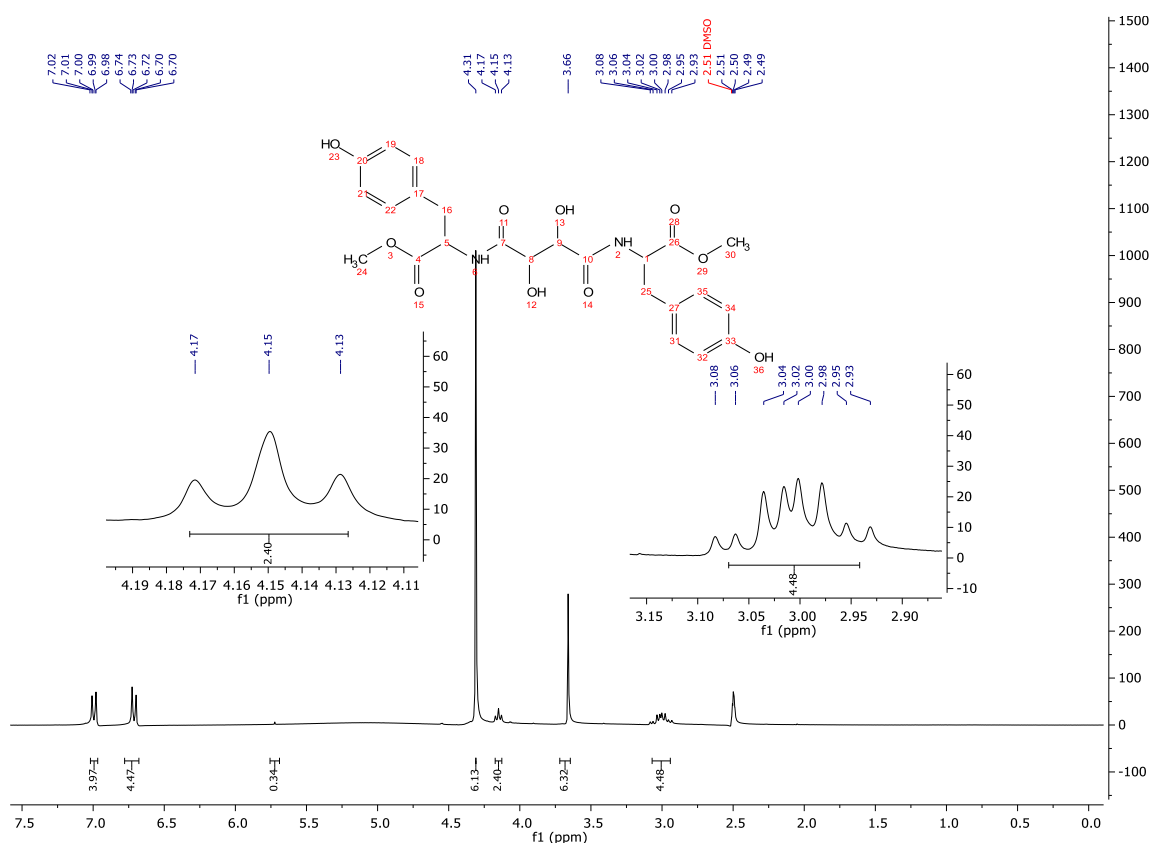


Figure 51: The ^1H NMR (300 MHz) spectrum for **113a** in $\text{DMSO}-d_6$.

The singlet at 3.66 ppm, integrating for 2x 3H corresponds to the methyl esters at both C-termini of the molecule, on carbons 24 and 30. This methyl ester was confirmed by the presence of the peak at 53.0 ppm in both ^{13}C NMR (Figure 52) and DEPT 135 NMR spectra (Figure 54), while showing correlation with the carbonyl carbon 26 at 157.1 ppm in the HMBC spectrum (Figure 53).

The multiplet at 3.03 ppm, integrating for 2x 2H corresponds to the diastereotopic protons on the methylene groups (carbon 25 and 16) of the tyrosine. Carbons 25 and 16 were confirmed as methylenes by the DEPT 135 NMR spectrum (35.5 ppm, Figure 54) where they appeared as the only negatively phased signal. This carbon correlates with the methine group (carbon 1), and the carbonyl carbon 26 as revealed by the HMBC spectrum (Figure 53). The triplet at 4.06 ppm integrating for 1x 2H with $J = 6$ Hz, corresponds to the only proton of the methine group (carbon number 1) next to the methylene group to which it correlates as shown by the HMBC spectrum.

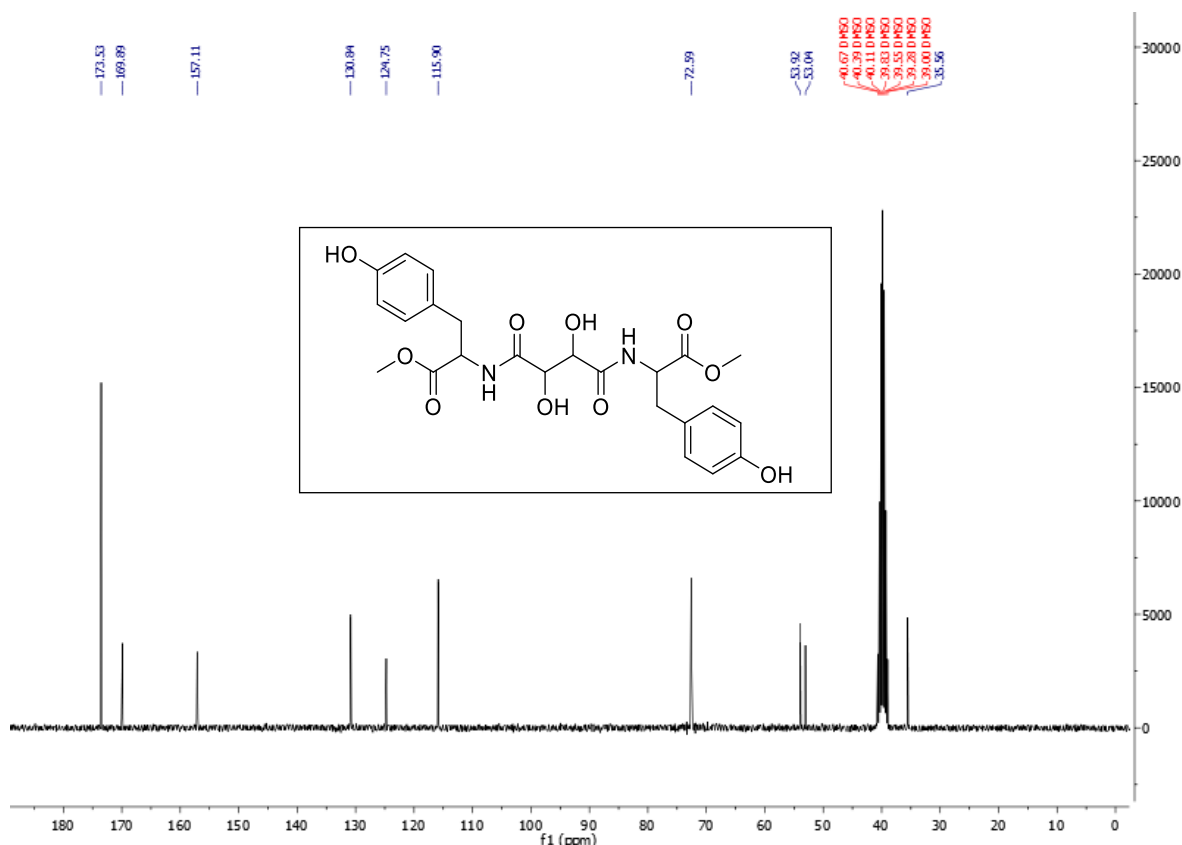


Figure 52: The ^{13}C NMR (75 MHz) spectrum for 113a in $\text{DMSO}-d_6$.

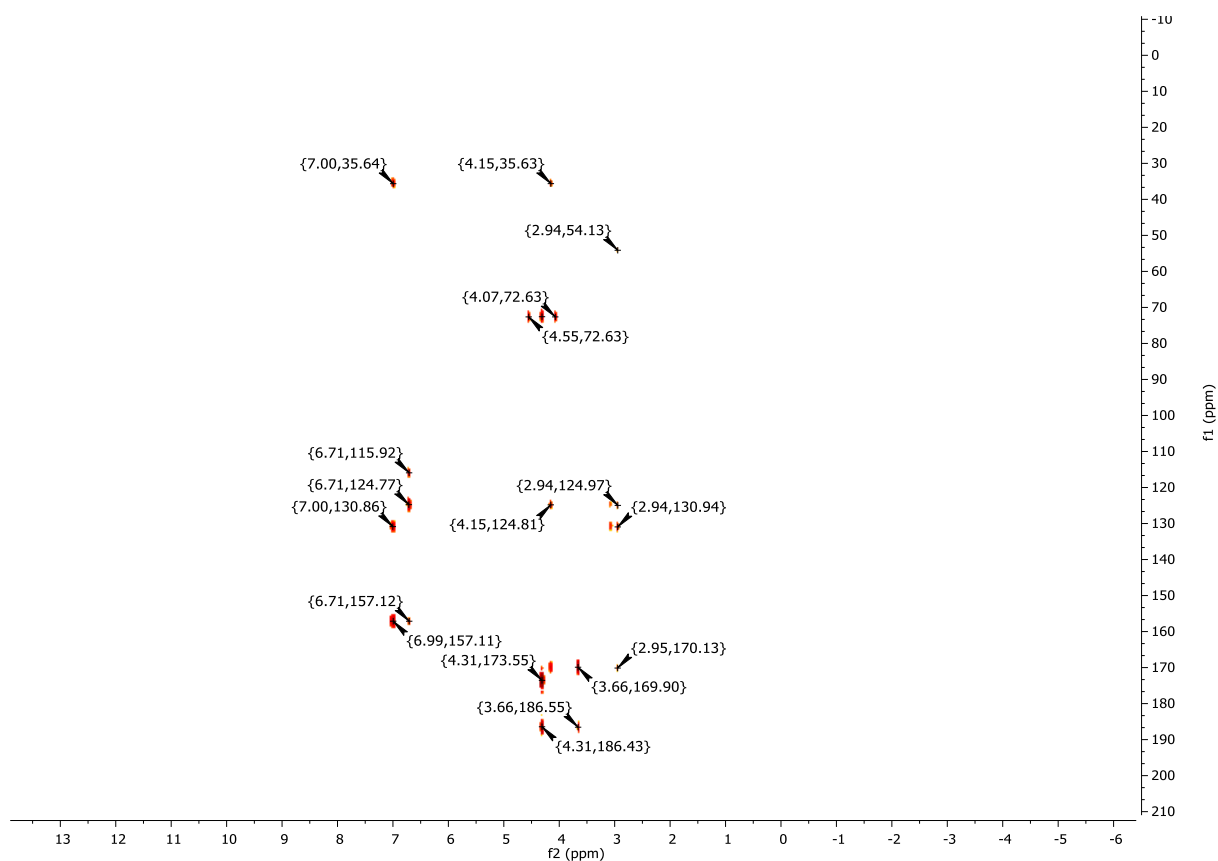


Figure 53: The HMBC NMR (300 MHz) spectrum for 113a in DMSO-d₆.

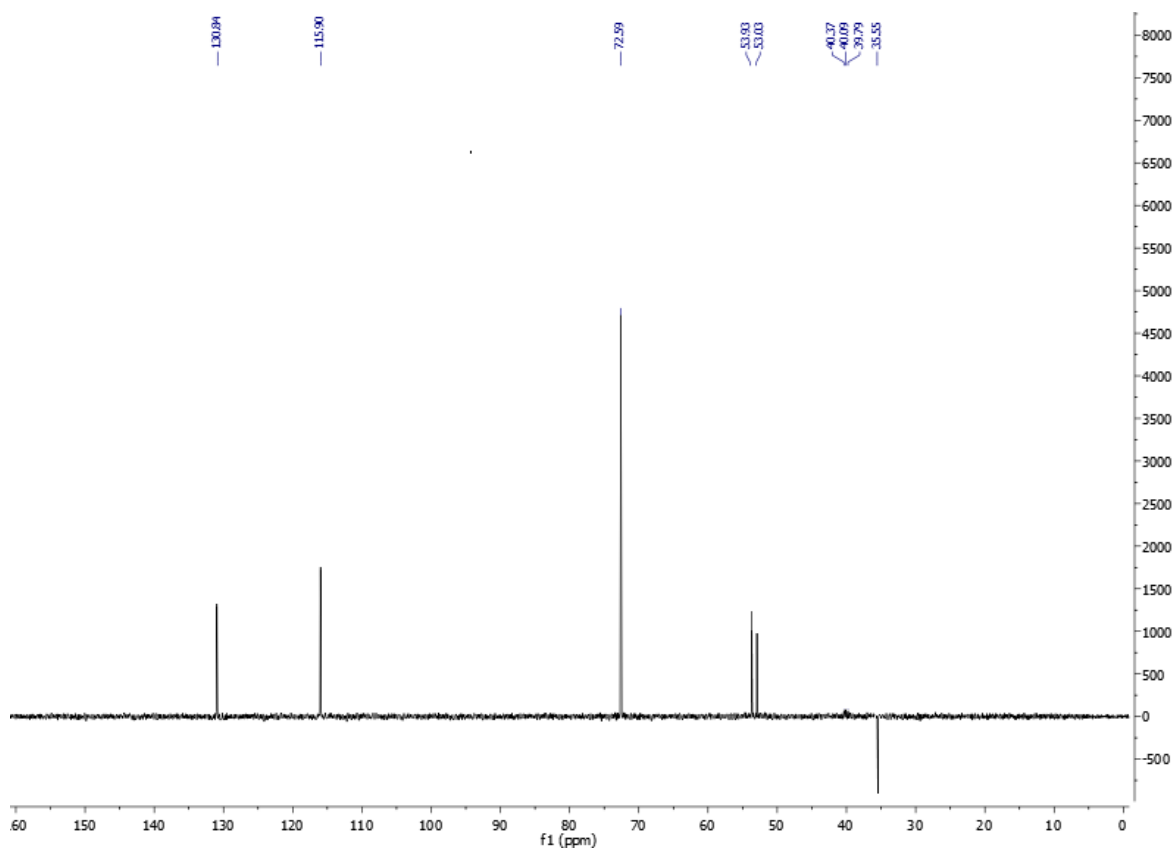
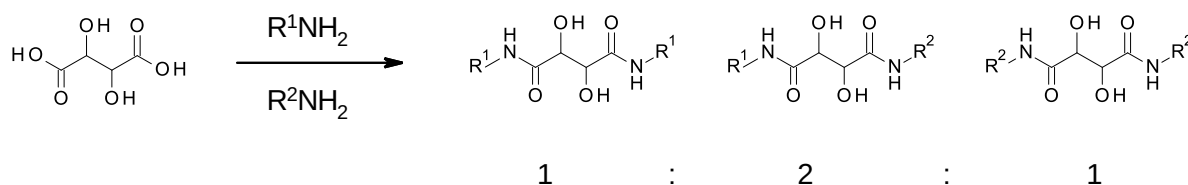


Figure 54: The DEPT 135 NMR spectrum for the compound 113a in DMSO.

Tartramides were synthesized in this current project by reacting tartaric acid and/or its derivatives with amines at high temperature (120°C), thus incurring potential undesired side reactions, especially for the unsymmetrically substituted compounds, and this seriously affected their yields. These by-products presented several signals in the NMR spectra (proton and carbon) of the crude mixtures that could not be accounted for.

2.2.1.3. Preliminary kinetics study of the formation of the C₂-symmetrical diamide tartrate

The synthesis of the symmetrically substituted diamide was much faster than the synthesis of the unsymmetrical analogs (2h vs. 12h) regardless of the amine groups used. Furthermore, the product distribution appeared to be different to what would have been predicted based on a statistical analysis alone (Scheme 27). A preliminary study was devised to provide insight into the mechanism.



Scheme 27: Different product distributions

Initially, the formation of the symmetrically substituted diamide was examined, to establish the parameters of the experiment. To monitor the formation of the C₂-symmetrically substituted compounds, the synthesis of the dibenzyl tartrate **111a** was repeated as described previously. After starting the reaction, aliquots of 1.0 ml were taken at intervals from the starting of the reaction until the final product formation and were labeled as time zero (T₀), ten minutes after (T₁₀), twenty (T₂₀), thirty (T₃₀), forty (T₄₀), sixty (T₆₀), ninety (T₉₀) and one hundred and twenty minutes after (T₁₂₀) respectively. After removal of toluene under reduced pressure, each sample was diluted with methanol (0.1 ml) and DMSO-d₆ (0.4 ml) and then characterized using ¹H NMR spectroscopy.

All the integrals were calibrated using methanol's methyl signal since it was used as internal standard of known concentration (4.12 mol.L⁻¹), which was multiplied by 3H

(from the three protons of the methyl group of CH_3OH), to get a final C_{MeOH} of 12.36 mol/L. Therefore, each signal was integrated relative to the methanol singlet as 12.36.

The triplet at 1.23 ppm on Figure 55 (integrating for 3H and corresponding to the terminal CH_3 of diethyl tartrate), the singlet at 3.38 ppm (integrating for 3H, and corresponding to the methyl group of methanol used as internal standard), and the triplet at 8.23 ppm (integrating for 2x 1H, corresponding to the amide protons of the product), served as a guage of the reaction progress.

The signal at 8.23 ppm was absent on the ^1H NMR spectrum at T_0 (Figure 56), confirming that no product had formed at that stage. This was also supported by the presence of the triplet at 1.23 ppm, which correspond to 3H of the terminal CH_3 of diethyl tartrate.

However, one hour later, (T_{60}), the ^1H NMR spectrum (Figure 55 below) revealed that the desired product started forming in the reaction flask. This was confirmed by the appearance of a signal at 8.23 ppm corresponding to the desired diamide tartrate (as shown in the Figure 55 below), although some starting materials were still present, as supported by TLC monitoring and the H-NMR spectra analysis (Figure 56 and Figure 57). Integration of the three signals indicated that the product and the starting materials were in 50/50 ratio.

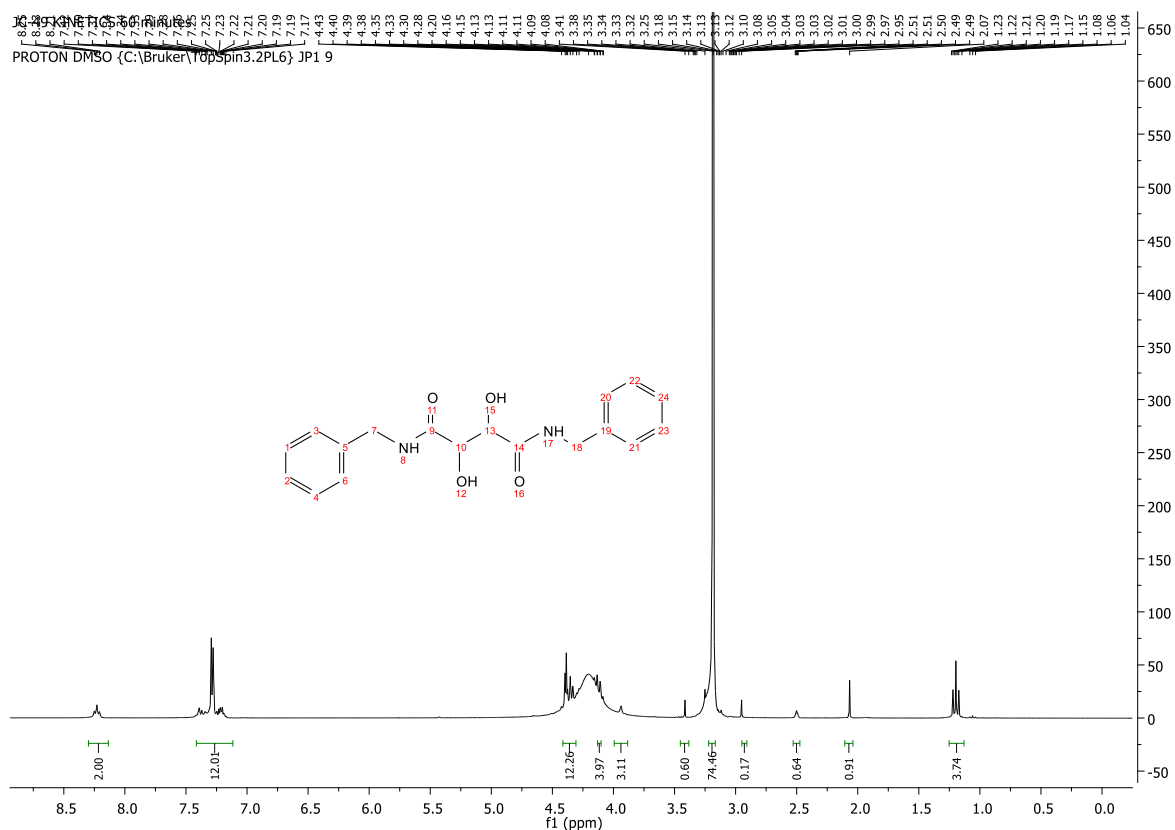


Figure 55: The ^1H NMR (300 MHz) spectrum of 111a at T_{60} in DMSO-d_6 and methanol as internal standard

Reaction progress was illustrated by a stacked plot of ^1H -NMR spectra from T_{10} to T_{120} as shown in Figure 57 below, which was generated using OriginPro software program.²²⁴

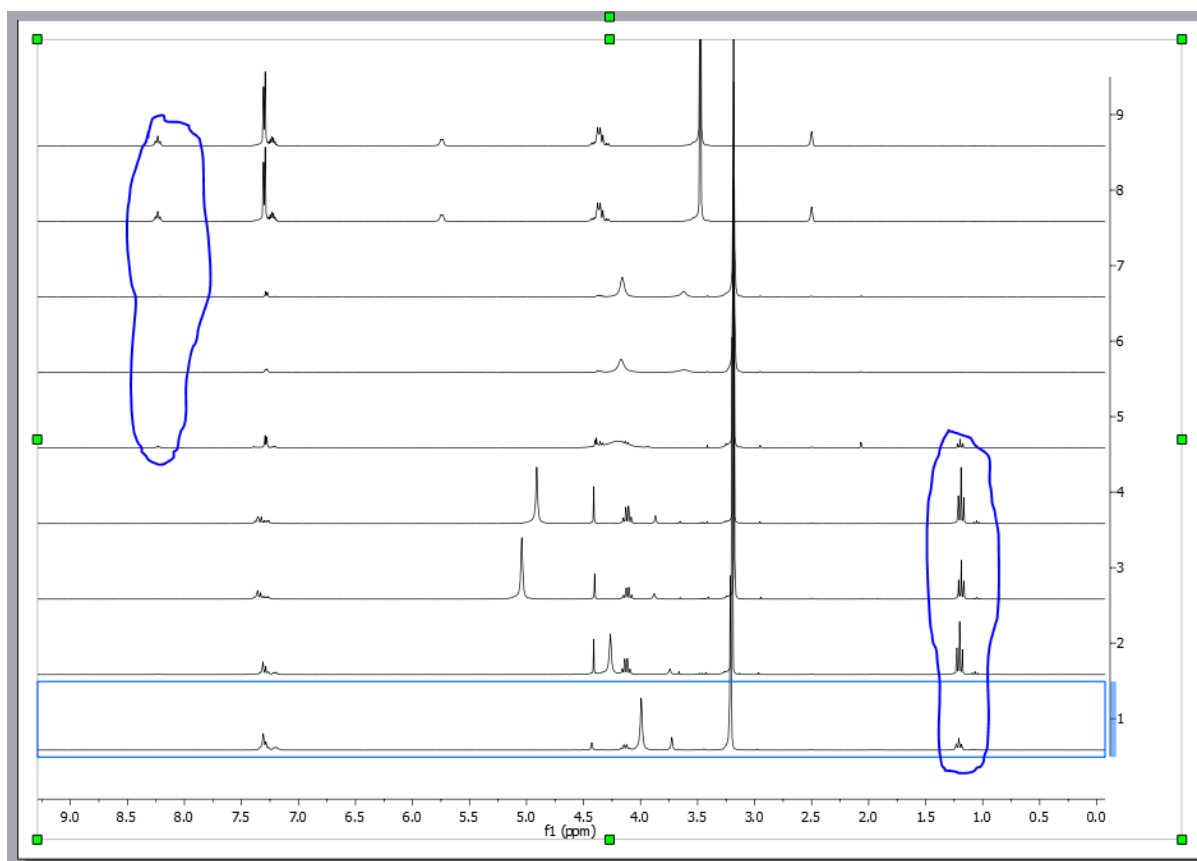


Figure 56: Stacked plot²²⁴ of ^1H NMR (300 MHz) spectra of 111a from T_{10} to T_{120} in DMSO- d_6 and methanol as internal standard.

The ^1H NMR spectrum of the sample collected at T_{120} indicated that the product formed in 99.9:0.1 ratios and this is shown in Figure 57 below.

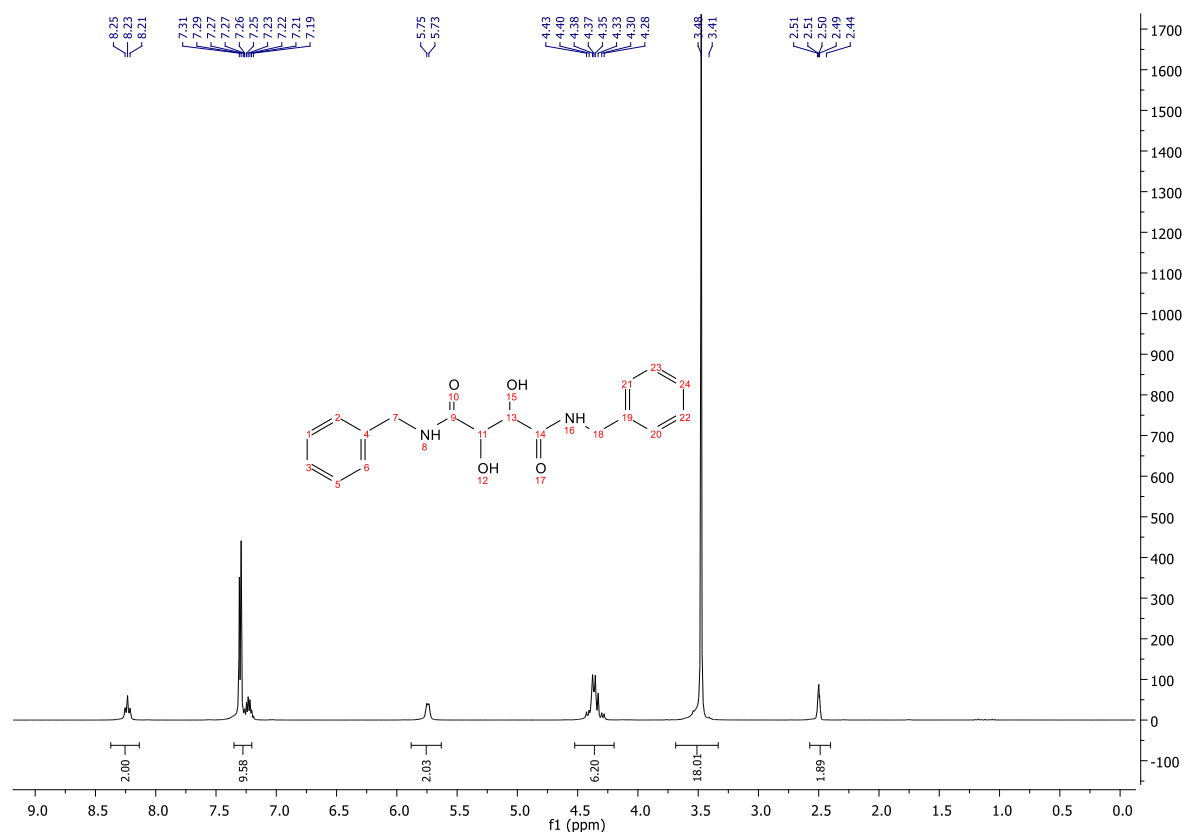


Figure 57: The ^1H NMR (300 MHz) spectrum of **111a** at T_{120} in $\text{DMSO}-d_6$ and methanol as internal standard.

The combination of all the ^1H NMR spectra allowed us to get different concentrations of both starting materials and the final diamide tartrate product **111a**. These are summarized in Table 11, which permitted us to plot different curves to elucidate clearly the observed changes such as the decreasing of the starting material (Figure 58) and the increasing of the product formation (Figure 59) as the time increased whereas Figure 60 combines both graphs.

Table 11 Concentrations of starting material and the final products at different times.

Time (minutes)	Concentration		
	NH (8.23 ppm): [amide]	CH_2CH_3 (1.23 ppm) [diethyl tartrate (DT)]	MeOH (3.38 ppm) [standard]
10	0.03	3.26	12.36
20	0.06	3.13	12.36
30	0.12	2.98	12.36
40	0.25	2.9	12.36
60	0.56	2.2	12.36

90	1.04	1.48	12.36
120	2.04	0.00	12.36

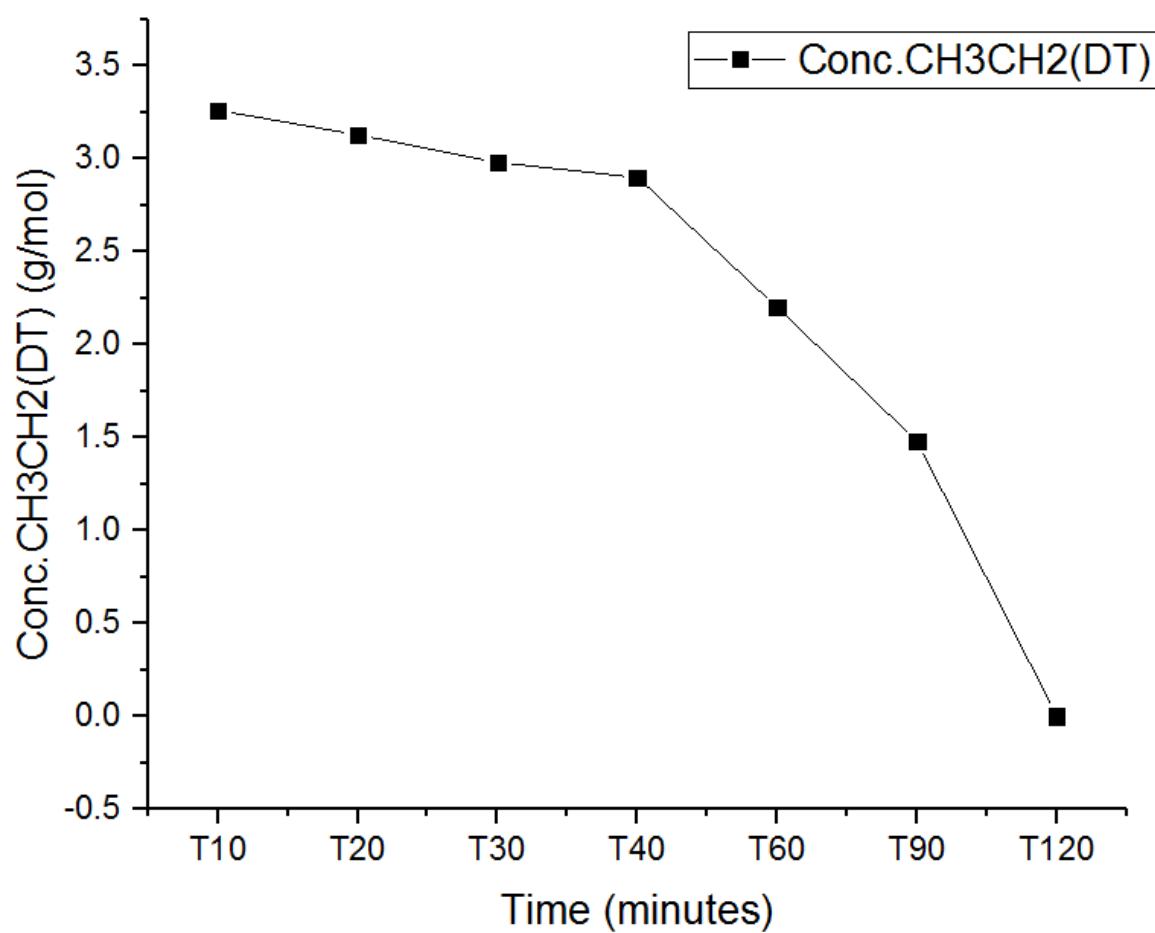


Figure 58: Single plot of the curve of concentration of the CH_3CH_2 signal of diethyl tartrate signal.

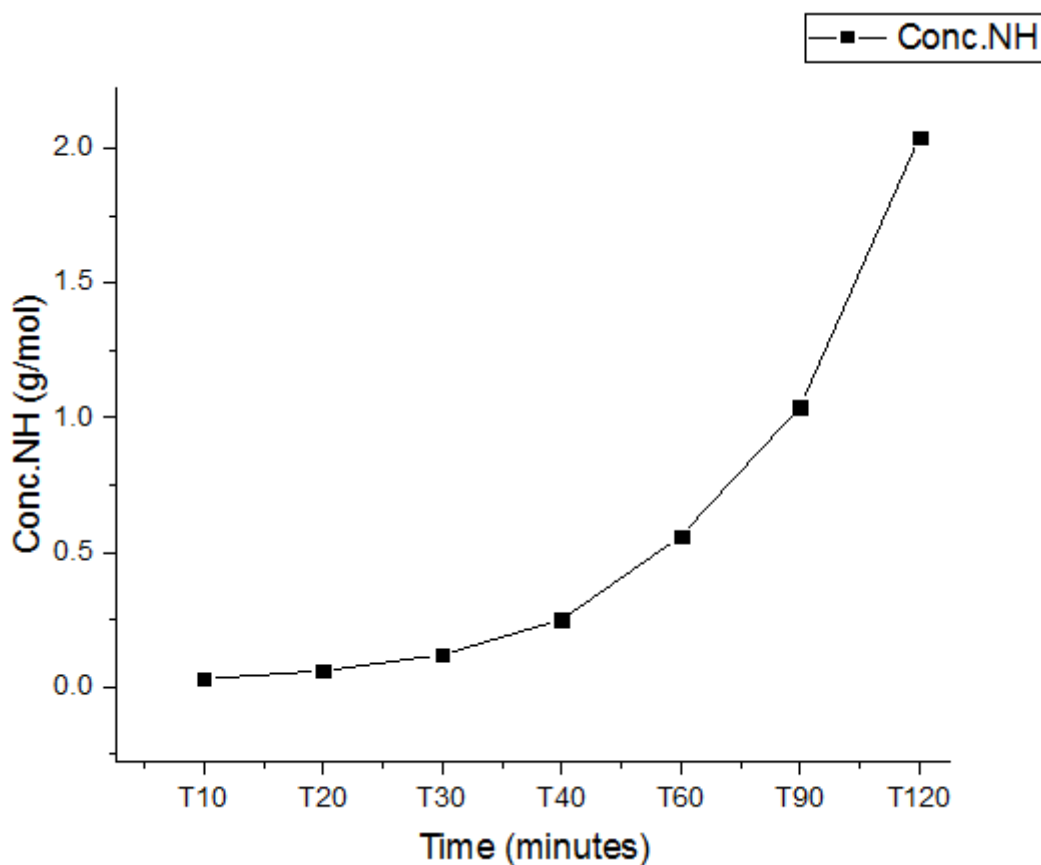


Figure 59: Single plot of the curve of concentration of the NH signal of product signal.

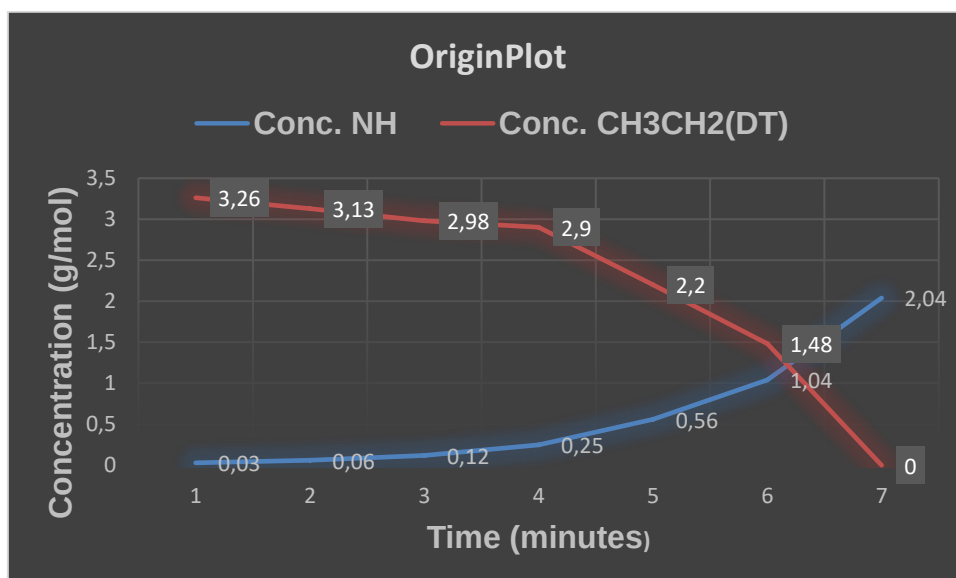


Figure 6550: Change in concentrations of starting materials and product as the reaction progressed.

Figure 60 correspond to a combined plot of starting materials and final product **111a**. The curve in red shows a progressive decrease of one of the diethyl tartrate from a

concentration of 3.26 g/mol to null whereas the curve in blue (bottom) indicates the increase in the formation of the product as followed using one of its characteristic signals, the triplet at 8.23 ppm. The concentration of this signal went from 0.03 g/mol at time 0 (T_0) to 2.04 g/mol after 120 minutes (T_{120}).

2.2.2. Synthesis of the C_2 -unsymmetrical ligands 123- 152(ab).

After exploring successfully the advantage of the C_2 -symmetrically substituted analogs, we sought also undertake and explore the unsymmetrical ones. As mentioned before that some of potential advantages of C_2 -symmetrically substituted drugs, designed as inhibitors of the HIV-1 PR enzyme includes their high potency, highselectivity, stability and their bioavailability.²²⁵ In this way, several pseudo- C_2 -symmetric monools as well as C_2 -symmetric diols (containing a central hydroxymethylene or dihydroxyethylene core units) incorporated with hydrophobic P1/P1' side chains have been explored.

However, Dreyer, G.B. and co-workers speculated in 1993 that even a C_2 -symmetric inhibitor still binding the HIV-1 PR in an asymmetric manner. This based on their findings that monools and diols HIV-1 PR inhibitors were potentially inhibited the porcine pepsin, which is an asymmetric monometric prototype of aspartic PR.²²⁵ These authors speculated that the X-ray crystal structure of the HIV-1 PR complexed with a C_2 -symmetric diol binds the HIV-1 PR asymmetrically and showed 2-folds disorder in the electron density map.²²⁵

Results from their molecular dynamics simulations conducted with model complexes also supported their hypothesis that the more stable trajectory came from the asymmetric complex. They used a four-point model to also argue that van der Waals and electrostatic force fields would generally lead to an asymmetric association between symmetric molecules.²²⁵ Therefore, this valuable work done by Dreyer, G.B. and co-workers and their findings/observations amongst others, motivated us to explore/investigate more C_2 -asymmetrically substituted diamides ligands.

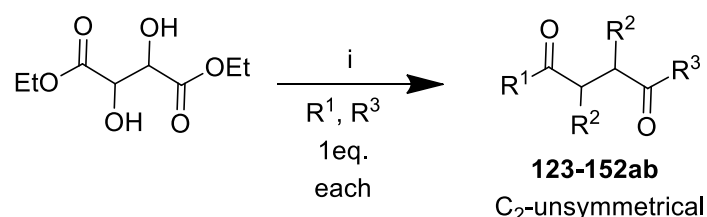
To the best of our knowledge, there is no literature up-to-date describing a one-step synthesis of both symmetrically and unsymmetrically substituted diamides from diacids using a direct amidation reaction as herein described. A close examination

of the supernatant obtained after synthesis of unsymmetrically substituted diamide tartrates' (after filtration of the pure diamide products after recrystallization), revealed some by-products, including monosubstituted amides, with some noticable racemization. However, only the major targeted products were isolated and considered for further analysis in this project.

The racemization was revealed by a quick comparison of peak areas on H-NMR analysis of some pure enantiomers (on standing), which revealed a mixture of more than one enantiomers. This was believed before as part of by-products). This can be also due to the fact that the one of the starting materials, being the major core unit, the tartaric acid, was a mixture of racemates as determined by Louis Pasteur in 1843.²²⁶

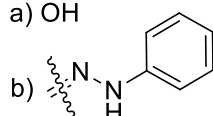
Therefore, a further study and/or analysis need to be conducted in continuation of this current project, using a polarimeter to determine and separate the Dextro and the Levo-moieties if possible and determine not only their optical rotation (or activity) but the rate of racemization also (for most of these new compounds).

The method used to prepare symmetrical compounds was also used for the unsymmetrically substituted 2,3-dihydroxysuccinamides **123-152(ab)**, applying a slight variation of the original method described in the literature for symmetrical amidation.¹⁸⁰ In the synthesis of unsymmetrical amides, after refluxing the diethyl tartrate or tartaric acid osazone for about two hours, both corresponding amine and amino acid were added at the same time in 1:1:1 ratio (Scheme 28). All the results are combined in Table 12.



R¹ and R³ = two different amines/amino acids

R² : a) OH



Scheme 28: Synthetic route for the preparation of the C₂-unsymmetrical analogues 123-152(ab): Reaction conditions: i) Toluene, reflux, 2 h; then R¹ and simultaneously, reflux 24 h.

The purification of these complex mixtures was most challenging. It was observed that for the same combinations of amine/amino acid, in most cases, the substitution of diethyl tartrate by tartaric acid osazone, resulted in a dramatic drop in the yields of isolated compounds. All the unsymmetrical diamides were generally isolated in very low to moderate yields, ranging between 20% and 57% (Table 12); much lower than the yields of the symmetrical analogues. The lowest yields were observed with the sulfaguanidine containing scaffolds. This could be associated with the presence of two extra free amine groups at the other end of Sulfaguanidine – leading to poor recovery from the column chromatography purification.

TLC analysis indicated the formation of several by-products, including mono-substituted products and some symmetrically substituted products. The appearance of these products, while unsurprising, necessitated extensive and complex chromatographic purification, contributing to the decreased yields of isolated products.

Table 12: Combined results of the direct amidation of unsymmetrically substituted analogues 123-152(ab).

ID	R ¹	R ²	R ³	% YIELDS
123	a	Iso-Leucine	DOPA	51
124	a	Tryptophan	DOPA	46
125	a	Tryptophan	Proline-OMe	57
126	a	Tryptophan	Tyrosine	54
127	a	Tryptophan	Iso-Leucine	53
128	a	Tryptophan	Sulfadiazine	41
129	a	Tyrosine-OMe	DOPA	40
130	a	Tyrosine-OMe	Sulfadiazine	51
131	a	Tryptophan	Sulfamethizole	48
132	a	Tryptophan	Sulfamethoxazole	38

133	a	Sulfamethoxazole	DOPA	49
134	a	Sulfaguanidine	Sulfadiazine	21
135	a	Sulfaguanidine	Iso-Leucine	24
136	b	Leucine	DOPA	40
137	b	Tryptophan	DOPA	55
138	b	Tryptophan	Proline-OMe	52
139	b	Tryptophan	Tyrosine-OMe	49
140	b	Tryptophan	Leucine	50
141	b	Tryptophan	Sulfadiazine	38
142	b	Tyrosine-OMe	DOPA	55
143	b	Tyrosine-OMe	Sulfadiazine	43
144	b	Tryptophan	Sulfamethizole	31
145	b	Tryptophan	Sulfamethoxazole	20
146	b	Sulfamethoxazole	DOPA	25
147	b	Sulfamethoxazole	Sulfadiazine	22
148	b	Sulfamethoxazole	Leucine	30
149	b	DOPA	Sulfadiazine	52
150	b	DOPA	Sulfaguanidine	20
151	b	Sulfaguanidine	Tyrosine OMe	30
152	a	Benzylamine	Glycine (amino acid)	78

To confirm the formation of compound **152a**, since the main reaction involved amide bond formation between the carboxylic acid or its carboxylates and a corresponding amine/amino acid, it was therefore imperative to use other characterization means such as the Fourier-transform infrared spectroscopy (FTIR) technique. As shown in Figure 61, the amide carbonyl bond was confirmed by the presence of a strong band at 1645 cm^{-1} while the characteristic carboxylic acid bands were observed at 3000--

3398 (OH) and 1714 cm^{-1} (carboxylic acid from glycine). A characteristic N-H stretch was observed at 2603 cm^{-1} in agreement with the literature.¹⁹³

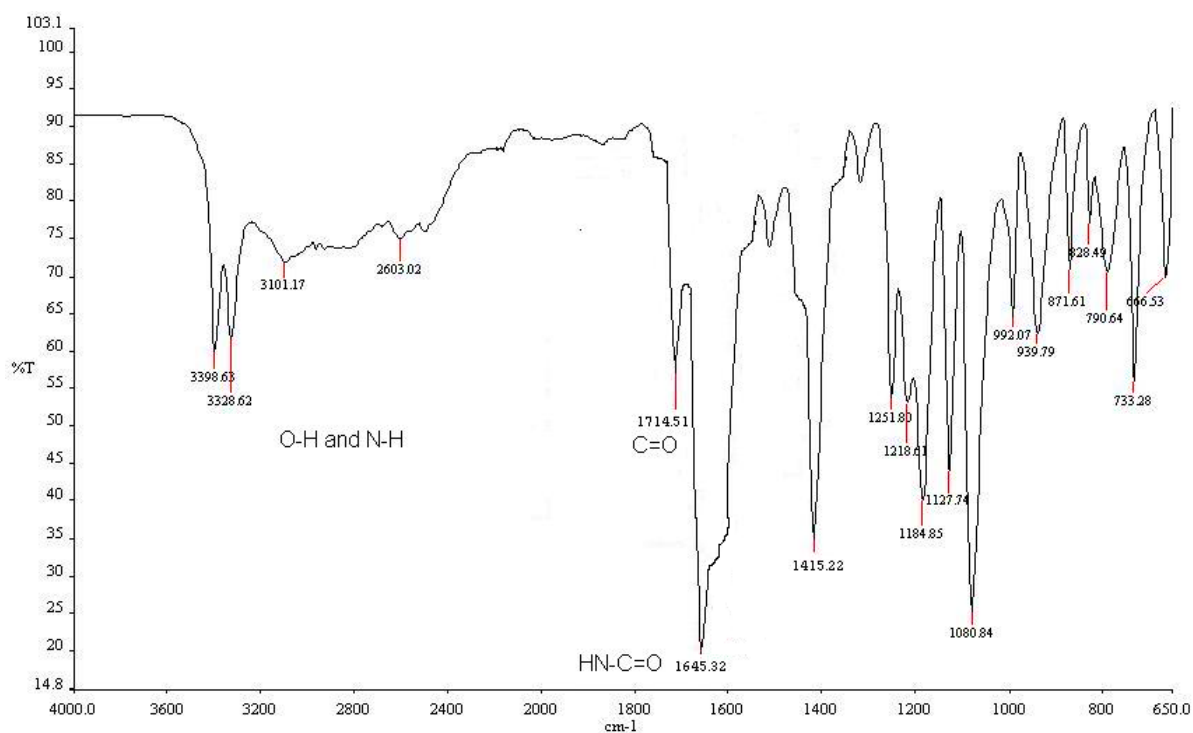


Figure 61: The FT-IR spectrum of 152a.

For comparison, both starting materials were also analyzed and are presented in Figure 62 for the diethyl tartrate, Figure 63 for benzylamine and Figure 64 for glycine. It was clear that the amide carbonyl band was the only new band while others were present, although shifted slightly, confirming the formation of the new product **152a**.

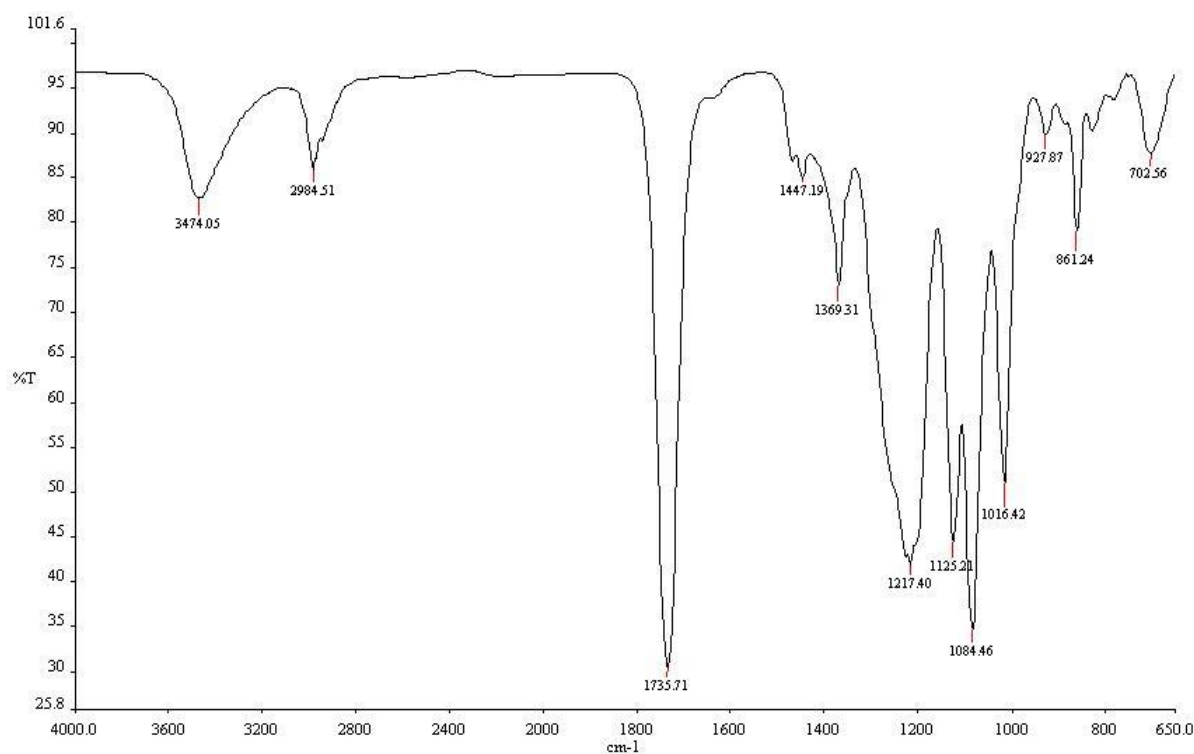


Figure 62: The FT-IR spectrum of diethyl tartrate starting material.

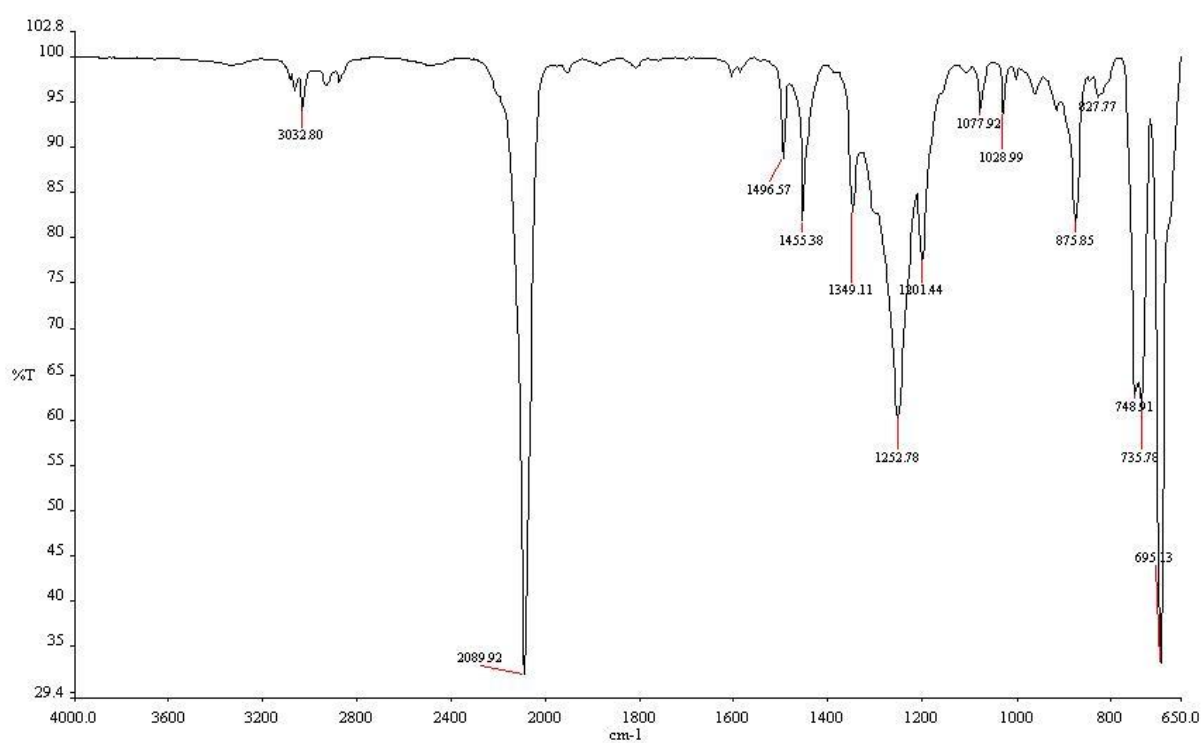


Figure 63: The FT-IR spectrum of benzylamine starting material.

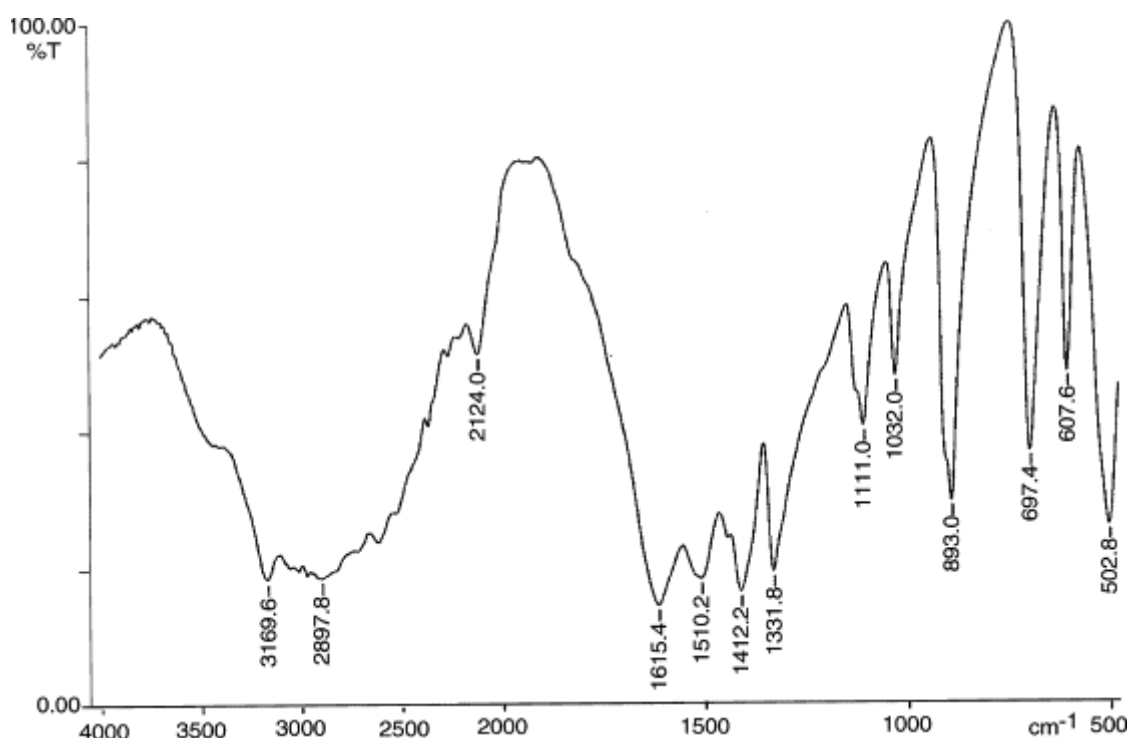


Figure 64: The FT-IR spectrum of glycine starting material.²²⁰

Since most of the unsymmetrical amides have several overlapping proton signals, which might make their conclusive elucidation identification difficult, a small molecule was chosen to demonstrate the effectiveness of the synthetic approach. Therefore, the unsymmetrically substituted diamide tartrate **152a** was synthesized and isolated in 78% yield, from a mixture of diethyl tartrate, benzylamine (amine) and glycine (amino acid) – each of which have clearly resolved proton and carbon NMR signals.

The structure of the product **152a** was analyzed and confirmed by different characterization techniques, including 1D and 2D NMR, IR, and elemental analysis. All the peaks related to the three different components of the compound **152a** were present and were identified accordingly, confirming the absence of a plane of symmetry within the scaffold. The full elucidation is described below.

As expected, a total of seven protons were observed in the aromatic region (Figure 65). These correspond to aromatic protons from benzylamine (5H) as well as the 2H on the nitrogen atoms of the amide bonds, N-8 and N-14 respectively. The amide protons appear as triplets at 7.77 ppm and 7.93 ppm, each integrating to 1H. This is

due to coupling with the neighboring methylene protons, the benzylic protons from benzylamine and the glycine methylene (Figure 65).

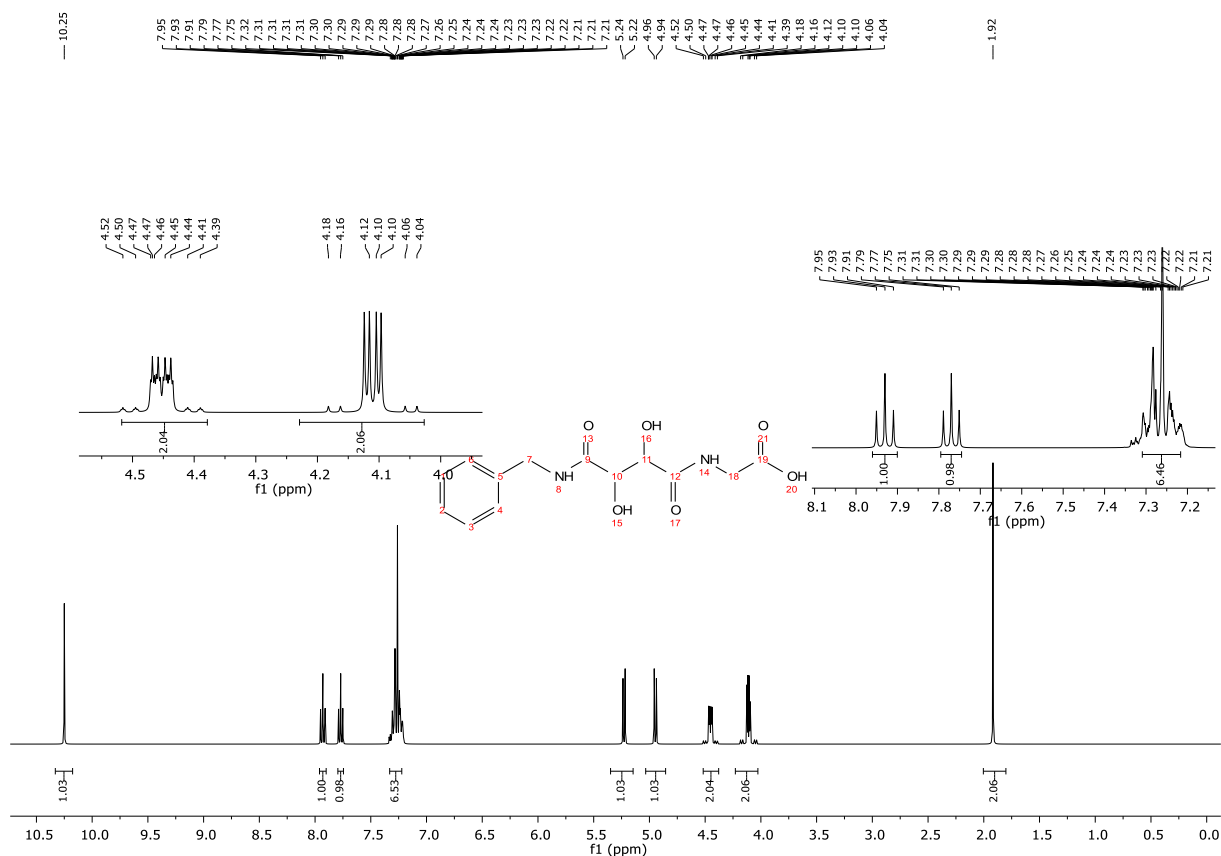


Figure 65: The ^1H NMR (300 MHz) spectrum of **152a** in CDCl_3 .

With regards to the splitting pattern of the symmetrically substituted compound **113a** (Figure 47), the fact that the two protons on N-8 and N-14 of compound **152a** appeared with a significant difference in terms of their splitting pattern [7.77 ppm and 7.93 ppm (Figure 65)], whereas for symmetrically substituted compound **113a** (Figure 47) they overlapped at 4.31 ppm), serve as further evidence of a successful synthesis of **152a** as unsymmetrically substituted compound. This was supported also by the ^{13}C NMR spectrum, which revealed the presence of two different carbonyl carbons at 162.0 ppm and 163.7 ppm (Figure 66), corresponding to the two amide bonds of our formed product **152a** whereas the acidic carbonyl of glycine was confirmed by the presence of the signal at 174.2 ppm (Figure 66).

The two doublets at 4.95 ppm and 5.23 ppm, each integrating to 1H, correspond to the methine protons (H-10 and H-11) adjacent to the hydroxyl groups on the tartrate, which appeared at 1.92 ppm. The remaining multiplets at 4.12 ppm and 4.48 ppm, integrating to 2H each correspond to the two benzylic protons from benzylamine and two methylene protons from glycine (amino acid).

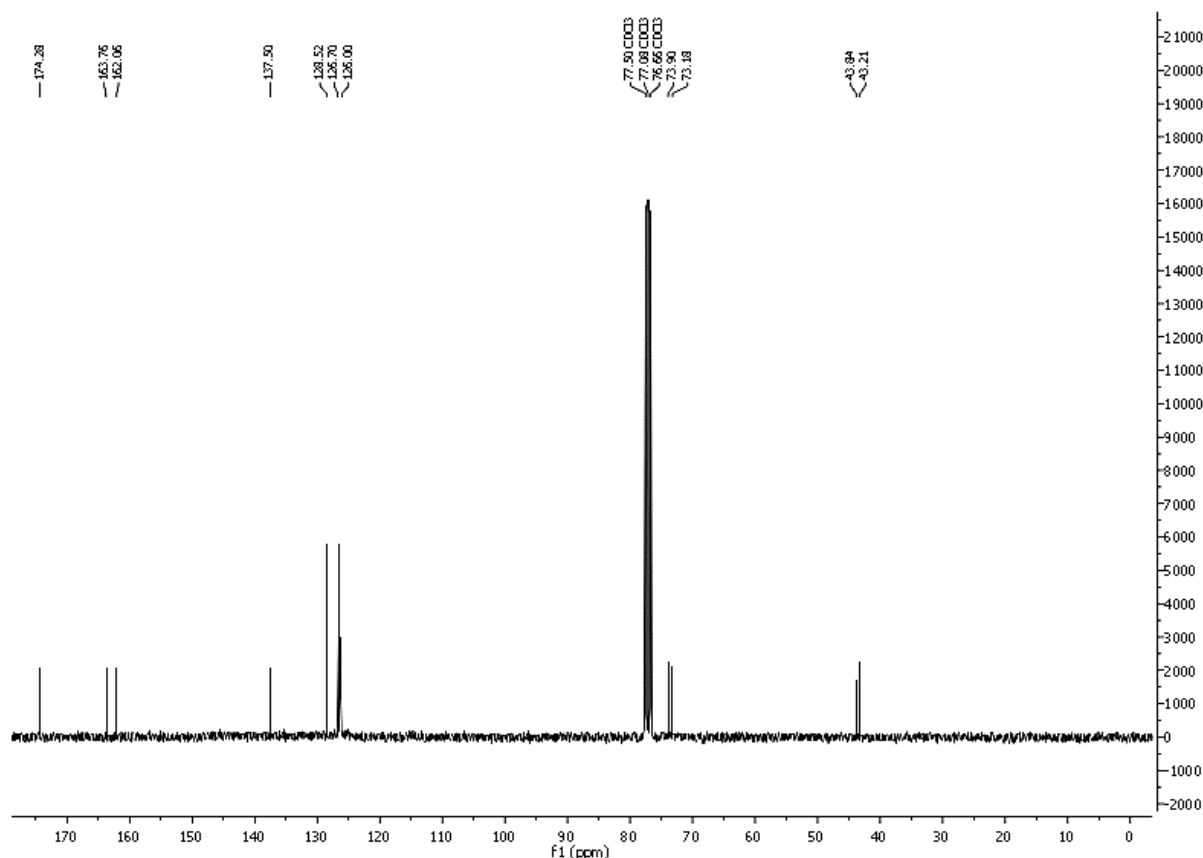


Figure 66: The 75 MHz ^{13}C NMR spectrum of **152a** in CDCl_3 .

The analysis of the HMBC spectrum gave more meaningful insight in the confirmation of the unsymmetrical product **152a**. Figure 67 represent an illustration used to assist in the explanation of the signals assignment of the HMBC correlations within **152a**. The HMBC spectrum (Figure 68) revealed expected correlations between the proton on the nitrogen-8 and the carbonyl carbon-9 (163.7 ppm, ^{13}C NMR), as well as both methine carbons C-10 and C-11 respectively in a long-range coupling. Another correlation was revealed between the proton on N-8 and the benzylic carbon-7 (43.8 ppm, ^{13}C NMR). The HMBC spectrum revealed also

correlations between the proton on the nitrogen 14 with the carbonyl carbon-12 (43.2 ppm, on the ^{13}C NMR, Figure 66), both methine carbons C-11 and C-10 [73.1 ppm and 73.9 ppm, (Figure 65)], as well as the carbonyl carbon-19 (174.2 ppm, ^{13}C NMR, Figure 66). These correlations were used to confirm with certainty the successful synthesis of the C_2 -unsymmetrical diamide tartrate **152a** in a one-step synthesis.

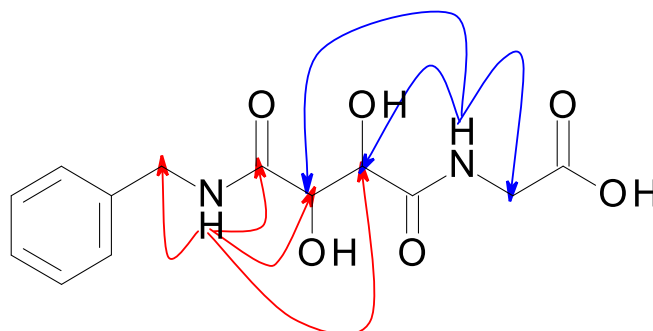


Figure 67: the illustration of the HMBC explanation of the compound 152a.

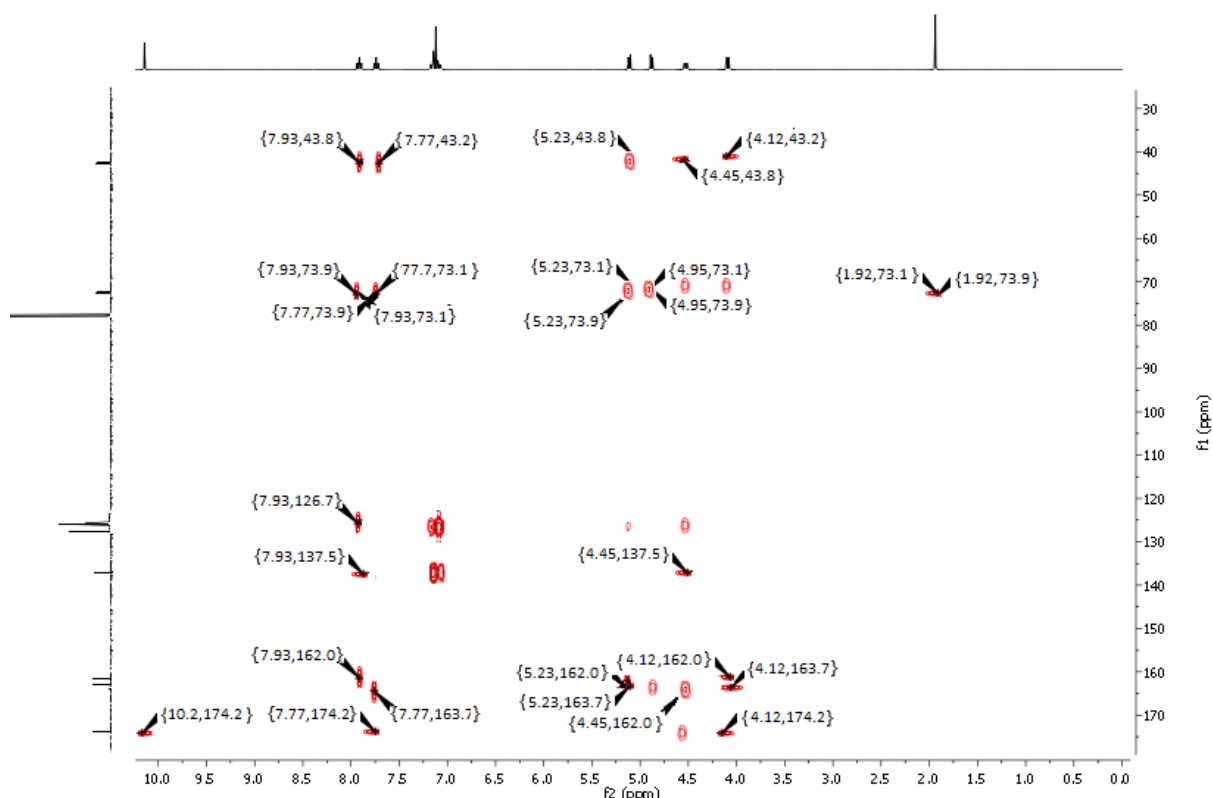


Figure 568: The HMBC NMR spectrum of the compound 152a in CDCl₃.

The DEPT 135 NMR (Figure 69), gave evidence of the formation of the unsymmetrical diamide tartrate **152a**. The spectrum revealed the presence of two negative signals ascribed to C-7 and C-8.

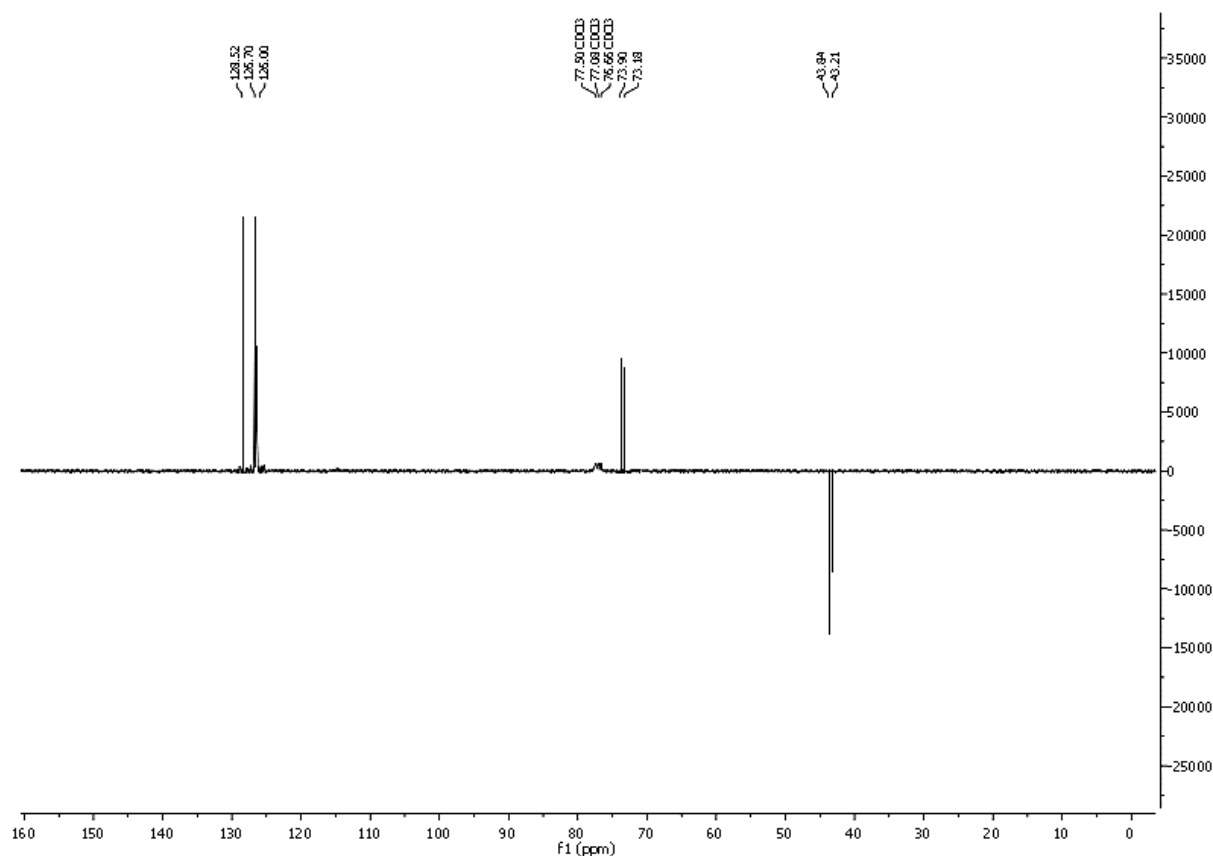


Figure 69: The DEPT 135 NMR spectrum of **152a** in CDCl_3 .

2.2.3. *In vitro* biological evaluation of tartrate amides

The tartrate amides **111-151(ab)** were examined for inhibition of HIV-1 protease (PR) (enzyme inhibition studies) as per the method described in the Appendix. A representative result is shown in Figure 70 (whereas all the remaining are presented in Appendix) and for cell toxicity (as determined by the viability of HeLa cells). Combined results are shown in Table 13.

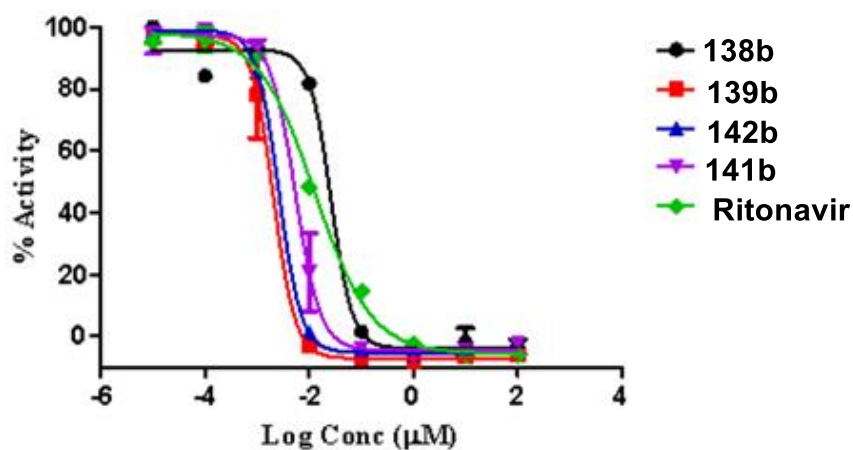


Figure 70: Plots of % activity of HIV-1 PR against concentration of ritonavir and ligands 138b, 139b, 141b and 142b.

Table 13: The % HeLa cell viability $\pm$ SD obtained for compounds 111-151(ab) at a concentration of 20 μ M

Compound	Substituents		Percentage viability	Percentae Inhibition	IC ₅₀ Values at 20 μ M
111a	Benzyl amine	Benzyl amine	113	98	0.08753
112a	DOPA	DOPA	106	98	0.04461
113a	Tyrosine-OMe	Tyrosine-OMe	108	96	0.02437
114b	Sulfaguanidine	Sulfaguanidine	96	97	0.01494
115b	Sulfadiazine	Sulfadiazine	123	96	0.04971
116b	Tryptophan	Tryptophan	98	98	0.0186
117b	Tyrosine	Tyrosine	127	93	0.0488
118a	Sulfamethoxazole	Sulfamethoxazole	106	104	0.001007
119b	Sulfamethizole	Sulfamethizole	117	93	0.001753
120b	Glycine-OEt	Glycine-OEt	110	1001	0.1618
121a	Proline-OMe	Proline-OMe	104	102	0.1217
122a	Iso-leucine	Iso-leucine	109	98	0.3704
123a	Iso-Leucine	DOPA	105	98	0.3060
124a	Tryptophan	DOPA	123	107	0.1035
125a	Tryptophan	Proline-OMe	102	99	1.019
126b	Tryptophan	Tyrosine	119	100	1.709

127a	Tryptophan	Iso-Leucine	97	96	0.7658
128a	Tryptophan	Sulfadiazine	114	85	0.3658
129b	Tyrosine-OMe	DOPA	92	95	0.7386
130b	Tyrosine-OMe	Sulfadiazine	104	95	0.9277
131b	Tryptophan	Sulfamethizole	116	98	0.9767
132a	Tryptophan	Sulfamethoxazole	111	97	0.2125
133b	Sulfamethoxazole	DOPA	97	98	3.159
134a	Sulfaguanidine	Sulfadiazine	119	98	0.7305
135b	Sulfaguanidine	Iso-Leucine	127	99	49.91
136a	Leucine	DOPA	130	104	0.8156
137b	Tryptophan	DOPA	98	106	0.9736
138a	Tryptophan	Proline-OMe	109	107	2.145
139a	Tryptophan	Tyrosine-OMe	120	102	0.3234
140a	Tryptophan	Leucine	121	106	2.914
141b	Tryptophan	Sulfadiazine	115	102	0.6885
142a	Tyrosine-OMe	DOPA	112	102	1.448
143b	Tyrosine-OMe	Sulfadiazine	87	94	0.02341
144a	Tryptophan	Sulfamethizole	112	94	0.02959
145b	Tryptophan	Sulfamethoxazole	103	94	0.008644
146b	Sulfamethoxazole	DOPA	97	94	0.02628
147a	Sulfamethoxazole	Sulfadiazine	92	94	0,002064
148a	Sulfamethoxazole	Leucine	98	94	0.01104
149b	DOPA	Sulfadiazine	107	94	0.005409
150b	DOPA	Sulfaguanidine	91	94	0.002611
151b	Sulfaguanidine	Tyrosine OMe	92	94	0.01612

All the synthetic ligands exhibited moderate to very high inhibition of HIV-1 PR in a range of 75-99% at a concentration of 20 μ M, when compared to Ritonavir used as standard and none of them showed significant toxicity against HeLa cells. Based on

the IC₅₀ values observed, it appeared that the variation of the amino/amine moieties appear to have a major effect on the activity of our ligands.

All of the compounds reflected greater inhibition of HIV-1 PR at 20 μ M compared to ritonavir as shown in Figure 70. The osazone-containing scaffolds exhibited the most exiting IC₅₀ values when compared to the dihydroxy compounds, making tartaric acid osazone a promising building block in the search and synthesis of highly active adducts against the HIV-1 protease. When looking at Table 13, the last nine compounds revealed to be more active. The ligands **138b**, **139b**, **141b** and **142b**, exhibited a very high activity (90-99% inhibition activity). IC₅₀ values of these selected representative compounds **138b**, **139b**, **141b** and **142b** are presented in Figure 63. However, **118a** and **119b** showed nanomolar inhibition whereas **120b**, **121b** and **122b** were ~ 100 xs worse. This could be due to the small amino acids attached to them, resultin in a poor fitting and binding affinity to the active site of the HIV-1 PR enzyme.

It was assumed that the presence of the two extra benzene groups as well as the two nitrogen atoms from tartaric acid osazone starting material were very critical to fit the S1/S1' pockects of the HIV-1 PR enzyme and that they have contributed to the enhancement of the activity of our ligands. This could suggest that tartaric acid osazone would be also a good starting point in the identification of potential lead compounds as HIV-1 PR inhibitors. Furthermore, none of the ligands showed significant toxicity against HeLa cells at 20 μ M as shown in Table 13.

A structural comparison between the symmetrically and unsymmetrically substituted ligands, has shown that the selected combination "amine-amino acid" at both end-sides of the unsymmetrically substituted ligands revealed to increase the binding of these ligands to the active site of the HIV-1 PR enzyme than the "amino acid-amino acid" combination. This resulting in very high inhibition activity with IC₅₀ values in nanomolar range (for example the last nine compounds in Table 13 including selected representative shown in Figure 70).

It was observed that for the same combinations, substituents were more active on the unsymmetrical diamides than they were on the symmetrical ones. In addition,

when comparing osazone containing scaffolds with dihydroxyl-containing scaffolds (from tartaric acid/diethyl tartrate), it is not surprising that with the osazone revealed to be the best candidates with much heavier molecular weight, owing to its two extra phenyl function group, intended to fit the S1/S1' pocket of the HIV-1 PR enzyme.

Selection of some of the used amines specifically including sulfamethoxazole, sulfaguanidine, sulfamethizole and sulfadiazine and amino acids such as DOPA, tryptophan, tyrosine methyl ester etc., was based of their structural features. It was anticipated or intended that combination of these amines/amino acids to the readily available features of tartrate derivatives (including tartaric acid osazone) they would provide rigid ligands with increased binding activity and capable of fitting the exactly the active site of the HIV-1 PR enzyme.

Most of these selected amines have been known and/or used as antibacterials against several infections.²²⁷⁻²³⁰ Therefore, when looking at the last nine compounds in Table 12, it is not surprising that scaffolds containing these amines stand out as very active. This is due to their structural features that make them seem unusually effective.

Structurally these selected amines/amino acids' key futures includes the sulphonamides antibiotic moieties, heterocycles containing sulfur or oxygen as P1/P1' substituents, both electron-donating and electron-withdrawing substituents, the hydroxyl-moitie amongst key functional groups, which would provide an efficient readily binding network to the active site of the HIV-1 PR enzyme.

Chapter 3: Concluding remarks and future work

3.1. Concluding remarks

Here is presented a one-step, two and three-steps synthesis approaches to four series of fully characterized novel truncated, coumarin and both symmetrical and unsymmetrical peptidomimetics as potential HIV-1 protease inhibitors accessed from shortest and most cost effective synthetic routes.

Elaboration of MBH methodology in this research project afforded a range of β -hydroxypropionate ester conjugates as potential HIV-1 PR inhibitors. Two series comprising a total of forty four β -hydroxypropionate ester (aza-Michael) conjugates were synthesised. In the first series, *O*-benzylated salicylaldehydes, methyl acrylate (as the activated alkene) and DABCO (as catalyst) were used to access six MBH adducts in yields ranging from 26 to 61%.

These were reacted through a Michael addition reaction to afford the final twenty-four aza-Michael analogues in 50-95% yields.

In the second series, to circumvent the low-yields and very long reaction times encountered with the first series (for the MBH adducts), unprotected salicylaldehydes, *tert*-butyl acrylate (as the activated alkene), and aqueous TEA were used to afford MBH adducts (77-97% yields) in just 3-6 hours. The TEA method has permitted dramatic improvement of the MBH method in terms of both yields and reaction running time. These were also reacted through a Michael addition reaction to afford twenty aza-Michael analogs in 81-99% yields. Here, except where both reagents were solids where a few milliliters of a dried THF were used to dissolve them, the neat reaction mixtures were stirred at room temperature for about six hours.

Base-catalyzed cyclization of the second series of non-benzylated aza-Michael adducts afforded a third series of coumarin-3-hydroxy-2-methylenepropanoate esters in moderate yields (53-78%) in three-steps using different reaction conditions than previously described in the literature. This gave access to only a single scaffold: coumarin and not chromene. Only four out of forty-four aza-Michael analogues

showed reasonable IC₅₀ values whereas coumarin analogues did not. These three MBH derived series failed to provide any promising HVI-1 PR inhibitor candidates.

In an exploratory study, a structure-based design was used to design symmetrical and unsymmetrical tartrate amides in a simple and cost-effective one-step synthesis. Uncatalyzed direct amidation of diethyl tartrate/tartaric acid or tartaric acid osazone with selected amines/amino acids afforded a fourth series of forty symmetrical (twelve compounds) and unsymmetrical (twenty eight compounds) derivatives. The symmetrical analogs were accessed in 40-90% whereas the unsymmetrical analogs were obtained in only 20-57% in a one-step synthesis.

The synthesis of this series of compounds was very simple and rapid since products precipitated after the reaction was cooled. However, especially for the C₂-unsymmetrical analogues, low-yields issue was among the challenging issues encountered. Many by-products formed during the reaction, making purification difficult. Additionally, the isolation of sulfamethoxazole, sulfamethizole, sulfadiazine and sulfaguanidine-containing analogs was further complicated by the two free amine groups.

In the tartrates series, although all of them reflected greater inhibition of HIV-1 PR at 20 µM compared to ritonavir as shown in Figure 70, the osazone-containing scaffolds exhibited the most exiting IC₅₀ values when compared to the dihydroxy compounds. This suggested that tartaric acid osazone would be the best promising building block in the search and synthesis of highly active adducts against the HIV-1 protease. Based on the IC₅₀ values observed, it appeared that the variation of the amino/amine moieties significantly improved the activity of our ligands. A few compounds such as **118a** and **119b** showed nanomolar inhibition whereas **120b**, **121b** and **122b** were ~ 100 xs worse. This could be due to the small amino acids attached to them, resultin in a low molecular weight, poor fitting and therefore a poor binding affinity to the active site of the HIV-1 PR enzyme. It was observed that the presence of the two extra benzene groups as well as the two nitrogen atoms from tartaric acid osazone starting material were very critical to fit the S1/S1' pockects of the HIV-1 PR enzyme and that they have contributed to the enhancement of the activity of our ligands. This could suggest that tartaric acid osazone would be also a

good starting point in the identification of potential lead compounds as HIV-1 PR inhibitors.

A structural comparison between the symmetrically and unsymmetrically substituted ligands, has shown that the selected combination “amine-amino acid” at both ends of the unsymmetrically substituted ligands revealed to increase the binding of these ligands to the active site of the HIV-1 PR enzyme than the “amino acid-amino acid” combination. This resulting in very high inhibition activity with IC₅₀ values in nanomolar range. It was observed that for the same combinations, substituents were more active on the unsymmetrical diamides than they were on the symmetrical ones. In addition, when comparing osazone containing scaffolds with dihydroxyl-containing scaffolds (from tartaric acid/diethyl tartrate), it is not surprising that with the osazone revealed to be the best candidates with much heavier molecular weight, owing to its two extra phenyl function group, intended to fit the S1/S1' pocket of the HIV-1 PR enzyme.

Selection of some of the used amines specifically including sulfamethoxazole, sulfaguanidine, sulfamethizole and sulfadiazine and amino acids such as DOPA, tryptophan, tyrosine methyl ester etc., was based of their structural features. It was anticipated or intended that combination of these amines/amino acids to the readily available features of tartrate derivatives (including tartaric acid osazone) they would provide rigid ligands with increased binding activity and capable of fitting the exactly the active site of the HIV-1 PR enzyme.

Most of these selected amines have been known and/or used as antibacterials against several infections. Structurally these selected amines/amino acids' key features includes the sulphonamides antibiotic moieties, heterocycles containing sulfur or oxygen as P1/P1' substituents, both electron-donating and electron-withdrawing substituents, the hydroxyl-moitie amongst key functional groups, which would provide an efficient readily binding network to the active site of the HIV-1 PR enzyme. Therefore, it is not surprising that scaffolds containing these amines stand out as very active. This is due to their structural features that make them seem unusually effective.

Over one hundred and four new compounds have been synthesized and fully characterised using spectroscopic techniques, including the FT-IR, 1- and 2D-NMR, elemental analysis (combustion) and HR-MS.

These conjugates, were tested against the HIV-1 protease enzyme and several of them exhibited inhibition levels and IC₅₀ values comparable to drugs in clinical use, permitting therefore their identification as lead compounds for the development of novel action inhibitors. Although both symmetrical and unsymmetrical analogues were very active against HIV-1 protease enzyme, tartaric acid osazone-containing analogues were the most active, when compared to those accessed from diethyl tartrate.

3.2 Future work

This project represents a new direction of research and has uncovered some interesting questions.

- ❖ A thorough kinetic study of both symmetrical and unsymmetrical diamide tartrates required in order to establish the mechanism by which the unsymmetrical substitution occurs, and thus facilitate the optimization of reaction conditions;
- ❖ A computational study of the docking of tartrate amides into the active site of the HIV-1 protease enzyme will facilitate an understanding of the structure activity relationships;
- ❖ Validation of the computational studies by experimental exploration of the conformational effects through STD NMR of symmetrical and unsymmetrical diamide tartrates.
- ❖ A further study and/or analysis of all tartramides need to be conducted in continuation of this current project, using a polarimeter to determine and separate the Dextro and the Levo-moieties if possible and determine not only their optical rotation (or activity) but their rate of racemization.

Chapter 4: Experimental section

4.1. General

Low-resolution mass spectra were obtained on a Finnegan Mat GCQ spectrometer. High resolution mass spectra were recorded on Bruker Daltonics Compact QTOF mass spectrometer at Rhodes University. NMR spectra were recorded on Bruker 300 MHz, 400 MHz or Bruker 600 MHz spectrometers and were referenced using solvent signals (δ_{H} 7.25 ppm for CDCl_3 , 2.50 ppm and 3.40 ppm for $\text{DMSO}-d_6$; δ_{C} : 77.0 ppm for CDCl_3 and 39.4 ppm for $\text{DMSO}-d_6$).

IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum 2000 spectrometer using KBr discs, nujol mulls or thin films. Elemental Analysis (CHN) was done on a Vario MICRO VI.6.2 elemental analysen systeme GmbH. Melting points were determined using a Reichert hot-stage apparatus and are uncorrected.

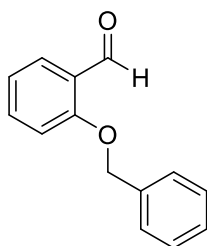
The reagents used in this research were supplied by Aldrich® and were used without further purification. Starting materials used in this thesis were mostly racemates. Thin layer chromatography (TLC) was run on pre-coated Merck silica gel F254 plates visualized under UV light (254/365 nm). Well-known and previously synthesized compounds have been appropriately referenced.

The preparation and characterisation of new compounds, including resolved diastereomers and/or racemates of the previously reported compounds in some cases, are described and well referenced. NMR data for all synthesized compounds and bioassay procedures are described. All the multiplets are reported as the mean of the highest and the lowest values and not as a range.

4.2. General procedure for the synthesis of the ortho-benzylated salicylaldehydes 96a-e.^{165-168, 201-203}

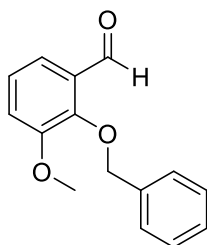
To a 250 ml round bottomed flask equipped with a stirrer bar, was added a mixture of a substituted or unsubstituted 2-hydroxybenzaldehydes (1 eq.), and 10% NaOH aqueous, and benzyl bromide (1 eq.). The mixture was refluxed for 3 hrs. When the TLC showed no starting materials, the reaction mixture was cooled to room temperature (RT) and water was then added. The combined organic extracts were washed with brine and the product was extracted 3 times with chloroform and dried over anhydrous MgSO_4 to afford after recrystallization from hexane the pure *O*-benzylated ethers:

4.2.1. 2-(Benzyloxy)-benzaldehyde **96a**.



The general procedure as described above in section 3.2 was followed, using salicylaldehyde (0.10 ml, 0.81 mmol), benzyl bromide (0.10 ml, 0.81 mmol), and 10% NaOH (15.0 ml) to afford 2-(Benzyloxy)-benzaldehyde **96a** as a brown viscous liquid²⁰⁹ (16 mg, 95%). $U_{\text{max}} / \text{cm}^{-1}$: 1672 (C=O), δ_{H} (400 MHz, CDCl_3): 5.22 (s, 2H, OCH_2Ph), 7.22 (m, 8H, *ArH*), 7.52 (td, $J = 7.5, 1.5$ Hz, 1H, *ArH*), 7.78 (dd, $J = 7.3, 1.4$ Hz, 1H, *ArH*), 10.21 (s, 1H, COH); δ_{C} (100 MHz, CDCl_3) 70.8 (OCH_2Ph), 114.7, 126.0, 126.7, 127.3, 128.0, 128.5, 128.8, 135.4 and 135.5 (*Ar-C*), 159.4 (C-OBn) and 188.4 (C=O).

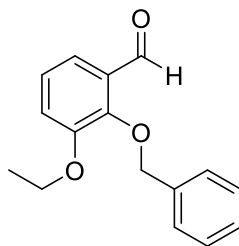
4.2.2. Synthesis of 2-(Benzyloxy)-3-methoxybenzaldehyde **96b**.



The general procedure as described above in section 3.2 was followed, using 3-methoxysalicylaldehyde (10 mg, 0.66 mmol), benzyl bromide (0.10 ml, 0.66 mmol) and 10 % NaOH (5.00 ml) to afford 2-benzyloxy-3-methoxybenzaldehyde **96b** as yellow viscous liquid (14 mg, 89%). $U_{\text{max}} / \text{cm}^{-1}$: 1688 (C=O), δ_{H} (400 MHz; CDCl_3)

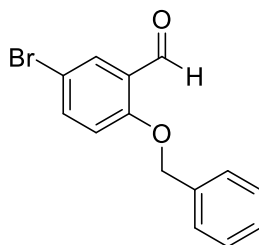
3.84 (s, 3H, OCH₃), 5.27 (s, 2H, OCH₂Ph), 7.17 (m, 2H, ArH), 7.23 (d, *J* = 1.7 Hz, 1H, ArH), 7.26 (m, 3H, ArH), 7.31 (dd, *J* = 7.8, 1.8 Hz, 1H, ArH), 7.59 (dd, *J* = 7.3, 1.7 Hz, 1H, ArH), and 10.25 (s, 1H, COH), δ_c (100 MHz, CDCl₃) 54.8 (OCH₃), 70.8 (OCH₂Ph), 114.7, 126.0, 126.7, 127.3, 128.0, 128.5, 128.8, 135.4, 135.5, 159.4 and 188.4 (C=O).

4.2.3. Synthesis of 2-(Benzyloxy)-3-ethoxybenzaldehyde **96c**.



The general procedure as described above in section 3.2 was followed, using 3-ethoxysalicylaldehyde (3.00 g, 18.1 mmol), benzyl bromide (2.14 ml, 18.0 mmol), and 10% NaOH (15.0 ml) to afford 2-benzyloxy-3-ethoxybenzaldehyde **96c** as brown viscous liquid (430 mg, 93.0 %). $U_{\max}$ / cm⁻¹: 1683 (C=O), δ_H (400 MHz, CDCl₃) 1.42 (t, *J* = 6.0 Hz, 3H, CH₃CH₂), 4.07 (q, *J* = 6.0 Hz, 2H, CH₂CH₃), 5.26 (s, 2H, OCH₂Ph), 7.20 (m, 2H, ArH), 7.25 (m, 1H, ArH), 7.28 (m, 3H), 7.32 (dd, *J* = 7.9, 1.2 Hz, 1H, ArH), 7.59 (d, *J* = 9.0 Hz, 1H), 10.26 (s, 1H, COH); δ_c (100 MHz; CDCl₃) 14.1 (CH₃), 64.7 (OCH₂CH₃), 70.8 (OCH₂Ph), 113.8, 115.1, 126.3, 127.3, 128.2, 128.5, 128.6, 128.8, 131.0, 135.5, 138.2 (ArC(OEt)), 159.9 (ArC-OBn), 188.3 (C=O).

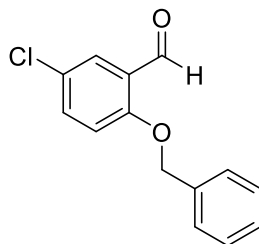
4.2.4. Synthesis of 2-(Benzyloxy)-5-bromobenzaldehyde **96d**.



The general procedure as described above in section 3.2 was followed using 5-bromosalicylaldehyde (6.40 g, 32.0 mmol), benzyl bromide (3.80 ml, 32.0 mmol), and 10% NaOH (5.00 ml) to afford 2-benzyloxy-5-bromobenzaldehyde **96d** as a light-yellow solid (790 mg, 85.0 %). mp. 71-75 °C, (reported 73-74 °C by Musa, M.A. *et*

*al.*¹⁹¹⁻¹⁹²); $\nu_{\max}$ / cm^{-1} : 1683 (C=O), δ_{H} (300 MHz; CDCl_3): 5.20 (s, 2H, OCH_2Ph), 6.96 (t, $J = 8.0$ Hz, 1H, ArH), 7.35 (m, 5H, ArH), 7.62 (d, $J = 8.9$ Hz, 1H, ArH), 7.96 (s, 1H, ArH), and 10.48 (s, 1H, COH); δ_{C} (75 MHz, CDCl_3) 70.8 (OCH_2Ph), 113.8, 115.1, 126.3, 127.3, 128.5, 128.8, 131.0, 135.5 and 138.2 (Ar-C), 159.8, [ArC(OBn)] and 188.3 (C=O).

4.2.5. Synthesis of 2-(Benzyloxy)-5-chlorobenzaldehyde 96e.

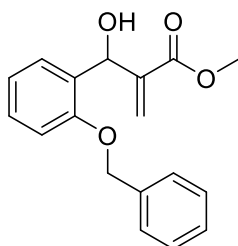


The general procedure as described above in section 3.2 was followed using 5-chlorosalicylaldehyde (5.00 g, 32.0 mmol), benzyl bromide (3.80 ml, 34 mmol) and 10% NaOH (15.0 ml) to afford 2-benzyloxy-5-chlorobenzaldehyde **96e** as a yellow solid (7.34, 93.0 %), m.p.: 70-74°C (reported 70-72 °C by Musa, M.A. et al.¹¹⁹⁻¹²¹), $\nu_{\max}$ / cm^{-1} : 1677 (C=O), δ_{H} (400 MHz, CDCl_3) 5.21 (s, 2H, OCH_2Ph), 7.03 (d, $J = 8.9$ Hz, 1H, ArH), 7.35 (m, 6H), 7.82 (d, $J = 2.8$ Hz, 1H), 10.50 (s, 1H, COH); δ_{C} (100 MHz, CDCl_3) 70.0 (OCH_2Ph), 114.7, 126.0, 126.7, 127.3, 128.0, 128.8, 128.8, 135.4 and 135.5, 159.4 and 188.7 (C=O).

4.3. General procedure for the synthesis of the MBH adducts (97a-f) from the ortho-benzylated salicylaldehydes with methyl acrylate.^{165-168,201-203}

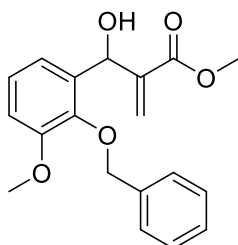
A mixture of substituted or unsubstituted salicylaldehyde benzyl ethers (1 eq.) methyl acrylate and/or tert-butyl acrylate (2 eq.) and DABCO (2 eq.), in CHCl_3 was stoppered in a round-bottomed flask and stirred at room temperature for specific number of days depending on the substrate used (10-31 days). The mixture washed with brine followed by water and the organic layer extracted with chloroform, dried on MgSO_4 , and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (on silica gel; eluting from hexane: EtOAc (7:3) to afford desired MBH adducts as follows:

4.3.1. Methyl 2-((2-(benzyloxy)phenyl)(hydroxy)methylenepropanoate 97a.



The general procedure described above in the section 4.3 was followed, using 2-(Benzyloxy)-benzaldehyde **96a** (4.0g, 18.80 mmol), methyl acrylate (3.60 ml, 37.60 mmol) and DABCO (4.21 g, 37.6 mmol) to afford methyl 2-((2-(benzyloxy)phenyl)(hydroxy)methyl) acrylate **97a** as a viscous liquid (145 mg, 26.0 %). $U_{\max}$ / cm^{-1} : 1714 (C=O), 3470 (OH); δ_{H} (300 MHz, CDCl_3) 3.89 (s, 3H, OCH_3), 5.17 (s, 2H, OCH_2Ph), 5.42 (s, 1H, $=\text{CHa}$), 5.76 (s, 1H, $\text{CH}(\text{OH})$), 6.02 (s, 1H, $=\text{CHb}$), 6.99 (t, $J = 7.2$ Hz, 1H, ArH), 7.02 (d, $J = 1.3$ Hz, 1H, ArH), 7.28 (m, 6H, ArH), 7.42 (dd, $J = 6.9, 0.8$ Hz, 1H, ArH); (75 MHz, CDCl_3) 51.0 (OCH_3), 68.1 (CHOH), 70.8 (OCH_2Ph), 113.8 ($\text{C}=\text{CH}_2$), 115.1, 126.3, 127.3, 128.5, 128.8, 131.0, 135.5, 138.3 ($\text{C}=\text{CH}_2$), 159.9 (Ar-C) and 170.0 (C=O).

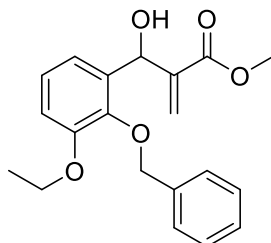
4.3.2. Methyl 2-((2-(benzyloxy)-3-methoxyphenyl)(hydroxy) methylene propanoate **97b**.



The general procedure described above in the section 4.3 was followed, using 2-(Benzyloxy)-3-methoxybenzaldehyde **96b** (4.0g, 16.5 mmol), methyl acrylate (3.20 ml, 33.0 mmol) and DABCO (3.70g, 33.0 mmol, 2eq.) to afford methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-methylenepropanoate **97b** as a brown viscous liquid which solidified on standing (130 mg, 23%). $U_{\max}$ / cm^{-1} : 1719 (C=O), 3455 (OH); δ_{H} (300 MHz, CDCl_3) 3.81 (s, 3H, PhOCH_3), 3.84 (s, 3H, CO_2CH_3), 4.26 (s, 1H, OH), 5.23 (s, 2H, CH_2Ph), 5.42 (s, 1H, $=\text{CHa}$), 5.73 (s, 1H, $\text{CH}(\text{OH})$), 6.02 (s, 1H, $=\text{CHb}$), 6.98 (m, 3H, ArH), 7.24 (m, 6H, ArH); δ_{C} (75 MHz, CDCl_3) 51.8 (OCH_3), 55.8 (OCH_3), 67.5 (CHOH), 70.8 (OCH_2Ph), 111.9, 114.7, 119.3, 124.1, 128.0,

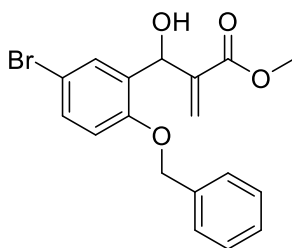
128.5, 128.8, 135.4, 135.5, 141.3, 152.3, 152.7 (C=CH₂), 159.4, 169.9, and 188.4 (C=O).

4.3.3. Methyl 2-((2-(benzyloxy)-3-ethoxyphenyl)(hydroxy)methylenepropanoate 97c.



The general procedure described above in the section 4.3 was followed, using 2-(Benzyloxy)-3-ethoxybenzaldehyde **96c** (4.00 g, 15.6 mmol), methyl acrylate (3.10 ml, 31.20 mmol) and DABCO (350 mg, 31.2 mmol) to afford methyl 2-((2-(benzyloxy)-3-ethoxyphenyl)(hydroxy) methyl) acrylate **97c** as a brown viscous liquid (1.06 g, 20.0%). $U_{\max} / \text{cm}^{-1}$: 1720 (C=O), 3450 (OH); δ_{H} (300 MHz, CDCl₃) 1.41 (t, J = 5.9 Hz, 1H, CH₃CH₂), 3.89 (s, 1H, OCH₃), 4.06 (q, J = 5.9 Hz, 2H, CH₂CH₃), 5.23 (s, 2H, OCH₂Ph), 5.42 (s, 1H, =CHb), 5.76 (s, 1H, CHOH), 6.02 (dd, J = 1.9, 0.8 Hz, 1H, =CH), 7.00 (dd, J = 7.5, 1.6 Hz, 1H, ArH), 7.10 (t, J = 7.4 Hz, 1H, ArH), 7.21 (dd, J = 7.5, 1.6 Hz, 1H, ArH), 7.26 (m, 1H, ArH), 7.33 (m, 1H, ArH); δ_{H} (75 MHz; CDCl₃) 14.2 (OCH₂CH₃), 51.8 (OCH₃), 64.2 (OCH₂CH₃), 67.8 (CHOH), 70.8 (OCH₂Ph), 113.8, (C=CH₂), 115.1, 119.3, 127.3, 128.5, 128.8, 135.5, 138.3, 141.6, 145.9 and 151.5 (C=CH₂) Ar-C) and 169.4 (C=O).

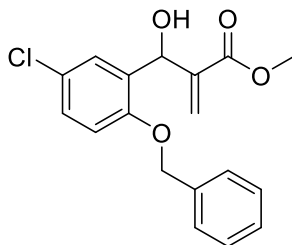
4.3.4. Methyl 2-((2-(benzyloxy)-5-bromophenyl)(hydroxy) methylenepropanoate 97d.



The general procedure as described above in the section 4.3 was followed, using 2-(Benzyloxy)-5-bromobenzaldehyde **96d** (2.90 g, 10 mmol), methyl acrylate (5.40 ml, 60 mmol), and DABCO (0.30 g, 2.61 mmol) to afford the methyl 2-((2-(benzyloxy)-5-bromophenyl)(hydroxy)methyl)acrylate **97d** (356 mg, 94 %). m.p.: 113-115°C, $U_{\max} / \text{cm}^{-1}$: 1712 (C=O), 3215 (OH), δ_{H} (400 MHz, CDCl₃) 3.67 (s, 3H, OCH₃), 4.98 (s, 2H,

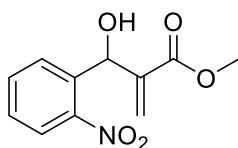
OCH₂Ph), 5.58 (s, 1H, =CHa), 5.81 (d, *J* = 5.1 Hz, 1H, CH(OH)), 6.22 (s, 1H, =CHb), 6.72 (d, *J* = 8.7 Hz, 1H, ArH), 7.18 (s, 1H, ArH), 7.29 (m, 6H, ArH, ArH), 7.47 (d, *J* = 2.4 Hz, 1H, ArH); δ_c (100 MHz, CDCl₃) 52.7 (OCH₃), 63.9 (CHOH), 71.8 (OCH₂Ph), 112.8, 114.4, 126.4, 127.9, 132.4, 140.7, 155.0 (Ar-C), 167.6 (C=O).

4.3. 5. Methyl 2-((2-(benzyloxy)-5-chlorophenyl)(hydroxy) methylenepropanoate 97e.



The general procedure described above in the section 4.3 was followed, using 2-(benzyloxy)-5-chlorobenzaldehyde **96e** (3.00 g, 12.2 mmol), methyl acrylate (2.10 ml, 24.4 mmol) and DABCO (2.73g, 24.4 mmol) to afford Methyl 2-((2-(benzyloxy)-5-chlorophenyl)(hydroxy) methyl) acrylate **97e** as a off-white solid (223 mg, 55.0 %). U_{max} / cm⁻¹: 1712 (C=O), 3389 (OH); δ_H (300 MHz, CDCl₃) 3.88 (s, 3H, OCH₃), 5.21 (s, 2H, OCH₂Ph), 5.42 (s, 1H, =CHa), 5.76 (s, 1H, CH(OH)), 6.02 (s, 1H, =CHb), 7.01 (d, *J* = 7.5 Hz, 1H, ArH), 7.26 (m, 5H, ArH), 7.33 (d, *J* = 4.8 Hz, 1H, ArH), 7.52 (d, *J* = 1.3 Hz, 1H, ArH); δ_c (75 MHz, CDCl₃) 51.9 (OCH₃), 67.8 (CHOH), 70.8 (OCH₂Ph), 113.8, 115.13, 126.3, 127.1, 127.3, 128.5, 128.7, 128.8, 131.0, 135.5, 138.3, 140.9 (ArC), 154.2 (C=CH₂), and 166.7 (C=O).

4.3. 6. Methyl 2-(hydroxy(2-nitrophenyl)methylenepropanoate 97f



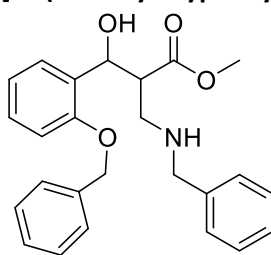
The general procedure described above in the the section 4.3 was followed using 2-nitrobenzaldehyde (3.00 g, 20.0mmol), methyl acrylate (3.80 ml, 40.0mmol) and DABCO (4.50g, 40.0 mmol). This afforded methyl 2-(hydroxyl (2-nitrophenyl) methyl) acrylate **97f** as a brown viscous liquid (335 Mg, 60.0 %). U_{max} / cm⁻¹: 1770 (C=O) 3480 (OH). δ_c (300 MHz, CDCl₃) 3.88 (s, 3H, OCH₃), 5.42 (s, 1H, =CHa), 5.76 (s, 1H, CH(OH)), 6.02 (s, 1H, =CHb), 7.47 (m, 1H, ArH), 7.68 (dd, *J* = 10.4, 2.5 Hz, 2H, ArH), 8.11 (dd, *J* = 8.2, 7.6 Hz, 1H, ArH); δ_c (75 MHz, CDCl₃) 52.7 (OCH₃), 65.5,

(CH(OH), 125.2, (C=CH) 126.0, 128.1, 128.5, 135.1, 136.7, 141.1, 147.6 (=CH), and 166.9 (C=O).

4.4. General procedure for the synthesis of the aza-Michael adducts (98-101a-f).²⁰¹⁻²⁰²

A mixture of the MBH adduct (**97a-f**) (1.0 mmol), the amino nucleophile (benzylamine or, as their hydrochloride salts, methyl glycine, ethyl glycine or (S)-proline; 3.0 mmol), sodium acetate (0.32 g, 3.0 mmol) and TBAB (0.64 g, 2.0 mmol) in dry MeOH (5.0 mL) was stirred in a stoppered flask for 10 d. The resulting mixture was concentrated *in vacuo* and purified by preparative layer chromatography (PLC) [on silica; elution with EtOAc-hexane (1:3)] to isolate single the major diastereomer product(s) as follows:

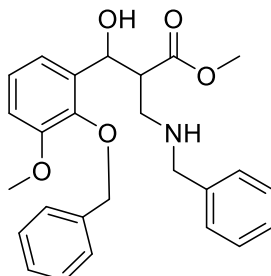
4.4.1. Methyl 2-[(benzylamino)methyl]-3-(2-benzyloxyphenyl)-3-hydroxy-propanoate **98a**.



The general procedure as described above in the section 4.4 was followed, using methyl 2-((2-(benzyloxy) phenyl)(hydroxyl)methyl)acrylate **97a** (0.50g, 1.67mmol), benzyl amine (0.18ml, 1.69mmol, 1.01eq), TBAB (0.81g, 2.50mmol, 1.5eq) and sodium acetate (0.20g, 2.50mmol, 1.5eq) to afford methyl 2-[(benzylamino)methyl]-3-(2-benzyloxyphenyl)-3-hydroxy-propanoate **98a** as pale-yellow solid (690 mg, 71%); mp. 91-94 °C; $U_{\max}$ / cm^{-1} : 1769 (C=O), 3254 (OH), 1279 (N-C); $\text{C}_{25}\text{H}_{27}\text{NO}_4$ CHN: calculated: C(74.05%) H(6.71%) N(3.45%), found C(74.16%) H(6.75%) N(3.41%); (400 MHz, CDCl_3) δ_{H} : 2.02 (s, 1H, N-H), 2.81 (dd, J = 12.3, 7.4 Hz, 1H, CHCH₂N), 3.06 (dd, J = 12.5, 7.5 Hz, 1H, CHCH₂N), 3.39 (td, J = 7.5, 1.7 Hz, 1H, CHCH(CH₂)), 3.69 (s, 3H, OCH₃), 3.82 (s, 2H, NCH₂Ph), 5.16 (s, 2H, OCH₂Ph), 5.47 (d, J = 1.7 Hz, 1H, CH(OH)), 6.91 (m, 2H, ArH), 7.00 (m, 1H, ArH), 7.28 (t, J = 3.2 Hz, 1H, ArH), 7.29 (t, J = 1.8 Hz, 1H, ArH), 7.32 (m, 5H, ArH), 7.30 (s, 1H, ArH), 7.40 (t, J = 7.4 Hz, 2H, ArH), 7.48 (dd, J = 7.5, 1.3 Hz, 2H, ArH) and 7.48 (dd, J = 7.5, 1.3 Hz, 2H, ArH); δ_{C} (100 MHz; CDCl_3) 46.2 (CH₂), 49.1 (CH), 50.6 (OCH₃), 53.1, 66.6, (CHOH) 70.8

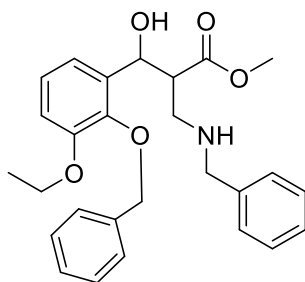
(OCH₂Ph), 113.8, 115.1, 126.3, 127.3, 128.5, 128.8, 131.0, 135.5, 138.3, 159.9, and 174.2 (C=O).

4.4.2. Methyl 2-[(benzylamino)methyl]-3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxypropanoate **98b**.



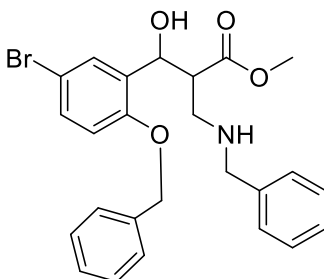
The general procedure as described above in the section 4.4 was followed, using Methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-methylenepropanoate **97b** (1.00 ml g, 3.60 mmol), benzyl amine (0.50 ml, 4.32 mmol), TBAB (1.74 g, 5.40 mmol) and sodium acetate (0.44 g, 5.40 mmol) to afford methyl 2-((benzyl amino) methyl)-3-(2-(benzyloxy)-3-methoxyphenyl)-3-hydroxypropanoate **98b** as a brown viscous oil (780 mg, 50%). $U_{\max}$ / cm⁻¹: 1682 (C=O), 3331 (OH); 1274 (N-C); C₂₆H₂₉NO₅ CHN: calculated: C(71.70%) H(6.71%) N(3.22%), found C(71.68%) H(6.75%) N(3.26%); (400 MHz, CDCl₃) δ_H : 2.02 (s, 1H, NH), 2.81 (dd, J = 12.3, 8.0 Hz, 1H, CH(CH_b)), 3.06 (dd, J = 12.3, 8.0 Hz, 1H, CH(CH_a)), 3.66 (td, J = 8.0, 2.5 Hz, 1H, CHCH(CH₂)), 3.82 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 5.16 (s, 2H, OCH₂Ph), 5.67 (d, J = 2.3 Hz, 1H, CH(OH)), 6.92 (d, J = 2.1 Hz, 1H, Ar-*H*), 6.94 (d, J = 7.5 Hz, 1H, Ar-*H*), 7.02 (dd, J = 6.8, 2.1 Hz, 1H, Ar-*H*), 7.29 (m, 1H, Ar-*H*), 7.30 (s, 1H, Ar-*H*), 7.32 (s, 1H, Ar-*H*), 7.33 (d, J = 1.6 Hz, 1H, Ar-*H*), 7.34 (d, J = 1.6 Hz, 1H, Ar-*H*), 7.36 (d, J = 1.7 Hz, 1H, Ar-*H*), 7.40 (t, J = 7.4 Hz, 2H, Ar-*H*), 7.47 (d, J = 1.3 Hz, 1H, Ar-*H*), 7.49 (d, J = 1.2 Hz, 1H, Ar-*H*), δ_C (100 MHz; CDCl₃) 46.7 (CH₂), 48.8 (CH), 52.0 (OCH₃), 53.7 (CH₂), 56.9 (OCH₃), 71.1 (CHOH), 74.6 (OCH₂Ph), 115.1, 120.8, 126.0, 126.7, 128.0, 128.5, 128.8, 135.4, 135.5, 139.5, 143.5, 146.6, 159.4, and 174.6 (C=O).

4.4.3. Methyl 2-[(benzylamino)methyl]-3-(2-benzyloxy-3-ethoxyphenyl)-3-hydroxypropanoate **98c**.



The general procedure as described above in the section 4.4 was followed, using Methyl 2-((2-(benzyloxy)-3-ethoxyphenyl)(hydroxy) methyl) acrylate **97c** (1.00 ml g, 3.40 mmol), benzyl amine (0.44 ml, 4.32 mmol), TBAB (1.64 g, 5.10 mmol) and sodium acetate (0.42 g, 5.10 mmol) to afford methyl 2-((benzyl amino) methyl)-3-(2-(benzyloxy)-3-ethoxyphenyl)-3-hydroxypropanoate **98c** as brown viscous oil (810 mg, 53.5 %). ν_{max} / cm^{-1} : 1690 (C=O), 3411 (OH); 1250 (N-C), $\text{C}_{27}\text{H}_{31}\text{NO}_5$ CHN calculated: C(72.14%) H(6.95%) N(3.12%), found: C(72.17%) H(6.23%) N(3.20%); δ_{H} (400 MHz, CDCl_3) 1.42 (t, $J = 5.9$ Hz, 3H, CH_3CH_2), 2.39 (s, 1H, NH), 2.83 (dd, $J = 12.5, 7.5$ Hz, 1H, CHCHaN), 3.04-3.07 (dd, $J = 12.5, 7.5$ Hz, 1H, CHCHbN), 2.73 (s, 1H, OH), 3.69 (s, 3H, OCH_3), 3.81 (td, $J = 7.4, 2.2$ Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 3.94 (s, 1H, NCHaPh), 4.02 (s, 1H, NCHbPh), 4.03-4.07 (q, 2H, $J = 5.8$ Hz, 2H, CH_3CH_2), 5.24 (s, 2H, OCH_2Ph), 5.33 (d, $J = 2.3$ Hz, 1H, $\text{CH}(\text{OH})$), 6.92 (d, $J = 7.5$ Hz, 1H, ArH), 7.00 (dd, $J = 6.8, 2.1$ Hz, 1H, ArH), 7.18 (t, $J = 3.2$ Hz, 1H, ArH), 7.27 (m, 10H, ArH); δ_{C} (100 MHz; CDCl_3) 14.9 (OCH_2CH_3), 46.1, 48.0, 51.8 (OCH_3), 54.1 (CH_2), 60.0 (OCH_2CH_3), 72.1 (CHOH), 74.5 (OCH_2Ph), 112.1, 123.8, 127.1, 127.9, 2x 128.2, 2x 128.4, 137.1, 137.6, 139.0, 143.8, and 151.6 (Ar-C) and 173.8 (C=O).

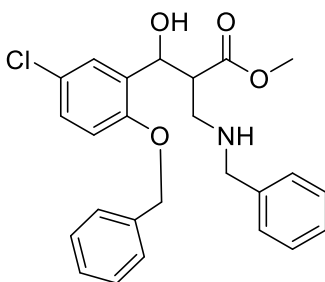
4.4.4. Methyl 2-[(benzylamino)methyl]-3-(2-benzyloxy-5-bromophenyl)-3-hydroxypropanoate **98d**.



The general procedure as described above in the the section 4.4 was followed, using Methyl 3-(2-benzyloxy-5-bromophenyl)-3-hydroxy-2-methylenepropanoate **97d** (0.50 g, 1.32 mmol), benzyl amine (0.17 ml, 1.60 mmol), TBAB (0.21 g, 0.66 mmol) and sodium acetate (1.16 g, 0.98 mmol) to afford methyl 2-((benzylamino) methyl)-3-(2-

(benzyloxy)-5-bromophenyl)-3-hydroxypropanoate **98d** as off-white viscous oil (310 mg, 49.0 %); $U_{\max} / \text{cm}^{-1}$: 1681 (C=O), 3454 (OH); 1306 (N-C); $\text{C}_{25}\text{H}_{26}\text{NO}_4\text{Br}$: CHN calculated: C(61.99%) H(5.41%) N(2.89%), found: C(61.95%) H(5.48%) N(2.21%); δ_{H} (400 MHz, CDCl_3) 1.90 (s, 1H, NH), 2.93 (ddd, $J = 125.6, 12.3, 7.3$ Hz, 2H, $\text{CHCH}_{2\text{abN}}$), 3.74 (s, 3H, OCH_3), 3.92 (s, 2H, NCH_2Ph), 4.20 (m, 1H, $\text{CHCH}_2(\text{CH}_2)$), 5.22 (s, 2H, OCH_2Ph), 5.33 (d, $J = 11.0$ Hz, 1H, $\text{CH}(\text{OH})$), 6.86 (d, $J = 7.5$ Hz, 1H, ArH), 7.20 (m, 11H, ArH), 7.39 (dd, $J = 7.5, 1.6$ Hz, 1H, ArH), 7.46 (d, $J = 1.4$ Hz, 1H, ArH); δ_{C} (100 MHz; CDCl_3) 46.7 (CH_2), 48.8 (CH), 52.0 (OCH_3), 53.7 (CH_2), 71.1(CHOH), 72.9 (OCH_2Ph), 115.1, 120.8, 126.0, 126.7, 128.05, 128.07, 128.5, 128.8, 135.4, 135.5, 139.5, 143.5, 146.6, 159.4 (Ar-C), and 174.6 (C=O).

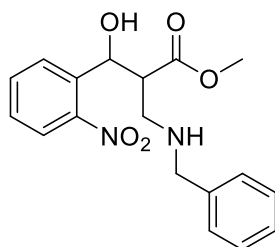
4.4.5. Methyl 2-[(benzylamino)methyl]-3-[2-(benzyloxy)-5-chlorophenyl]-3-hydroxypropanoate **98e**.



The general procedure as described above in the the section 4.4 was followed, using Methyl 2-((2-(benzyloxy)-5-chlorophenyl)(hydroxy) methyl) acrylate **97e** (0.30 g, 0.98 mmol), benzyl amine (0.13 ml, 1.21 mmol), TBAB (0.16 g, 0.49 mmol) and sodium acetate (4.12 g, 1.47 mmol) to afford Methyl 2-[(benzylamino)methyl]-3-[2-(benzyloxy)-5-chlorophenyl]-3-hydroxypropanoate **98e** as a colourless, viscous oil (220 mg, 53%); $\text{C}_{25}\text{H}_{26}\text{ClNO}_4$ CHN: calculated: C (68.25%), H (5.96%), N (3.18%); Found: C (68.27%), H (5.94%), N (3.19%); δ_{H} (400 MHz; CDCl_3) 1.17 (s, 1H, NH), 1.57 (s, 1H, OH), 2.81 (dd, 1H, $J = 12.3, 6.7$ Hz, CHCHN CH_a), 3.06 (dd, 1H, $J = 12.3, 6.9$ Hz, CHCHN CH_b), 3.12 (m, 1H, $\text{CHCH}(\text{CH}_2\text{ 2-CH})$), 3.69 (s, 3H, OCH_3), 3.82 (s, 2H, NCH_2Ph), 5.16 (s, 2H OCH_2Ph), 5.83 (d, 1H, $J = 9.8$ Hz, CHOH), 6.90 (d, 1H, $J = 7.5$ Hz, Ar-H), 7.28 (d, 1H, $J = 3.2$ Hz, Ar-H), 7.29 (t, 1H, $J = 1.8$ Hz, Ar-H), 7.30 s, (1H, Ar-H), 7.32 (s, 2H, Ar-H overlapping), 7.33 (t, 1H $J = 2.5$ Hz, Ar-H), 7.34 (d, 1H, $J = 1.6$ Hz, Ar-H), 7.35 (m, 1H, Ar-H), 7.36 (d, 1H, $J = 1.8$ Hz, Ar-H), 7.37 (d, 1H, $J = 1.4$ Hz, Ar-H), 7.40 (m, 3H, Ar-H overlapping), 7.46 (d, 1H, $J = 1.4$ Hz, Ar-H), 7.47 (d, 1H, $J = 1.3$ Hz, Ar-H) and 7.49 (d, 1H, $J = 1.2$ Hz, Ar-H); δ_{C} (100 MHz;

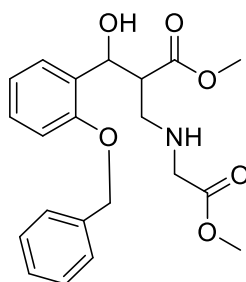
CDCl_3) 48.8, 52.0, 53.7, 71.1, 72.9, 115.2, 120.8, 126.1, 126.7, 128.04, 128.06, 128.5, 128.8, 135.4, 135.5, 139.5, 143.5, 146.6, 159.4 (Ar-C), and 174.6 (C=O).

4.4.6. Methyl 2-[(benzylamino)methyl]-3-[2-(benzyloxy)-6-nitrophenyl]-3-hydroxypropanoate **98f**.



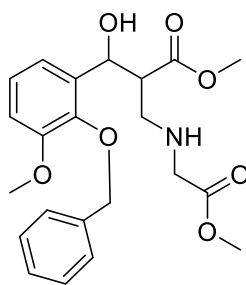
The general procedure as described above in the section 4.4 was followed, using Methyl 2-(hydroxy(2-nitrophenyl) methyl) acrylate **97f** (0.50 g, 1.80 mmol), benzylamine (0.23 ml, 2.16 mmol), TBAB (0.29 g, 0.90 mmol) and sodium acetate (0.01 g, 0.15 mmol) to afford Methyl 2-[(benzylamino)methyl]-3-[2-(benzyloxy)-6-nitrophenyl]-3-hydroxypropanoate **98f** as a yellow viscous oil (410 mg, 67.0%); $\text{C}_{25}\text{H}_{26}\text{N}_2\text{O}_6$; CHN: Calculated: C (62.78%) H (5.85%) N (8.13%); Found: C (62.50%) H (5.33%) N (8.79%); δ_{H} (400 MHz, CDCl_3) 1.55 (s, 1H, NH), 2.54 (s, 1H, OH), 2.81 (dd, 1H, $J = 12.3, 6.8$ Hz, CHCHN CH_a), 3.06 (dd, 1H, $J = 12.4, 6.9$ Hz, CHCHN CH_b), 3.30 (dt, 1H, $J = 10.7, 6.8$ Hz, CHCH(CH_2)), 3.69 (s, 3H, OCH_3), 3.82 (s, 2H, NCH_2Ph), 5.16 (s, 2H, OCH_2Ph), 5.64 (d, 1H, $J = 10.7$ Hz, CH(OH)), 7.27 (m, 1H, Ar-H), 7.29 (d, 1H, $J = 3.7; 2.0$ Hz, Ar-H), 7.31 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.35 (m, 3H, Ar-H), 7.61 (dd, 2H, $J = 12.0, 4.4$ Hz, Ar-H), 7.71 (td, 1H, $J = 7.5, 1.5$ Hz, Ar-H), 8.08 (dd, 1H, $J = 7.2; 1.4$ Hz, Ar-H); δ_{C} (100 MHz; CDCl_3) 46.7, 48.8, 52.0, 53.7, 71.1, 72.9, 115.1, 126.0, 126.7, 128.1, 128.2, 128.5, 128.8, 135.4, 135.5, 159.4 and 174.6 (C=O).

4.4.7. Methyl 3-(2-benzyloxyphenyl)-3-hydroxy-2-[[[methoxycarbonyl]methyl-amino] methyl] propanoate **99a**.



The general procedure as described above in the section 4.4 was followed, using Methyl 2-((2-(benzyloxy) phenyl) (hydroxy) methyl) acrylate **97a** (0.42 ml, 1.67 mmol), glycine methyl ester (0.31 g, 2.51 mmol) TBAB (0.26 g, 0.83 mmol) and sodium acetate (0.20 g, 2.51 mmol) to afford methyl 3-(2-(benzyloxy) phenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **99a** as brown viscous oil (640 mg, 94.8%). $U_{\max} / \text{cm}^{-1}$: 1625 (C=O), 3451 (OH); 1311 (N-C); $\text{C}_{21}\text{H}_{25}\text{NO}_6$: CHN calculated: C(65.10%) H(6.50%) N(3.62%), found C(65.09%) H(6.55%) N(3.53); δ_{H} (400 MHz, CDCl_3): 2.81 (dd, $J = 12.5, 7.0$ Hz, 1H, CHCHaN), 2.81 (dd, $J = 12.5, 7.0$ Hz, 1H, CHCHbN), 3.06 (dd, $J = 12.5, 7.0$ Hz, 1H), 3.26 (s, NH), 3.57 (d, $J = 10.1$ Hz, 2H, $\text{NCH}_{2\text{ab}}\text{Ph}$), 3.66 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.77 (td, $J = 7.3, 2.9$ Hz, 1H, CHCH(CH₂)), 5.16 (s, 2H, OCH_2Ph), 5.90 (d, $J = 2.6$ Hz, 1H, CH(OH)), 6.92 (m, 2H, ArH), 7.01 (m, 1H, ArH), 7.26 (s, 2H, ArH), 7.30 (t, $J = 1.5$ Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.33 (t, $J = 2.1$ Hz, 1H, ArH), 7.35 (s, 1H, ArH), 7.40 (t, $J = 7.4$ Hz, 2H, ArH), 7.47 (d, $J = 1.3$ Hz, 1H, ArH), 7.49 (d, $J = 1.2$ Hz, 1H, ArH); δ (100 MHz, CDCl_3) 46.7, 49.8, 53.3 (OCH_3), 56.9 (OCH_3), 61.3, 66.7 (CHOH), 71.1 (OCH_2Ph), 115.2, 126.0, 126.7, 128.03, 128.04, 128.5, 128.8, 135.4, 135.5, 159.4, 171.5 (C=O) and 174.6 (C=O).

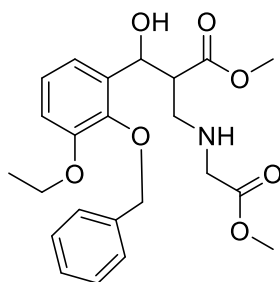
4.4.8. Methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-[[{methoxy-carbonyl} methylamino] methyl]propanoate 99b.



The general procedure as described above in the section 4.4. was followed, using Methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-methylenepropanoate **97b** (0.43 ml, 1.52 mmol), glycine methyl ester (0.30 g, 2.28 mmol) TBAB (0.25 g, 0.76 mmol) and sodium acetate (0.18 g, 2.28 mmol), to afford Methyl 3-(2-(benzyloxy)-3-methoxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **99b** as a yellowish oil (560 mg, 89.0 %). $U_{\max} / \text{cm}^{-1}$: 1702 (C=O), 3453 (OH); 1280 (N-C); $\text{C}_{22}\text{H}_{27}\text{NO}_7$: CHN calculated: C(63.30%) H(6.52%) N(3.36%), found: C(63.61%) H(6.12%) N(3.77%); δ_{H} (400 MHz, CDCl_3) 1.96 (s, 1H, NH), 2.44 (m, 1H, CHCH₂N), 3.30 (m, 1H, CHCH(CH₂)), 3.55 (d, $J = 20.6$ Hz, 2H, $\text{NCH}_{2\text{ab}}\text{Ph}$), 3.66 (s, 3H,

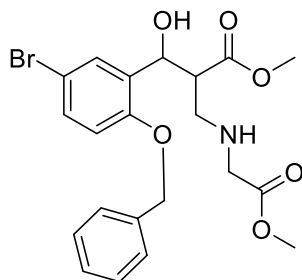
PhOCH₃)), 3.69 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 5.16 (s, 1H, OCH₂Ph), 5.85 (d, *J* = 10.7 Hz, 1H, CH(OH)), 6.93 (m, 5H, ArH), 7.01 (d, *J* = 2.3 Hz, 1H, ArH), 7.40 (s, 1H, ArH), 7.47 (d, *J* = 1.4 Hz, 1H, ArH); δ_c (100 MHz; CDCl₃) 2x 46.7, (2x CH₂), 50.0 (CH₂), 53.3 (OCH₃), 56.0 (OCH₃), 57.3 (OCH₃), 61.3, 71.1 (CHOH), 74.2 (OCH₂Ph), 115.1, 126.0, 126.7, 128.0, 128.1, 128.5, 128.8, 135.4, 135.5, 159.4, 171.5 and 174.6 (both C=O).

4.4.9. Methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-[[{ethoxy-carbonyl} methylamino] methyl]propanoate **99c**.



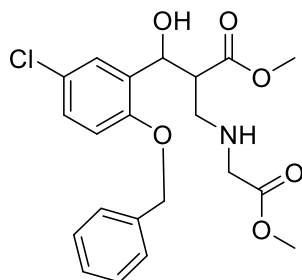
The general procedure as described above in the section 4.4. was followed, using Methyl 2-((2-(benzyloxy)-3-ethoxyphenyl) (hydroxy) methyl) acrylate **97c** (2.00 ml, 6.80 mmol), glycine methyl ester (1.28 g, 10.2 mmol) TBAB (1.09 g, 3.40 mmol) and sodium acetate (0.83 g, 10.2 mmol) to afford methyl 3-(2-(benzyloxy)-3-ethoxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **99c** as brown viscous oil (239 mg, 81.6 %). $U_{\max}$ / cm⁻¹: 1670 (C=O), 3298 (OH); 1301 (N-C); C₂₃H₂₉NO₇: CHN calculated: C(64.02%) H(6.77%) N(3.25%), found: C(64.18%) H(6.70%) N(3.19%); δ_H (400 MHz, CDCl₃) 1.42 (t, *J* = 5.9 Hz, 3H, CH₃CH₂), 2.81 (dd, *J* = 12.5, 7.5 Hz, 1H, CHCHaN), 3.06 (dd, *J* = 12.5, 7.5 Hz, 1H, CHCHbN), 3.16 (s, 1H, NH), 3.66 (s, 3H, NH), 3.57 (d, *J* = 9.3 Hz, 2H, NCH₂Ph), 3.69 (s, 3H, OCH₃), 3.70 – 3.75 (m, 1H, CHCH(CH₂)), 4.13 (m, 2H, CH₂CH₃), 5.16 (s, 2H, OCH₂Ph), 6.05 (d, *J* = 1.4 Hz, 1H, CH(OH)), 6.91 (dd, *J* = 7.6, 2.1 Hz, 1H, ArH), 7.02 (dd, *J* = 6.8, 2.1 Hz, 1H, ArH), 7.30 (t, *J* = 1.5 2Hz, 1H), ArH), 7.32 (s, 1H, ArH), 7.33 (t, *J* = 1.5 Hz, 1H, ArH), 7.40 (t, *J* = 7.4 Hz, 1H, ArH), 7.47 (d, *J* = 1.3 Hz, 1H, ArH), 7.49 (d, *J* = 1.2 Hz, 1H, ArH); δ_c (100 MHz, CDCl₃) 14.5 (OCH₂CH₃), 46.7 (CH₂), 50.0 (CH), 53.3, 56.0, 57.3, 61.3, 71.1 (OCH₂CH₃), 74.2 (OCH₂Ph), 115.1, 126.0, 126.7, 128.06, 128.08, 128.5, 128.8, 135.4, 135.5, 159.4, 171.5 and 174.6 (both C=O).

4.4.10. Methyl 3-(2-benzyloxy-5-bromophenyl)-3-hydroxy-2-[[{methoxy-carbonyl} methylamino] methyl]propanoate 99d.



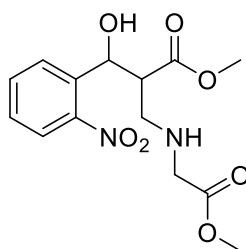
The general procedure as described above in the section 4.4. was followed, using methyl 3-(2-benzyloxy-5-bromophenyl)-3-hydroxy-2-methylenepropanonate **97d** (0.30 g, 0.80 mmol), glycine methyl ester (0.30g, 2.40 mmol) TBAB (0.50 g, 1.60 mmol) and sodium acetate (0.20 g, 2.40 mmol), to afford Methyl3-(2-(benzyloxy)-5-bromophenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **99d** as off-white viscous oil (gel) which solidify on standing m.p.: 74-79 °C, (370 mg, 83.0 %). $\nu_{\max}$ / cm^{-1} : 1690 (C=O), 3423 (OH); 1279 (N-C), $\text{C}_{21}\text{H}_{24}\text{NO}_6\text{Br}$: CHN calculated: C(54.09%) H(5.19%) N(3.00%), found: C(54.11%) H(5.15%) N(3.07%); δ_{H} (400 MHz, CDCl_3), 2.81 (dd, $J = 12.5, 7.0$ Hz, 1H, CHCHaN), 3.06 (dd, $J = 12.5, 7.0$ Hz, 1H, CHCHbN), 3.54 (d, $J = 10.1$ Hz, 2H, NCH_2Ph), 3.66 (s, 3H, OCH_3), 3.70 (4H overlapping multiplets: 3.69 (s, 3H, OCH_3 and 3.71 (td, 1H, $J = 5.1$ Hz, $\text{CHCH}(\text{CH}_2)$), 5.16 (s, 2H, OCH_2Ph), 5.88 (d, $J = 10.4$ Hz, 1H, $\text{CH}(\text{OH})$), 7.30 (t, $J = 1.5$ Hz, 1H, ArH), 7.32 (d, $J = 1.9$ Hz, 1H, ArH), 7.33 – 7.34 (m, 2H, ArH), 7.40 (t, $J = 7.4$ Hz, 3H, ArH), 7.44 (d, $J = 1.4$ Hz, 1H, ArH), 7.47 (d, $J = 1.3$ Hz, 1H, ArH), 7.49 (d, $J = 1.2$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 46.7, 50.0, 53.3, 56.0, 2x 71.1 (CHOH) and (OCH_2Ph), 115.2, 124.1, 126.0, 126.7, 128.0, 128.1, 128.5, 128.8, 135.4, 135.5, 171.5, and 174.6 from both C=O.

4.4.11. Methyl 3-(2-benzyloxy-5-chlorophenyl)-3-hydroxy-2-[[{methoxy-carbonyl}methylamino] methyl]propanoate 99e.



The general procedure as described above in the section 4.4. was followed, using Methyl 2-((2-(benzyloxy)-5-chlorophenyl) (hydroxy) methyl) acrylate **97e** (0.50 g, 1.50 mmol), glycine methyl ester (0.28 g, 2.25 mmol) TBAB (0.24 g, 0.75 mmol) and sodium acetate (0.18 g, 2.25 mmol), to afford Methyl 3-(2-(benzyloxy)-5-chlorophenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **99e** as brown viscous oil (610 mg, 95.0 %); $C_{21}H_{24}ClNO_6$ CHN: Calculated: C (59.79%) H (5.73%) Cl (8.40%) N (3.32%); Found: C(59.82%) H(5.79%), Cl (8.43%), N(3.37%); δ_H (400 MHz, $CDCl_3$) 2.32 (1H, s, OH), 2.81 (dd, 1H, J 12.3, 7.4 Hz, CHCHaN), 3.06 (dd, 1H, J = 12.2 and 7.3 Hz, CHCHbN), 3.38 (td, 1H, J = 7.2, 4.7 Hz, CHCH(CH₂)), 3.54 (s, 2H, NHCH₂CO), 3.66 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 3.77 (s, 1H, NH), 5.16 (s, 2H, CH₂Ph), 5.69 (d, 1H, J = 4.6 Hz, CHOH), 6.90 (d, 1H, J = 7.5 Hz, Ar-H), 7.32 (ddd, 1H, J = 6.7, 3.0, 1.5 Hz, Ar-H), 7.35 (d, 1H, J = 1.4 Hz, Ar-H), 7.37 (d, 1H, J = 1.4 Hz, Ar-H), 7.40 (t, 2H, J = 7.4 Hz, Ar-H), 7.46 (d, 1H, J = 1.4 Hz, Ar-H), 7.47 (d, 1H, J = 1.3 Hz, Ar-H), 7.49 (1H, d, J 1.2 Hz, Ar-H); δ_C (100 MHz, $CDCl_3$) 46.7, 50.0, 53.3, 56.0, 71.1, 115.1, 126.0, 126.7, 128.0, 128.5, 128.8, 135.4, 135.5, 171.5 and 174.6.

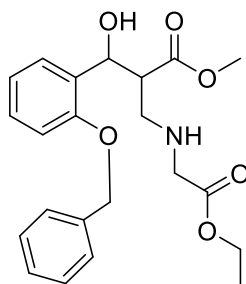
4.4.12. Methyl 3-(2-benzyloxy-6-nitrophenyl)-3-hydroxy-2-[[methoxy-carbonyl]methylamino] methyl]propanoate 99f



The general procedure as described above in the section 4.4. was followed, using 2-(hydroxy(2-nitrophenyl) methyl) acrylate **97f** (3.948g or 3.00 ml, ($d = 1.316 \text{ g/cm}^3$), 16.64 mmol), glycine methyl ester (6.27g, 49.9 mmol) TBAB (10.7 g, 33.3 mmol) and sodium acetate (4.10 g, 49.9 mmol), to afford tert-butyl 3-(5-chloro-2-hydroxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **99f** as a brown, viscous oil (3.21 ml, 78.0 %); $C_{21}H_{24}N_2O_8$ CHN: calculated: C(51.53%) H(5.56%) N(8.58%); Found: C(51.57%) H(5.51%) N(8.55%); δ_H (400 MHz; $CDCl_3$) 1.69 (1H, s, NH), 2.77 (1H, s, CHOH), 2.93 (1H, ddd, J 12.6, 12.5 and 6.4 Hz, CHCH₂N), 3.54 (2H, d, J 13.0 Hz, NCH₂CO), 3.66 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.84 (dt, 1H, J = 11.0,

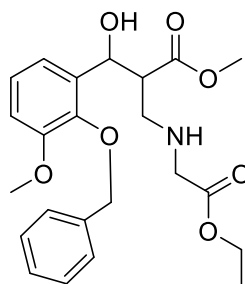
6.4 Hz, CHCH(CH₂)), 5.63 (d, 1H, *J* = 10.7 Hz, CHOH), 7.61 (dd, 1H, *J* = 12.0 and 4.4 Hz, Ar-H), 7.71 (td, 1H, *J* = 7.5, 1.5 Hz, Ar-H) and 8.08 (dd, 1H, *J* = 7.2, 1.4 Hz, Ar-H); δ_{C} (100 MHz; CDCl₃) 46.2, 49.2, 53.5, 57.0, 62.4, 71.1, 73.7, 115.13, 126.0, 128.0, 128.52, 128.8, 135.5, 159.3, 172.2 and 175.0.

4.4.13. Methyl 3-(2-benzyloxyphenyl)-2-[[ethoxycarbonyl]methylamino]-methyl}-3-hydroxypropanoate 100a.



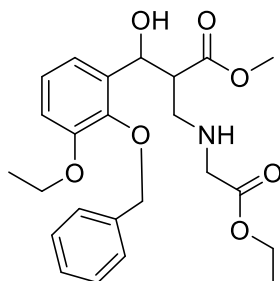
The general procedure as described above in the section 4.4. was followed, using Methyl 2-((2-(benzyloxy) phenyl) (hydroxy) methyl) acrylate **97a** (0.42 ml, 1.67 mmol), glycine ethyl ester (0.35 g, 2.51 mmol), TBAB (0.26 g, 0.83 mmol) and sodium acetate (0.20 g, 2.51 mmol), to afford methyl 3-(2-(benzyloxy) phenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **100a** as a brown viscous oil (620 mg, 92.3 %). $U_{\text{max}} / \text{cm}^{-1}$: 1699 (C=O), 3334 (OH), 1221 (N-C); C₂₂H₂₇NO₆: CHN calculated C(65.82%) H(6.78%) N(3.49%), found: C(65.81%) H(6.77%) N(3.48%); δ_{H} (400 MHz, CDCl₃) 1.21 (t, *J* = 5.9 Hz, 3H, CH₃CH₂), 2.13 (s, 1H, NH), 2.81 (dd, *J* = 12.5, 7.0 Hz, 1H, CHCHaN), 3.06 (dd, *J* = 12.5, 7.0 Hz, 1H, CHCHbN), 3.45 (s, 1H, NCHaPh), 3.50 (s, 1H, NCHbPh), 3.55 (td, *J* = 7.0, 3.6 Hz, 1H, CHCH(CH₂), 3.69 (s, 3H, OCH₃), 4.16 (q, *J* = 5.8 Hz, 2H, CH₂CH₃), 5.16 (s, 2H, OCH₂Ph), 5.80 (d, *J* = 3.8 Hz, 1H, CH(OH)), 6.92 (m, 2H, ArH), 6.98 (m, 1H, ArH), 7.31 (dd, *J* = 5.3, 3.6 Hz, 1H, ArH), 7.33 (d, *J* = 1.1 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.35 (s, 1H, ArH), 7.47 (d, *J* = 1.3 Hz, 1H, ArH), and 7.49 (d, *J* = 1.2 Hz, 1H, ArH), δ_{C} (100 MHz; CDCl₃) 14.2, (CH₂CH₃), 49.8, (CH₂), 53.3 (OCH₃), 56.0, 57.3, 61.3, 67.1, (CHOH) 71.1, (OCH₂Ph), 115.0, 126.0, 126.7, 128.11, 128.12, 128.5, 128.8, 135.4, 135.5, 159.4, 171.5, and 174.6 both C=O.

4.4.14. Methyl 3-(2-benzyloxy-3-methoxyphenyl)-2-[[ethoxy-carbonyl]methyl-amino]methyl}-3-hydroxypropanoate 100b.



The general procedure as described above in the section 4.4. was followed, using Methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-methylenepropanoate **97b** (2.00 ml, 7.10 mmol), glycine ethyl ester (0.148 g, 10.8 mmol) TBAB (1.18 g, 3.55 mmol) and sodium acetate (0.87 g, 10.8 mmol), to afford Methyl 3-(2-(benzyloxy)-3-methoxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **100b** as brown viscous oil (263 mg, 85.0 %). $U_{\max} / \text{cm}^{-1}$: 1666 (C=O), 3377 (OH); 1275 (N-C); $\text{C}_{23}\text{H}_{30}\text{NO}_7$ CHN calculated: C(64.02%) H(6.77%) N(3.25%), found: C(64.03%) H(6.72%) N(3.21%); δ_{H} (400 MHz, CDCl_3) 1.21 (t, $J = 5.9$ Hz, 3H, CH_3), 1.89 (s, 1H, NH), 2.94 (m, 1H, $\text{CHCH}_{2\text{abN}}$), 3.56 (s, 1H, NCHaPh), 3.58 (s, 1H, NCHbPh), 3.69 (s, 3H, OCH_3), 3.82 (s, 3H, PhOCH_3), 4.16 (q, $J = 5.8$ Hz, 2H, CH_2CH_3), 5.16 (s, 2H, OCH_2Ph), 5.76 (d, $J = 10.1$ Hz, 1H, $\text{CH}(\text{OH})$), 6.92 (m, 2H, ArH), 7.02 (dt, $J = 9.6, 4.6$ Hz, 1H, ArH), 7.26 (s, 1H, ArH), 7.31 (m, 1H), 7.40 (t, $J = 7.4$ Hz, 1H, ArH), 7.48 (dd, $J = 7.5, 1.3$ Hz, 2H, ArH), δ_{C} (100 MHz; CDCl_3) 14.2 (CH_2CH_3), 46.7, 53.3, 56.0, 57.3, 61.3, 71.1(CHOH), 74.2 (OCH_2Ph), 115.3, 126.0, 126.7, 128.01, 128.05, 128.5, 128.8, 135.4, 135.5, 159.4, 171.51 and 174.6, (2x C=O).

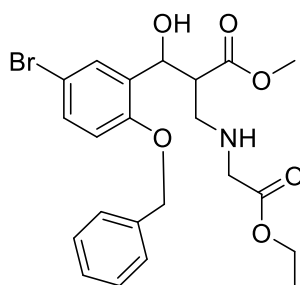
4.4.15. Methyl 3-(2-benzyloxy-3-ethoxyphenyl)-2-[[ethoxycarbonyl)methyl-amino]methyl]-3-hydroxypropanoate **100c**.



The general procedure as described above in the section 4.4. was followed, using Methyl 2-((2-(benzyloxy)-3-ethoxyphenyl) (hydroxy) methyl) acrylate **97c** (2.00 ml, 6.81 mmol), glycine ethyl ester (1.42 g, 10.2 mmol) TBAB (1.09 g, 3.40 mmol) and

sodium acetate (3.29 g, 10.2 mmol), to afford methyl 3-(2-(benzyloxy)-3-ethoxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **100c** as brown viscous oil (266 mg, 88.0 %). $U_{\max} / \text{cm}^{-1}$: 1675 (C=O), 3347 (OH); 1260 (N-C); $\text{C}_{24}\text{H}_{31}\text{NO}_7$ CHN calculated: C(64.70%) H(7.01%) N(3.14%); found: C(64.68%) H(7.04%) N(3.15%); δ_{H} (400 MHz, CDCl_3) 1.21 (t, $J = 5.9$ Hz, 3H, CH_2CH_3), 1.42 (t, $J = 5.9$ Hz, 3H, PhOCH_3), 1.84 (s, 1H, NH), 2.93 (ddd, $J = 125.6, 12.5, 5.9$ Hz, 1H), 3.17 (dt, $J = 10.4, 6.1$ Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 3.53 (d, $J = 31.3$ Hz, 2H, NCH_2Ph), 3.69 (s, 1H, OCH_3), 4.15 (q, $J = 15.1, 5.8$ Hz, 2H, CH_2CH_3), 5.16 (s, 1H, OCH_2Ph), 5.84 (d, $J = 10.3$ Hz, 1H, $\text{CH}(\text{OH})$), 6.92 (m, 1H, ArH), 7.02 (dd, $J = 6.8, 2.2$ Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.40 (t, $J = 7.4$ Hz, 1H, ArH), 7.48 (dd, $J = 7.6, 1.4$ Hz, 4H, ArH); δ_{H} (100 MHz, CDCl_3) 14.5 (CH_2CH_3), 15.8 (OCH_2CH_3), 46.7, 50.0 (OCH_3), 53.3 (CH_2CH_3), 56.0, 61.3, 64.4, 71.1, 115.2, 124.1, 126.0, 126.7, 128.01, 128.02, 128.5, 128.8, 135.4, 135.5, 171.5, and 174.6 (C=O).

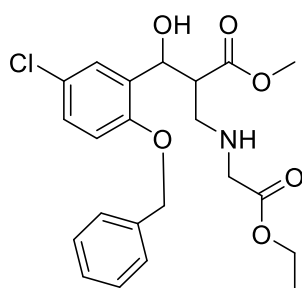
4.4.16. Methyl 3-(2-benzyloxy-5-bromophenyl)-2-[(ethoxycarbonyl)methyl-amino]methyl]-3-hydroxypropanoate 100d.



The general procedure as described above in the section 4.4. was followed, using methyl 3-(2-benzyloxy-5-bromophenyl)-3-hydroxy-2-methylenepropanoate **97d** (0.50 g, 1.32 mmol), glycine ethyl ester (0.55 g, 3.96 mmol) TBAB (0.85 g, 2.64 mmol) and sodium acetate (0.32g, 3.96 mmol), to afford methyl 3-(2-(benzyloxy)-5-bromophenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **100d** as yellow-off viscous liquid which solidify by standing (500 mg, 79.0 %). $U_{\max} / \text{cm}^{-1}$: 1682 (C=O), 3480 (OH); 1278 (N-C); m.p.: 84-87 °C; $\text{C}_{22}\text{H}_{26}\text{NO}_6\text{Br}$ CHN calculated: C(55.01%) H(5.46%) N(2.92%), found: C(55.04%) H(5.40%) N(2.95%); δ_{H} (400 MHz, CDCl_3) 1.21 (t, $J = 5.9$ Hz, 3H), 2.81 (dd, $J = 12.5, 7.5$ Hz, 1H), 3.06 (dd, $J = 12.5, 7.5$ Hz, 1H), 3.57 (s, 1H), 3.60 (s, 1H), 3.69 (s, 3H, OCH_3), 4.16 (q, $J = 5.8$ Hz, 2H, CH_2CH_3), 4.36 (td, $J = 7.5, 1.8$ Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 5.16 (s, 2H, OCH_2Ph), 5.83

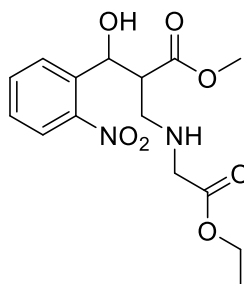
(d, $J = 1.7$ Hz, 1H, CH(OH)), 6.95 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.12 (s, 1H, ArH), 7.30 (t, $J = 1.5$ Hz, 1H, ArH), 7.32 (d, $J = 1.9$ Hz, 1H, ArH), 7.33 (m, 1H, ArH), 7.40 (t, $J = 7.4$ Hz, 2H ArH), 7.44 (d, $J = 1.4$ Hz, 1H ArH), 7.47 (d, $J = 1.3$ Hz, 1H ArH), and 7.49 (d, $J = 1.2$ Hz, 1H ArH); δ_c (100 MHz; $CDCl_3$) 14.5, 46.2, 48.5, 50.8, 52.4, 54.9, 70.8, 73.4, 113.7, 115.1, 126.3, 127.3, 127.6, 128.0, 128.5, 128.7, 2x 128.8, 131.0, 135.5, 138.3, 159.9 and 173.2.

4.4.17. Methyl 3-(2-benzyloxy-5-chlorophenyl)-2-[[{ethoxycarbonyl}methyl-amino]methyl]-3-hydroxypropanoate 100e.



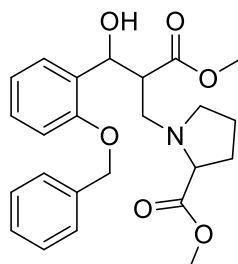
The general procedure as described above in the section 4.4. was followed, using Methyl 2-((2-(benzyloxy)-5-chlorophenyl) (hydroxy) methyl) acrylate **97e** (0.50 g, 1.50 mmol), glycine ethyl ester (0.31 g, 2.25 mmol) TBAB (0.24 g, 0.75 mmol) and sodium acetate (0.18 g, 2.25 mmol), to afford Methyl 3-(2-(benzyloxy)-5-chlorophenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **100e** as a yellow-off viscous liquid (600 mg, 93.0%); $C_{22}H_{26}ClNO_6$ CHN calculated: C (60.62%) H (6.01%) N (3.21%), found: C (60.68%) H (6.08%) N (3.20%); δ_H (400 MHz, $CDCl_3$) 1.21 (3H, t, $J = 5.9$ Hz, CH_3CH_2), 2.15 (1H, s, CHOH), 2.81 (1H, dd, $J = 12.5$ and 7.5 Hz, CHCHaN), 3.06 (1H, dd, $J = 12.3$ and 7.4 Hz, CHCHbN), 3.69 (3H, s, OCH_3), 3.88-3.94 (1H, m, CH(OH)CH(CH_2)), 4.16 (2H, q, $J = 5.8$ Hz, CH_3CH_2), 5.16 (2H, s, CH_2Ph), 5.18 (d, 1H, $J = 10.2$ Hz, CHOH), 6.90 (d, 1H, $J = 7.5$ Hz, Ar-H), 7.30 (1H, t, $J = 1.5$ Hz, Ar-H), 7.32 (s, 1H, Ar-H), 7.33 (t, 1H, $J = 1.5$ Hz, Ar-H), 7.35 (d, 1H, $J = 1.4$ Hz, Ar-H), 7.37 (d, 1H, $J = 1.4$ Hz, Ar-H), 7.40 (t, 2H, $J = 7.4$ Hz, Ar-H), 7.46 (d, 1H, $J = 1.4$ Hz, Ar-H), 7.47 (d, 1H, $J = 1.3$ Hz, Ar-H) and 7.49 (d, 1H, $J = 1.2$ Hz, Ar-H); δ_c (100 MHz, $CDCl_3$) 15.0, 46.2, 48.5, 50.8, 52.4, 54.9, 70.8, 73.4, 113.7, 115.1, 126.3, 127.3, 127.6, 128.0, 128.5, 128.7, 128.8, 131.05, 135.52, 138.3, 159.9 and 173.2.

4.4.18. Methyl 3-(2-benzyloxy-6-nitrophenyl-2-[[ethoxycarbonyl]methyl-amino]methyl)-3-hydroxypropanoate 100f



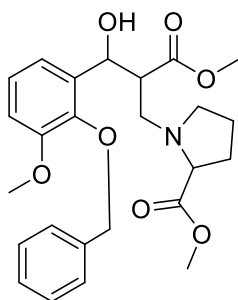
The general procedure as described above in the section 4.4. was followed, using 2-(hydroxy(2-nitrophenyl) methyl) acrylate **97f** (2.00 ml, 11.1 mmol), glycine ethyl ester (4.20g, 33.2 mmol) TBAB (7.15 g, 22.2 mmol) and sodium acetate (2.72 g, 33.2 mmol), to afford tert-butyl 3-(5-chloro-2-hydroxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **100f** as brown viscous liquid (2.64 ml, 70.0 %); $C_{22}H_{26}N_2O_8$ CHN calculated: C(52.94%), H(5.92%), N(8.23%); found: C (52.95%), H(5.95%), N(8.21%); δ_H (400 MHz, $CDCl_3$) 1.21 (t, 3H, $J = 5.9$ Hz, CH_3CH_2), 1.85 (1H, s, NH), 2.81 (dd, 1H, $J = 12.3, 6.8$ Hz, $CHCHaNH$), 3.06 (d, 1H, $J = 12.3, 6.8$ Hz, $CHCHbNH$), 3.55 (s, 2H, $NHCH_2CO$), 3.61 (td, 1H, $J = 6.7, 3.5$ Hz, $CHCH(CH_2)$), 3.69 (3H, s, OCH_3), 4.16 (q, 2H, $J = 5.8$ Hz, CH_2CH_3), 5.70 (d, 1H, $J = 3.5$ Hz, $CHOH$), 7.61 (dd, 2H, $J = 12.0, 4.4$ Hz, Ar-H), 7.71 (td, 1H, $J = 7.5$ and 1.5 Hz, Ar-H), 8.08 (dd, 1H, $J = 7.2, 1.4$ Hz, Ar-H); δ_C (100 MHz, $CDCl_3$) 14.2, 46.2, 49.2, 53.5, 57.0, 62.4, 71.1, 73.7, 115.1, 126.0, 128.5, 128.8, 135.5, 159.3, 172.26 and 175.0.

4.4.19. Methyl 3-(2-benzyloxyphenyl)-3-hydroxy-2-[[2-(methoxycarbonyl)-(1S)-pyrrolidin-1-yl]methyl]propanoate 101a.



The general procedure as described above in the section 4.4. was followed, using methyl 2-((2-(benzyloxy) phenyl) (hydroxy) methyl) acrylate **97a** (1.00 ml, 3.94 mmol), proline methyl ester (0.98 g, 5.92 mmol), TBAB (1.90 g, 5.92 mmol) and sodium acetate (0.48 g, 5.92 mmol), to afford Methyl 1-(3-(2-(benzyloxy) phenyl)-3-hydroxy-2-(methoxycarbonyl) propyl) pyrrolidine-2-carboxylate **101a** as yellow-off oil (770 mg, 46.0 %). $U_{\max} / \text{cm}^{-1}$: 1760 (C=O), 3310 (OH); 1250 (N-C); $\text{C}_{24}\text{H}_{29}\text{NO}_6$ CHN calculated: C(67.43%) H(6.84%) N(3.28%), found: C(67.65%) H(6.81%) N(3.20%); (400 MHz, CDCl_3) δ_{H} : 1.53 (p, $J = 6.4$ Hz, 2H, ArH), 1.80 (td, $J = 13.1, 6.5$ Hz, 1H, ArH), 2.14 (dq, $J = 13.1, 6.7$ Hz, 1H, ArH), 2.26 (dt, $J = 12.2, 6.0$ Hz, 1H, ArH), 2.40 (dd, $J = 12.5, 6.1$ Hz, 1H, ArH), 2.60 (t, $J = 6.4$ Hz, 1H, ArH), 3.07 (m, 1H, ArH), 3.22 (dd, $J = 12.5, 6.1$ Hz, 1H, ArH), 3.66 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 4.47 (dt, $J = 11.1, 6.4$ Hz, 1H, $\text{CH}(\text{OH})\text{CH}(\text{CH}_2)$), 5.16 (s, 2H, CH_2Ph), 5.37 (d, $J = 10.7$ Hz, 1H, $\text{CH}(\text{OH})$), 6.91 (m, 2H, ArH), 7.01 (m, 1H, ArH), 7.30 (t, $J = 1.5$ Hz, 1H, ArH), 7.33 (dd, $J = 7.8, 6.7$ Hz, 2H, ArH), 7.40 (t, $J = 7.4$ Hz, 2H, ArH), 7.47 (d, $J = 1.3$ Hz, 1H, ArH), and 7.49 (d, $J = 1.2$ Hz, 1H, ArH); (100 MHz, CDCl_3) δ 24.9, 28.4, 47.0 ($\text{CH}(\text{OH})$), 52.0, 54.2, 55.7, 58.3, 66.8, 70.8, 115.1, 121.7, 126.4, 127.3, 128.5, 128.8, 131.0, 131.0, 135.5, 138.2, 159.9, 170.3 (C=O), and 173.5 (C=O).

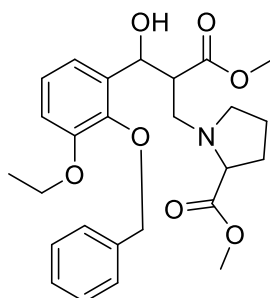
4.4.20. Methyl 3-(2-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-[[2-(methoxy-carbonyl)-(1S)-pyrrolidin-1-yl]methyl]propanoate **101b.**



The general procedure as described above in the section 4.4. was followed, using methyl 2-((2-(benzyloxy)-3-methoxyphenyl)(hydroxy) methyl) acrylate **97b** (1.00 ml, 3.60 mmol), proline methyl ester (0.89 g, 5.40 mmol), TBAB (1.74 g, 5.40 mmol) and sodium acetate (0.44 g, 5.40 mmol), to afford Methyl 1-(3-(2-(benzyloxy)-3-methoxyphenyl)-3-hydroxy-2-(methoxycarbonyl) propyl) pyrrolidine-2-carboxylate **101b** as grey-off oil that solidified on standing (380 mg, 55.0 %). $U_{\max} / \text{cm}^{-1}$: 1654 (C=O), 3314 (OH), 1277 (N-C); $\text{C}_{25}\text{H}_{31}\text{NO}_7$; CHN calculated: C(65.63%) H(6.83%)

N(3.06%), found: C(65.62%) H(6.87%) N(3.01%); δ_{H} (400 MHz, CDCl_3) 1.65 (p, J = 6.6 Hz, 2H, ArH), 1.81 (td, J = 13.0, 6.6 Hz, 1H, ArH), 2.10 (td, J = 12.8, 6.6 Hz, 1H, ArH), 2.25 (dt, J = 12.0, 6.0 Hz, 1H, ArH), 2.60 (t, J = 6.1 Hz, 1H, ArH), 2.78 (dd, J = 12.3, 3.3 Hz, 1H, ArH), 2.99 (dt, J = 12.0, 5.9 Hz, 1H, ArH), 3.47 (dd, J = 12.3, 3.3 Hz, 1H, ArH), 3.66 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.82 (s, 3H, PhOCH_3), 4.25 (dt, J = 10.4, 3.3 Hz, 1H, CHCH_2N), 5.16 (s, 2H, OCH_2Ph), 5.83 (d, J = 10.4 Hz, 1H, $\text{CH}(\text{OH})$), 6.92 (m, 1H, ArH), 7.02 (dt, J = 10.1, 4.9 Hz, 1H, ArH), 7.30 (t, J = 1.5 Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.33 (t, J = 1.5 Hz, 1H, ArH), 7.40 (t, J = 7.4 Hz, 3H, ArH), 7.47 (d, J = 1.3 Hz, 1H, ArH), 7.49 (d, J = 1.2 Hz, 1H, ArH); δ_{C} (100 MHz; CDCl_3) 24.9, 28.4, 47.0, 52.0, 54.2, , 55.7, 56.7 , 58.3 , 66.8, 70.8, 115.1, 121.7 , 126.4, 127.3, 128.5, 128.8, 131.0, 131.0, 135.5, 138.2, 153.9, 170.3, and 173.5.

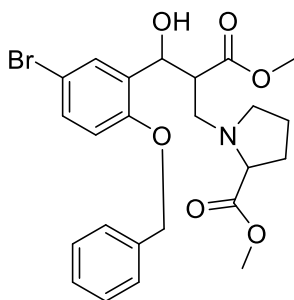
4.4.21. Methyl 3-(2-benzyloxy-3-ethoxyphenyl)-3-hydroxy-2-[(2-(methoxy-carbonyl)-(1S)-pyrrolidin-1-yl)methyl]propanoate 101c.



The general procedure as described above in the section 4.4. was followed, using methyl 2-((2-(benzyloxy)-3-ethoxyphenyl) (hydroxy) methyl) acrylate **97c** (1.00 ml, 3.40 mmol), proline methyl ester (0.84 g, 5.10 mmol), TBAB (1.64 g, 5.10 mmol) and sodium acetate (0.41 g, 5.10 mmol), to afford Methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl) (hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **101c** as brown oil (640 mg, 40.5 %). U_{max} / cm^{-1} : 1624 ($\text{C}=\text{O}$), 3360 (OH); 1300 ($\text{N}-\text{C}$); $\text{C}_{26}\text{H}_{33}\text{NO}_7$; CHN calculated: C(66.22%) H(7.05%) N(2.97%), found: C (66.25%), H (7.06%), N (2.99%); δ_{H} (400 MHz, CDCl_3) 1.21 (t, J = 5.9 Hz, 3H, CH_3CH_2), 1.42 (t, J = 5.9 Hz, 3H, CH_3CH_2), 1.84 (s, 1H, OH), 2.07 (s, 1H, NH), 2.81 (dd, J = 12.5, 5.8 Hz, 1H, CHCHaN), 3.06 (dd, J = 12.3, 5.9 Hz, 1H, CHCHbN), 3.17 (dt, J = 10.5, 6.1 Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 3.50 (s, 2H, NCH_2CO), 3.69 (s, 3H, OCH_3), 4.15 (dq, J = 15.1, 5.8 Hz, 4H, 2x CH_2CH_3), 5.16 (s, 2H, OCH_2Ph), 5.84 (d, J = 10.3 Hz, 1H, $\text{CH}(\text{OH})$), 6.92 (m, 1H, ArH), 7.02 (dt, J = 10.1, 4.9 Hz, 1H, ArH), 7.30 (t, J = 1.5 Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.33 (t, J = 1.5 Hz, 1H, ArH), 7.40 (t, J = 7.4 Hz, 2H, ArH), 7.47 (d,

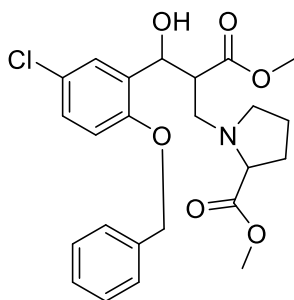
$J = 1.3$ Hz, 1H, ArH), and 7.49 (d, $J = 1.2$ Hz, 1H, ArH); δ_{C} (100 MHz; CDCl_3) 15.0, 24.9, 28.4, 47.0, 52.0, 54.2, 55.7, 58.3, 66.8, 70.8, 115.1, 121.7, 126.4, 127.3, 128.5, 128.8, 131.0, 131.0, 135.5, 138.2, 153.9, 170.3 and 173.5.

4.4.22. Methyl 3-(2-benzyloxy-5-bromophenyl)-3-hydroxy-2-[[2-(methoxy-carbonyl)-(1S)-pyrrolidin-1-yl]methyl]propanoate 101d.



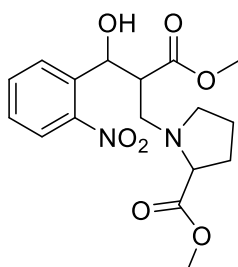
The general procedure as described above in the section 4.4. was followed, using methyl 2-((2-(benzyloxy)-5-bromophenyl)(hydroxy) methyl) acrylate **97d** (0.5 g, 1.32 mmol), proline methyl ester (0.33 g, 1.98 mmol), TBAB (0.64 g, 1.98 mmol) and sodium acetate (0.16 g, 1.98 mmol), to afford Methyl 1-(3-(2-(benzyloxy)-5-bromophenyl)-3-hydroxy-2-(methoxycarbonyl) propyl) pyrrolidine-2-carboxylate **101d** as off-white viscous oil that solidified on standing (340 mg, 51.2 %). $U_{\text{max}} / \text{cm}^{-1}$: 1669 ($\text{C}=\text{O}$), 3350 (OH); 1330 ($\text{N}-\text{C}$); $\text{C}_{24}\text{H}_{29}\text{NO}_6\text{Br}$; CNH calculated: C(56.92%) H(5.57%) N(2.77%), found: C(56.33%) H(5.80%) N(2.13%); δ_{H} (400 MHz, Cl_3) 1.53 (m, 2H); 1.84 (dq, $J = 11.6, 7.2$ Hz, 1H), 2.04 (t, $J = 7.5$ Hz, 1H, ArH), 2.11 (ddd, $J = 14.4, 11.9, 7.3$ Hz, 1H, ArH), 2.30 (dt, $J = 12.5, 6.1$ Hz, 1H, ArH), 2.74 (m, 3H, ArH), 3.32 (dd, $J = 12.5, 6.1$ Hz, 1H), 3.69 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.92 (td, $J = 6.1, 4.1$ Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 5.16 (s, 2H, OCH_2Ph), 5.72 (d, $J = 4.3$ Hz, 1H, $\text{CH}(\text{OH})$), 6.95 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.31 (d, $J = 1.7$ Hz, 1H, ArH), 7.32 (d, $J = 1.9$ Hz, 1H, ArH), 7.34 (m, 1H, ArH), 7.40 (t, $J = 7.4$ Hz, 3H, ArH), 7.44 (d, $J = 1.4$ Hz, 1H, ArH), 7.47 (d, $J = 1.3$ Hz, 2H, ArH), 7.49 (d, $J = 1.2$ Hz, 1H, ArH); δ_{C} (100 MHz; CDCl_3) 24.9, 28.4, 47.1, 51.8, 54.2, 55.4, 62.7, 66.7, 70.8, 115.1, 121.7, 126.4, 127.3, 28.5, 128.8, 131.0, 131.0, 135.5, 138.2, 160.0, 170.3 ($\text{C}=\text{O}$) and 173.5 ($\text{C}=\text{O}$).

4.4.23. Methyl 3-(2-benzyloxy-5-chlorophenyl)-3-hydroxy-2-[[2-(methoxy-carbonyl)-(1S)-pyrrolidin-1-yl]methyl]propanoate 101e.



The general procedure as described above in the section 4.4. was followed, using methyl 2-((2-(benzyloxy)-5-chlorophenyl) (hydroxy) methyl) acrylate **97e** (1.00 ml, 3.77 mmol), proline methyl ester (0.93 g, 5.65 mmol), TBAB (1.82 g, 5.65 mmol) and sodium acetate (0.46 g, 5.65 mmol), to afford Methyl 1-(3-(2-(benzyloxy)-5-chlorophenyl)-3-hydroxy-2-(methoxycarbonyl) propyl) pyrrolidine-2-carboxylate **101e** as yellow-off oil that solidified on standing (920 mg, 53.0 %). $U_{\max}$ / cm^{-1} : 1730 (C=O), 3499 (OH), 1290 (N-C); $\text{C}_{24}\text{H}_{28}\text{ClNO}_6$ CHN: calculated: C (56.92%), H (5.57%), N (2.77%); found: C (56.95%), H (5.53%), N (2.79%); δ_{H} (400 MHz; CDCl_3) 1.57-1.50 (2H, m, Ar-H), 1.84 (d, J = 11.6 and 7.1 Hz, 1H, Ar-H), 2.11 (ddd, 1H, J = 14.2, 11.6, 7.1 Hz, Ar-H), 2.05 (1H, t, J = 7.5 Hz, Ar-H), 2.30 (dt, 1H, J = 12.5, 6.2 Hz, Ar-H), 2.74 (m, 2H, Ar-H), 3.32 (dd, 1H, J = 12.5, 6.1 Hz, Ar-H), 3.66 (s, 3H, OCH_3), 3.92 (d, 1H, J = 6.2 and 4.1 Hz, $\text{CHCH}(\text{CH}_2)$), 3.99 (s, 3H, OCH_3), 5.16 (s, 2H, CH_2Ph), 5.74 (d, 1H, J = 4.1 Hz, CHOH), 6.90 (1H, d, J = 7.5 Hz, Ar-H), 7.32 (m, 1H, Ar-H), 7.35 (d, 1H, J = 1.4 Hz, Ar-H), 7.37 (d, 1H, J = 1.4 Hz, Ar-H), 7.40 (t, 2H, J = 7.4 Hz, Ar-H), 7.46 (d, 1H, J = 1.4 Hz, Ar-H), 7.47 (d, 1H, J = 1.3 Hz, Ar-H) and 7.49 (d, 1H, J = 1.2 Hz, Ar-H); δ_{C} (100 MHz; CDCl_3) 24.9, 28.4, 46.0, 51.8 (OCH_3), 54.2, 55.4, 64.6(CHOH), 70.8 (OCH_2Ph), 73.8, 115.1, 121.7, 126.4, 127.3, 128.5, 128.8, 131.0, 131.0, 135.5, 138.2, 157.0, 170.3 (C=O) and 173.5 (C=O).

4.4.24. Methyl 3-(2-benzyloxy-6-nitrophenyl)-3-hydroxy-2-[[2-(methoxy-carbonyl)-(1S)-pyrrolidin-1-yl]methyl]propanoate **101f**



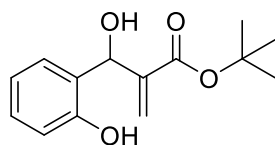
The general procedure as described above in the section 4.4. was followed, using methyl 2-(hydroxy(2-nitrophenyl) methyl) acrylate **97f** (1.00 ml, 5.54 mmol), proline methyl ester (1.35 g, 8.32 mmol), TBAB (2.68 g, 8.32 mmol) and sodium acetate (0.68 g, 8.32 mmol), to afford Methyl 1-(3-hydroxy-2-(methoxycarbonyl)-3-(2-nitrophenyl) propyl) pyrrolidine-2-carboxylate **101f** as yellow viscous oil/gel (107 mg, 53.0 %) $C_{24}H_{28}N_2O_8$ CHN: calculated: C (55.73%), H (6.05%), N (7.65%); found: C (55.70%), H (6.03%), N (7.69%); δ_H (400 MHz; $CDCl_3$) 1.68-1.72 (2H, q, J 7.5 Hz, Ar-*H*), 1.76 (m, 2H, Ar-*H*), 2.10 (dtd, 1H, J = 12.1, 7.6; 4.4 Hz, Ar-*H*), 2.36 (1H, dt, J = 12.3, 7.1 Hz, Ar-*H*), 2.70 (dd, 1H, J = 12.2, 6.1 Hz, Ar-*H*), 2.87 (m, 2H, Ar-*H*), 3.34 (t, 1H, J = 4.4 Hz, Ar-*H*), 3.64 (m, 4H, overlapping from OCH_3 and $CHCH(CH_2)$), 3.69 (s, 3H, OCH_3), 5.59 (d, 1H, J = 11.0 Hz, $CHOH$), 7.61 (d, 2H, J = 12.0; 4.4 Hz, Ar-*H*), 7.71 (td, 1H, J = 7.5, 1.5 Hz, Ar-*H*) and 8.08 (dd, 1H, J = 7.2; 1.4 Hz, Ar-*H*); δ_C (100 MHz; $CDCl_3$) 24.4, 29.4, 46.5, 50.1, 52.0, 54.1, 56.1, 70.5, 126.2, 128.3, 130.1, 131.4, 136.3, 145.2, 172.0 (C=O) and 175.4 (C=O).

4.5. General procedure for the synthesis of the MBH adducts (102a-e) from the non-benzylated salicylaldehydes with tert-buthyl acrylate.

140,208-209

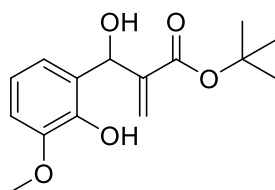
This method was addaped with some modifications from Basavaiah, D. *et al.*²¹⁰ To a solution of substituted or unsubstituted salicylaldehyde **43** (1 eq.), and tert-butyl acrylate (2 eq.), was added 20 ml of aqueous triethylamine (TEA) (20% in methanol: water (1:1 ratio)). The mixture was stirred at room temperature for 0.5 to 3 days or until TLC showed no-starting materials. The reaction mixture was stopped, quenched with cold water and the product extracted with chloroform (3 times). It was dried over anhydrous $MgSO_4$ and the solvent evaporated to afford the crude, which was recrystallized or purified via flash column chromatofraphy (on silica gel; eluting from hexane: EtOAc (7:3) to afford the pure products.

4.5.1. Tert-butyl 2-((2-hydroxyphenyl)(hydroxy) methyl)acrylate 102a.



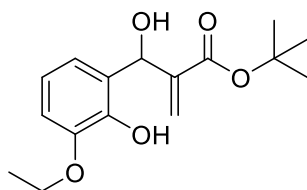
The general procedure as described above in the in the section 4.5 was followed using 2-hydroxybenzaldehyde **43** (1.15 g, 9.41 mmol), and tert-butyl acrylate (2.41 g, 18.8 mmol), to afford tert-butyl 2-(hydroxy(2-hydroxyphenyl) methyl) acrylate **102a** as brown solid (1820 mg, 77 %), m.p.: 106-109°C (reported value in the literature: 108-110 °C¹⁹³⁻¹⁹⁴); $U_{\max}$ / cm^{-1} : 1685 (C=O), 3340 (OH); δ_{H} (300 MHz, CDCl_3) 1.09 (s, 1H, NH), 1.48 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.85 (s, 1H, OH), 5.42 (s, 1H, =CHa), 5.47 (s, 1H, CH(OH)), 6.02 (s, 1H, =CHb), 6.81 (dd, J = 7.4, 1.4 Hz, 1H, ArH), 6.90 (td, J = 7.5, 1.5 Hz, 1H, ArH), 7.13 (m, 2H, ArH), δ_{C} (75 MHz, CDCl_3) 28.4 ($\text{C}(\text{CH}_3)_3$), 61.1 (CH(OH)), 82.5 ($\text{C}(\text{CH}_3)_3$), 115.5, 119.6 (ArC), 124.3, 126.4, 127.6, 129.5, 141.5 (C=CH), 155.2 (ArC(OH)), 166.3 (C=O)

4.5.2. Tert-butyl 2-(hydroxy(2-hydroxy-3-methoxyphenyl) methyl) acrylate **102b**.



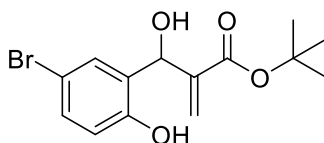
The general procedure as described above in the in the section 4.5 was followed, using 2-hydroxy-3-methoxybenzaldehyde **43** (1 g, 6.57 mmol), and tert-butyl acrylate (1.68 g, 13.1 mmol), to afford tert-butyl 2-(hydroxy(2-hydroxy-3-methoxyphenyl) methyl) acrylate **102b** as brown liquid (1545 mg, 84%). $U_{\max}$ / cm^{-1} : 1790 (C=O), 3292 (OH); δ_{H} (300 MHz, CDCl_3) 1.16 (s, 1H, OH). 1.45 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.82 (s, 3H, OCH_3), 5.42 (s, 1H, C=CHa), 5.69 (s, 1H, CH(O)), 6.01 (s, 1H, =CHb), 6.88 (dd, J = 7.5, 1.4 Hz, 1H, ArH), 7.01 (dd, J = 7.5, 1.4 Hz, 1H, ArH), 7.25 (m, 2H, ArH); δ_{C} (75 MHz, CDCl_3) 27.8 ($\text{C}(\text{CH}_3)_3$), 55.9 (OCH_3), 69.5 (CH(OH)), 82.9 $\text{C}(\text{CH}_3)_3$, 114.5, 119.0, 119.5, 125.4 (C=CH), 126.6, 142.4 (C=CH), 143.7 (ArC(OH)) 148.0, 166.3 (C=O).

4.5.3. Tert-butyl 2-((-2-hydroxy-3-ethoxyphenyl)(hydroxy) methyl)acrylate **102c**.



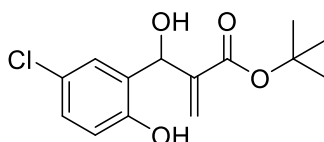
The general procedure as described above in the in the the section 4.5.was followed using 2-hydroxy-3-ethoxybenzaldehyde **43** (1 g, 6.01 mmol), tert-butyl acrylate (1.54 g, 12.0 mmol), to afford tert-butyl 2-((3-ethoxy-2-hydroxyphenyl) (hydroxy) methyl) acrylate **102c** as brown liquid (1432 mg, 81%). $U_{\max} / \text{cm}^{-1}$: 1714 (C=O), 3331 (OH). δ_{H} (400 MHz, CDCl_3) 1.34 (t, $J = 5.8$ Hz, 3H, CH_3CH_2), 1.42 (s, 9H, 3x CCH_3), 4.07 (q, $J = 5.9$ Hz, 2H, CH_2CH_3), 5.40 (s, 1H, C=CHa), 6.12 (s, 1H, CH(OH)), 6.52 (s, 1H, $=\text{CHb}$), 6.79 (dd, $J = 7.4, 1.5$ Hz, 1H, ArH), 6.91 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.19 (t, $J = 7.4$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 14.4 (CH_2CH_2), 27.8 ($\text{C}(\text{CH}_3)_3$), 55.9, 64.1 (CH_2CH_3), 69.5, 82.9 ($\text{C}(\text{CH}_3)_3$), 114.5, 119.07, 119.5, 125.4, 126.6, 142.4, 143.7, 148.0, 166.3 (C=O).

4.5.4. Tert-butyl 2-((5-bromo-2-hydroxyphenyl)(hydroxy) methyl)acrylate **102d**.



The general procedure as described above in the the section 4.5 was followed using 5-bromo-2-hydroxybenzaldehyde **43** (1 g, 4.97 mmol), and tert-butyl acrylate (1.27 g, 9.94 mmol) to afford tert-butyl 2-((2-(benzyloxy)-5-bromophenyl) (hydroxy) methyl) acrylate **102d** as a white solid (1586 mg, 97 %), m.p: 178-183; $U_{\max} / \text{cm}^{-1}$: 1681 (C=O), 3310 (OH); δ_{H} (300 MHz, DMSO) 1.26 (s, 9H, $\text{C}(\text{CH}_3)_3$), 5.63 (s, 1H, $=\text{CHa}$), 5.65 (s, 1H, CH(OH)), 6.05 (s, 2H, $=\text{CHb}$), 6.73 (d, $J = 8.5$ Hz, 1H, ArH), 7.14 (d, $J = 2.6$ Hz, 1H, ArH), 7.18 (d, $J = 2.6$ Hz, 1H, ArH); δ_{C} (75 MHz, DMSO) 27.8 ($\text{C}(\text{CH}_3)_3$), 65.0, 80.8 ($\text{C}(\text{CH}_3)_3$), 110.1, 117.5, 123.7, 130.1, 131.0, 132.1, 145.0, 154.2 and 165.5 (C=O)

4.5.5. Tert-butyl 2-((5-chloro-2-hydroxyphenyl)(hydroxy) methyl)acrylate **102e**.

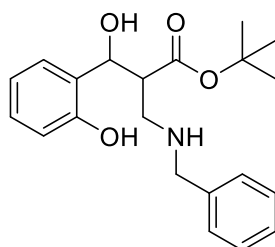


The general procedure as described above in the in the section 4.5 was followed, using 5-chloro-2-hydroxybenzaldehyde **43** (1 g, 6.38 mmol), and tert-butyl acrylate (1.63 g, 12.77 mmol), to afford tert-butyl 2-((5-chloro-2-hydroxyphenyl) (hydroxy) methyl) acrylate **102e** as white solid (1725 mg, 95%), m.p.: 179-182 °C²¹⁰, $U_{\max}$ / cm^{-1} : 1683 (C=O), 3352 (OH); δ_{H} (300 MHz, DMSO) 1.26 (s, 9H, 3x OCH₃), 5.65 (s, 2H, overlapping CH(OH) and C=CHa), 6.06 (s, 1H, C=CHb), 6.77 (d, J = 8.5 Hz, 1H, ArH), 7.02 (d, J = 2.7 Hz, 1H, ArH), 7.07 (dd, J = 8.5, 2.7 Hz, 1H, ArH); δ_{C} (75 MHz, DMSO) 27.8 (C(CH₃)₃), 65.1 (CH(OH)), 80.8 (C(CH₃)₃), 117.0, 122.6, 123.7, 127.3, 128.1, 131.5, 145.0, 153.7, and 165.5 (C=O).

4.6. General procedure for the synthesis of the aza-Michael adducts 52-55a-e from the MBH (103-106a-e).^{165-168,201-203}

A mixture of MBH adducts (**102a-e**) (1 eq) and the appropriate amino acid/amine (1 eq) was stirred at room temperature as a neat mixture for 0.5 h – 3 days, monitoring with TLC. In some cases, a few drops of dried THF solvent were used to dissolve solid reactants. The reaction mixture was then subject to short column chromatography over silica gel (hexane/ethyl acetate 3:1) to provide pure desired products as follows:

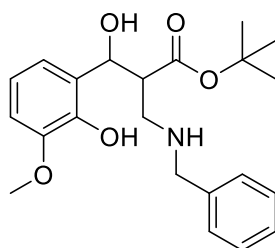
4.6.1. Tert-butyl 2-((benzylamino)methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **103a**.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxyphenyl)methyl)acrylate **102a** (1.0 g, 3.99 mmol), and benzyl amine (0.42 g, 3.99 mmol) to afford tert-butyl 2-((benzylamino)methyl)-3-hydroxy-3-(2-hydroxyphenyl)propanoate **103a** as yellow oil (1240 mg, 87%). $U_{\max}$ /

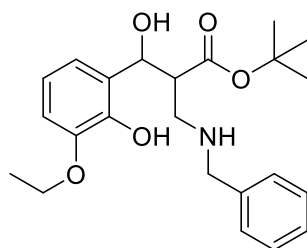
cm⁻¹: 1672 (C=O), 3420 (OH); 1234 (N-C); C₂₁H₂₇N₃O₄, CHN: calculated: C(70.56%), H(7.61%), N(3.92%), O(17.90%), found: C(70.54%), H(7.63%), N(3.91%), O(17.91%). δ_{H} (300 MHz, CDCl₃) 1.49 (s, 9H, C(CH₃)₃), 2.65 (s, 1H, NH), 2.81 (td, J = 3.3, 1.3 Hz, 1H, CHCH(CH₂)), 3.07 (ddd, J = 9.0, 5.3, 2.9 Hz, 2H, CH(CH₂N)), 3.76 (s, 1H, OH), 3.93 (d, J = 6.7 Hz, 2H, NCH₂Ph), 5.33 (d, J = 10.0 Hz, 1H, CH(OH)), 6.79 (d, J = 7.5 Hz, 1H, ArH), 6.88 (t, J = 7.5 Hz, 1H, ArH), 7.08 (t, J = 8.2 Hz, 1H, ArH), 7.16 (d, J = 7.4 Hz, 1H, ArH), 7.28 (m, 5H, ArH); δ_{C} (75 MHz, CDCl₃) 3x 29.9 (C(CH₃)₃), 44.4 (CH(CH₂N)), 51.9 (NCH₂Ph), 55.1 (CH(CH₂N)), 70.8 (CH(OH)), 81.3 (C(CH₃)₃), 114.7, 126.0, 126.7, 2x 127.3, 2x 128.0, 128.5, 128.8, 135.4, 135.5 NCH₂-ArC, 159.4 (ArC(OH)), 174.3 (C=O).

4.6.2. Tert-butyl 2-((benzylamino)methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **103b**.



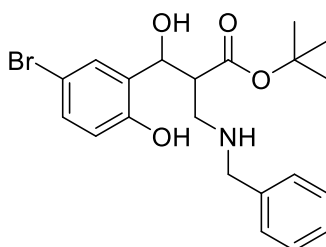
The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxy-3-methoxyphenyl)methyl)acrylate **102b** (1 g, 3.56 mmol), benzyl amine (0.38 g, 3.56 mmol), to afford tert-butyl 2-((benzylamino)methyl)-3-hydroxy-3-(2-hydroxy-3-methoxyphenyl)propanoate **103b** as brown viscous oil (1172 mg, 85%). $U_{\text{max}} / \text{cm}^{-1}$: 1691 (C=O), 3325 (OH), 1293 (N-C); C₂₂H₂₉N₃O₅, CHN calculated: C(68.20%), H(7.54%), N(3.61%), O(20.65%), found: C(68.21%), H(7.52%), N(3.62%), O(20.65%); δ_{H} (300 MHz, CDCl₃): 1.50 (s, 9H, C(CH₃)₃), 2.72 (m, 2H, overlapping CHCH(CH₂) with NH), 3.07 (ddd, J = 8.7, 5.2, 2.8 Hz, 2H, CH(CH₂N)), 3.82 (s, 3H, OCH₃), 3.92 (d, J = 9.5 Hz, 1H NCH₂Ph), 4.41 (s, 1H, OH), 5.33 (d, J = 10.6 Hz, 1H, CH(OH)), 6.68 (dd, J = 6.7, 2.3 Hz, 1H, ArH), 6.82 (m, 1H, ArH), 7.25 (m, 1H, ArH); δ_{C} (75 MHz, CDCl₃) 29.9 (C(CH₃)₃), 44.4 (CH(CH₂N)), 51.9 (NCH₂Ph), 53.5, 55.1, 57.0, 70.8, 81.3, 115.1, 126.3, 127.4, 128.6, 128.9, 129.1, 129.5, 130.2, 135.4, 140.5, 159.9, (ArC(OH)), 174.3 (C=O).

4.6.3. Tert-butyl 2-((benzylamino) methyl)-3-(3-ethoxy-2-hydroxyphenyl)-3-hydroxypropanoate **103c**.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((3-ethoxy-2-hydroxyphenyl)(hydroxy)methyl)acrylate **102c** (1 g, 3.39 mmol), benzyl amine (0.36 g, 3.39 mmol), to afford tert-butyl 2-((benzylamino)methyl)-3-(3-ethoxy-2-hydroxyphenyl)-3-hydroxypropanoate **103c** as brown viscous oil (1075 mg, 79%). $U_{\max}$ / cm^{-1} : 1630 (C=O), 3320 (OH), 1288 (N-C); $\text{C}_{23}\text{H}_{31}\text{NO}_5$, CHN calculated: C(68.80%), H(7.78%), N(3.49%), O(19.92%), found: C(68.84%), H(7.79%), N(3.45%), O(19.90%); δ_{H} (400 MHz, CDCl_3): 1.40 (t, J = 6.0 Hz, 8H, CH_3CH_2), 1.52 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.20 (s, 1H, NH), 2.93 (ddd, J = 125.6, 12.5, 6.4 Hz, 2H, $\text{CH}(\text{CH}_2\text{N})$), 3.94 (s, 1H, OH), 4.05 (m, 3H, overlapping $\text{CHCH}(\text{CH}_2)$ with CH_2CH_3), 4.20 (dt, J = 11.4, 6.4 Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 5.33 (d, J = 11.4 Hz, 1H, $\text{CH}(\text{OH})$), 6.89 (ddd, J = 48.2, 7.4, 1.4 Hz, 2H, ArH), 7.21 (ddd, J = 7.1, 4.2, 1.6 Hz, 1H, ArH), 7.23 (d, J = 1.1 Hz, 1H, ArH), 7.24 (s, 1H, ArH), 7.28 (d, J = 7.5 Hz, 4H), 7.30 (s, 1H, ArH), 7.31 (t, J = 1.9 Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 15.0 (CH_2CH_3), 29.9 ($\text{C}(\text{CH}_3)_3$), 44.4 ($\text{CH}(\text{CH}_2\text{N})$), 51.9 (NCH_2Ph), 55.1 ($\text{CH}(\text{CH}_2\text{N})$), 64.4 (CH_2CH_3), 71.8 ($\text{CH}(\text{OH})$), 81.3 ($\text{C}(\text{CH}_3)_3$), 113.8, 115.1, 126.3, 127.3, 127.4, 128.5, 128.6, 128.8, 131.0, 135.5, 138.2, 159.9, 174.3 (C=O).

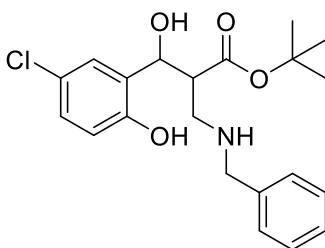
4.6.4. Tert-butyl 2-((benzylamino)methyl)-3-(5-bromo-2-hydroxyphenyl)-3-hydroxypropanoate **103d**.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((5-bromo-2-hydroxyphenyl)(hydroxy)methyl)acrylate **102d** (1 g, 3.03 mmol) and benzylamine (0.32 g, 3.04 mmol) to afford tert-butyl 2-((benzylamino)methyl)-3-(5-bromo-2-hydroxyphenyl)-3-hydroxypropanoate **103d** as

yellow viscous oil (1282 mg, 97%). $U_{\max} / \text{cm}^{-1}$: 1677 (C=O), 3410 (OH); 1280 (N-C); $\text{C}_{21}\text{H}_{26}\text{BrNO}_4$, CHN: calculated: C(57.81%), H(6.01%), N(3.21%), O(14.67%), Br(18.31), found: C(57.10%), H(6.12%), N(3.10%), O(14.18%), Br(18.28); δ_{H} (400 MHz, CDCl_3) 1.49 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.89 (s, 1H, NH), 2.93 (m, 3H, overlapping 2H, $\text{CH}(\text{CH}_2\text{N})$ and 1H, $\text{CHCH}(\text{CH}_2)$), 3.94 (d, $J = 13.4$ Hz, 2H, NCH_2Ph) 5.36 – 5.30 (d, 1H, $\text{CH}(\text{OH})$), 7.23 (dt, $J = 3.4, 2.1$ Hz, 1H, ArH), 7.25 (s, 1H, ArH), 7.27 (s, 1H, ArH), 7.30 (d, $J = 7.5$ Hz, 2H, ArH), 7.32 (s, 1H, ArH), 7.35 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.43 (d, $J = 1.4$ Hz, 1H, ArH), 7.47 (d, $J = 7.5$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 29.9 ($\text{C}(\text{CH}_3)_3$), 44.4 ($\text{CH}(\text{CH}_2\text{N})$), 51.1 (NCH_2Ph), 71.0($\text{CH}(\text{OH})$), 81.3 ($\text{C}(\text{CH}_3)_3$), 113.8, 115.1, 126.3, 127.3, 127.4, 128.5, 128.6, 128.8, 131.0, 135.5, 138.2, 159.9, (ArC(OH)), 174.3 (C=O).

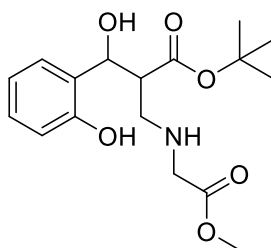
4.6.4. Tert-butyl 2-((benzylamino)methyl)-3-(5-bromo-2-hydroxyphenyl)-3-hydroxypropanoate **103e**.



The general procedure as described above in the the section 4.6 was followed, using Tert-butyl 2-((5-chloro-2-hydroxyphenyl)(hydroxy) methyl) acrylate **102e** (1 g, 3.51 mmol), and benzyl amine (0.37 g, 3.51 mmol) to afford Tert-butyl 2-((benzyl amino) methyl)-3-(5-chloro-2-hydroxyphenyl)-3-hydroxypropanoate **103e** as white viscous oil (1362 mg, 99%). $U_{\max} / \text{cm}^{-1}$: 1680 (C=O), 3440 (OH); 1300 (N-C); $\text{C}_{21}\text{H}_{26}\text{ClNO}_4$, CHN calculated: C(64.36%), H(6.69%), N(3.57%), O(16.33%), Cl(9.03%), found: C(64.31%), H(6.66%), N(3.51%), O(16.28%), Cl(9.21%); δ_{H} (400 MHz, CDCl_3) 1.50 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.93 (ddd, $J = 125.6, 12.4, 8.2$ Hz, 2H, $\text{CH}(\text{CH}_2\text{N})$), 3.98 (d, $J = 60.4$ Hz, 2H, NCH_2Ph), 4.20 (td, $J = 8.2, 2.5$ Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 4.57 (s, 1H, OH), 5.33 (d, $J = 2.5$ Hz, 1H, $\text{CH}(\text{OH})$), 7.14 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.22 (m, 1H, ArH), 7.28 (m, 3H), 7.31 (d, $J = 7.4$ Hz, 1H, ArH), 7.40 (d, $J = 1.4$ Hz, 1H, ArH), 7.42 (d, $J = 7.5$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 29.9 ($\text{C}(\text{CH}_3)_3$), 45.7 ($\text{CH}(\text{CH}_2\text{N})$), 50.1 (NCH_2Ph), 52.6 ($\text{CH}(\text{CH}_2\text{N})$), 71.8 ($\text{CH}(\text{OH})$), 82.7 ($\text{C}(\text{CH}_3)_3$), 113.6, 115.3, 126.3,

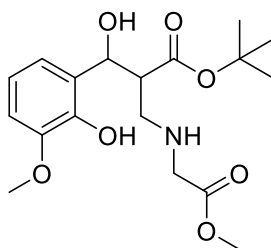
127.3, 127.4, 128.5, 128.6, 128.8, 131.0, 135.6, 138.3, 159.8 (ArC(OH)), 174.3 (C=O).

4.6.5. Tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104a.**



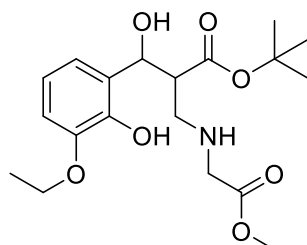
The general procedure as described above in the in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxyphenyl) methyl) acrylate **102a** (1.0 g, 3.99 mmol), glycine methyl ester (0.50 g, 3.99 mmol) to afford tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104a** as brown gel (1151 mg, 85%). $U_{\max}$ / cm^{-1} : 1711 (C=O), 3412 (OH), 1273 (N-C); $\text{C}_{17}\text{H}_{25}\text{NO}_6$, CHN calculated: C(60.16%), H(7.43%), N(4.13%), O(28.28%), found: C(60.33%), H(7.20%), N(4.36%), O(28.52%); δ_{H} (400 MHz, CDCl_3) 1.51 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.27 (s, 1H, NH), 3.11 – 2.77 (m, 3H, overlapping 2H, $\text{CH}(\text{CH}_2\text{N})$ and 1H, $\text{CHCH}(\text{CH}_2)$), 3.54 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 5.33 (m, 1H, $\text{CH}(\text{OH})$), 7.23 (m, 1H, ArH), 7.27 (m, 1H, ArH), 7.29 (d, $J = 2.1$ Hz, 1H, ArH), 7.31 (d, $J = 7.4$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 28.8, 41.5, 52.3, 52.7, 56.3, 64.2, 82.2, 115.1, 121.8, 126.3, 127.3, 128.8, 155.2, 173.2,

4.6.6. Tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104b.**



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxy-3-methoxyphenyl) methyl) acrylate **102b** (1 g, 3.56 mmol), glycine methyl ester (0.44 g, 3.56 mmol) to Tert-butyl 3-hydroxy-3-(2-hydroxy-3-methoxyphenyl)-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104b** as brown viscous (1157 mg, 88%). $U_{\max} / \text{cm}^{-1}$: 1702 (C=O), 3440 (OH); 1230 (N-C); $\text{C}_{18}\text{H}_{27}\text{NO}_7$, CHN calculated C(58.52%), H(7.37%), N(3.79%), O(30.32%), found: C(58.47%), H(7.18%), N(3.28%), O(30.52%); δ_{H} : (300 MHz, CDCl_3) 1.55 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.12 (s, 1H, NH), 2.93 (ddd, 2H, $J = 125.6, 12.4, 9.1$ Hz, $\text{CH}(\text{CH}_2\text{N})$), 3.59 (d, $J = 52.0$ Hz, 2H, NCH_2Ph), 3.75 (s, 1H, OCH_3), 3.82 (s, 1H, OCH_3), 4.20 (s, 1H, (dt, $J = 11.5, 9.2$ Hz, $\text{CHCH}(\text{CH}_2)$), 5.33 (m, 1H, (d, $J = 11.7$ Hz, $\text{CH}(\text{OH})$), 6.80 ((ddd, 2H, $J = 43.6, 7.5, 1.6$ Hz ArH), 7.21 (t, $J = 7.5$ Hz, 1H, ArH), δ_{C} (75 MHz, CDCl_3) 28.8 ($\text{C}(\text{CH}_3)_3$), 41.5 (CHCH_2N), 50.1 (CO_2CH_3), 52.3 (NCH_2CO_2), 52.7 ($\text{CH}(\text{CH}_2\text{N})$), 56.4 (PhOCH_3), 64.2, 82.2 ($\text{C}(\text{CH}_3)_3$), 109.3 (ArC), 120.0 (ArC), 122.1 (ArC), 127.5 (ArC), 144.5 ($\text{ArC}(\text{OH})$), 148.8 ($\text{ArC}(\text{OCH}_3)$), 169.0 ($\text{ArC}=\text{O}$), 173.2 (CO_2CH_3).

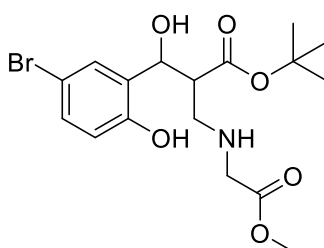
4.6.7. Tert-butyl 3-(3-ethoxy-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino)methyl) propanoate 104c.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl) acrylate **102c** (1 g, 3.39 mmol), glycine methyl ester (0.042 g, 3.39 mmol) to afford tert-butyl 3-(3-ethoxy-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104c** as yellow viscous oil (1078 mg, 83%). $U_{\max} / \text{cm}^{-1}$: 1699 (C=O), 3362 (OH); 1278 (N-C); $\text{C}_{19}\text{H}_{29}\text{NO}_7$ CHN calculated C(59.52%), H(7.62%), N(3.65%), O(29.21%), found: C(59.11%), H(7.13%), N(3.25%), O(29.51); δ_{H} (400 MHz, CDCl_3) 1.40 (t, $J = 6.0$ Hz, 3H, CH_3CH_2), 1.48 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.91 (dd, $J = 12.4, 8.0$ Hz, 1H, CHCHaN), 2.95 (s, 1H, NH), 3.06 (dd, $J = 12.4, 8.0$ Hz, 1H, CHCHbN), 3.58 (d, $J = 17.2$ Hz, 2H, NCH_2Ph), 3.78 (s, 3H, OCH_3), 4.05 (q, $J = 6.0$ Hz, 1H, CH_3CH_2), 4.20 (dt, $J = 10.8$,

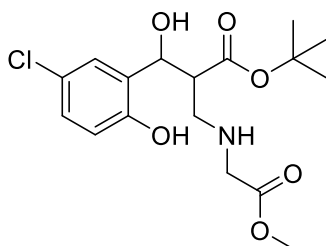
8.1 Hz, 1H, CHCH(CH₂)), 5.33 (d, *J* = 10.6 Hz, 1H, CH(OH)), 6.77 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar), 6.86 (dd, *J* = 7.5, 1.4 Hz, 1H, ArH), 7.22 (t, *J* = 7.5 Hz, 1H, ArH), δ_c (100 MHz, CDCl₃) 14.2 (CH₃CH₂), 28.8 (C(CH₃)₃), 41.5 (CHCH₂N), 50.1 (CO₂CH₃), 52.3 (NCH₂CO₂), 52.7 (CH(CH₂N), 56.4 (PhOCH₃), 64.2 (CH(OH), 66.7 (CH₃CH₂), 82.2 (C(CH₃)₃), 113.8, 118.2, 122.4, 127.8, 144.1(ArC(OH)), 148.3 (ArC(OCH₂CH₃)), 169.0 (ArC=O), 173.2 (CO₂CH₃).

4.6.8. Tert-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate 104d.



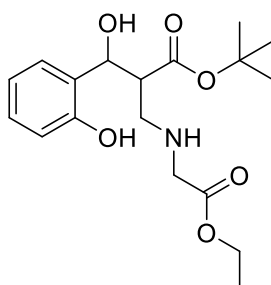
The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((5-bromo-2-hydroxyphenyl) (hydroxy) methyl) acrylate **102d** (1 gg, 3.03 mmol), glycine methyl ester (0.38 g, 3.04 mmol) to afford Tert-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104d** as yellow-off viscous liquid (1241 mg, 98%). U_{max} / cm⁻¹: 1671 (C=O), 3489 (OH); 1277 (N-C); C₁₇H₂₄BrNO₆, CHN calculated C(48.82%), H(5.78%), N(3.35%), O(22.95%), Br(19.10%), found: C(48.13%), H(5.17%), N(3.14%), O(22.26), Br(19.33%); δ_H (400 MHz, CDCl₃) 1.48 (s, 9H, C(CH₃)₃), 1.76 (s, 1H, NH), 2.93 (ddd, *J* = 125.6, 12.5, 8.2 Hz, 2H, CHCH₂N), 3.50 (s, 1H, NCHaPh), 3.79 (s, 3H, OCH₃), 3.83 (s, 1H, NCHbPh), 4.20 (dt, *J* = 10.8, 8.3 Hz, 1H, CHCH(CH₂)), 5.33 (d, *J* = 10.8 Hz, 1H, CH(OH)), 7.30 (dd, *J* = 7.5, 1.4 Hz, 1H, ArH), 7.38 (d, *J* = 7.5 Hz, 1H, ArH), 7.43 (d, *J* = 1.4 Hz, 1H, ArH), δ_c (100 MHz, CDCl₃) 28.8 (C(CH₃)₃), 41.5 (CHCH₂N), 50.1 (CO₂CH₃), 52.3 (NCH₂CO₂), 52.7 (CH(CH₂N), 56.4 (PhOCH₃), 64.2 (CH(OH), 82.2 (C(CH₃)₃), 115.1, 119.1 (ArC(Br)), 122.8, 128.7, 133.6, 155.0, 169.0, 173.2 (CO₂^tBu).

4.6.9. Tert-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate 104e.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((5-chloro-2-hydroxyphenyl) (hydroxy)methyl) acrylate **102e** (1 g, 3.52 mmol), glycine methyl ester (0.44 g, 3.52 mmol) to afford tert-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104e** as yellow-off solid (1289 mg, 98%). $U_{\max}$ / cm^{-1} : 1650 (C=O), 3420 (OH), 1277 (N-C); $\text{C}_{17}\text{H}_{24}\text{ClNO}_6$, CHN calculated C(54.62%), H(6.47%), N(3.75%), O(25.68%), Cl(9.48%), found: C(54.14%), H(6.94%), N(3.24%), O(25.96), Cl(9.48%); δ_{H} : (400 MHz, CDCl_3) 1.51 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.38 (s, 1H, NH), 2.93 (ddd, $J = 125.6, 12.5, 8.2$ Hz, 2H, CHCH_2N), 3.54 (d, $J = 5.5$ Hz, 2H, NCH_2Ph), 3.78 (s, 3H, OCH_3), 4.20 (dt, $J = 10.8, 8.2$ Hz, 1H, $\text{CHCH}(\text{CH}_2)$), 5.33 (d, $J = 11.0$ Hz, 1H, $\text{CH}(\text{OH})$), 7.14 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.28 (m, 2H, ArH), 7.43 (d, $J = 7.5$ Hz, 1H, ArH), δ_{C} (100 MHz, CDCl_3) 28.8 ($\text{C}(\text{CH}_3)_3$), 41.5, 52.3 ($\text{CHCH}(\text{CH}_2)$), 52.7 (OCH_3), 56.3, 64.2 ($\text{CH}(\text{OH})$), 82.2 ($\text{C}(\text{CH}_3)_3$), 115.1 ($\text{ArC}(\text{Br})$), 119.1, 122.8, 128.7, 133.6, 155.0 ($\text{ArC}(\text{OH})$), 169.0 (C=O glycine OMe), 173.2 (C=O).

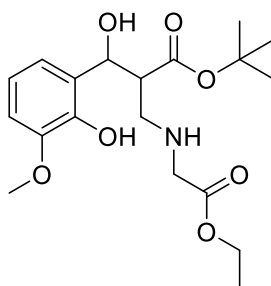
4.6.10. Tert-butyl 2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **105a**.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxyl (2-hydroxyphenyl) methyl) acrylate **102a** (1.0 g, 3.99 mmol), glycine ethyl ester (0.55 g, 3.99 mmol) to afforded tert-butyl 2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **105a** as brown viscous oil (1128 mg, 80%). $U_{\max}$ / cm^{-1} : 1711 (C=O), 3412 (OH) 1273 (N-C);

$C_{18}H_{27}NO_6$, CHN calculated C(61.17%), H(7.70%), N(3.96%), O(27.16%), found: C(61.25%), H(7.27%), N(3.15%), O(27.09); δ_H (400 MHz, $CDCl_3$) 1.37 (t, $J = 6.0$ Hz, 3H, CH_3CH_2), 1.47 (s, 9H, $C(CH_3)_3$), 2.47 (s, 1H, NH), 2.93 (ddd, $J = 125.6, 12.5, 8.2$ Hz, 2H, $CHCH_2N$), 3.63 (d, $J = 92.1$ Hz, 2H, NCH_2Ph), 4.20 (m, 3H, overlapping 2H, CH_2CH_3 and 1H, $CHCH(CH_2)$), 5.33 (d, $J = 10.6$ Hz, 1H, $CH(OH)$), 7.13 (td, $J = 7.4, 1.6$ Hz, 1H, ArH), 7.19 (dd, $J = 7.4, 1.6$ Hz, 1H, ArH), 7.24 (dd, $J = 7.4, 1.4$ Hz, 1H, ArH), 7.49 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH); δ_C (100 MHz, $CDCl_3$) 14.2 CH_3CH_2 , 28.8 ($C(CH_3)_3$), 41.5 ($CHCH_2N$), 50.1 (NCH_2Ph), 52.3 $CHCH(CH_2)$, 60.7 CH_2CH_3 , 64.2 ($CH(OH)$), 82.2 ($C(CH_3)_3$), 115.1, 122.0, 126.3, 127.3, 128.5, 155.6 (ArC(OH)), 169.0 ($C=O$), 173.2 ($C=O$).

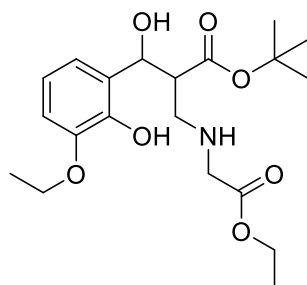
4.6.11. Methyl 1-(3-tert-butoxy-2-(hydroxyl (2-hydroxy-3-methoxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **105b.**



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxy-3-methoxyphenyl) methyl) acrylate **102b** (1 g, 3.56 mmol), glycine ethyl ester (0.49 g, 3.56 mmol) to afford Methyl 1-(3-tert-butoxy-2-(hydroxyl (2-hydroxy-3-methoxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **105b** as off-white gel (1215 mg, 89%). U_{max} / cm^{-1} : 1654 ($C=O$), 3330 (OH); 1298 (N-C); $C_{19}H_{29}NO_7$, CHN calculated C(59.52%), H(7.62%), N(3.65%), O(29.21%), found: C(59.30%), H(7.27%), N(3.47%), O(29.13%); δ_H : (400 MHz, $CDCl_3$) 1.38 (t, $J = 6.0$ Hz, 3H, CH_3CH_2), 1.48 (s, 9H, $C(CH_3)_3$), 2.18 (s, 1H, NH), 2.93 (m, 2H, $CHCH_2N$), 3.62 (d, $J = 58.4$ Hz, 2H, NCH_2Ph), 3.82 (s, 3H, $PhOCH_3$), 4.14 (q, $J = 6.0$ Hz, 2H CH_3CH_2), 4.20 (dt, $J = 11.0, 8.8$ Hz, 1H, $CHCH(CH_2)$), 5.33 (d, $J = 11.0$ Hz, 1H, $CH(OH)$), 6.76 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 6.85 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.21 (t, $J = 7.5$ Hz, 1H, ArH); δ_C (100 MHz, $CDCl_3$) 14.2 CH_3CH_2 , 28.8 ($C(CH_3)_3$), 41.5 ($CHCH_2N$), 50.1 (NCH_2Ph), 52.3 $CHCH(CH_2)$, 60.7 CH_2CH_3 ,

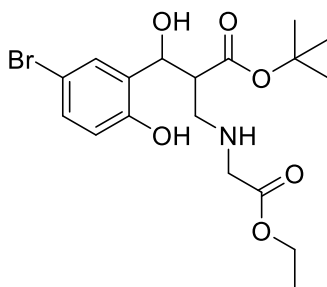
64.2 (CH(OH)), 82.2 ((C(CH₃)₃), 109.2, 121.3, 123.5, 127.3, 143.4 (ArC(OCH₃)), 148.5 (ArC(OH)), 169.0 (C=O), 173.2 (C=O).

4.6.12. Methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate 105c.



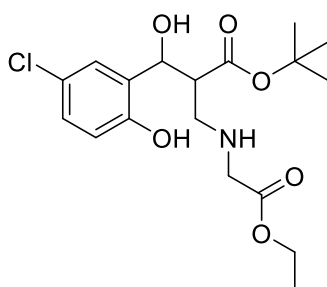
The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((3-ethoxy-2-hydroxyphenyl) (hydroxy) methyl) acrylate **102c** (1 g, 3.39 mmol), glycine ethyl ester (0.47 g, 3.39 mmol), to afford methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl) (hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **105c** as grey gel (1145 mg, 85%). $U_{\max}$ / cm⁻¹: 1678 (C=O), 3253 (OH); 1287 (N-C); C₂₀H₃₁N₂O₇, CHN calculated C(60.44%), H(7.86%), N(3.52%), O(28.18%), found: C(60.53%), H(7.17%), N(3.35%), O(28.09%) δ_H (400 MHz, CDCl₃) 1.37 (t, J = 5.9 Hz, 3H, CH₃CH₂OPh), 1.42 (t, J = 5.9 Hz, 3H CH₃CH₂OC(O)), 1.48 (s, 9H, C(CH₃)₃), 2.49 (s, 1H, NH), 2.93 (m, 2H, CHCH₂N), 3.53 (d, J = 46.5 Hz, 2H, NCH₂Ph), 4.03 (q, J = 5.9 Hz, 2H, PhCH₂CH₃), 4.19 (m, 3H, overlapping 1H, CHCH(CH₂)), and 2H, CH₃CH₂OC(O)), 5.33 (d, J = 10.8 Hz, 1H, C_H(OH)), 6.87 (dd, J = 7.5, 1.4 Hz, 1H, ArH), 6.95 (dd, J = 7.5, 1.4 Hz, 1H, ArH), 7.62 (m, 2H, ArH, overlapping 1H, CDCl₃ and 1H, ArH); δ_C (100 MHz, CDCl₃) 14.0 (CH₃CH₂OC(O)), 14.2 PhCH₂CH₃, 28.8 (C(CH₃)₃), 41.5 (CHCH₂N), 50.6 (NCH₂Ph), 52.3, 56.3, 60.7, 64.2, 82.2, 115.1, 119.2, 122.5, 127.3, 143.4, 148.5, 169.0, 173.2.

4.6.13. Methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate 105d.



The general procedure as described above in the section 4.6 was followed, using tert-butyl 2-((5-bromo-2-hydroxyphenyl) (hydroxy) methyl) acrylate **102d** (1 gg, 3.03 mmol), glycine ethyl ester (0.42 g, 3.03 mmol), to afford tert-butyl 3-(5-bromo-2-hydroxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **105d** as yellow-off viscous liquid (1270 mg, 97%). $U_{\max}$ / cm^{-1} : 1668 (C=O), 3475 (OH); 1279 (N-C); $\text{C}_{18}\text{H}_{26}\text{BrNO}_6$, CHN calculated C(50.01%), H(6.06%), N(3.24%), O(22.20%), Br(18.48%), found: C(50.11%), H(6.36%), N(3.88%), O(22.54%), Br(18.88%); δ_{H} (400 MHz, CDCl_3): 1.39 (t, J = 6.0 Hz, 3H, CH_3CH_2), 1.48 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.53 (s, 1H, NH), 2.93 (ddd, J = 125.6, 12.5, 8.6 Hz, 2H, CHCH_2N), 3.62 (d, J = 93.2 Hz, 2H, NCH_2Ph), 4.23 – 4.15 (m, 3H, overlapping 1H, $\text{CHCH}(\text{CH}_2)$), and 2H, $\text{CH}_3\text{CH}_2\text{OC}(\text{O})$), 5.33 (d, J = 10.3 Hz, 1H, $\text{CH}(\text{OH})$), 7.31 (dd, J = 7.4, 1.5 Hz, 1H, ArH), 7.38 (d, J = 7.5 Hz, 1H, ArH), 7.43 (d, J = 1.4 Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 14.2, 28.8, 41.5, 50.1, 52.3, 60.7, 64.2, 82.2, 115.3, 119.2 (ArC(Br)), 123.5, 127.3, 143.4, 154.7, 169.0 (C=O), 173.2 (C=O).

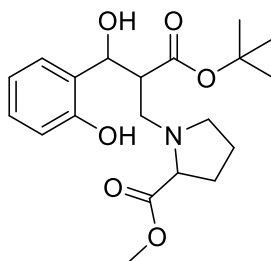
4.6.14. Tert-butyl 3-(5-chloro-2-hydroxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **105e**.



The general procedure as described above in the section 4.6 was followed, using tert-butyl 2-((5-chloro-2-hydroxyphenyl) (hydroxy)methyl) acrylate **102e** (1 g, 3.52 mmol), glycine ethyl ester (0.49 g, 3.52 mmol). This afforded tert-butyl 3-(5-chloro-2-hydroxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **105e** as yellow-off powder (1337 mg, 98%). $U_{\max}$ / cm^{-1} : 1650 (C=O), 3420 (OH), 1276 (N-

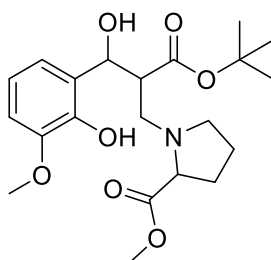
C); $C_{17}H_{26}ClNO_6$, CHN calculated C(55.74%), H(6.76%), N(3.61%), O(24.75%), Cl(9.14%), found: C(55.13%), H(6.17%), N(3.83%), O(24.97%), Cl(9.54%); δ_H (400 MHz, $CDCl_3$) 1.34 (t, $J = 6.0$ Hz, 3H, CH_3CH_2), 1.51 (s, 1H, NH), 1.52 (s, 9H, $C(CH_3)_3$), 2.93 (ddd, $J = 125.6, 12.5, 8.6$ Hz, 2H, $CHCH_2N$), 3.62 (d, $J = 66.1$ Hz, 2H, NCH_2Ph), 4.18 (m, 3H, overlapping 1H, $CHCH(CH_2)$), and 2H, $CH_3CH_2OC(O)$), 5.33 (d, $J = 10.6$ Hz, 1H, $CH(OH)$), 7.14 (dd, $J = 7.5, 1.6$ Hz, 1H, ArH), 7.25 (d, $J = 1.4$ Hz, 1H, ArH), 7.43 (d, $J = 7.5$ Hz, 1H, ArH); δ_C (100 MHz, $CDCl_3$) 14.2, 28.8, 41.5, 50.6, 52.3, 60.7, 64.2, 82.2, 119.7, 127.3, 128.0, 129.4, 130.4, 153.7, 169.0, 173.2,

4.6.15. Methyl 1-(3-tert-butoxy-2-(hydroxy(2-hydroxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate 106a.



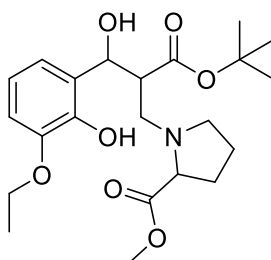
The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxyphenyl) methyl) acrylate **102a** (1.0 g, 3.99 mmol), proline methyl ester (0.66 g, 3.99 mmol) to afford Methyl 1-(3-tert-butoxy-2-(hydroxy(2-hydroxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **106a** as yellow-off oil (1362 mg, 90%). U_{max} / cm^{-1} : 1690 (C=O), 3336 (OH); 1321 (N-C); $C_{20}H_{29}NO_6$, CHN calculated C(63.31%), H(7.70%), N(3.69%), O(25.30%), found: C(63.66%), H(7.22%), N(3.51%), O(25.71%); δ_H (400 MHz, $CDCl_3$) 1.48 (s, 9H, $C(CH_3)_3$), 1.73 (m, 3H, ArH), 2.07 (m, 1H, ArH), 2.35 (dt, $J = 12.4, 7.6$ Hz, 1H, ArH), 3.20 (m, 5H, overlapping 4H, ArH and 1H, $CHCH(CH_2)$), 3.77 (s, 3H, OCH_3), 5.33 (d, $J = 10.8$ Hz, 1H, $CH(OH)$), 7.10 (td, $J = 7.5, 1.5$ Hz, 1H, ArH), 7.19 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.27 (m, 2H, ArH and $CDCl_3$), 7.39 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH); δ_C (100 MHz, $CDCl_3$) 25.1 (ArC), 28.8 ($C(CH_3)_3$), 29.8 (ArCHCH₂), 49.2 $CHCH_2N$, 50.4 $CHCH_2N$, 51.3 (OCH_3), 55.6 (NCH_2), 65.4 ($CH(OH)$), 71.2 ($NCHCO_2$), 82.9 ($C(CH_3)_3$), 116.6, 121.9, 126.5, 127.7, 128.7, 152.8 (ArC(OH)), 173.2 (CO_2tBu), 173.4 (C=O, proline OCH_3).

4.6.16. Methyl 1-(3-tert-butoxy-2-(hydroxyl (2-hydroxy-3-methoxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate 106b.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-(hydroxy(2-hydroxy-3-methoxyphenyl) methyl) acrylate **102b** (1 g, 3.56 mmol), proline methyl ester (0.58 g, 3.56 mmol) to afford Methyl 1-(3-tert-butoxy-2-(hydroxyl (2-hydroxy-3-methoxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **106b** as brown gel (1180 mg, 81%). $U_{\max} / \text{cm}^{-1}$: 1654 (C=O), 3330 (OH); 1298 (N-C); $\text{C}_{21}\text{H}_{31}\text{NO}_7$, CHN calculated C(61.60%), H(7.63%), N(3.42%), O(27.35%), found: C(61.32%), H(7.17%), N(3.87%), O(27.74%); δ_{H} (400 MHz, CDCl_3) 1.50 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.72 (m, 3H, ArH), 2.52 (m, 3H, ArH and 2H, $\text{CH}(\text{CH}_2\text{N})$), 3.23 (t, $J = 6.6$ Hz, 1H, $\text{NCH}(\text{CO})\text{CH}_2$), 3.79 (s, 3H, OCH_3 (proline OMe)), 3.84 (s, 3H, PhOCH_3), 4.20 (dt, $J = 10.4, 6.5$ Hz, 1H, $\text{CHCH}(\text{CH}_2\text{N})$), 5.33 (d, $J = 10.3$ Hz, 1H, $\text{CH}(\text{OH})$), 6.80 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 6.89 (dd, $J = 7.5, 1.6$ Hz, 1H, ArH), 7.22 (t, $J = 7.5$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 25.1 (ArC), 28.8 ($\text{C}(\text{CH}_3)_3$), 29.8 (ArCHCH_2), 49.2 CHCH_2N , 50.4 CHCH_2N , 51.3, (PhOCH_3), 55.6 (proline OCH_3), 57.6 (NCH_2), 65.4 ($\text{CH}(\text{OH})$), 71.2, 82.9, 109.0, 120.9, 122.9, 127.6, 144.1, 147.9, 170.5, 173.5,

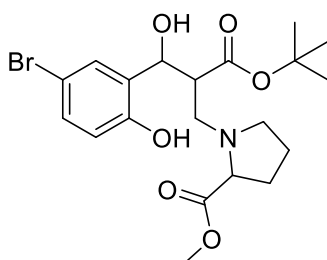
4.6.17. Methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate 106c.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((3-ethoxy-2-hydroxyphenyl)(hydroxy)methyl)acrylate **102c** (1 g, 3.39 mmol), proline methyl ester (0.66 g, 3.39 mmol), to afford Methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl) (hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-

carboxylate **106c** as grey gel (1148 mg, 80%). $U_{\max} / \text{cm}^{-1}$: 1678 (C=O), 3253 (OH); 1287 (N-C); $\text{C}_{22}\text{H}_{33}\text{NO}_7$, CHN calculated C(62.39%), H(7.85%), N(3.31%), O(26.44%), found: C(62.09%), H(7.99%), N(3.40%), O(26.29%); δ_{H} (400 MHz, CDCl_3) 1.42 (t, $J = 5.9$ Hz, 3H, $\text{CH}_3\text{CH}_2\text{OPh}$), 1.48 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.73 (m, 3H, ArH), 2.62 (m, 5H from 3H, ArH and 2H, $\text{CH}(\text{CH}_2\text{N})$), 3.38 (t, $J = 6.4$ Hz, 1H, $\text{NCH}(\text{CO})\text{CH}_2$), 3.82 (s, 3H, OCH_3 (proline OMe)), 4.04 (q, $J = 5.9$ Hz, 2H, $\text{CH}_3\text{CH}_2\text{OPh}$), 4.20 (dt, $J = 10.6, 6.8$ Hz, 1H, $\text{CHCH}(\text{CH}_2\text{N})$), 5.33 (d, $J = 10.8$ Hz, 1H, $\text{CH}(\text{OH})$), 6.78 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 6.92 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.24 (t, $J = 7.5$ Hz, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 25.1 (ArC), 14.4 (CH_3CH_2), 28.8 ($\text{C}(\text{CH}_3)_3$), 29.8 (CHCH_2), 49.2 CHCH_2N , 50.4 CHCH_2N , 51.3, (PhOCH_3), 55.6 (NCH_2), 63.8 (CH_3CH_2), 65.4($\text{CH}(\text{OH})$), 71.2 ($\text{NCH}(\text{CO}_2)$), 82.9 ($\text{C}(\text{CH}_3)_3$), 114.2, 119.4, 122.0, 127.0, 144.6, 147.8, 170.5 (C=O), 173.5 (C=O).

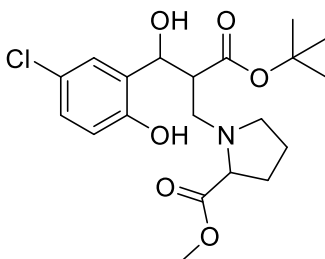
4.6.18. Methyl 1-(3-(5-bromo-2-hydroxyphenyl)-2-(tert-butoxycarbonyl)-3-hydroxypropyl)pyrrolidine-2-carboxylate 106d.



The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((5-bromo-2-hydroxyphenyl)(hydroxy)methyl)acrylate **102d** (1 gg, 3.03 mmol), proline methyl ester (0.50 g, 3.03 mmol), to afford methyl 1-(3-(5-bromo-2-hydroxyphenyl)-2-(tert-butoxycarbonyl)-3-hydroxypropyl) pyrrolidine-2-carboxylate **106d** as grey-off oil that solidified on standing (1374 mg, 99%). $U_{\max} / \text{cm}^{-1}$: 1700(C=O), 3457 (OH); 1340 (N-C); $\text{C}_{20}\text{H}_{28}\text{BrNO}_6$, CHN calculated C(52.41%), H(6.16%), N(3.06%), O(20.94%), Br(17.43%), found: C(52.62%), H(6.05%), N(3.64%), O(20.16%), Br(17.74%); δ_{H} (400 MHz, CDCl_3) 1.47 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.73 (m, 3H, ArH), 2.50 (m, 3H, ArH), 3.35 (t, $J = 8.3$ Hz, 1H, $\text{NCH}(\text{CO})\text{CH}_2$), 3.59 (dd, $J = 12.5, 8.1$ Hz, 2H, $\text{CH}(\text{CH}_2\text{N})$), 3.80 (s, 3H, OCH_3), 4.20 (dt, $J = 11.5, 8.0$ Hz, 1H, $\text{CH}(\text{CH}_2\text{N})$), 5.33 (d, $J = 11.5$ Hz, 1H, $\text{CH}(\text{OH})$), 7.26 (d, $J = 4.5$ Hz, 3H including 2H, ArH and 1H, s, CDCl_3), 7.44 (s, 1H, ArH); δ_{C} (100 MHz, CDCl_3) 25.1, 28.8 ($\text{C}(\text{CH}_3)_3$), 29.8, 49.2, 50.4 (CHCH_2N), 51.8 (proline OCH_3), 55.6 (ArNCH_2), 65.4 ($\text{CH}(\text{OH})$),

71.29, 82.9 ($C(CH_3)_3$), 115.9, 119.2, 122.8, 128.6, 135.0 ($ArCHC(Br)$), 153.8 ($ArC(OH)$), 170.5 ($C=O$), 173.5 ($C=O$).

4.6.19. Methyl 1-(3-tert-butoxy-2-((5-chloro-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **106e.**



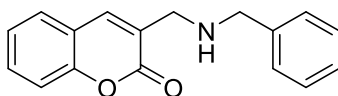
The general procedure as described above in the the section 4.6 was followed, using tert-butyl 2-((5-chloro-2-hydroxyphenyl) (hydroxy) methyl) acrylate **102e** (1 g, 3.52 mmol), proline methyl ester (0.58 g, 3.52 mmol), to afford methyl 1-(3-tert-butoxy-2-((5-chloro-2-hydroxyphenyl) (hydroxy)methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **106e** as off-white solid (1427 mg, 98%). U_{max} / cm^{-1} : 1690 ($C=O$), 3420 (OH), 1273 ($N-C$); $C_{20}H_{28}ClNO_6$, CHN calculated C(58.04%), H(6.82%), N(3.38%), O(23.19%), Cl(8.56%), found: C(58.11%), H(6.29%), N(3.17%), O(23.87%), Cl(6.33%); δ_H (400 MHz, $CDCl_3$) 1.46 (s, 9H, $C(CH_3)_3$), 1.76 (m, 3H, ArH), 2.43 (m, 3H, ArH), 3.38 (dd, $J = 16.8, 7.6$ Hz, 1H, $CH(CH_2N)$), 4.20 (dt, $J = 11.4, 5.5$ Hz, 1H $CH(CH_2N)$), 5.33 (d, $J = 11.7$ Hz, 1H, $CH(OH)$), 7.10 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.30 (d, $J = 1.6$ Hz, 1H, ArH), 7.44 (d, $J = 7.3$ Hz, 1H, ArH); δ_C (100 MHz, $CDCl_3$) 25.1, 28.8 ($C(CH_3)_3$), 29.8, 49.2, 50.4 ($CHCH_2N$), 51.8 (proline OCH_3), 55.6 ($ArNCH_2$), 65.4 ($CH(OH)$), 71.29, 82.9 ($C(CH_3)_3$), 118.1, 127.0, 127.9, 129.7, 132.4 ($ArC(Cl)$), 154.2 ($ArC(OH)$), 171.2 ($C=O$), 173.5 ($C=O$).

4.7. General procedure for the cyclization of aza-Michael adduct to coumarin **107-110a-e.** ^{201-202, 213}

A mixture of the aza-Michael adducts **103-105** (1 mmol), and 15% ethanolic KOH (20 ml) was refluxed for 5 h. The resulting mixture was washed with water and brine and the organic layer extracted 3 times with ethyl acetate, dried over $MgSO_4$ and the solvent removed in vacuo to give a a crude, which was purified by preparative layer

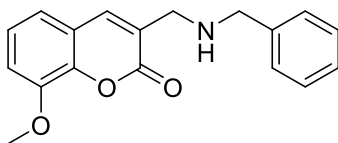
chromatography on silica; elution with CHCl_3 -EtOAc (3:1) to afford the final pure products.²¹³

4.7.1. Synthesis of 3-[(benzylamino)methyl]coumarin **107a**.



The general procedure as described above in the section 4.7 was followed, using Tert-butyl 2-((benzylamino)methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **103a** (1.00 g, 2.73 mmol), to afford 3-[(benzylamino)methyl]coumarin **107a** as off-white solid (461.8 mg, 66%); m.p. 72-75 °C (reported values: 70-74° C²⁰¹⁻²⁰²); ν_{max} / cm^{-1} : 1715 (C=O), 3324 (N-H); $\text{C}_{17}\text{H}_{15}\text{NO}_2$, CHN calculated C(76.96%), H(5.70%), N(5.28%), O(12.06%), found: C(76.33%), H(5.51%), N(5.64%), O(12.26%); δ_{H} (300 MHz, CDCl_3) 2.26 (s, 1H, NH), 3.37 (dd, $J = 3.6, 0.8$ Hz, 2H, $\text{NCH}_2\text{C(O)}$), 3.99 (s, 2H, PhCH_2N), 7.19 (m, 3H, ArH), 7.30 (m, 4H, ArH), 7.42 (td, $J = 7.5, 1.5$ Hz, 1H, ArH), 7.67 (dd, $J = 7.3, 1.5$ Hz, 1H, ArH), 7.94 (s, 1H, ArH), δ_{C} (75 MHz; CDCl_3) 48.3 (PhCH_2N), 53.2 ($\text{NCH}_2\text{C(O)}$), 116.5, 120.2, 126.0, 126.4, 127.0, 127.5, 127.7, 128.3, 128.5, 128.8, 139.6 (ArCH at the 4 position), 140.5 (NCH_2ArC), 153.8 (ArC-O-C=O), and 160.5 (C=O).

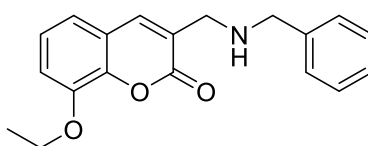
4.7.2. 3-[(benzylamino)methyl]-8-methoxycoumarin **107b**.



The general procedure as described above in the section 4.7 was followed, using Tert-butyl 2-((benzylamino)methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **103b** (1.00 g, 2.50 mmol), to afford 3-[(benzylamino)methyl]-8-methoxycoumarin as **107b** as pale yellow viscous oil (443 mg, 60%); ν_{max} / cm^{-1} 3380 (N-H) and 1720 (C=O); $\text{C}_{18}\text{H}_{17}\text{NO}_3$, CHN calculated C(73.20%), H(5.80%), N(4.74%), O(16.25%), found: C(73.69%), H(5.18%), N(4.13%), O(16.83%); δ_{H} (300 MHz, CDCl_3) 2.11 (s, 1H, NH), 3.38 (d, $J = 0.8$ Hz, 2H, $\text{NCH}_2\text{C(O)}$), 3.85 (s, 3H, PhOCH_3), 3.98 (d, $J = 3.1$

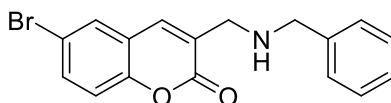
Hz, 2H, PhCH₂N), 7.07 (dd, *J* = 7.5, 1.5 Hz, 1H, ArH), 7.18 (dd, *J* = 9.9, 5.0 Hz, 1H, ArH), 7.23 (m, 2H, ArH), 7.27 (d, *J* = 1.3 Hz, 1H, ArH), 7.31 (m, 2H, ArH), 7.42 (dd, *J* = 7.4, 1.5 Hz, 1H, ArH), 7.97 (s, 1H, ArH); δ_c (75 MHz; CDCl₃) 51.3 (PhCH₂N) and 53.2 (NCH₂C(O)), 55.7 (OCH₃), 114.4, 120.0, 121.7, 126.3, 127.7, 127.8, 128.1, 128.4, 128.6, 128.7, 139.6 (ArCH at the 4 position), 140.5 (NCH₂ArC), 141.7 (ArC-O-C=O), 149.1 (ArC(OCH₃), 162.5 (C=O).

4.7.3. 3-[(benzylamino)methyl]-8-ethoxycoumarin **107c**.



The general procedure as described above in the section 4.7 was followed, using tert-butyl-2-((benzylamino)methyl)-3-(3-ethoxy-2-hydroxyphenyl)-3-hydroxypropanoate **103c** (1.00 g, 2.51 mmol), to afford 3-[(benzylamino)methyl]-8-ethoxycoumarin **107c** as a pale yellow solid (450.3 mg, 58%), m.p. 95-98 °C; ν_{max} / cm⁻¹ 3410 (N-H) and 1710 (C=O); C₁₉H₁₉N₃O₃, CHN calculated C(73.77%), H(6.19%), N(4.53%), O(15.51%), found: C(73.34%), H(6.77%), N(4.38%), O(15.10%); δ_H (300 MHz; CDCl₃) 1.41 (t, *J* = 5.9 Hz, 3H, CH₃CH₂), 2.27 (s, 1H, NH), 3.36 (dd, *J* = 7.4, 0.8 Hz, 2H, NCH₂C(O)), 3.98 (s, 2H, PhCH₂N), 4.05 (q, *J* = 5.9 Hz, 2H, CH₃CH₂), 6.98 (dd, *J* = 7.5, 1.5 Hz, 1H, ArH), 7.14 (t, *J* = 7.5 Hz, 1H, ArH), 7.31 (m, 6H, ArH), 7.88 (s, 1H, ArH); δ_c (75 MHz; CDCl₃) 14.2 (CH₃CH₂), 51.3 and 53.2 (CH₂), 64.2 (OCH₂CH₃), 114.4, 120.0, 121.7, 126.3, 127.7, 127.8, 128.1, 128.4, 128.6, 128.7, 139.6 (ArCH at the 4 position), 140.5, 141.7, 153.6 (Ar-C(OCH₂CH₃), 162.5(C=O).

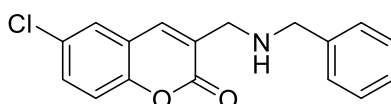
4.7.4. 3-[(benzylamino)methyl]-6-bromocoumarin **107d**.²⁰¹⁻²⁰¹



The general procedure as described above in the section 4.7 was followed, using tert-butyl 2-((benzylamino)methyl)-3-(5-bromo-2-hydroxyphenyl)-3-hydroxypropanoate

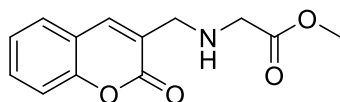
hydroxypropanoate propanoate **103d** (1.00 g, 2.35 mmol), to afford 3-[(benzylamino)methyl]-6-bromocoumarin **107d** as a pale-yellow solid (631 mg, 78%), m.p. 104-107 °C (reported as 107-110°C)²⁰¹⁻²⁰²; $U_{\text{max.}} / \text{cm}^{-1}$ 3314 (N-H) and 1730 (C=O); $\text{C}_{17}\text{H}_{14}\text{BrNO}_2$, CHN calculated C(59.32%), H(4.10%), Br(23.21%), N(4.07%), O(9.30%), found: C(59.98%), H(4.20%), Br(23.65%), N(4.18%), O(9.21%); δ_{H} (300 MHz; CDCl_3) 2.81 (s, 1H, NH), 3.33 (s, 2H, NCH_2Ph), 3.94 (s, 2H, PhCH_2N), 7.10 (d, $J = 7.5$ Hz, 1H, ArH), 7.22 (m, 5H, ArH), 7.61 (dd, $J = 7.5, 1.5$ Hz, 1H, ArH), 7.84 (s, 1H, ArH), 7.94 (s, 1H, ArH at the 4th position, on carbon 7); δ_{C} (75 MHz; DMSO-d_6) 52.4 (PhCH_2N), 53.6 (NCH_2Ph), 118.3, 121.2 (ArC(Br)), 122.9, 124.2, 125.0, 126.1, 126.8, 127.0, 128.6, 130.7, 134.0, 138.7 (ArCH at the 4th position), 143.2, 151.7 ArC-O-C=O), 161.2 (C=O).

4.7.5. 3-[(benzylamino)methyl]-6-chlorocoumarin **107e**.²⁰¹⁻²⁰²



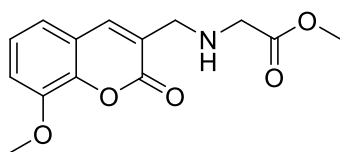
The general procedure as described above in the section 4.7 was followed, using tert-butyl 2-((benzylamino)methyl)-3-(5-bromo-2-hydroxyphenyl)-3-hydroxypropanoate **103e** (1.00 g, 2.55 mmol), to afford 3-[(benzylamino)methyl]-6-chlorocoumarin **107e** as a yellow solid (573.6 mg, 75%), m.p. 105-108 °C; $U_{\text{max.}} / \text{cm}^{-1}$ 3340 (N-H) and 1720 (C=O); $\text{C}_{17}\text{H}_{14}\text{ClNO}_2$, CHN calculated C(68.12%), H(4.71%), Cl(11.83%), N(4.67%), O(10.67%), found: C(68.62%), H(4.51%), Cl(11.63%), N(4.68%), O(10.19%); δ_{H} (300 MHz; CDCl_3) 1.99 (s, 1H, NH), 3.37 (s, 1H, $\text{NCH}_2\text{C(O)}$), 3.98 (d, $J = 4.6$ Hz, 2H, PhCH_2N), 7.16 (d, $J = 7.5$ Hz, 1H, ArH), 7.25 (m, 2H, ArH and CDCl_3), 7.29 (m, 2H), 7.30 (d, $J = 3.3$ Hz, 1H), 7.35 (m, 1H, ArH), 7.45 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.68 (d, $J = 1.5$ Hz, 1H, ArH), 7.88 (s, 1H, ArH at the 4th position); δ_{C} (75 MHz; CDCl_3) 51.3 (PhCH_2N), 53.2 ($\text{NCH}_2\text{C(O)}$), 118.0 (ArC(Cl)), 123.0, 124.2, 127.1, 127.2, 127.5, 127.7, 128.3, 128.5, 131.5, 134.6, 139.6, 141.2, 152.3, 162.5 (C=O).

4.7.6. Methyl ((2-oxo-2H-coumarin-3-yl)methyl)glycinate **108a**.



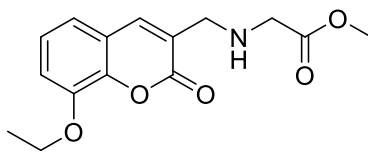
The general procedure as described above in the section 4.7 was followed, using tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104a** (1.00 g, 2.94 mmol), to afford methyl ((2-oxo-2H-coumarin-3-yl)methyl)glycinate **108a** as a brown viscous liquid (437.1 mg, 60%), $U_{\max}$. / cm^{-1} 3350 (N-H) and 1719 (C=O); $\text{C}_{13}\text{H}_{13}\text{NO}_4$, CHN calculated C(63.15%), H(5.30%), N(5.67%), O(25.88%), found: C(63.35%), H(5.13%), N(5.16%), O(25.59%); δ_{H} (300 MHz, DMSO), 3.33 (dd, $J = 3.2, 0.8$ Hz, 2H, NCH_2Ph), 3.55 (s, 2H, $\text{PhCH}_2\text{C(O)}$), 3.65 (s, 1H, NH), 3.73 (s, 3H, OCH_3), 7.18 (dd, $J = 10.7, 4.6$ Hz, 2H, ArH), 7.42 (td, $J = 7.6, 1.5$ Hz, 1H, ArH), 7.61 (m, 1H, ArH), 7.75 (s, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 47.5 (NCH_2CO_2), 52.4 (PhCH_2N), 53.6 (CO_2CH_3), 115.2, 121.1, 126.9, 127.8, 128.3, 129.4, 139.8, 152.4, 162.5 (ArC(O)), 172.3 (CO_2CH_3).

4.7.7. Methyl ((8-methoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **108b**.



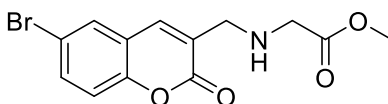
The general procedure as described above in the section 4.7 was followed, using tert-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104b** (1.00 g, 2.74 mmol), to afford Methyl ((8-methoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **108b** as a brown viscous liquid (403.2 mg, 53%), $U_{\max}$. / cm^{-1} 3348 (N-H) and 1732 (C=O); $\text{C}_{14}\text{H}_{15}\text{NO}_5$, CHN calculated C(60.64%), H(5.45%), N(5.05%), O(28.85%), found: C(60.66%), H(5.74%), N(5.25%), O(28.35.%); δ_{H} (300 MHz, DMSO- d_6) 2.36 (s, 1H, NH), 3.36 (dd, $J = 5.6, 0.8$ Hz, 1H, $\text{NCH}_2\text{C(O)}$), 3.56 (s, 2H, PhCH_2N), 3.78 (s, 3H, OCH_3), 3.80 (s, 3H, PhOCH_3), 6.99 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.17 (t, $J = 7.5$ Hz, 1H, ArH), 7.44 (dd, $J = 7.5, 1.5$ Hz, 1H, ArH), 8.01 (s, 1H, ArH); δ_{C} (75 MHz, DMSO d_6) 47.5 (NCH_2CO_2), 52.4 (PhCH_2N), 53.6 (CO_2CH_3), 56.5 (PhOCH_3), 113.1, 120.6, 121.5, 126.1, 127.6, 139.8 (ArCH), 140.4, 148.5, 162.4 (ArC(O)), 172.3 (CO_2CH_3).

4.7.8. Methyl ((8-ethoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **108c**.



The general procedure as described above in the section 4.7 was followed, using tert-butyl 3-(3-ethoxy-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl)propanoate **104c** (1.00 g, 2.60 mmol), to afford methyl ((8-ethoxy-2-oxo-2H-coumarin-3-yl)methyl) glycinate **108c** as a yellow viscous liquid (417.8 mg, 55%), $U_{\text{max.}} / \text{cm}^{-1}$ 3344 (N-H) and 1717 (C=O); $\text{C}_{15}\text{H}_{17}\text{NO}_5$, CHN calculated C(61.85%), H(5.88%), N(4.81%), O(27.46%), found: C(61.14%), H(5.19%), N(4.61%), O(27.86%); δ_{H} (300 MHz, DMSO- d_6) 2.36 (s, 1H, NH), 1.41 (t, $J = 5.9$ Hz, 3H, CH_2CH_3), 2.71 (s, 1H, NH), 3.37 (dd, $J = 28.4, 0.8$ Hz, 2H, $\text{NCH}_2\text{C}(\text{O})$), 3.65 (d, $J = 11.8$ Hz, 2H, PhCH_2N), 3.76 (s, 3H, OCH_3), 4.04 (q, $J = 5.9$ Hz, 2H, CH_2CH_3), 6.99 (dd, $J = 7.4, 1.6$ Hz, 1H, ArH), 7.16 (t, $J = 7.5$ Hz, 1H, ArH), 7.26 (m, 2H, overlapping CDCl_3 and ArH), 7.63 (s, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 14.1 (CH_3CH_2), 47.5(NCH_2CO_2), 52.4 (PhCH_2N), 53.6 (CO_2CH_3), 64.6 (CH_3CH_2), 115.0, 119.0, 120.4, 126.5, 127.8, 138.9, 140.7, 154.1, 161.6 (ArC(O)), 172.3 (CO_2CH_3).

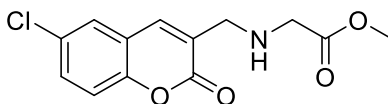
4.7.9. Methyl ((6-bromo-2-oxo-2H-coumarin-3-yl)methyl)glycinate **108d**.



The general procedure as described above in the section 4.7 was followed, using tert-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104d** (1.00 g, 2.39 mmol), to afford methyl ((6-bromo-2-oxo-2H-coumarin-3-yl)methyl)glycinate **108d** as a grey viscous gel (623.7 mg, 80%), $U_{\text{max.}} / \text{cm}^{-1}$ 3330 (N-H) and 1715 (C=O); $\text{C}_{13}\text{H}_{12}\text{BrNO}_4$, CHN calculated C(47.88%), H(3.71%), Br(24.50%), N(4.29%), O(16.62%), found: C(47.67%), H(3.55%), Br(24.37%), N(4.18%), O(16.97%); δ_{H} (300 MHz, DMSO- d_6) 3.33 (dd, $J = 17.6, 0.8$ Hz, 2H, $\text{NCH}_2\text{C}(\text{O})$), 3.57 (d, $J = 16.7$ Hz, 2H, PhCH_2N), 3.73 (s, 3H, OCH_3), 4.47 (s, 1H, NH), 7.09 (d, $J = 7.5$ Hz, 1H, ArH), 7.57 (m, 1H, ArH), 7.72 (d, $J = 1.5$ Hz, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 47.5(NCH_2CO_2), 52.4 (PhCH_2N), 53.6 (CO_2CH_3),

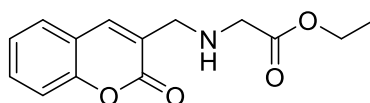
118.0, 119.8 (ArC(Br)), 123.9, 128.0, 131.3, 134.7, 139.8, 153.5, 162.5, (ArC(O)), 172.3 (CO₂CH₃).

4.7.10. Methyl ((6-chloro-2-oxo-2H-chromen-3-yl)methyl)glycinate **108e**.



The general procedure as described above in the section 4.7 was followed, using tert-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-((2-methoxy-2-oxoethylamino) methyl) propanoate **104e** (1.00g, 2.67 mmol) to afford methyl((6-chloro-2-oxo-2H-chromen-3-yl)methyl)glycinate **108e** as a grey viscous liquid which solidified on standing (527.4 mg, 70%), U_{max}. / cm⁻¹ 3340 (N-H) and 1722 (C=O); C₁₃H₁₂ClNO₄, CHN calculated C(55.43%), H(4.29%), Cl(12.58%), N(4.97%), O(22.72%), found: C(55.14%), H(4.18%), Cl(12.64%), N(4.67%), O(22.55%); δ_H (300 MHz, DMSO-d₆) 2.50 (m, DMSO d₆), 3.33 (d, *J* = 17.4 Hz, 2H, NCH₂C(O)), 3.57 (d, *J* = 16.8 Hz, 1H, PhCH₂N), 3.77 (s, 3H, OCH₃), 4.48 (s, 1H, NH), 7.14 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.58 (2x d, *J* = 12.7 Hz, 2H, ArH); δ_C (75 MHz, DMSO-d₆) 47.5(NCH₂CO₂), 52.4 (PhCH₂N), 53.6 (CO₂CH₃), 119.8 (ArC(Cl)), 122.8, 126.5, 127.5, 129.9, 132.7, 139.8, 152.3, 162.5 (ArC(O)), 172.3 (CO₂CH₃).

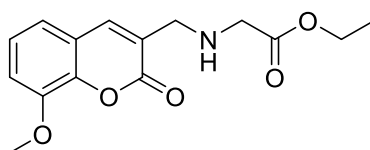
4.7.11. Ethyl ((2-oxo-2H-ccoumarin-3-yl)methyl)glycinate **109a**.



The general procedure as described above in the section 4.7 was followed, using tert-butyl 2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxy-3-(2-hydroxyphenyl) propanoate **105a** (1.00 g, 2.82 mmol) to afford ethyl ((2-oxo-2H-ccoumarin-3-yl)methyl)glycinate **109a** as a grey viscous liquid which solidified on standing (458.3 mg, 62%); U_{max}. / cm⁻¹ 3345 (N-H) and 1726 (C=O); C₁₄H₁₅N₂O₄, CHN calculated C(64.36%), H(5.79%), N(5.36%), O(24.49%), found: C(64.30%), H(5.55%), N(5.67%), O(24.62%); δ_H (300 MHz, DMSO-d₆) 1.36 (t, *J* = 6.0 Hz, 3H, CH₃CH₂), 3.34 (dd, *J* = 10.0, 0.8 Hz, 2H, PhCH₂N), 3.45 (s, 1H, NH), 3.58 (d, *J* = 5.0 Hz, 2H,

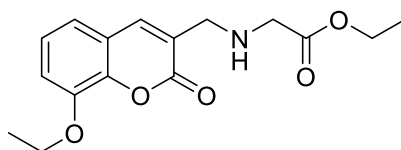
NCH₂C(O)), 4.14 (q, *J* = 6.0 Hz, 2H, CH₂CH₃), 7.15 (m, 2H, ArH), 7.42 (td, *J* = 7.5, 1.4 Hz, 1H ArH), 7.57 (m, 1H ArH), 7.67 (s, 1H ArH); δ_C (75 MHz, DMSO-d₆) 14.1 (CH₃CH₂), 48.3 (NCH₂CO₂), 53.6 PhCH₂N), 61.7 (CH₃CH₂), 115.1, 122.3, 126.1, 127.6, 128.1, 129.0, 138.9, 154.0 (ArCOC(O)), 162.7 (ArC=O), 173.2 (C=O of the ethyl ester).

4.7.12. Ethyl ((8-methoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109b**.



The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-tert-butoxy-2-(hydroxyl (2-hydroxy-3-methoxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **105b** (1.00g, 2.60 mmol) to afford ethyl ((8-methoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109b** as a grey viscous liquid which solidified on standing (440.6 mg, 58%), U_{max}. / cm⁻¹ 3355 (N-H) and 1715 (C=O); C₁₅H₁₇N₂O₅, CHN calculated C(61.85%), H(5.88%), N(4.81%), O(27.46%), found: C(61.15%), H(5.17%), N(4.99%), O(27.71%); δ_H (300 MHz, DMSO-d₆) 1.37 (t, *J* = 6.0 Hz, 3H, CH₃CH₂), 2.34 (s, 1H, NH), 3.75 (m, 3H, overlapping NCH₂C(O) and the water in DMSO-d₆), 3.58 (d, *J* = 14.3 Hz, 2H, PhCH₂N), 3.78 (s, 3H, OCH₃), 4.18 (q, *J* = 6.0 Hz, 2H, CH₂CH₃), 6.97 (dd, *J* = 7.4, 1.5 Hz, 1H, ArH), 7.13 (t, *J* = 7.5 Hz, 1H, ArH), 7.21 (dd, *J* = 7.5, 1.6 Hz, 1H, ArH), 7.51 (s, 1H, ArH); δ_C (75 MHz, DMSO d₆) 14.1 (CH₃CH₂), 48.3 (NCH₂CO₂), 53.6 PhCH₂N), 55.6 (PhOCH₃), 61.7 (CH₃CH₂), 114.1, 120.2, 122.0, 126.2, 127.6, 139.9, 141.3 (ArCOC(O)), 148.5 (ArCOCH₃), 162.7 (ArC=O), 173.2 (C=O of the ethyl ester).

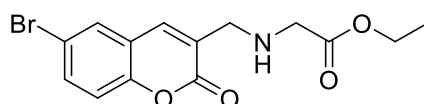
4.7.13. Ethyl ((8-ethoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109c**.



The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl)-3-

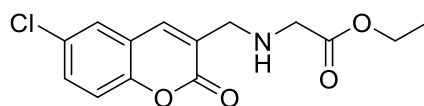
oxopropyl)pyrrolidine-2-carboxylate **105c** (1.00 g, 2.51 mmol) to afford ethyl ((8-ethoxy-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109c** as a grey viscous liquid which solidified on standing (422.7 mg, 55%), $U_{\text{max.}} / \text{cm}^{-1}$ 3341 (N-H) and 1724 (C=O); $\text{C}_{16}\text{H}_{19}\text{NO}_5$, CHN calculated C(62.94%), H(6.27%), N(4.59%), O(26.20%), found: C(62.72%), H(6.66%), N(4.45%), O(26.77%); δ_{H} (300 MHz, DMSO- d_6) 1.38 (td, J = 6.0, 2.6 Hz, 6H, overlapping 2x CH_3CH_2), 2.34 (s, 1H, NH), 3.36 (m, 4H, overlapping $\text{NCH}_2\text{C}(\text{O})$, and water in DMSO- d_6), 3.59 (d, J = 11.7 Hz, 2H, NCH_2Ph), 4.03 (q, J = 6.0 Hz, 2H, $\text{CH}_3\text{CH}_2\text{OPh}$), 4.18 (q, J = 6.0 Hz, 2H, $\text{CH}_3\text{CH}_2\text{OC}(\text{O})$), 7.16 (m, 2H, ArH), 7.37 (dd, J = 7.0, 1.9 Hz, 1H, ArH), 7.54 (s, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 14.1 ($\text{CH}_3\text{CH}_2\text{CO}_2$), 14.8 ($\text{CH}_3\text{CH}_2\text{OPh}$), 48.3 (NCH_2CO_2), 53.6 PhCH_2N), 61.7 (CH_3CH_2), 64.7 ($\text{CH}_3\text{CH}_2\text{OPh}$), 115.0, 118.4, 121.0, 126.6, 128.5, 139.9, 141.2 (ArCOC(O)), 154.1 (ArC(OCH $_2$ CH $_3$), 162.7 (ArC=O), 173.2 (C=O of the ethyl ester).

4.7.14. Ethyl ((6-bromo-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109d**.



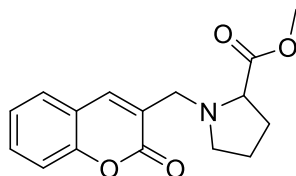
The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl)pyrrolidine-2-carboxylate **105d** (1.00g, 2.31 mmol) to afford ethyl ((6-bromo-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109d** as a grey viscous liquid which solidified on standing (534.3 mg, 68%), $U_{\text{max.}} / \text{cm}^{-1}$ 3401 (N-H) and 1724 (C=O); $\text{C}_{14}\text{H}_{14}\text{BrNO}_4$, CHN calculated C(49.43%), H(4.15%), Br(23.49%), N(4.12%), O(18.81%), found: C(49.15%), H(4.12%), Br(23.13%), N(4.25%), O(18.55); δ_{H} (300 MHz, DMSO- d_6) 1.36 (t, J = 6.0 Hz, 3H, CH_3CH_2), 3.34 (dd, J = 10.0, 0.8 Hz, 2H, $\text{NCH}_2\text{C}(\text{O})$), 3.45 (s, 1H, NH), 3.58 (d, J = 5.0 Hz, 2H, PhCH_2N), 4.14 (q, J = 6.0 Hz, 2H, CH_3CH_2), 7.17 (m, 1H, ArH), 7.42 (td, J = 7.5, 1.4 Hz, 1H, ArH), 7.60 (m, 1H, ArH), 7.67 (s, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 14.1 (CH_3CH_2), 48.3 (NCH_2CO_2), 53.6 PhCH_2N), 61.7 (CH_3CH_2), 116.0, 119.2 (ArC(Br)), 123.8, 127.4, 131.5, 135.0, 139.8, 153.3 (ArCOC(O)), 162.7 (ArC=O), 173.2 (C=O of the ethyl ester).

4.7.15. Ethyl ((6-chloro-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109e**.

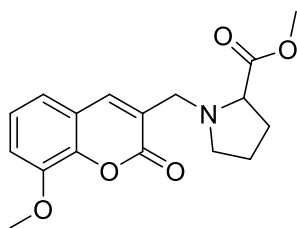


The general procedure as described above in the section 4.7 was followed, using tert-butyl 3-(5-chloro-2-hydroxyphenyl)-2-((2-ethoxy-2-oxoethylamino) methyl)-3-hydroxypropanoate **105e** (1.00g, 2.57 mmol) to afford ethyl ((6-chloro-2-oxo-2H-coumarin-3-yl)methyl)glycinate **109e** as a grey viscous liquid which solidified on standing (564.2 mg, 74%), $\nu_{\text{max.}} / \text{cm}^{-1}$ 3347 (N-H) and 1713 (C=O); $\text{C}_{14}\text{H}_{14}\text{ClN}\text{O}_4$, CHN calculated C(56.86%), H(4.77%), Cl(11.99%), N(4.74%), O(21.64%), found: C(56.66%), H(4.38%), Cl(11.78%), N(4.64%), O(21.99%); δ_{H} (300 MHz, DMSO- d_6) 1.36 (t, $J = 6.0$ Hz, 3H, CH_3CH_2), 3.33 (dd, $J = 17.8, 0.8$ Hz, 1H, NCH_2CO_2), 3.56 (d, $J = 16.9$ Hz, 2H, PhCH_2N), 4.15 (q, $J = 6.0$ Hz, 2H, CH_3CH_2), 4.29 (s, 1H, NH), 7.15 (2x d, $J = 7.5$ Hz, 2H, ArH), 7.45 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.58 (d, $J = 1.5$ Hz, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 14.1 (CH_3CH_2), 48.3 (NCH_2CO_2), 53.6 (PhCH_2N), 61.7 (CH_3CH_2), 117.6, 122.3, 126.5, 127.7, 129.3, 130.9 (ArC(Cl)), 139.8, 153.3 (ArCOC(O)), 162.7 (ArC=O), 173.2 (C=O of the ethyl ester).

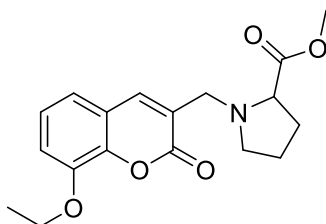
4.7.16. Methyl ((2-oxo-2H-coumarin-3-yl)methyl)prolinate **110a**.



The general procedure as described above in the section 4.7 **was** followed, using methyl 1-(3-tert-butoxy-2-(hydroxy(2-hydroxyphenyl) methyl)-3-oxopropyl)pyrrolidine-2-carboxylate **106a** (1.00g, 2.63 mmol) to afford methyl ((2-oxo-2H-coumarin-3-yl)methyl)prolinate **110a** as a grey viscous liquid which solidified on standing (477 mg, 63%), $\nu_{\text{max.}} / \text{cm}^{-1}$ 3356 (N-H) and 1722 (C=O); $\text{C}_{16}\text{H}_{17}\text{NO}_4$, CHN calculated C(66.89%), H(5.96%), N(4.88%), O(22.27%), found: C(66.59%), H(5.16%), N(4.72%), O(22.54%); δ_{H} (300 MHz, DMSO- d_6) 1.85 (m, 4H, ArH); 2.79 (qt, $J = 12.5, 6.4$ Hz, 2H, ArH), 3.20 (m, 3H, ArH), 3.69 (s, 3H, OCH_3), 7.16 (dd, $J = 11.3, 4.4$ Hz, 2H, ArH), 7.38 (m, 2H, ArH), 7.55 (m, 1H, ArH); δ_{C} (75 MHz, DMSO- d_6) 24.4 (ArC), 29.0 (ArC), 51.1 (OCH_3), 56.3 (PhCH_2N), 57.5 (ArC), 71.7 (ArCHCO $_2$), 115.1, 121.5, 125.46, 127.3, 128.5, 129.9, 139.8, 153.0, 163.2 (ArCO), 173.5 (C=O)

4.7.17. Methyl ((8-methoxy-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110b**.

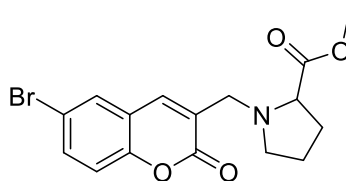
The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-tert-butoxy-2-(hydroxyl (2-hydroxy-3-methoxyphenyl) methyl)-3-oxopropyl) pyrrolidine-2-carboxylate **106b** (1.00g, 2.44 mmol) to afford methyl ((8-methoxy-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110b** as a grey viscous liquid which solidified on standing (464.9 mg, 60%), ν_{max} / cm^{-1} 3350 (N-H) and 1716 (C=O); $\text{C}_{17}\text{H}_{19}\text{NO}_5$, CHN calculated C(64.34%), H(6.04%), N(4.41%), O(25.21%), found: C(64.15%), H(6.11%), N(4.25%), O(25.84%); δ_{H} (300 MHz, DMSO-d_6) 1.84 (m, 4H, ArH), 2.80 (m, 2H, ArH), 3.20 (m, 3H, ArH), 3.67 (s, 3H, PhOCH_3), 3.85 (s, 3H, OCH_3 proline), 7.00 (dd, $J = 7.4, 1.6$ Hz, 1H, ArH), 7.14 (t, $J = 7.5$ Hz, 1H, ArH), 7.22 (dd, $J = 7.4, 1.6$ Hz, 1H, ArH), 7.39 (s, 1H, ArH); δ_{C} (75 MHz, DMSO-d_6) 24.4 (ArC), 29.0 (ArC), 51.1 (CO_2CH_3), 54.9 (PhOCH_3), 56.3 (PhCH_2N), 57.5 ArC), 71.7 (CHCO_2), 114.8, 120.4, 122.3, 126.4, 128.6, 138.9, 141.8, 147.74, 163.2 (CO), 173.5 (C=O).

4.7.18. Methyl ((8-ethoxy-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110c**.

The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-tert-butoxy-2-((3-ethoxy-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl)pyrrolidine-2-carboxylate **106c** (1.00g, 2.36 mmol) to afford methyl ((8-ethoxy-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110c** as a grey viscous liquid which solidified on standing (477.2 mg, 61%), ν_{max} / cm^{-1} 3343 (N-H) and 1729 (C=O); $\text{C}_{18}\text{H}_{21}\text{NO}_5$, CHN calculated C(65.24%), H(6.39%), N(4.23%), O(24.14%), found: C(65.57%), H(6.44%), N(4.09%), O(24.36%); δ_{H} (300 MHz, DMSO-d_6) 1.40 (t, $J = 6.0$ Hz, 3H, CH_3CH_2), 1.86 (m, 4H, ArH), 2.82 (m, 2H, ArH), 3.20 (m, 3H, overlapping

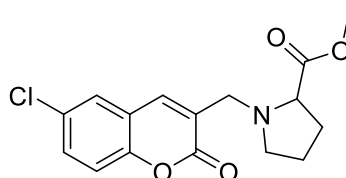
ArH with $\text{PhCH}_2\text{C(O)}$), 3.72 (s, 3H, CO_2CH_3), 4.06 (q, $J = 6.0$ Hz, 2H, CH_3CH_2), 7.00 (dd, $J = 7.4, 1.6$ Hz, 1H, ArH), 7.12 (t, $J = 7.4$ Hz, 1H, ArH), 7.22 (dd, $J = 7.5, 1.6$ Hz, 1H, ArH), 7.39 (s, 1H, ArH); δ_{C} (75 MHz, DMSO-d_6) 14.1 ($\text{CH}_3\text{CH}_2\text{OPh}$), 24.4 (ArC), 29.0 (ArC), 51.1 (CO_2CH_3), 56.3 (PhCH_2N), 57.5 (ArC), 71.7 (CHCO_2), 64.3 ($\text{CH}_3\text{CH}_2\text{OPh}$), 71.7 (CHCO_2), 115.2, 119.5, 121.3, 126.2, 128.2, 138.9, 141.73, 153.3, 163.2 (ArCO), 173.5 (C=O).

4.7.19. Methyl ((6-bromo-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110d**.



The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-(5-bromo-2-hydroxyphenyl)-2-(tert-butoxycarbonyl)-3-hydroxypropyl)pyrrolidine-2-carboxylate **106d** (1.00g, 2.18 mmol) to afford methyl ((6-bromo-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110d** as a off-white gel (639.1 mg, 80%), ν_{max} / cm^{-1} 3410 (N-H) and 1725 (C=O); $\text{C}_{16}\text{H}_{16}\text{BrNO}_4$, CHN calculated C(52.48%), H(4.40%), Br(21.82%), N(3.82%), O(17.48%), found: C(52.17%), H(4.32%), Br(21.53%), N(3.61%), O(17.33%); δ_{H} (300 MHz, DMSO-d_6) 1.72 (m, 3H, ArH), 2.06 (m, 1H, ArH), 2.83 (m, 2H, ArH), 3.19 (m, 3H, ArH), 3.68 (s, 3H, OCH_3), 7.05 (d, $J = 7.5$ Hz, 1H, ArH), 7.40 (s, 1H, ArH), 7.57 (dd, $J = 7.5, 1.5$ Hz, 1H, ArH), 7.64 (d, $J = 1.4$ Hz, 1H, ArH); δ_{C} (75 MHz, DMSO-d_6) 24.4 (ArC), 29.0 (ArC), 51.1 (NCH_2CH_2), 56.3 (PhCH_2N), 57.5 (ArC), 71.7 (CHCO_2), 118.0, 119.2 (ArC(Br)), 124.8, 127.6, 131.0, 135.1, 139.8, 152.0 (COC(O)), 162.7 (ArCO), 173.5 (C=O).

4.7.20. Methyl ((6-chloro-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110e**.



The general procedure as described above in the section 4.7 was followed, using methyl 1-(3-tert-butoxy-2-((5-chloro-2-hydroxyphenyl)(hydroxy) methyl)-3-oxopropyl)pyrrolidine-2-carboxylate **106e** (1.00g, 2.41 mmol) to afford methyl ((6-chloro-2-oxo-2H-coumarin-3-yl)methyl)prolinate **110e** as a grey viscous liquid which solidified on

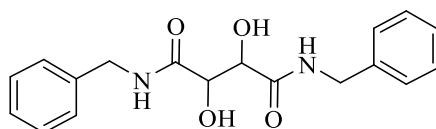
standing (598.5 mg, 77%), $\nu_{\text{max.}} / \text{cm}^{-1}$ 3421 (N-H) and 1719 (C=O); $\text{C}_{16}\text{H}_{16}\text{ClNO}_4$, CHN calculated C(59.73%), H(5.01%), Cl(11.02%), N(4.35%), O(19.89%), found: C(59.98%), H(5.10%), Cl(11.11%), N(4.51%), O(19.19%); δ_{H} (300 MHz, DMSO- d_6) 1.70 (m, 2H, ArH), 2.06 (m, 1H, ArH), 2.44 (dt, $J = 9.1, 6.1$ Hz, 1H, ArH), 2.82 (dt, $J = 12.2, 6.0$ Hz, 1H, ArH), 3.34 (m, 3H, ArH), 3.76 (s, 3H, OCH_3), 7.14 (d, $J = 7.5$ Hz, 1H, ArH), 7.44 (dd, $J = 7.5, 1.4$ Hz, 1H, ArH), 7.70 (s, 1H, ArH); δ_{C} (75 MHz, DMSO d_6) 24.4 (ArC), 29.0 (ArC), 51.1 (NCH_2CH_2), 56.3 (PhCH_2N), 57.5 (ArC), 71.7 (CHCO_2), 119.2, 123.8, 126.0, 127.9, 129.8, 131.5 (ArC(Cl)), 139.8, 152.0 (ArCOC(O)), 162.7 (CO), 173.5 (C=O).

4.8. General procedure for the catalyst-free one-step synthesis of tartrate diamides 111-153ab.²¹⁵

4.8.1. Method A: Synthesis of symmetrical tartrate diamides

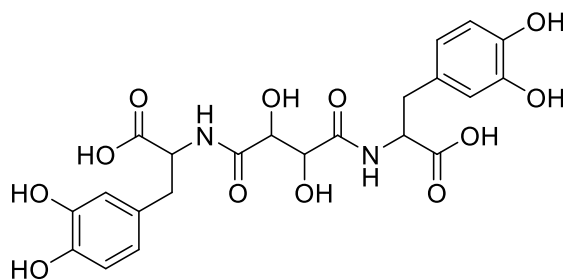
This method was used as described in the literature by Charville, H. and co-workers²¹⁵ for the synthesis of symmetrical diamides. To a round bottomed flask equipped with a Dean-Stark receiver and stirrer bar was added as solution of diethyl tartrate (0.5 g, 2.42 mmol, 1eq), (or tartaric acid osazone (0.2 g, 0.54 mmol, 1eq.) dissolved in toluene (20 ml). The mixture was refluxed for 2h after which the corresponding amine/primary amino acid (2.0 eq.) was added. The final mixture was refluxed for 2-3 hours. When TLC (eluting in EtOAc:Hexan/1:1) showed no starting materials, the reaction mixture was cooled to room temperature. The precipitate formed was then filtrated off and dissolved in Ethyl acetate, quenched water, washed with saturated NH_4Cl and then transferred to the separating funnel. The organic layers were dried over MgSO_4 and the solvent removed under reduced pressure to afford the titled compound in high yields after column chromatography and prep-TLC in some cases.

4.8.1. 1. N^1, N^4 -dibenzyl-2,3-dihydroxysuccinamide 111a.²²³



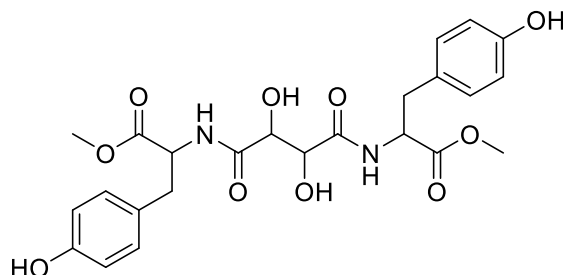
The general procedure as described above in the section 4.8.1 was followed using Benzyl amine (0.54 g, 5.09 mmol, 2.1 eq.) to afford the product *N*¹,*N*⁴-dibenzyl-2,3-dihydroxysuccinamide **111a** as a off-white powder (0.76 g, 96% yields). m.p. 64-69 °C, (literature reported 65-68°C)²²³, (HRMS: m/z calculated for C₁₈H₂₀N₂O₄ (ESI M+H) 328.1423. Found 329.1491); U_{max}./cm⁻¹ 3450 (N-H) and 1625 (C=O), 3500 (OH); C₁₈H₂₀N₂O₄, CHN calculated C(65.84%), H(6.14%), N(8.53%), O(19.49%), found: C(65.33%), H(6.22%), N(8.34%), O(19.65%); δ_H (300 MHz, DMSO-d₆): 3.42 (s, OH in DMSO d₆), 4.35 (m, 6H, overlapping 4H, CH₂Ph and 2H, CH(OH)), 5.74 (d, *J* = 5.2 Hz, 2H, OH), 7.23 (m, 1H, ArH), 7.30 (d, *J* = 4.4 Hz, 3H, ArH), 8.23 (t, *J* = 6.2 Hz, 2H, NH); δ_C (75 MHz, DMSO-d₆) 42.3 (2x CH₂Ph), 73.2 (2x CH(OH)), 2x 127.0, 2x 127.5, 2x 127.9, 2x 128.6, 2x 139.8, 2x 172.7 (C=O).

4.8.1.2. 2,2'-((2,3-dihydroxysuccinyl)bis(azanediyl))bis(3-(3,4-dihydroxyphenyl) propanoic acid) **112a**



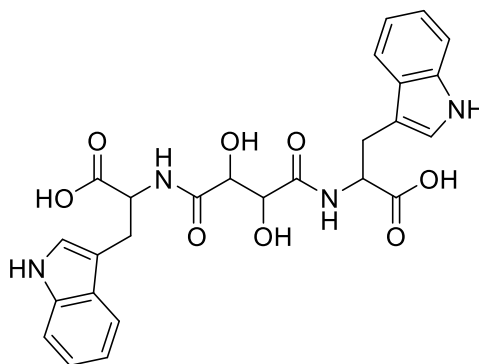
The general procedure as described above in the section 4.8.1 was followed using DOPA (1.0 g, 5.09 mmol, 2.1 eq.) to afford the product 2,2'-((2,3-dihydroxysuccinyl)bis(azanediyl))bis(3-(3,4-dihydroxyphenyl) propanoic acid) **112a** as a off-white powder (1.11 g, 91% yields). m.p. 256-258°C; HRMS: m/z calculated for C₂₂H₂₄N₂O₁₂ (M+H) 508.1329, found 509.1325; U_{max}. / cm⁻¹ 3520 (OH), 3430 (N-H), 1630 (C=O amide), 1780 (C=O, carboxylic acid); CHN calculated C(51.97%), H(4.76%), N(5.51%), O(37.76%), found: C(51.17%), H(4.85%), N(5.77%), O(37.52%); δ_H (300 MHz, CDCl₃) 2.26 (m, 6H: overlapping 4H from PhCH₂CH and 2x OH), 4.09 (dd, *J* = 4.4, 2.2 Hz, 2H, 2x CH(OH)), 4.75 (dt, *J* = 9.3, 7.7 Hz, 2H, PhCH₂CHNH), 6.92 (d, *J* = 9.3 Hz, 2H, 2x NH), 7.13 (ddt, *J* = 8.4, 2.1, 1.0 Hz, 2H, ArH), 7.38 (dt, *J* = 2.0, 1.0 Hz, 2H, ArH), 7.43 (d, *J* = 8.5 Hz, 2H, ArH); δ_C (75 MHz, CDCl₃) 36.4 (2x CH₂), 59.7 (2x CHCH₂), 73.4 (2x CH(OH)), 2x 115.0, 2x 116.9, 2x 122.5, 2x 130.5 (ArC), 144.4 and 145.7 (4x ArC(OH)), 172.2 (C=O, amide), 174.4 (C=O, carboxylic acid).

4.8.1.3. Dimethyl 2,2'-((2,3-dihydroxysuccinyl) bis (azanediy))bis(3-(4-hydroxyphenyl) propanoate) **113a**



The general procedure as described above in the section 4.8.1 was followed using tyrosine methyl ester (0.99 g, 5.09 mmol, 2.1 eq.) to afford Dimethyl 2,2'-((2,3-dihydroxysuccinyl) bis (azanediy))bis(3-(4-hydroxyphenyl) propanoate) **113a** as a off-white powder (1.09 g, 90% yields); m.p. 178-204°C, (HR-MS: m/z calculated for $C_{24}H_{28}N_2O_{10}$ (ESI MH⁺) 504.1744, found: 505.1747; ν_{max} / cm^{-1} 3500 (OH), 3424 (N-H), 1628 (C=O amide), 1740 (C=O, ester); $C_{24}H_{28}N_2O_{10}$ CHN calculated (57.14%), H(5.59%), N(5.55%), O(31.71%), found: C(57.52%), H(5.19%), N(5.84%), O(31.66%); δ_H (300 MHz, DMSO- d_6) δ 6.99 (d, J = 8.5 Hz, 4H), 6.71 (d, J = 8.4 Hz, 4H), 4.31 (m, 6H including 2x CH(OH), 2x CH(OH), and 2x NHCH), 4.15 (t, J = 6.4 Hz, 2x 2H, PhCH₂CHNH), 3.66 (s, 6H, s, 6H, OCH₃), 3.02 (m, 4H, 2x CHCH₂Ph). δ_C (75 MHz, DMSO- d_6) 35.5 (2x CHCH₂Ph), 53.0 (2x OCH₃), 53.9 (2x CHCH₂Ph), 72.5 (2x (CH(OH))), 115.9 (4xArCH), 124.7 (2x CH₂ArC), 130.8 (4x ArC), 157.1 (2x ArC(OH)), 169.9 (2x C=O, methy ester carbonyl), 173.5 (2x C=O, amide carbonyl).

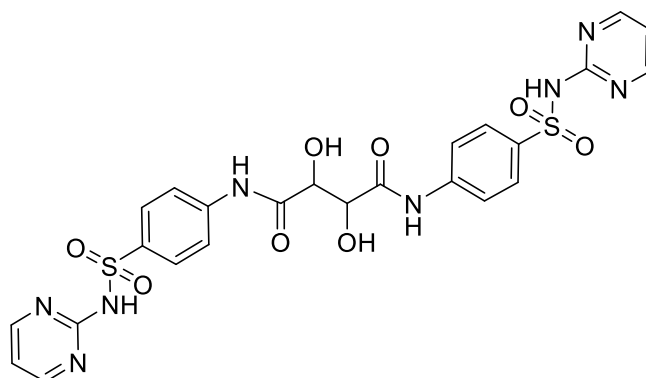
4.8.1.4. 2,2'-((2,3-dihydroxysuccinyl)bis(azanediy))bis(3-(1H-indol-3-yl)propanoic acid) **114a**



The general procedure as described above in the section 4.8.1 was followed using tryptophan (1.03 g, 5.09 mmol, 2.1 eq.) to afford the product 2,2'-((2,3-

dihydroxysuccinyl)bis(azanediyl))bis(3-(1H-indol-3-yl)propanoic acid) **114a** as a off-white powder (0.98 g, 77% yields). m.p. 324-327°C, (HR-MS: m/z calculated for $C_{26}H_{26}N_4O_8$ (MH⁺) 522.1026, found 523.1025; $U_{max.}/cm^{-1}$ 3490 (OH), 3462 (N-H), 1633 (C=O amide), 1760 (C=O, acid); $C_{26}H_{26}N_4O_8$, CHN calculated C(59.77%), H(5.02%), N(10.72%), O(24.50%), found: C(59.25%), H(5.22%), N(10.58%), O(24.69%); δ_H (300 MHz, $CDCl_3$) 2.99 (m, 6H, overlapping 4H from $PhCH_2CH$ and 2x OH), 4.17 (dd, $J = 4.4, 2.3$ Hz, 2H, 2x CH(OH)), 4.85 (m, 2H, $PhCH_2CHNH$), 6.92 (d, $J = 9.3$ Hz, 2H, 2x NH), 7.13 (m, 3H, ArH), 7.19 (d, $J = 2.6$ Hz, 3H, ArH), 7.35 (m, 2H, ArH), 7.61 (dd, $J = 7.4, 1.3$ Hz, 2H, ArH), 8.24 (t, $J = 2.4$ Hz, 2H, ArNH); δ_C (75 MHz, $CDCl_3$) 28.6 (2x CH_2CH), 61.0 (2x $CHCH_2$), 73.5 (2x CH(OH)), 109.5 (2x $ArCCH_2$), 113.9 (2x ArC), 118.0 (2x ArC), 119.7 (2x ArC), 122.2 (2x ArC), 124.1 (2x ArC), 128.3 (2x ArC), 135.0 (2x ArC), 170.1 (2x C=O, amide), 173.4 (2x C=O carboxylic acid).

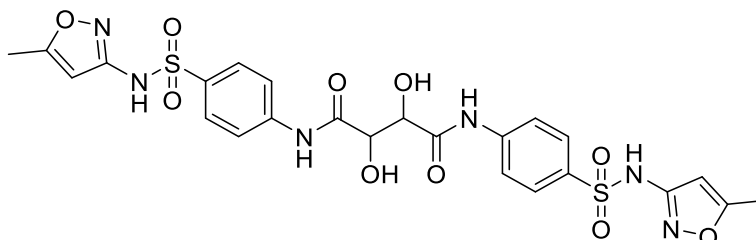
4.8.1.5. 2,3-dihydroxy-*N*¹,*N*⁴-bis(4-(*N*-(pyrimidin-2-yl) sulfamoyl) phenyl) succinamide **115a**



The general procedure as described above in the section 4.8.1 was followed using sulfadiazine (1.27 g, 5.09 mmol, 2.1 eq.) to afford the product 2,3-dihydroxy-*N*¹,*N*⁴-bis(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)succinamide **115a** as a pale-yellow gel (1.30 g, 88% yields). (HR-MS: m/z calculated for $C_{24}H_{22}N_8O_4$ (MH⁺) 613.9893, found 614.9895; $U_{max.}/cm^{-1}$ 3487 (OH), 3410 (N-H), 1634 (C=O amide); $C_{24}H_{22}N_8O_4S_2$, CHN calculated C(46.90%), H(3.61%), N(18.23%), O(20.82%), S(10.43%), found: C(46.88%), H(3.70%), N(18.16%), O(20.77%), S(10.59%); δ_H (300 MHz, $CDCl_3$) 3.14 (dd, $J = 4.4, 2.3$ Hz, 2H, 2x CH(OH)), 4.20 (dd, $J = 4.4, 2.3$ Hz, 2H, 2x CH(OH)), 7.32 (t, $J = 3.4$ Hz, 2H, ArH), 7.68 (m, 8H, ArH and 2x NH), 8.57 (m, 6H, ArH); δ_C (75 MHz, $CDCl_3$) 73.6 (2x CH(OH)), 115.2 (2x ArC), 118.4 (4x

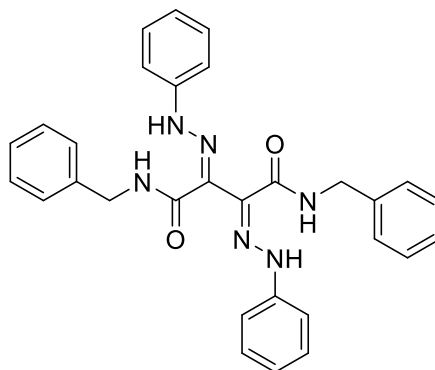
ArC), 128.7 (4x ArCH), 136.2 (2x S(O₂)ArC), 143.5 (2x ArCN), 156.1 (4x ArNCH), 169.2 (2x ArC(N)₃), 170.3 (2x C=O).

4.8.1.6. 2,3-dichloro-*N*¹,*N*⁴-bis(4-(*N*-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) succinamide **116a**



The general procedure as described above in the section 4.8.1 was followed using sulfmethoxazole (1.29 g, 5.09 mmol, 2.1 eq.) to afford 2,3-dichloro-*N*¹,*N*⁴-bis(4-(*N*-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) succinamide **116a** as a grey viscous gel (0.64 g, 43% yields (HR-MS: *m/z* calculated for C₂₄H₂₆N₆O₁₀S₂ (MH⁺) 622.1152, found 623.1154; U_{max}/ cm⁻¹ 3500 (OH), 3408 (N-H), 1651 (C=O amide); C₂₄H₂₄N₆O₁₀S₂, CHN calculated C(46.30%), H(4.21%), N(13.50%), O(25.70%), S(10.30%), found: C(46.26%), H(4.11%), N(13.61%), O(25.52%), S(10.21%); δ_H (300 MHz, CDCl₃) 2.29 (d, *J* = 0.6 Hz, 6H, ArCCH₃), 3.14 (dd, *J* = 4.4, 2.3 Hz, 2H, 2x CH(OH)), 4.37 (dd, *J* = 4.4, 2.3 Hz, 2H, 2x CH(OH)), 6.09 (s, 2H, NH), 7.69 (m, 8H, ArH), 8.57 (s, 2H, NH); δ_C (75 MHz, CDCl₃) 24.2 (2x CH₃), 73.6 (2x CH(OH)), 96.1 (2x ArCHCCH₃), 119.0 (4x ArCHCN), 128.6 (4x ArCHCS), 135.9 (2x ArC), 142.2 (2x ArCN), 151.2 (2x NCN), 169.1 (2x ArC(CH₃)), 173.1 (2x C=O).

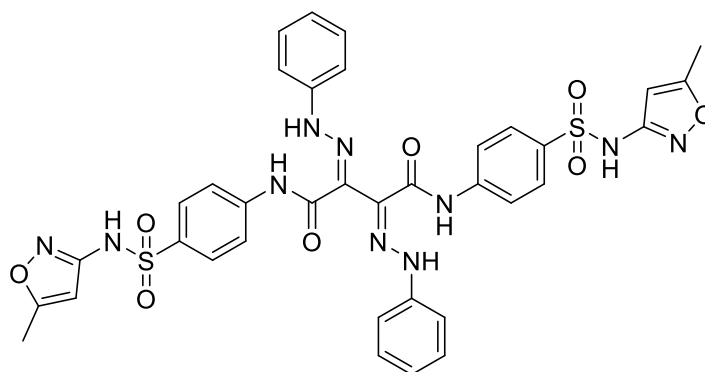
4.8.1.7. (2*Z*,3*Z*)-*N*¹,*N*⁴-dibenzyl-2,3-bis(2-phenylhydrazono)succinamide **117b**



The general procedure as described above in the section 4.8.1 was followed using benzylamine (0.12 g, 1.13 mmol, 2.1 eq.), to afford (2*Z*,3*Z*)-*N*¹,*N*⁴-dibenzyl-2,3-bis(2-

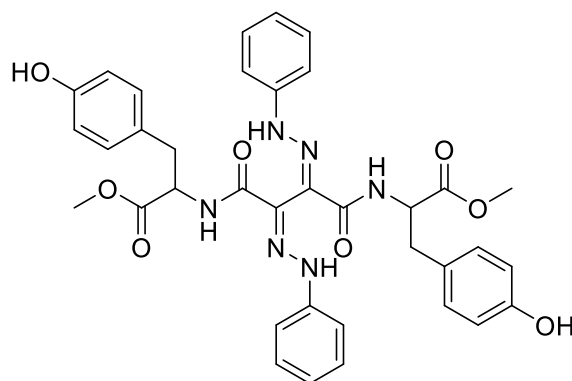
phenylhydrazono)succinamide **117b** as a yellow solid (0.24 g, 89% yields). m.p. 104-108°C, (HR-MS: m/z calculated for $C_{30}H_{28}N_6O_2$ (MH⁺) 503.2096, found 504.2094; $U_{max.}/cm^{-1}$ 3398 (N-H), 1631 (C=O amide), 2365 (C=N); CHN calculated C(71.41%), H(5.59%), N(16.66%), O(6.34%), found: C(71.31%), H(5.67%), N(16.51%), O(6.52%); δ_H (300 MHz, $CDCl_3$) 4.33 (d, J = 16.5 Hz, 4H, NCH_2Ph), 7.22 (m, 17H, ArH), 7.85 (m, 3H, ArH), 9.84 (s, 2H, NH); δ_C (375 MHz, $CDCl_3$) 43.8 (2x $PhCH_2N$), 114.3 (4x ArCH), 122.6 (2x ArCH), 125.9 (2x ArCH), 127.5 (4x ArCH), 128.8 (4x ArCH), 129.7 (4x ArCH), 133.7 (2x C=N), 137.2 (2x $ArCCH_2$), 143.7 (2x $ArCNH$), 162.1 (2x C=O).

4.8.1.9. (2Z,3Z)-N¹,N⁴-bis(4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl)-2,3-bis(2-phenylhydrazono) succinamide **118b**



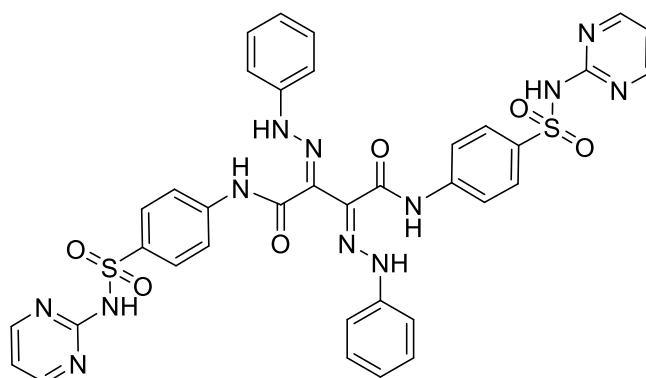
The general procedure as described above in the section 4.8.1 was followed using sulfamethoxazole (0.28 g, 1.13 mmol, 2.1 eq.) to afford (2Z,3Z)-N¹,N⁴-bis(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-2,3-bis(2-phenylhydrazono)succinamide **118b** as a viscous gel (0.17 g, 40% yields). (HR-MS: m/z calculated for $C_{36}H_{32}N_{10}O_8S_2$ (MH⁺) 795.7359, found 796.7357; $U_{max.}/cm^{-1}$ 3398 (N-H), 1631 (C=O amide), 2358 (C=N); CHN calculated C(54.26%), H(4.05%), N(17.58%), O(16.06%), S(8.05%), found: C(54.34%), H(4.11%), N(17.63%), O(16.22%), S(8.20%); δ_H (300 MHz, $CDCl_3$) 2.29 (s, 6H, 2x CH_3), 6.07 (s, 2H, ArH), 7.24 (m, 7H, ArH, including 1H of the $CDCl_3$), 7.52 (m, 8H, ArH), 8.62 (d, J = 7.4 Hz, 4H, ArH), 10.4 (s, 2H, NH), 11.1 (s, 1H, NH); δ_C (75 MHz, $CDCl_3$) 13.6 (2x CH_3), 95.0 (2x ArCH), 114.6 (4x ArCH), 118.3 (4x ArCH), 122.1 (2x ArCH), 127.8 (4x ArCH), 129.8 (4x ArCH), 133.3 (2x ArCH), 135.9 (2x ArCH), 141.7 (2x ArCH), 144.0 (2x ArCH), 152.4 (2x ArCH), 161.6 (2x C=O), 168.1 (2x $ArNCN$).

4.8.1.10. Dimethyl 2,2'-(((2E,3E)-2,3-bis(2-phenylhydrazono)succinyl) bis(azanediyl)) bis(3-(4-hydroxyphenyl) propanoate) **119b**



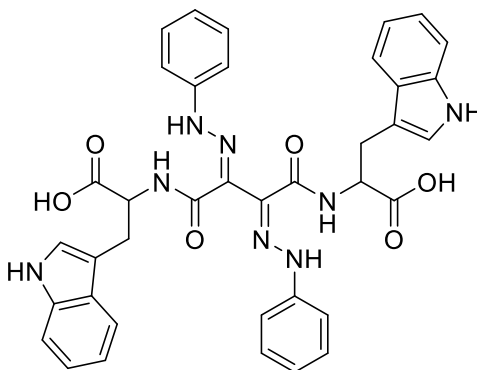
The general procedure as described above in the section 4.8.1 using tyrosine methyl ester (0.22 g, 1.13 mmol, 2.1 eq.), to afford *dimethyl 2,2'-(((2E,3E)-2,3-bis(2-phenylhydrazono)succinyl) bis(azanediyl)) bis(3-(4-hydroxyphenyl)propanoate)* **119b** as a yellow powder (0.35 g, 97% yields); m.p.: 153-156°C; (HR-MS: m/z calculated for $C_{35}H_{34}N_6O_8$ (MH⁺) 680,2595 found: 681,2597; U_{max}/cm^{-1} 3501 (OH), 3488 (N-H), 1653 (C=O amide), 1758 (C=O, ester), 2355 (C=N); CHN calculated C(63.52%), H(5.33%), N(12.35%), O(18.80%), found: C(63.88%), H(5.40%), N(12.59%), O(18.99%); δ_H (300 MHz, $CDCl_3$) 3.14 (ddd, $J = 124.8, 14.0, 7.7$ Hz, 2H, $PhCH_2CH$), 3.70 (s, 6H, 2x OCH_3), 5.04 (dt, $J = 9.3, 7.7$ Hz, 2H, 2x $CH(OH)$), 7.04 (m, 2H, ArH), 7.09 (m, 2H, ArH), 7.12 (t, $J = 1.0$ Hz, 4H, ArH), 7.20 (s, 2H, NH), 7.34 (d, $J = 4.1$ Hz, 8H, ArH), 7.41 (d, $J = 1.2$ Hz, 2H, ArH), 7.43 (d, $J = 1.6$ Hz, 3H, ArH), 7.46 (s, 1H, ArH); δ_C (75 MHz, $CDCl_3$) 35.5 (2x $CHCH_2$), 52.2 (2x OCH_3), 58.8 (2x $CHCH_2$), 112.6 (4x $ArCH$), 115.8 (4x $ArCH$), 123.5 (2x $ArCH$), 128.5 (2x $ArCCH_2$), 129.7 (4x $ArCH$), 131.6 (4x $ArCH$), 134.8 (2x $C=N$), 143.6 (2x $ArCNH$), 155.5 (2x $C(OH)$), 162.3 (2x $C=O$, amide), 174.5 (2x $C=O$, carboxylate).

4.8.1.11. (2Z,3Z)-2,3-bis(2-phenylhydrazono)-N1,N4-bis(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)succinamide 120b



The general procedure as described above in the section 4.8.1 was followed using sulfadiazine (0.28 g, 1.13 mmol, 2.1 eq.), to afford (2Z,3Z)-2,3-bis(2-phenylhydrazono)-N¹,N⁴-bis(4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl)succinamide **120b** as a yellow powder (0.26 g, 63% yields). m.p. 296-299°C, (HR-MS: m/z calculated for C₃₆H₃₀N₁₂O₆S₂ (MH⁺) 790.1853, found 791.1851; U_{max}./cm⁻¹ 3334 (N-H), 1620 (C=O amide), 3300 (C=N); CHN calculated C(54.68%), H(3.82%), N(21.25%), O(12.14%), S(8.11%), found: C(54.15%), H(3.70%), N(21.33%), O(12.22%), S(8.48%); δ_H (300 MHz, CDCl₃) 6.75 (t, J = 7.5 Hz, 1H, ArH), 7.00 (s, 1H, ArH), 7.31 (m, 8H, ArH), 7.50 (t, J = 7.4 Hz, 2H, ArH), 7.67 (dt, J = 3.7, 1.6 Hz, 2H, ArH), 7.74 (d, J = 7.4 Hz, 2H, ArH), 7.82 (d, J = 7.4 Hz, 2H, ArH), 8.07 (d, J = 7.5 Hz, 4H, ArH), 8.44 (d, J = 7.5 Hz, 2H, ArH), 8.75 (d, J = 7.5 Hz, 2H, ArH); δ_C (75 MHz, CDCl₃) 113.5 (4x ArCH), 116.5 (2x ArCH), 119.1 (4x ArCH), 122.7 (2x ArCH), 127.9 (4x ArCH), 129.4 (4x ArCH), 134.2 (2x C=O), 136.8 (2x ArCS), 140.6 (2x ArCN), 144.2 (2x ArCNH), 157.2 (4x ArCH), 161.6 (2x C=O), 168.1(2x ArC(N)₃).

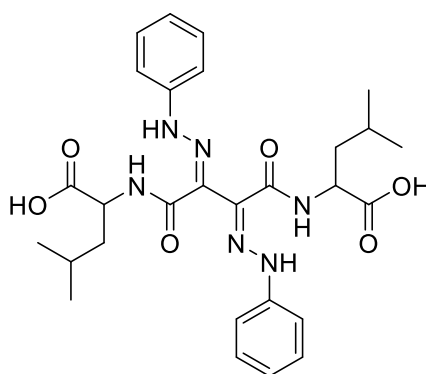
4.8.1.12. 2,2'-(((2E,3E)-2,3-bis(2-phenylhydrazono) succinyl)bis(azanediyl))bis (3-(1H-indol-3-yl) propanoic acid) 121b



The general procedure as described above in the section 4.8.1 was followed using tryptophan (0.23 g, 1.13 mmol, 2.1 eq.) to afford 2,2'-(((2E,3E)-2,3-bis(2-phenylhydrazono)succinyl)bis(azanediyl))bis(3-(1H-indol-3-yl)propanoic acid) **121b** as a pale-yellow powder (0.29 g, 79% yields). m.p. 323-326°C, (HR-MS: m/z calculated for C₃₈H₃₄N₈O₆ (MH⁺) 698.2601, found: 699.2604; U_{max}./cm⁻¹ 3330 (N-H), 3470 (OH), and 1630 (C=O amide), 3250 (C+N), 1753 (carboxylic acid); CHN calculated C(65.32%), H(4.90%), N(16.04%), O(13.74%), found: C(65.51%), H(4.54%), N(16.66%), O(13.85%); δ_H (300 MHz, CDCl₃) 3.16 (m, 2H, PhCH₂CH),

4.72 (m, 2H, NCHCH₂), 6.92 (s, 2H, ArH), 7.08 (ddd, *J* = 8.9, 2.8, 1.4 Hz, 2H, ArH), 7.19 (m, 2H, ArH), 7.30 (m, 4H, CDCl₃ and ArH), 7.43 (t, *J* = 7.4 Hz, 2H, ArH), 9.52 (s, 2H, ArNH), 9.87 (s, 2H, NH), 10.2 (s, 2H, NH), 11.0 (s, 2H, CO₂H); δ_C (75 MHz, CDCl₃) 28.2 (2x CH₂CH), 61.9 (2x CH₂CH), 109.3 (2x ArCCH₂), 111.9 (2x ArC), 113.3 (4x ArCH), 117.5 (2x ArCH), 119.5 (2x ArCH), 122.1 (2x ArCH), 123.5 (2x ArCH), 125.8 (2x ArCH), 126.7 (2x ArCH), 127.6 (4x ArCH), 134.0 (2x ArC=N), 137.8 (2x ArC), 142.8 (2x ArCNH), 159.0 (2x C=O amide), 173.3 (2x C=O carboxylic acid).

4.8.1.13. 2,2'-(((2Z,3Z)-2,3-bis(2-phenylhydrazono)succinyl)bis(azanediyl))bis(4-methylpentanoic acid) 122b



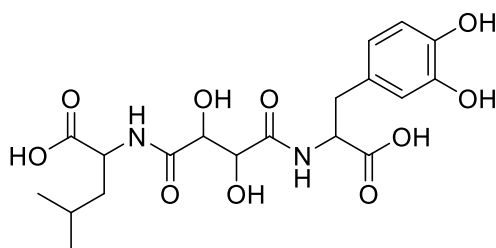
The general procedure as described above in the section 4.8.1 was followed using Leucine (0.14 g, 1.13 mmol, 2.1 eq.) to afford 2,2'-(((2Z,3Z)-2,3-bis (2-phenylhydrazono)succinyl)bis (azanediyl)) bis (4-methylpentanoic acid) **72b** as a yellow powder (0.22 g, 75% yields). m.p. 305-308°C, (HRMS: *m/z* calculated for C₂₈H₃₆N₆O₆ (MH⁺) 552.2696, found: 553.2699); U_{max}./cm⁻¹ 3360 (N-H), and 1627 (C=O amide), 1780 (C=O, acid), 3005 (C=N); CHN calculated C(60.86%), H(6.57%), N(15.21%), O(17.37%), found: C(60.77%), H(6.24%), N(15.35%), O(17.48%); δ_H (300 MHz, CDCl₃) 1.02 (d, *J* = 6.0, 6H, 2x CH₃), 1.68 (m, 4H, CHCH₂CH), 2.30 (m, 2H, CH₂CH(CH₃)₂), 5.11 (m, 2H, CHCH₂CH), 7.20 (m, 7H, CDCl₃ and ArH), 9.39 (s, 2H, NH), 10.7 (s, 2H, CO₂H); δ_C (75 MHz, CDCl₃) 22.3 (4x CHCH₃), 25.0 (2x CHCH₃), 41.3 (2x CHCH₂CH), 53.7 (2x NCHCH₃), 115.0 (4x ArCH), 122.6 (2x ArCH), 128.8 (4x ArCH), 135.1 (2x C=N), 142.7 (2x ArCNH), 159.5 (2x C=O, amide), 174.4 (2x C=O, acid).

4.8.2. Method B: Synthesis of unsymmetrical tartrate diamides

This method was used as described above, with some modifications.²¹⁵ To a round bottomed flask equipped with a Dean-Stark receiver and stirrer bar was added a

solution of diethyl tartrate (0.1 g, 0.48 mmol, 1eq.) (or tartaric acid osazone (0.1 g, 0.27 mmol, 1 eq.), dissolved in toluene (20 ml). The mixture was refluxed for 2h after which, two different corresponding amine/amino acid (1.0 eq. each), were added in one portion. The final mixture was refluxed for 24 hours. When TLC (eluting in EtOAc:Hexan/1:1) showed no starting materials, the reaction mixture was cooled to room temperature. The precipitate formed was then filtrated off and dissolved in Ethyl acetate, quenched water, washed with saturated NH_4Cl and then transferred to the separating funnel. The organic layers were dried over MgSO_4 and the solvent removed under reduced pressure to afford the titled compound in high yields after column chromatography and prep-TLC in some cases.

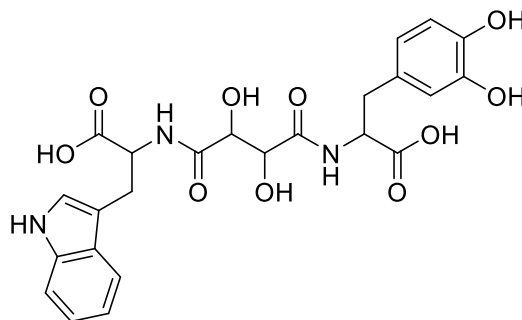
4.8.2.1. (4-((1-carboxy-2-(3,4-dihydroxyphenyl) ethyl)amino)-2,3-dihydroxy-4-oxobutanoyl) leucine 123a



The general procedure as described above in the section 4.8.2 was followed using DOPA (0.09 g, 0.48 mmol), and Leucine (0.06 g, 0.48 mmol, 1 eq.) to afford the product (4-((1-carboxy-2-(3,4-dihydroxyphenyl)ethyl)amino)-2,3-dihydroxy-4-oxobutanoyl)leucine **123a** as a off-white powder (0.10 g, 51% yields). m.p. 287-300°C, (HRMS: m/z calculated for $\text{C}_{19}\text{H}_{26}\text{N}_2\text{O}_{10}$ (MH^+) 442.1644, found: 443.1646; Umax./cm^{-1} 3651 (N-H), 3550 (OH, broad), 1780 (carboxylic acid C=O) and 1632 (amide N-C=O); CHN calculated C(51.58%), H(5.92%), N(6.33%), O(33.16%), found: C(51.17%), H(5.13%), N(6.66%), O(33.24%); δ_{H} (300 MHz, CDCl_3) 0.90 (d, J = 6.9 Hz, 3H, CHCH_3), 0.95 (d, J = 7.0 Hz, 3H, CHCH_3), 1.51 (dq, J = 13.9, 6.9 Hz, 1H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$), 1.89 (m, 2H, CHCH_2CH), 3.17 (m, 2H, CHCH_2Ph), 4.41 (dt, J = 9.3, 6.8 Hz, 1H, $\text{CH}(\text{CH}_2)\text{NH}$), 4.94 (m, 1H, $\text{CH}(\text{CH}_2)\text{NH}$), 5.08 (d, J = 5.9 Hz, 1H, $\text{CH}(\text{OH})$), 5.40 (d, J = 5.9 Hz, 1H, $\text{CH}(\text{OH})$), 6.99 (m, 1H, ArH), 7.32 (d, J = 9.3 Hz, 1H, NH), 7.39 (s, 1H, ArH), 7.41 (m, 2H, ArH), 7.52 (d, J = 9.3 Hz, 1H, NH), 10.5 (s, 1H, CO_2H), 11.7 (s, 1H, CO_2H); δ_{C} (75 MHz, CDCl_3) 22.8 (CHCH_3), 22.9 (CHCH_3), 24.5 ($\text{CH}(\text{CH}_3)_2$), 36.9 (CH_2Ph), 41.5 (CHCH_2CH), 56.7 ($\text{CH}(\text{CH}_2)\text{N}$), 58.7 ($\text{CH}(\text{CH}_2)\text{N}$), 70.8 ($\text{CH}(\text{OH})$), 73.5 ($\text{CH}(\text{OH})$), 115.2 (ArH), 117.4 (ArH), 122.6 (ArH),

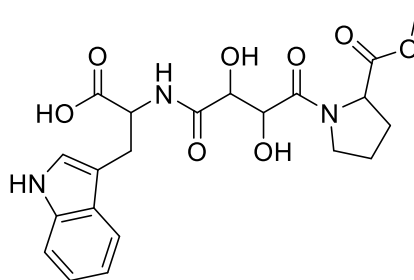
130.6 (ArC(CH₂), 145.1 (ArC(OH)), 146.8 (ArC(OH)), 172.3 (NC=O), 173.5 (NC=O), 174.2 (C=O), 175.8 (C=O).

4.8.2.2. 2-(4-((1-carboxy-2-(1H-indol-3-yl)ethyl) amino)-2,3-dihydroxy-4-oxobutanamido)-3-(3,4-dihydroxyphenyl) propanoic acid 124a



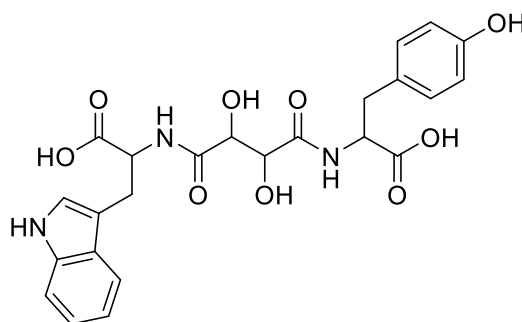
The general procedure as described above in the section 4.8.2 was followed using DOPA (0.09 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford 2-(4-((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-2,3-dihydroxy-4-oxobutanamido)-3-(3,4-dihydroxyphenyl)propanoic acid **124a** as a off-white powder (0.11 g, 46% yields). m.p. 283-286°C, (HRMS: m/z calculated for C₂₄H₂₅N₃O₁₀ (MH⁺) 551.1540, found 552.1545; U_{max}./cm⁻¹ 3641 (N-H), 3520 (OH, broad), 1781 (carboxylic acid C=O) and 1630 (amide N-C=O); CHN calculated C(55.92%), H(4.89%), N(8.15%), O(31.04%), found: C(55.77%), H(4.65%), N(8.87%), O(31.51%); δ_H (300 MHz, CDCl₃); 3.11 (m, 4H, 2x CHCH₂), 4.64 (dt, J = 16.3, 9.3, 7.6 Hz, 2H, 2x CHCH₂), 4.95 (d, J = 5.8 Hz, 1H, CH(OH)), 5.26 (d, J = 5.9 Hz, 1H, CH(OH)), 7.15 (m, 4H, ArH), 7.30 (s, 1H, ArH), 7.35 (m, 2H, ArH, NH), 7.50 (m, 2H, ArH, NH), 7.62 (m, 1H, ArH), 8.24 (t, J = 2.3 Hz, 1H, ArH), 14.2 (s, 1H, CO₂H), 14.6 (s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 25.4, 36.5, 56.2, 68.2, 73.1, 74.1, 109.2, 111.8, 115.9, 117.2, 119.3, 120.4, 121.6, 122.1, 123.3, 127.6, 130.5, 136.9, 144.5, 145.7, 168.2 (NC=O), 169.2 (NC=O), 172.7 (C=O), 174.7 (C=O).

4.8.2.3. (2,3-dihydroxy-4-(2-(methoxycarbonyl) pyrrolidin-1-yl) -4-oxobutanoyl) tryptophan 125a



The general procedure as described above in the section 4.8.2 was followed using proline methyl ester (0.07 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford (2,3-dihydroxy-4-(2-(methoxycarbonyl)pyrrolidin-1-yl)-4-oxobutanoyl)tryptophan **125a** as a grey gel which solidified on standing (0.12 g, 57% yields). m.p. 283-286°C, (HRMS: m/z calculated for $C_{21}H_{25}N_3O_8$ (MH^+) 447.1642, found: 448.1644; U_{max}/cm^{-1} 3450 (N-H), 3365 (OH, broad), 1776 (carboxylic acid C=O), 1748 (carboxylate C=O), and 1637 (amide N-C=O); CHN calculated C(56.37%), H(5.63%), N(9.39%), O(28.61%), found: C(56.23%), H(5.19%), N(9.27%), O(28.24%); δ_H (300 MHz, $CDCl_3$) 2.05 (m, 4H, $ArCH_2$), 3.01 (ddd, $J = 76.5, 14.8, 7.7$ Hz, 2H, $CH(CH_2)NH$), 3.65 (m, 5H, $ArCH_2CH_2$, and OCH_3), 4.41 (t, $J = 8.7$ Hz, 1H, $NCHCO_2$), 4.75 (m, 1H, $CH(CH_2)N$), 5.49 (d, $J = 8.2$ Hz, 1H $CH(OH)$), 5.63 (d, $J = 8.2$ Hz, 1H, $CH(OH)$), 7.15 (m, 2H, ArH), 7.30 (s, 1H, ArH), 7.34 (d, $J = 1.3$ Hz, 1H, NH), 7.36 (dd, $J = 2.2, 1.2$ Hz, 1H, ArH), 7.59 (m, 1H, ArH), 8.22 (m, 1H, $ArNH$), 14.64 (s, 1H, CO_2H); δ_H (75 MHz, $CDCl_3$) 24.8 ($ArCH_2$ proline-OMe), 28.0 ($ArCCH_2CH$), 28.9 ($ArCHCH_2$ proline-OMe), 50.1 ($ArCH_2N$), 51.7 (OCH_3), 60.0 ($NCHCH_2$), 60.9 ($ArCHCO_2$), 71.6 ($CH(OH)$), 74.7($CH(OH)$), 109.5 ($ArCHCH_2CH$), 111.7, 118.3, 119.4, 120.63, 124.0, 128.0, 136.9 ($ArCNH$ tryptophan), 171.1 (CO_2Me), 172.8 ($HNC=O$), 173.9 ($HNC=O$), 174.2 (CO_2H).

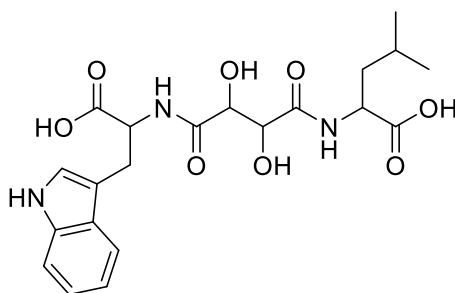
4.8.2.4. (4-((1-carboxy-2-(1H-indol-3-yl) ethyl) amino)-2,3-dihydroxy-4-oxobutanoyl) tyrosine **126a**



The general procedure as described above in the section 4.8.2 was followed using tyrosine (0.08 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford (4-

((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-2,3-dihydroxy-4-oxobutanoyl tyrosine **126a** as a grey gel which solidified on standing (0.12 g, 54% yields). m.p. 302-305°C, (HRMS: m/z calculated for $C_{24}H_{25}N_3O_9$ (MH⁺) 499.2128, found 500.2124; $U_{max.}/cm^{-1}$ 3422 (N-H), 3500 (OH, broad), 1786 (carboxylic acid C=O), 1775 (carboxylic acid C=O), and 1644 (amide N-C=O); CHN calculated C(57.71%), H(5.05%), N(8.41%), O(28.83%), found: C(57.50%), H(5.22%), N(8.54%), O(28.77%); δ_H (300 MHz, $CDCl_3$) 3.23 (dddd, $J = 161.1, 148.8, 12.5, 7.7$ Hz, 4H, 2x CH_2), 4.57 (s, NH), 4.83 (t, $J = 7.6$ Hz, 1H, NCH(CH_2)), 4.86 (s, 1H, NH), 4.93 (d, $J = 10.7$ Hz, 1H, CHa(OH)), 5.13 (d, $J = 10.7$ Hz, 1H, CHb(OH)), 5.32 (t, $J = 7.6$ Hz, 1H, (CH_2)CHb), 6.93 (s, 1H, ArH, NHCHC, Tryptophan), 7.12 (d, $J = 7.5$ Hz, 2H, ArH), 7.27 (m, 7H, ArH), 7.39 (s, 1H, ArH), 9.55 (s, 1H, CO_2H); δ_C (75 MHz, $CDCl_3$) 28.4 (ArCH₂CH, tryptophan), 36.2 (PhCH₂CH tyrosine), 60.0 (NCHCH₂Ph, tyrosine), 60.9 (NCHCH₂Ph, tryptophan), 71.6 (CH(OH)), 74.7(CH(OH)), 109.5 (ArCHCH₂CH), 111.7, 115.4 (2x ArCH), 118.3, 119.4, 120.6, 124.0, 128.0, 129.8, 130.7 (2x ArCH), 136.9 (ArCNH tryptophan), 156.0 (ArC(OH), 171.1 (NC=O), 172.8 (NC=O), 173.8 (CO_2H), 174.2 (CO_2H).

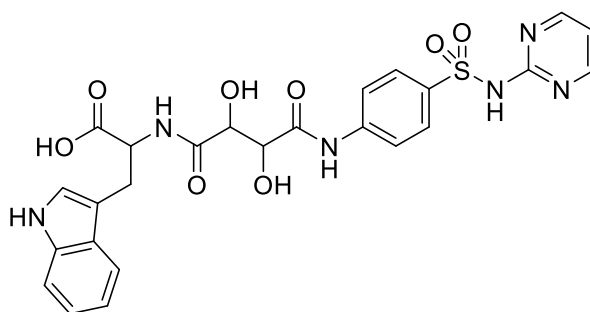
4.8.2.5. 4-((1-carboxy-2-(1H-indol-3-yl) ethyl) amino)-2,3-dihydroxy-4-oxobutanoyl leucine **127a**



The general procedure as described above in the section 4.8.2 was followed using Leucine (0.06 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford 4-((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-2,3-dihydroxy-4-oxobutanoyl leucine **127a** as a grey gel (0.11 g, 53% yields), (HRMS: m/z calculated for $C_{21}H_{27}N_3O_8$ (MH⁺) 449.1798, found: 450.1796; $U_{max.}/cm^{-1}$ 3461 (N-H), 3499 (OH, broad), 1784 (carboxylic acid C=O), 1781 (carboxylic acid C=O), and 1650 (amide N-C=O); CHN calculated C(56.12%), H(6.06%), N(9.35%), O(28.48%), found: C(56.30%), H(6.10%), N(9.51%), O(28.89%); δ_H (300 MHz, $CDCl_3$) δ_H (300 MHz, $CDCl_3$) 1.06 (d, $J = 6.2$ Hz, 6H, 2x CH_3), 1.62 (m, 3H, CH(CH_2)CH(CH_2)₂), Leucine), 3.20 (ddd, $J =$

135.1, 12.4, 7.8 Hz, 2H, ArCCH_2CH , Tryptophan), 4.54 (t, $J = 3.4$ Hz, 1H, $(\text{NCH}(\text{CH}_2))$, Leucine), 4.94 (m, 2H, $\text{CHa}(\text{OH})$ at 4.97 ppm and NH at 4.93 ppm), 5.13 (d, $J = 1.2$ Hz, 1H, $\text{CHb}(\text{OH})$), 6.05 (s, 1H, NH), 7.17 (m, 6H, ArH , and CDCl_3), 7.75 (s, 1H, ArNH , Tryptophan); δ_{C} (75 MHz, CDCl_3) 22.4 ($(\text{CHCH}_3)_2$), 24.3 ($(\text{CHCH}_3)_2$), 28.4 (ArCH_2CH , tryptophan), 41.3 (CHCH_2CH Leucine), 55.0 (CHCH_2CH Leucine), 60.0 (NCHCH_2Ph , tryptophan), 71.6 ($\text{CH}(\text{OH})$), 74.7($\text{CH}(\text{OH})$), 109.5 (ArCHCH_2CH), 111.5, 118.3, 119.4, 120.6, 124.0, 128.0, 136.9 (ArCNH tryptophan), 171.1 ($\text{NC}=\text{O}$), 172.8 ($\text{NC}=\text{O}$), 173.8 (CO_2H), 174.2 (CO_2H).

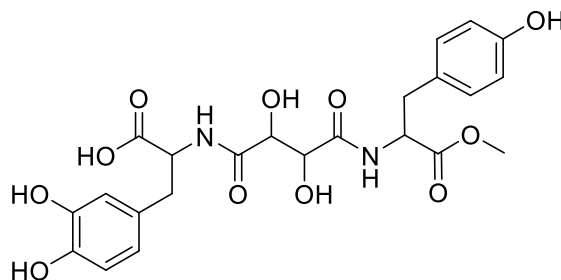
4.8.2.6. (2,3-dihydroxy-4-oxo-4-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) amino) butanoyl) tryptophan 128a



The general procedure as described above in the section 4.8.2 was followed using sulfadiazine (0.12 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford (2,3-dihydroxy-4-oxo-4-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) amino) butanoyl) tryptophan **128a** as a off-white solid (0.11 g, 41% yields). m.p. 312-315°C, (HRMS: m/z calculated for $\text{C}_{25}\text{H}_{24}\text{N}_6\text{O}_8\text{S}$ (MH^+) 568.1376, found: 569.1373; $U_{\text{max.}}/\text{cm}^{-1}$ 3461 (N-H), 3500 (OH, broad), 1770 (carboxylic acid $\text{C}=\text{O}$), and 1631 (amide $\text{N}-\text{C}=\text{O}$); CHN calculated C(52.81%), H(4.25%), N(14.78%), O(22.51%), S(5.64%), found: C(52.66%), H(4.52%), N(14.64%), O(22.20%), S(5.77%); δ_{H} (300 MHz, $\text{DMSO}-d_6$) 3.08 (ddd, $J = 70.9, 12.3, 7.8$ Hz, 2H, $\text{CH}(\text{CH}_2)\text{N}$), 4.85 (t, $J = 7.8$ Hz, 1H, $\text{CH}(\text{CH}_2)\text{N}$), 4.93 (d, $J = 3.4$ Hz, 1H, $\text{CHa}(\text{OH})$), 5.29 (d, $J = 3.5$ Hz, 1H, $\text{CHb}(\text{OH})$), 6.69 (t, $J = 7.5$ Hz, 1H, ArH , on C-36 of Sulfadiazine), 6.98 (s, 1H, ArNCHC , Tryptophan), 7.17 (m, 3H, ArH), 7.32 (m, 1H, ArH), 7.66 (m, 3H, ArH and NH), 7.73 (s, 1H, SO_2NH), 8.04 (d, $J = 7.4$ Hz, 2H, ArH , on Sulfadiazine ring), 8.31 (m, 3H, ArH , and NH), 10.4 (s, 1H, ArNH , Tryptophan); 11.1 (s, 1H, CO_2H); δ_{C} (75 MHz, $\text{DMSO}-d_6$) 28.4 (ArCH_2CH , tryptophan), 61.2 ($\text{NCH}(\text{CH}_2)$), 71.6 ($\text{CH}(\text{OH})$), 74.7($\text{CH}(\text{OH})$), 109.5 (ArCHCH_2CH), 111.5, 115.6, 118.3 (2x ArH), 118.7, 119.4, 120.6, 124.0,

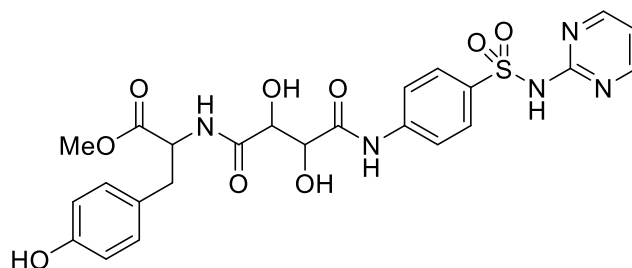
128.1, 128.8 (2x ArH), 135.9 (ArCS), 136.9, 141.7 (ArCN), 155.0 (2x ArC), 169.6 (ArC(N)₃), 171.1 (NC=O), 172.8 (NC=O), 174.2 (CO₂H).

4.8.2.7. 2-(2,3-dihydroxy-4-((3-(4-hydroxyphenyl)-1-methoxy-1-oxopropan-2-yl) amino)-4-oxobutanamido) -3-(3,4-dihydroxyphenyl) propanoic acid 129a



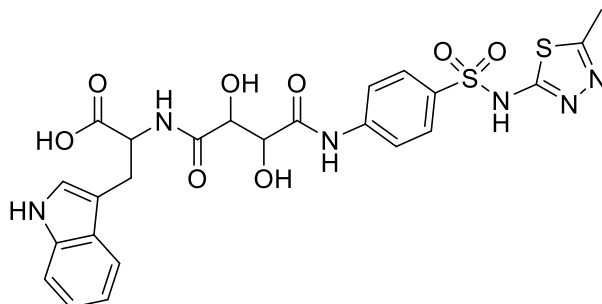
The general procedure as described above in the section 4.8.2 was followed using DOPA (0.09 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford 2-(2,3-dihydroxy-4-((3-(4-hydroxyphenyl)-1-methoxy-1-oxopropan-2-yl) amino)-4-oxobutanamido) -3-(3,4-dihydroxyphenyl) propanoic acid **129a** as a pale-yellow solid (0.09 g, 40% yields). m.p. 271-254°C, (HRMS: m/z calculated for C₂₃H₂₆N₂O₁₁ (MH⁺) 506.1537, found: 507.1539; U_{max}/cm⁻¹ 3399 (N-H), 3487 (OH, broad), 1780 (carboxylic acid C=O), and 1650 (amide N-C=O); CHN calculated C(54.55%), H(5.17%), N(5.53%), O(34.75%), found: C(54.14%), H(5.22%), N(5.13%), O(34.91%); δ_H (300 MHz, DMSO-d₆) 3.04 (m, 4H, overlapping 2x CHCH₂Ph), 3.72 (s, 3H, OCH₃), 4.59 (t, J = 5.1 Hz, 1H CH(CH₂)N, Tyrosine), 4.83 (t, J = 6.9 Hz, 1H, CH(CH₂)N, DOPA), 4.93 (d, J = 10.4 Hz, 1H, CHb(OH)), 5.13 (d, J = 10.4 Hz, 1H, CHa(OH)), 7.00 (dd, J = 7.5, 1.4 Hz, 1H, ArH), 7.10 (d, J = 7.4 Hz, 2H, ArH), 7.36 (d, J = 7.5 Hz, 1H, ArH), 7.44 (d, J = 7.4 Hz, 2H, NH and ArH), 7.50 (d, J = 1.4 Hz, 1H, ArH), 7.56 (s, 1H, ArH), 9.42 (s, 1H, NH), 10.3 (s, 1H, CO₂H); δ_C (75 MHz, DMSO-d₆) 36.1 (CHCH₂), 36.8 (CHCH₂), 51.4 (OCH₃), 56.9 (CHCH₂), 60.7 (CHCH₂), 71.6 (CH(OH)), 74.7 (CH(OH)), 115.0 (ArCHC(OH)), 115.9 (2x ArCH), 117.1, 122.6, 128.8 (ArCCH₂ tyrosine-OMe), 130.7, 130.9 (2x ArCHC, tyrosine-OMe), 141.3 (ArC(OH), DOPA), 141.9 (ArC(OH), DOPA), 155.0 (ArC(OH), tyrosine-OMe), 171.5 (NC=O), 172.6 (2x NC=O), 174.2 (CO₂H).

4.8.2.8. Methyl (2,3-dihydroxy-4-oxo-4-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl)amino) butanoyl) tyrosinate 130a



The general procedure as described above in the section 4.8.2 was followed using tyrosine methyl ester (0.09 g, 0.48 mmol), and sulfadiazine (0.12 g, 0.48 mmol, 1 eq.) to afford *Methyl (2,3-dihydroxy-4-oxo-4-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl)amino) butanoyl) tyrosinate* **130a** as a grey solid (0.13 g, 51% yields). m.p. 294-297°C, (HRMS: m/z calculated for $C_{24}H_{25}N_5O_9S$ (MH⁺) 559.1373, found: 560.1377; $U_{max.}/cm^{-1}$ 3450 (N-H), 3500 (OH, broad), 1740 (carboxylate C=O), and 1640 (amide N-C=O); CHN calculated C(51.20%), H(4.50%), N(12.52%), O(25.73%), S(5.73%), found: C(51.65%), H(4.25%), N(12.77%), O(25.86%), S(5.21%); δ_H (300 MHz, DMSO d_6): 3.09 (ddd, $J = 56.4, 12.5, 7.4$ Hz, 2H, $CHCH_2Ph$), 3.69 (s, 3H, OCH_3), 4.98 (t, $J = 7.4$ Hz, 1H, $CH(CH_2)N$), 5.19 (d, $J = 11.0$ Hz, 1H, $CHa(OH)$), 5.45 (d, $J = 10.8$ Hz, 1H $CHa(OH)$), 6.74 (t, $J = 7.5$ Hz, 1H, ArH), 6.81 (s, 1H, ArH), 6.92 (s, 1H, ArH), 7.11 (d, $J = 7.4$ Hz, 1H, ArH), 7.44 (d, $J = 7.4$ Hz, 1H, ArH), 7.61 (m, 3H, ArH), 8.06 (d, $J = 7.4$ Hz, 1H, ArH), 8.52 (d, $J = 7.5$ Hz, 1H, ArH); δ_C (75 MHz, DMSO d_6) 36.8 ($PhCH_2$), 51.2 (OCH_3), 56.5 ($NCH(CH_2)$), 71.1 ($CH(OH)$), 74.3 ($CH(OH)$), 115.0 (ArH), 115.4 (2x $ArCH$), 119.2 (2x ArH), 128.0 ($ArCCH_2$), 129.7 (2x ArH), 131.6 (2x $ArCH$), 135.6 ($ArCS$), 140.5 ($ArCNH$), 155.2 ($ArC(OH)$), 158.9 (2x $ArCH$), 169.1 ($ArC(N)_3$), 171.0 ($NC=O$), 172.5 ($NC=O$), 173.6 (CO_2Me).

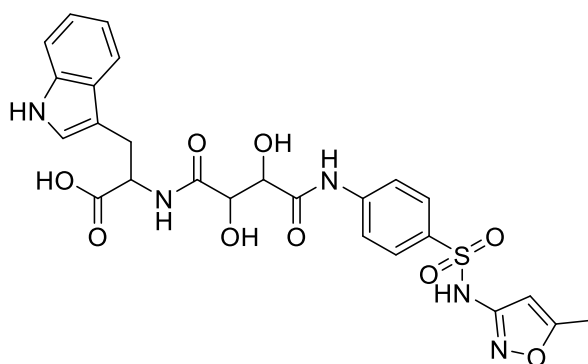
4.8.2.9. (2,3-dihydroxy-4-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl) sulfamoyl) phenyl) amino)-4-oxobutanoyl) tryptophan **131a**



The general procedure as described above in the section 4.8.2 was followed using sulfamethizole (0.13 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford

(2,3-dihydroxy-4-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl)amino)-4-oxobutanoyl)tryptophan **131a** as a brown solid (0.13 g, 48% yields). m.p. 281-284°C, (HRMS: m/z calculated for $C_{24}H_{24}N_6O_8S_2$ (MH⁺) 588.1097, found: 589.1095; $U_{max.}/cm^{-1}$ 3422 (N-H), 3500 (OH, broad), 1777 (carboxylic acid C=O), and 1624 (amide N-C=O); CHN calculated C(48.97%), H(4.11%), N(14.28%), O(21.74%), S(10.89%), found: C(48.77%), H(4.32%), N(14.47%), O(21.55%), S(10.79%); δ_H (300 MHz, DMSO- d_6) 2.22 (s, 3H, ArCCH₃), 3.31 (m, 2H, CHCH₂), 4.71 (t, J = 7.8 Hz, 1H, CHCH₂), 5.03 (d, J = 6.4 Hz, 1H, CHa(OH)), 5.19 (d, J = 6.4 Hz, 1H CHb(OH)), 7.03 (s, 1H, ArNCHC, Tryptophan), 7.16 (m, 2H, ArH), 7.31 (m, 2H, CH(CH₂N) and NH), 7.52 (m, 1H, ArH), 7.69 (d, J = 7.6 Hz, 2H, ArH), 7.85 (s, 1H, NH), 8.00 (d, J = 7.6 Hz, 2H, ArH), 8.53 (s, 1H, NH), 11.0 (s, 1H, CO₂H); δ_C (75 MHz, DMSO- d_6) 19.4 (CH₃), 28.5 (CHCH₂), 61.0 ((CHCH₂), 71.1(CH(OH)), 74.5 (CH(OH)), 109.4 (ArCCH₂), 110.6 (ArC), 118.0 (2x ArC), 118.7, 119.2, 122.4, 123.5 (ArCNH), 127.7, 128.9 (2x ArCH), 135.6 (ArCS), 136.6 (ArCNH), 140.1 ((ArCN), 143.5 ((ArCCH₃), 171.1(NC=O), 172.5 (NC=O), 173.6 (Ar(N)C(S)NH), 174.5 (C=O).

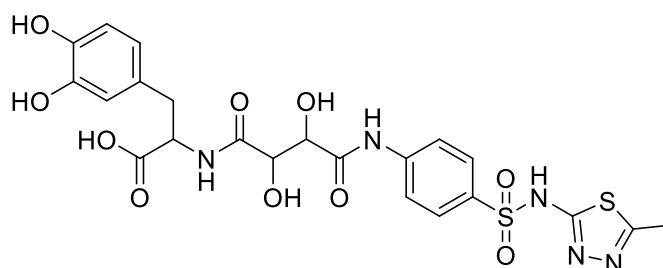
4.8.2.10. (2,3-dihydroxy-4-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-4-oxobutanoyl) tryptophan 132a



The general procedure as described above in the section 4.8.2 was followed using sulfamethoxazole (0.12 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford (2,3-dihydroxy-4-((4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)amino)-4-oxobutanoyl) tryptophan **132a** as a pale-yellow solid (0.07 g, 28% yields). m.p. 240-244°C, (HRMS: m/z calculated for $C_{25}H_{25}N_5O_9S$ (MH⁺) 571.1373, found: 572.1375;

U_{max}./cm⁻¹ 3455 (N-H), 3490 (OH, broad), 1780 (carboxylic acid C=O), and 1635 (amide N-C=O); CHN calculated C(52.54%), H(4.41%), N(12.25%), O(25.19%), S(5.61%), found: C(52.45%), H(4.66%), N(12.57%), O(25.20%), S(5.77%); δ_H (300 MHz, CDCl₃) 2.29 (s, 3H, CH₃), 3.01 (ddd, *J* = 76.5, 14.8, 7.7 Hz, 2H, CHCH₂), 4.84 (dt, *J* = 9.4, 7.7 Hz, 1H, CHCH₂), 4.90 (d, *J* = 4.4 Hz, 1H, CHCH(OH)), 5.07 (d, *J* = 4.4 Hz, 1H, CHCH(OH)), 6.09 (s, 1H, ArH heterocyclic ring), 7.15 (m, 3H, ArH), 7.35 (m, 1H, ArH), 7.52 (d, *J* = 9.4 Hz, 1H, NH), 7.61 (dd, *J* = 7.2, 1.4 Hz, 1H, ArH), 7.71 (m, 4H, ArH), 8.24 (m, 1H, NH), 9.17 (s, 1H, NH), 10.5 (s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 12.8 (ArCCH₃), 28.5 (CHCH₂), 61.0 (CHCH₂), 71.1(CH(OH)), 74.5 (CH(OH)), 94.6 (ArCH), 109.4 (ArCCH₂), 110.6 (ArC), 118.0 (2x ArC), 118.7, 119.2, 122.4, 123.5 (ArCNH), 127.7, 128.9 (2x ArCH), 135.6 (ArCS), 136.6 (ArCNH), 140.1 ((ArCN), 152.2 (ArCNHN), 168.8 (ArCCH₃), 171.1 (NC=O), 172.5 (NC=O), 173.6 (CO₂H).

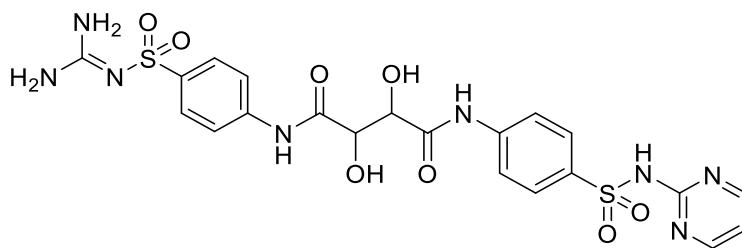
4.8.2.11. 2-(2,3-dihydroxy-4-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfamoyl)phenyl) amino)-4-oxobutanamido)-3-(3,4-dihydroxyphenyl) propanoic acid 133a



The general procedure as described above in the section 4.8.2 was followed using DOPA (0.12 g, 0.48 mmol), and sulfamethizole (0.13 g, 0.48 mmol, 1 eq.) to afford 2-(2,3-dihydroxy-4-((4-(N-(5-methyl-1,3,4-thiadiazol-2-yl) sulfamoyl) phenyl)amino)-4-oxobutanamido)-3-(3,4-dihydroxyphenyl) propanoic acid **133a** as a yellowish gel (0.13 g, 49% yields), (HRMS: *m/z* calculated for C₂₂H₂₃N₅O₁₀S₂ (MH⁺) 581.0886, found: 582.0883; U_{max}./cm⁻¹ 3500 (N-H), 3490 (OH, broad), 1795 (carboxylic acid C=O), and 1641 (amide N-C=O); CHN calculated C(45.44%), H(3.99%), N(12.04%), O(27.51%), S(11.03%), found: C(45.58%), H(3.75%), N(12.13%), O(27.65%), S(11.20%); δ_H (300 MHz, CDCl₃) 2.58 (s, 3H, CH₃), 3.13 (ddd, *J* = 126.2, 14.0, 7.7 Hz, 2H, CHCH₂), 4.84 (dt, *J* = 9.4, 7.7 Hz, 1H, CHCH₂), 4.91 (d, *J* = 4.4 Hz, 1H, CH(OH)), 5.03 (d, *J* = 4.4 Hz, 1H CH(OH)), 7.08 (m, 2H, ArH), 7.36– 7.60 (m, 2H, ArH, and NH), 7.58 – 7.80 (m, 5H, ArH), 9.17 (s, 1H, NH), 12.7 (s, 1H, CO₂H); δ_C (75

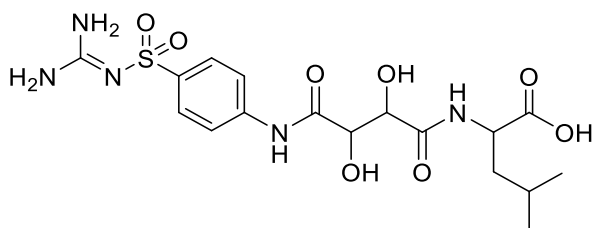
MHz, CDCl₃) 19.4 (CH₃), 36.2 (CHCH₂), 61.0 (CHCH₂), 71.1(CH(OH)), 74.5 (CH(OH)), 115.6, 116.8, 118.4 (2x ArCH), 122.5, 128.4 (2x (2x ArCH), 130.3, 135.6, 141.4, 143.6, 144.3 and 145.3 (ArC(OH)), 171.1(NC=O), 172.5 (NC=O), 173.6 (Ar(N)C(S)NH), 174.5 (C=O).

4.8.2.12. N¹-(4-(N-(diaminomethylene) sulfamoyl) phenyl)-2,3-dihydroxy-N⁴-(4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) succinamide 134a



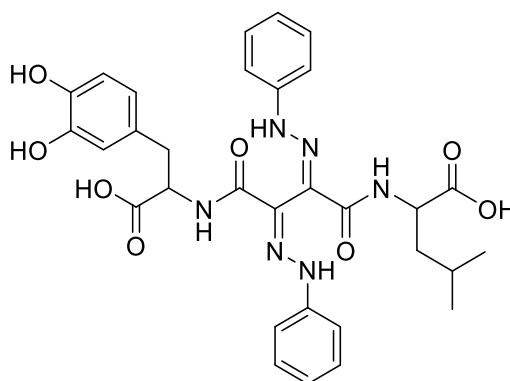
The general procedure as described above in the section 4.8.2 was followed using sulfaguanidine (0.01 g, 0.48 mmol), and sulfadiazine (0.12 g, 0.48 mmol, 1 eq.) to afford N¹-(4-(N-(diaminomethylene) sulfamoyl) phenyl)-2,3-dihydroxy-N⁴-(4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl) succinamide **134a** as a off-white powder (0.05 g, 21% yields). m.p. 205-207°C, (HRMS: m/z calculated for C₂₁H₂₂N₈O₈S₂ (MH⁺) 578.1002, found: 579.1005; U_{max}./cm⁻¹ 3250 (N-H), 3500 (OH, broad), and 1641 (amide N-C=O); CHN calculated C(43.60%), H(3.86%), N(19.37%), O(22.12%), S(11.08%), found: C(43.50%), H(3.80%), N(19.24%), O(22.44%), S(11.65%); δ_H (300 MHz, CDCl₃) 4.89 (d, J = 2.8 Hz, 1H, CH(OH)), 5.03 (d, J = 2.6 Hz, 1H CH(OH)), 6.73 (s, 4H, 2x NH₂), 7.32 (t, J = 3.5 Hz, 1H, ArH), 7.71 (q, J = 8.6 Hz, 4H, ArH), 7.85 (d, J = 1.3 Hz, 4H, ArH), 8.58 (d, J = 3.5 Hz, 2H, ArH), 9.17 (s, 1H, 2x NH); δ_C (75 MHz, CDCl₃) 71.1(CH(OH)), 74.5 (CH(OH)), 115.6, 118.4 (2x ArCH), 118.8 (2x ArCH), 126.4 (2x ArCH), 128.8 (2x ArCH), 135.5, 140.2, 142.4, 143.8, 157.6 (2x ArCH), 158.9 (C=N(NH₂)), 169.3, 171.1(NC=O), 172.5 (NC=O).

4.8.2.13. (4-((4-(N-(diaminomethylene)sulfamoyl)phenyl)amino)-2,3-dihydroxy-4-oxobutanoyl) leucine 135a



The general procedure as described above in the section 4.8.2 was followed using sulfaguanidine (0.10 g, 0.48 mmol), and tryptophan (0.09 g, 0.48 mmol, 1 eq.) to afford (4-((4-(N-(diaminomethylene) sulfamoyl) phenyl) amino)-2,3-dihydroxy-4-oxobutanoyl) leucine **135a** as a grey powder (0.12 g, 24% yields). m.p. 187-189°C, (HRMS: m/z calculated for $C_{17}H_{25}N_5O_5S$ (MH⁺) 459.1424, found: 460.1421; U_{max}/cm^{-1} 3255 (N-H), 3500 (OH, broad), and 1633 (amide N-C=O); CHN calculated C(44.44%), H(5.48%), N(15.24%), O(27.86%), S(6.98%), found: C(44.31%), H(5.40%), N(15.33%), O(27.68%), S(6.77%); δ_H (300 MHz, $CDCl_3$) 0.95 (d, J = 6.9 Hz, 3H, (CH_3)), 0.90 (d, J = 6.9 Hz, 3H, (CH_3)), 1.35 –1.73 (m, 1H, $CH(CH_3)_2$), 1.87 (m, 2H, $CH(CH_2)$), 4.62 (dt, J = 9.3, 6.8 Hz, 1H, $NHCH(CH_2)$), 5.19 (d, J = 5.9 Hz, 1H, $CH(OH)$), 5.34 (d, J = 5.9 Hz, 1H, $CH(OH)$), 6.73 (s, 4H, 2x NH_2), 7.30 (s, 1H, NH), 7.76 (m, 2H, ArH), 7.80 (d, J = 8.5 Hz, 2H, ArH), 9.17 (s, 1H, NH), 10.6 (s, 1H, CO_2H); δ_C (75 MHz, $CDCl_3$) 22.8 (2x CH_3), 24.5 ($CHCH_3$)₂, 41.5 ($CHCH_2CH$), 55.5 ($CHCH_2CH$), 71.1($CH(OH)$), 74.5 ($CH(OH)$), 118.8 (2x $ArCH$), 126.4 (2x $ArCH$), 140.2, 142.4, 158.9 ($C=N(NH_2)$), 171.1($NC=O$), 172.5 ($NC=O$), 174.3 ($C=O$).

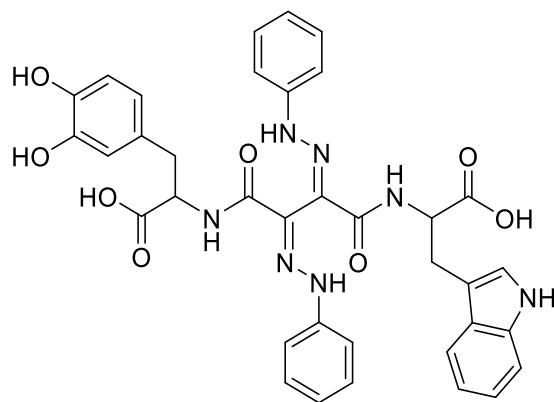
4.8.2.14. ((2E,3E)-4-((1-carboxy-2-(3,4-dihydroxyphenyl)ethyl)amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) leucine 136b



The general procedure as described above in the section 4.8.2 was followed using DOPA (0.05 g, 0.27 mmol, 1 eq.), and Leucine (0.03 g, 0.27 mmol, 1 eq.) to afford ((2E,3E)-4-((1-carboxy-2-(3,4-dihydroxyphenyl) ethyl) amino)-4-oxo-2,3-bis(2-

phenylhydrazono) butanoyl) leucine **136b** as a grey solid (0.07 g, 45% yields). m.p. 187-189°C, (HRMS: m/z calculated for $C_{31}H_{34}N_6O_8$ (MH⁺) 618.2438, found 619.2436; $U_{max.}/cm^{-1}$ 3368 (N-H), 3474 (OH, broad), and 1650 (amide N-C=O); CHN calculated C(60.19%), H(5.54%), N(13.58%), O(20.69%), found: C(60.32%), H(5.50%), N(13.39%), O(20.77%); δ_H (300 MHz, $CDCl_3$) 0.90 (d, J = 6.9 Hz, 3H, CH_3), 0.95 (d, J = 6.9 Hz, 3H, CH_3), 1.52 (m, 1H, $CHCH_3$), 1.86 (m, 2H, $CHCH_2CH$), 3.29 (m, 2H, $CHCH_2Ph$), 4.47 (dt, J = 9.2, 6.8 Hz, 1H, $NHCHCH_2$), 5.02 (dt, J = 9.2, 7.7 Hz, 1H, $NHCHCH_2$), 6.16 (dd, J = 9.3, 4.2 Hz, 2H, 2x NH), 70.6 (m, 3H, ArH), 7.13 (s, 1H, NH), 7.34 (dd, J = 4.1, 1.2 Hz, 9H, ArH), 7.44 (d, J = 8.4 Hz, 1H, ArH), 10.3 (s, 1H, NH), 10.5 (s, 1H, NH), 10.9 (s, 1H, CO_2H); 75 MHz, $CDCl_3$) 22.8 (2x CH_3), 24.5 ($CHCH_3$), 36.5 ($PhCH_2$), 41.5 ($CHCH_2CH$), 55.5 ($CHCH_2CH$), 58.5 ($NHCHCH_2$), 113.8 (4x $ArCH$), 115.4, 116.8, 121.8 (2x $ArCH$), 123.4, 128.7 (4x $ArCH$), 130.7, 134.3 and 134.7 (C=N), 143.5 (2x $ArCNH$), 144.8 and 145.6 ($ArC(OH)$), 159.5 and 160.4 (NC=O), 174.3 and 174.4 (C=O).

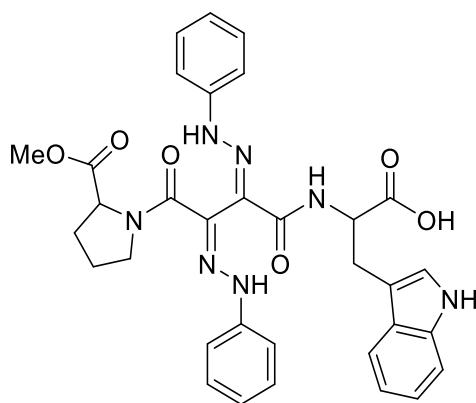
4.8.2.15. 2-((2E,3E)-4-((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-4-oxo-2,3-bis(2-phenyl hydrazono) butanamido)-3-(3,4-dihydroxyphenyl) propanoic acid 137b



The general procedure as described above in the section 4.8.2 was followed using DOPA (0.05 g, 0.27 mmol, 1 eq), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford 2-((2E,3E)-4-((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanamido)-3-(3,4-dihydroxyphenyl) propanoic acid **137b** as a grey powder (0.10 g, 55% yields). m.p. 187-189°C, (HRMS: m/z calculated for $C_{36}H_{33}N_7O_8$ (MH⁺) 691.2391, found: 692.2393; $U_{max.}/cm^{-1}$ 3455 (N-H), 3500 and 3000 (OH, broad, ad OH of the carboxylic acid), 2650 (C=N), and 1660 (amide N-C=O); CHN calculated C(62.51%), H(4.81%), N(14.18%), O(18.50%), found: C(62.43%), H(4.71%), N(14.28%), O(18.60%); δ_H (300 MHz, $CDCl_3$) 2.84 (m, 4H,

CHCH₂), 4.96 (m, 2x CHCH₂), 2H, CHCH₂), 7.06 (m, 1H, ArH), 7.14 (m, 1H, ArH), 7.21 (m, 3H, ArH), 7.35 (m, 12H, ArH), 7.61 (dd, *J* = 7.2, 1.3 Hz, 1H, ArH), 8.00 (dd, *J* = 9.4, 0.6 Hz, 2H, 2x NH), 10.2 (s, 2H, 2x CO₂H); δ_C (75 MHz, CDCl₃) 28.8 (CHCH₂), 36.5 (PhCH₂), 55.5 (CHCH₂CH), 58.5 (NHCHCH₂), 109.5, 111.4, 113.8 (4x ArCH), 115.4, 116.8, 118.4, 119.8, 120.9, 121.8 (2x ArCH), 122.7, 123.2, 126.7, 128.7 (4x ArCH), 130.7, 134.3 and 134.7 (C=N), 136.8, 143.5 (2x ArCNH), 144.8 and 145.6 (ArC(OH)), 159.5 and 160.4 (NC=O), 174.3 and 174.4 (C=O).

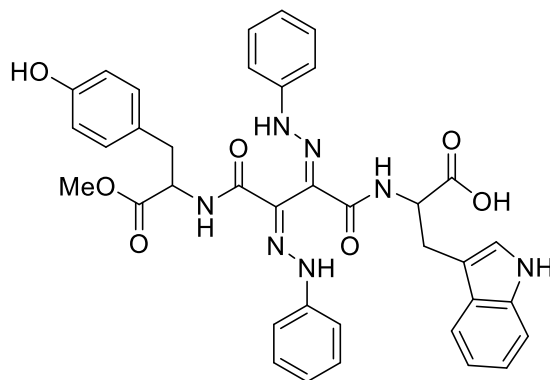
4.8.2.16. ((2E,3E)-4-(2-(methoxycarbonyl) pyrrolidin-1-yl)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl)tryptophan 138b



The general procedure as described above in the section 4.8.2 was followed using proline methyl ester (0.04 g, 0.27 mmol, 1 eq), and tryptophan (0.05 g, 0.27 mmol, 1 eq) to afford ((2E,3E)-4-(2-(methoxycarbonyl)pyrrolidin-1-yl)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tryptophan **138b** as a viscous gel (0.08 g, 52% yields). (HRMS: *m/z* calculated for C₃₃H₃₃N₇O₆ (MH⁺) 623.2492, found: 624.2494; U_{max}./cm⁻¹ 3250 (N-H), 3000 (OH carboxylic acid), 2260 (C=N), and 1632 (amide N-C=O); CHN calculated C(63.65%), H(5.33%), N(15.72%), O(15.39%), found: C(63.58%), H(5.29%), N(15.41%), O(15.44%); δ_H (300 MHz, CDCl₃) 1.81 (m, 4H, ArCH₂ overlapping on proline), 3.21 (ddd, *J* = 76.5, 14.8, 7.7 Hz, 2H, CH(CH₂)NH, tryptophan), 3.48 (m, 2H, ArCH₂), 3.69 (s, 3H, OCH₃), 4.51 (t, *J* = 5.9 Hz, 1H, ArNCHCH₂ on proline), 4.96 (dt, *J* = 9.4, 7.7 Hz, 1H, CH(CH₂)NH), 7.26 (m, 16H, ArH and CDCl₃), 7.61 (dd, *J* = 7.0, 1.5 Hz, 1H, ArH), 8.00 (d, *J* = 9.4 Hz, 1H, NH), 8.24 (m, 1H, ArNH, tryptophan), 10.2 (s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 24.5, (ArCH₂), 28.4 (ArCH₂), 28.8 (CHCH₂), 49.5 (CHCH₂), 51.5 (OCH₃), 60.5, 64.6 (ArCHCO₂), 109.5, 111.4, 113.8 (4x ArCH), 118.4, 119.8, 120.9, 121.8 (2x ArCH),

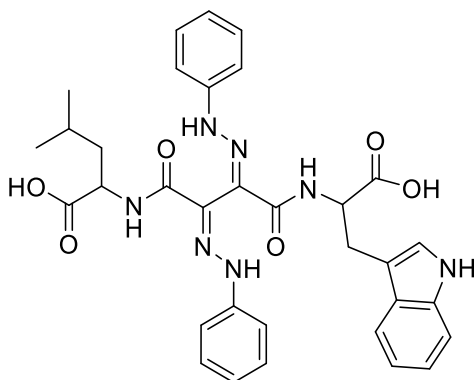
122.7, 126.7, 128.7 (4x ArCH), 134.3 and 134.7 (C=N), 136.8, 143.5 (2x ArCNH), 161.3 and 163.6 (NC=O), 171.5 (C=O ester) and 174.4 (C=O).

4.8.2.17. ((2E,3E)-4-((3-(4-hydroxyphenyl)-1-methoxy-1-oxopropan-2-yl)amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tryptophan **139b**



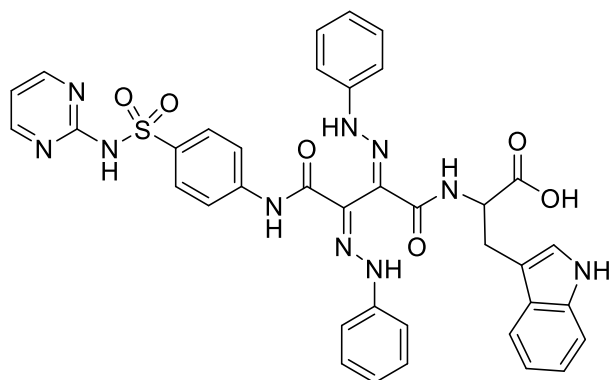
The general procedure as described above in the section 4.8.2 was followed using tyrosine methyl ester (0.05 g, 0.27 mmol, 1 eq.), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford ((2E,3E)-4-((3-(4-hydroxyphenyl)-1-methoxy-1-oxopropan-2-yl)amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tryptophan **139b** as a off-white powder (0.10 g, 49% yields). m.p. 277-300°C, (HRMS: m/z calculated for C₃₇H₃₅N₇O₇ (MH⁺) 689.2598, found: 690.2596; U_{max}/cm⁻¹ 3220 (N-H), 2900 (OH carboxylic acid), 2340 (C=N), and 1639 (amide N-C=O); CHN calculated C(64.43%), H(5.12%), N(14.22%), O(16.24%); found: C(64.52%), H(5.25%), N(14.32%), O(16.68%); δ_H (300 MHz, CDCl₃) 3.26 (m, 4H 2x CHCH₂), 3.68 (s, 3H, OCH₃), 4.83 (dt, *J* = 9.4, 7.7 Hz, 1H CHCH₂), 5.00 (dt, *J* = 9.4, 7.8 Hz, 1H, CHCH₂), 7.17 (m, 19H, CDCl₃ and ArH), 7.44 (m, 2H, ArH), 7.61 (m, 1H, ArH), 8.54 (d, *J* = 9.4 Hz, 1H, NH), 8.82 (s, 1H, NH), 9.16 (t, *J* = 2.4 Hz, 1H, NH), 9.26 (d, *J* = 9.2 Hz, 1H, NH), 9.43 (s, 1H, NH), 12.0 (s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 28.8 (CHCH₂), 36.5 (PhCH₂), 51.8 (OCH₃), 55.6, 60.5, 109.5, 111.4, 113.8 (4x ArCH), 115.5 (2x ArCH), 118.4, 119.8, 120.9, 121.8 (2x ArCH), 122.7, 126.7, 128.7 (4x ArCH), 129, (ArCCH₂), 130.6 (2x ArCH), 134.3 and 134.7 (C=N), 136.8, 143.5 (2x ArCNH), 155.4 (ArC(OH)), 161.3 and 163.6 (NC=O), 171.5 (C=O ester) and 174.4 (C=O).

4.8.2.18. ((2E,3E)-4-((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl)leucine **140b**



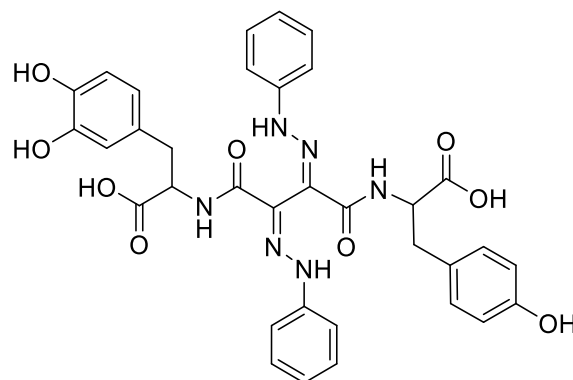
The general procedure as described above in the section 4.8.2 was followed using Leucine (0.03 g, 0.27 mmol, 1 eq.), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford *((2E,3E)-4-((1-carboxy-2-(1H-indol-3-yl)ethyl)amino)-4-oxo-2,3-bis(2-phenylhydrazono)butanoyl)leucine* **140b** as a brownish powder (0.08 g, 50% yields). m.p. 245-247°C, (HRMS: m/z calculated for $C_{33}H_{35}N_7O_6$ (MH^+) 625.2649, found: 626.2647; ν_{max}/cm^{-1} 3340 (N-H), 2970 (OH carboxylic acid), 2410 (C=N), and 1650 (amide N-C=O); CHN calculated C(63.35%), H(5.64%), N(15.67%), O(15.34%); found: C(63.13%), H(5.54%), N(15.19%), O(15.75%); δ_H (300 MHz, $CDCl_3$) 0.91 (d, $J = 6.8$ Hz, 3H, $CHCH_3$), 0.95 (d, $J = 6.7$ Hz, 3H, $CHCH_3$), 1.76 (m, 4H, $CHCHCH_2$ and $CHCH_2$ Leucine), 3.27 (m, 2H, $CHCH_2$), 4.44 (m, 1H, $CHCH_2$), 5.25 (dt, $J = 9.4, 7.7$ Hz, 1H, $CHCH_2$), 6.36 (d, $J = 9.4$ Hz, 1H, ArH), 6.67 (s, 1H, NH), 6.95 (d, $J = 9.2$ Hz, 1H, ArH), 7.11 (m, 6H, ArH), 7.34 (m, 11H, ArH), 7.61 (dd, $J = 7.2, 1.3$ Hz, 1H, ArH), 8.24 (m, 1H, NH), 9.08 (s, 1H, NH), 10.70 and 10.7 (s, 2H, CO_2H); δ_C (75 MHz, $CDCl_3$) 22.5 ($2 \times CH_3$), 24.3 ($CH(CH_3)_2$), 28.8, 41.2, 55.6, 61.0, 109.5, 111.4, 113.8 ($4 \times ArCH$), 118.4, 119.8, 120.9, 121.8 ($2 \times ArCH$), 122.7, 126.7, 128.7 ($4 \times ArCH$), 134.3 and 134.7 (C=N), 136.8, 143.5 ($2 \times ArCNH$), 161.3 and 163.6 (NC=O), 174.3 and 174.5 (C=O).

4.8.2.19. ((2E,3E)-4-oxo-2,3-bis(2-phenylhydrazono)-4-((4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl) amino) butanoyl)tryptophan 141b



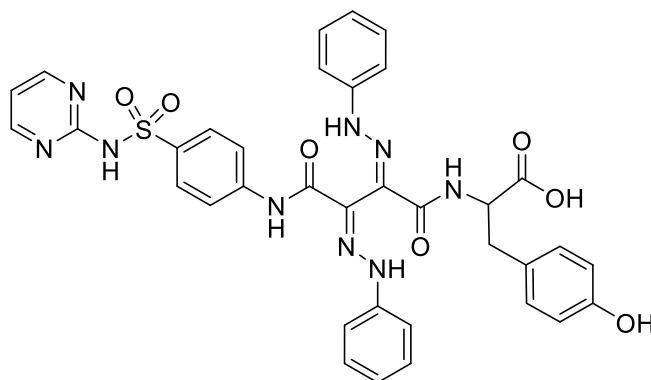
The general procedure as described above in the section 4.8.2 was followed using sulfadiazine (0.06 g, 0.27 mmol, 1 eq.), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford *((2E,3E)-4-oxo-2,3-bis(2-phenylhydrazono)-4-((4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl) amino) butanoyl) tryptophan* **141b** as a off-white powder (0.07 g, 38%); RMS: m/z calculated for $C_{37}H_{32}N_{10}O_8S$ (MH⁺) 744.2227, found: 745.2229; U_{max}/cm^{-1} 3377 (N-H), 3000 (OH carboxylic acid), 2670 (C=N), and 16350 (amide N-C=O); CHN calculated C(59.67%), H(4.33%), N(18.81%), O(12.89%), S(4.30%); found: C(59.77%), H(4.41%), N(18.99%), O(12.72%), S(4.41%); δ_H (300 MHz, $CDCl_3$) 3.23 (ddd, $J = 87.2, 14.8, 7.8$ Hz, 2H, CHCH₂), 4.78 (dt, $J = 9.2, 7.7$ Hz, 1H, CHCH₂), 7.05 (m, 15H, ArH), 7.25 (s, 1H, $CDCl_3$), 7.34 (m, 1H, ArH), 7.60 (m, 1H, ArH), 7.78 (m, 4H, ArH), 8.51 (d, $J = 3.5$ Hz, 2H, ArH), 9.12 (d, $J = 9.2$ Hz, 1H, NH), 9.16 (t, $J = 2.3$ Hz, 1H, NH), 9.98 (s, 1H, NH), 11.1 (s, 1H, CO₂H); δ_C (75 MHz, $CDCl_3$) 28.8 (CHCH₂), 61.0 (CHCH₂), 109.5, 111.4, 113.8 (4x ArCH), 115.4, 118.1 (2x ArCH), 118.7, 119.8, 120.9, 121.8 (2x ArCH), 122.7, 126.7, 128.7 (4x ArCH), 129.0 (2x ArCH), 134.3 and 134.7 (C=N), 135.5, 136.8, 141.8, 143.5 (2x ArCNH), 155.9 (2x ArCH), 159.9 and 162.6 (NC=O), 169.9 (ArC(N₃)), and 174.5 (C=O).

4.8.2.20. ((2E,3E)-4-((1-carboxy-2-(3,4-dihydroxyphenyl)ethyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tyrosine 142b



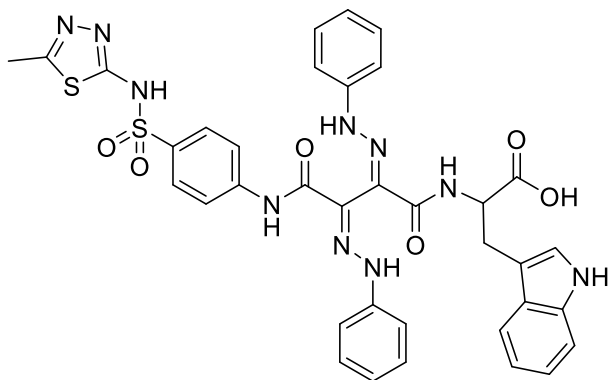
The general procedure as described above in the section 4.8.2 was followed using tyrosine (0.04 g, 0.27 mmol, 1 eq), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford ((2E,3E)-4-((1-carboxy-2-(3,4-dihydroxyphenyl)ethyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tyrosine **142b** as a yellow powder (0.12 g, 55% yields). m.p. 191-195°C, (HRMS: m/z calculated for $C_{34}H_{32}N_6O_9$ (MH⁺) 668.2231, found: 669.2230; $\nu_{\max}/\text{cm}^{-1}$ 3400 (N-H), 2957 (OH carboxylic acid), 2679 (C=N), and 1654 (amide N-C=O); CHN calculated C(61.07%), H(4.82%), N(12.57%), O(21.53%); found: C(61.10%), H(4.90%), N(12.48%), O(21.41); δ_H (300 MHz, $CDCl_3$) 3.09 (m, 4H, 2x $CHCH_2$), 4.78 (m, 2H, 2x $CHCH_2$), 7.08 (m, 5H, ArH), 7.26 (d, $J = 1.1$ Hz, 1H, ArH), 7.32 (m, 8H, ArH and $CDCl_3$), 7.14 (m, 3H, ArH), 9.18 (s, 1H, NH), 9.21 (s, 1H, NH), 9.26 (d, $J = 1.6$ Hz, 1H, NH), 9.29 (d, $J = 2.3$ Hz, 1H, NH), 12.0 (d, $J = 1.0$ Hz, 2H, CO_2H); δ_C (75 MHz, $CDCl_3$) 36.3 ($CHCH_2$), 36.7 ($CHCH_2$), 60.1 ($CHCH_2$), 60.5 ($CHCH_2$), 113.8 (4x ArCH), 115.4 (2x ArCH), 115.6, 116.8, 121.8 (2x ArCH), 122.5, 126.7, 128.7 (4x ArCH), 129.0, 130.3 (2x ArCH), 134.3 and 134.7 (C=N), 143.5 (2x ArCNH), 144.4 and 145.6 (ArC(OH)), 157.1 (ArC(OH)), 160.0 and 160.6 (NC=O), 174.5 (2x C=O).

4.8.2.21. ((2E,3E)-4-oxo-2,3-bis(2-phenylhydrazono)-4-((4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) amino) butanoyl) tyrosine 143b



The general procedure as described above in the section 4.8.2 was followed using sulfadiazine (0.06 g, 0.27 mmol, 1 eq.), and tyrosine (0.04 g, 0.27 mmol, 1 eq.) to afford ((2*E*,3*E*)-4-oxo-2,3-bis(2-phenylhydrazono)-4-((4-(*N*-(pyrimidin-2-yl)sulfamoyl) phenyl) amino) butanoyl) tyrosine **143b** as brown powder (0.08 g, 43% yields). m.p. 310-314°C, (HRMS: *m/z* calculated for C₃₅H₃₁N₉O₇S (MH⁺) 721.2067, found 722.2067; U_{max}./cm⁻¹ 3310 (N-H), 2860 (OH carboxylic acid), 2488 (C=N), and 1678 (amide N-C=O); CHN calculated C(58.25%), H(4.33%), N(17.47%), O(15.52%), S(4.44%); found: C(58.14%), H(4.47%), N(17.88%), O(15.43%), S(4.55%); δ_H (300 MHz, CDCl₃) 3.00 (dd, *J* = 18.2, 7.8 Hz, 1H, CHCH₂), 4.60 (dt, *J* = 9.3, 7.7 Hz, 1H CHCH₂), 7.08 (m, 5H, ArH), 7.30 (m, 9H, CDCl₃ and ArH), 7.47 (m, 2H, ArH), 7.75 (m, 4H, ArH), 8.36 (d, *J* = 3.4 Hz, 2H, ArH and NH), 9.15 (d, *J* = 9.4 Hz, 1H, NH), 9.64 (d, *J* = 5.5 Hz 2H, NH), 10.3 (s, 1H, NH), 12.03(s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 36.4 (CHCH₂), 60.1 (CHCH₂), 113.8 (4x ArCH), 115.2, 115.9 (2x ArCH), 118.2 (2x ArCH), 123.6 (2x ArCH), 127.0, 128.7 (4x ArCH), 129.0 (2x ArCH), 130.3 (2x ArCH), 134.3 and 134.7 (C=N), 135.8 (ArCS), 141.3 (ArCNH), 143.5 (2x ArCNH), 155.2 (ArC(OH)), 158.8 (2x ArCH), 159.9 and 162.6 (NC=O), 169.9 (ArC(N₃)), and 174.5 (C=O).

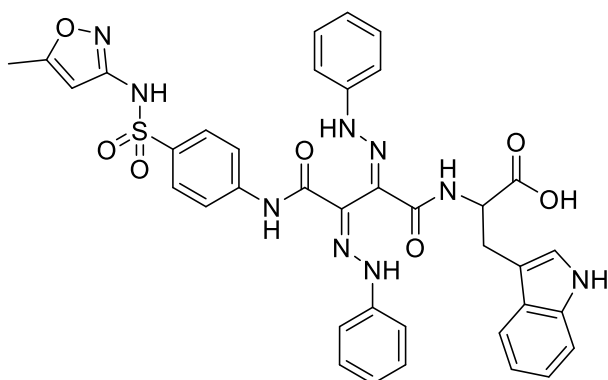
4.8.2.22. ((2*E*,3*E*)-4-((4-(*N*-(5-methyl-1,3,4-thiadiazol-2-yl) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl)tryptophan **144b**



The general procedure as described above in the section 4.8.2 was followed using sulfamethizole (0.07 g, 0.27 mmol, 1 eq.), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford ((2*E*,3*E*)-4-((4-(*N*-(5-methyl-1,3,4-thiadiazol-2-yl) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl)tryptophan **144b** as a pale-yellow powder (0.06 g, 31% yields). m.p. 213-217°C, (HRMS: *m/z* calculated for

$C_{36}H_{32}N_{10}O_6S_2$ (MH⁺) 764.1948, found: 765.1946; $U_{max.}/cm^{-1}$ 3340 (N-H), 2600 (OH carboxylic acid), 2300 (C=N), and 1631 (amide N-C=O); CHN calculated C(56.53%), H(4.22%), N(18.31%), O(12.55%), S(8.38%); found: C(56.33%), H(4.14%), N(18.51%), O(12.44%), S(8.65%); δ_H (300 MHz, $CDCl_3$) 2.62 (s, 3H, CH_3), 3.23 (ddd, J = 87.2, 14.8, 7.8 Hz, 2H, $CHCH_2$), 4.78 (dt, J = 9.2, 7.7 Hz, 1H, $CHCH_2$), 6.91 (m, 8H, ArH), 7.16 (m, 6H, ArH), 7.25 (s, 1H, $CDCl_3$), 7.34 (m, 1H, ArH), 7.60 (m, 1H, ArH), 7.78 (m, 4H, ArH), 9.12 (d, J = 9.2 Hz, 1H, NH), 9.16 (t, J = 2.3 Hz, 1H, NH), 9.79 (s, 1H, NH), 9.9 (s, 1H, NH), 11.9 (s, 1H CO_2H); δ_C (75 MHz, $CDCl_3$) 19.6 ($ArCH_3$), 28.8 ($CHCH_2$), 60.1 ($CHCH_2$), 109.5, 111.4, 113.8 (4x $ArCH$), 118.1 (2x $ArCH$), 118.7, 119.8, 120.9, 121.8 (2x $ArCH$), 122.7, 126.7, 128.7 (4x $ArCH$), 129.7 (2x $ArCH$), 134.3 and 134.7 (C=N), 135.5, 136.8, 141.8, 142.5 ($ArC(CH_3)$), 143.5 (2x $ArCNH$), 159.9 and 162.6 (NC=O), 172.6 ($ArC(SN_2)$), and 174.3 (C=O).

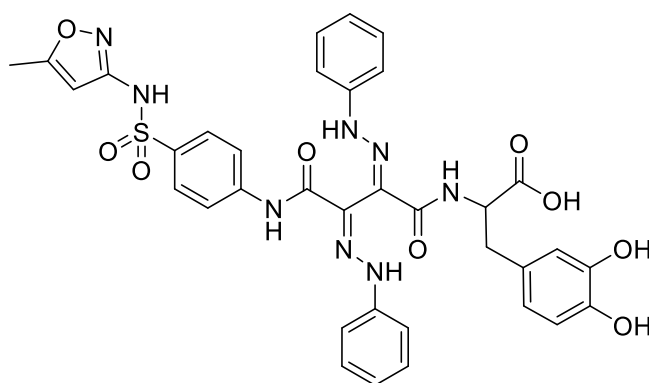
4.8.2.23. ((2E,3E)-4-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tryptophan **145b**



The general procedure as described above in the section 4.8.2 was followed using sulfamethoxazole (0.06 g, 0.27 mmol, 1 eq), and tryptophan (0.05 g, 0.27 mmol, 1 eq.) to afford ((2E,3E)-4-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tryptophan **145b** as a grey powder (0.04 g, 20% yields). m.p. 287-291°C, (HRMS: m/z calculated for $C_{37}H_{33}N_9O_7S$ (MH⁺) 747.2224, found: 748.2221; $U_{max.}/cm^{-1}$ 3440 (N-H), 2682 (OH carboxylic acid), 3200 (C=N), and 1690 (amide N-C=O); CHN calculated C(59.43%), H(4.45%), N(6.86%), O(14.98%), S(4.29%); found: C(59.50%), H(4.52%), N(6.73%), O(14.69%), S(4.66%); δ_H (300 MHz, $CDCl_3$) 2.29 (s, 3H, CH_3), 3.12 (m, 2H, $CHCH_2$), 4.96 (dt, J = 9.4, 7.7 Hz, 1H, $CHCH_2$), 6.09 (s, 1H, ArH), 7.24 (m, 15H, ArH and $CDCl_3$), 7.64 (m, 5H, ArH), 8.00 (d, J = 9.4 Hz, 1H, ArH), 8.23 (m, 1H, ArH), 8.70 (s, 1H, NH), 9.75

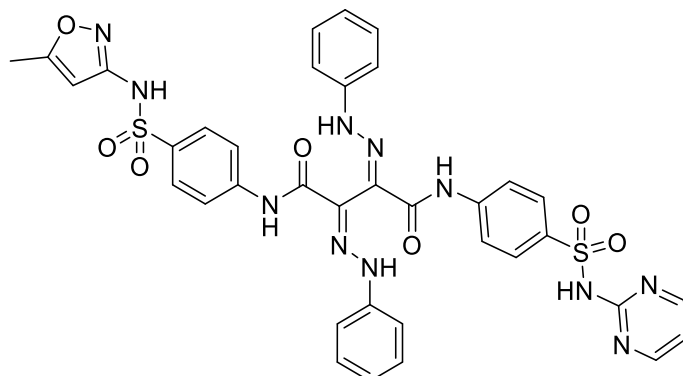
(s, 1H, NH), 10.2 (s, 1H, CO₂H); δ_c (75 MHz, CDCl₃) 12.6 (ArCCH₃), 28.8 (CHCH₂), 60.1 (CHCH₂), 95.6 (ArCH), 109.5, 111.4, 113.8 (4x ArCH), 118.1 (2x ArCH), 118.7, 119.8, 120.9, 121.8 (2x ArCH), 122.7, 126.7, 128.7 (4x ArCH), 129.7 (2x ArCH), 134.3 and 134.7 (C=N), 135.5, 136.8, 141.8, 143.5 (2x ArCNH), 151.3 (ArC(N₂)(CH)), 158.8 and 161.4 (NC=O), 168.9 (ArC(CH₃)), and 174.3 (C=O).

4.8.2.24. 3-(3,4-dihydroxyphenyl)-2-((2E,3E)-4-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2- phenylhydrazono) butanamido) propanoic acid 146b



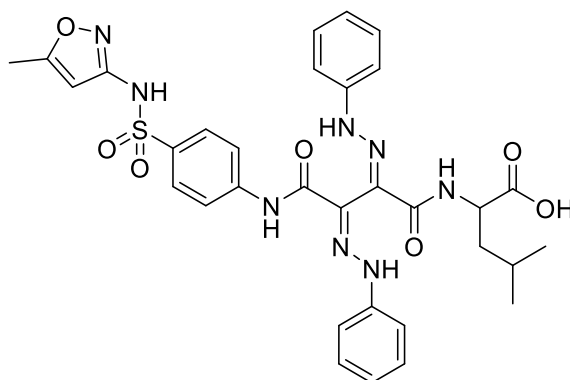
The general procedure as described above in the section 4.8.2 was followed using sulfamethoxazole (0.06 g, 0.27 mmol, 1 eq.), and DOPA (0.05 g, 0.27 mmol, 1 eq.) to afford 3-(3,4-dihydroxyphenyl)-2-((2E,3E)-4-((4-(N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2- phenylhydrazono) butanamido) propanoic acid **146b** as a yellowish powder (0.04 g, 25% yields). m.p. 287-291°C, (HRMS: m/z calculated for C₃₅H₃₂N₈O₉S (MH⁺) 740.2013, found: 741.2015; U_{max}/cm⁻¹ 3390 (N-H), 2541 (OH carboxylic acid), 3254 (C=N), and 1645 (amide N-C=O); CHN calculated C(56.75%), H(4.35%), N(15.13%), O(19.44%), S(4.33%); found: C(56.17%), H(4.45%), N(15.33%), O(19.52%), S(4.12%); δ_H (300 MHz, CDCl₃) 2.29 (s, 3H, CH₃), 3.27 (m, 2H, CHCH₂), 4.96 (dt, J = 9.2, 7.7 Hz, 1H, CHCH₂), 5.95 (d, J = 9.3 Hz, 1H, NH), 6.09 (s, 1H, ArH), 6.42 (s, 1H, ArH), 7.08 (m, 3H, ArH), 7.34 (m, 11H, ArH), 7.47 (d, J = 8.4 Hz, 1H, ArH), 7.71 (m, 5H, ArH), 7.87 (s, 1H, NH), 9.75 (s, 1H, NH), 12.4 (s, 1H, CO₂H); δ_c (75 MHz, CDCl₃) 12.6 (ArCCH₃), 36.3 (CHCH₂), 60.1 (CHCH₂), 95.6 (ArCH), 113.8 (4x ArCH), 115.4, 116.7, 118.1 (2x ArCH), 121.8 (2x ArCH), 122.7, 128.7 (4x ArCH), 129.7 (2x ArCH), 130.8, 134.3 and 134.7 (C=N), 135.5, 141.8, 143.5 (2x ArCNH), 144.3 and 144.6 (ArCH(OH)), 151.3 (ArC(N₂)(CH)), 158.8 and 161.4 (NC=O), 168.9 (ArC(CH₃)), and 174.3 (C=O).

4.8.2.25. (2E,3E)-N¹-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-2,3-bis(2-phenylhydrazono)-N⁴-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)succinamide **147b**



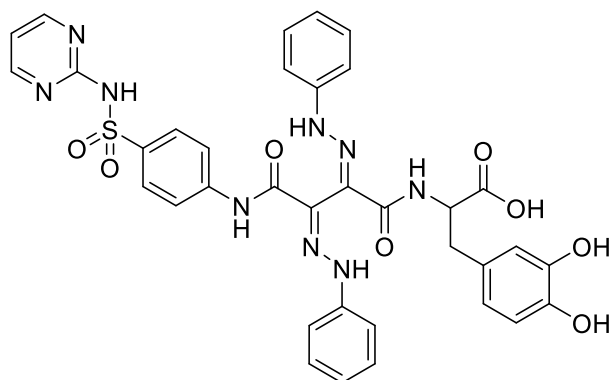
The general procedure as described above in the section 4.8.2 was followed using sulfadiazine (0.06 g, 0.27 mmol, 1 eq.), and sulfamethoxazole (0.06 g, 0.27 mmol, 1 eq.) to afford (2E,3E)-N¹-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)-2,3-bis(2-phenylhydrazono)-N⁴-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)succinamide **147b** as a off-white powder (0.04 g, 22% yields). m.p. 166-169°C, (HRMS: m/z calculated for C₃₆H₃₁N₁₁O₇S₂ (MH⁺) 793.7359, found 794.7359; U_{max}./cm⁻¹ 3220 (N-H), 2450 (OH carboxylic acid), 3480 (C=N), and 1682 (amide N-C=O); CHN calculated C(54.47%), H(3.94%), N(19.41%), O(14.11%), S(8.08%); found: C(54.75%), H(3.80%), N(19.66%), O(14.33%), S(8.89%); δ_H (300 MHz, CDCl₃) 2.29 (s, 3H, CH₃), 6.09 (s, 1H, ArH), 7.24 (m, 12H, ArH), 7.68 (m, 8H, ArH), 8.58 (d, J = 3.5 Hz, 2H, NH), 8.70 (s, 2H, NH), 9.75 (d, J = 0.6 Hz, 2H, NH); δ_C (75 MHz, CDCl₃) 12.6 (ArCCH₃), 95.6 (ArCH), 113.8 (4x ArCH), 115.4, 118.1 (4x ArCH), 121.8 (2x ArCH), 128.7 (4x ArCH), 129.7 (4x ArCH), 134.3 and 134.7 (C=N), 135.5 (2x ArCS), 141.8 (2x ArCNH), 143.5 (2x ArCNH), 151.3 (ArC(N₂)(CH)), 158.5 (2x ArCH), 160.9 and 161.4 (NC=O), 168.9 (ArC(N₃)), 169.4 (ArC(CH₃)).

4.8.2.26. ((2E,3E)-4-((4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)amino)-4-oxo-2,3-bis(2-phenylhydrazono)butanoyl)leucine **148b**



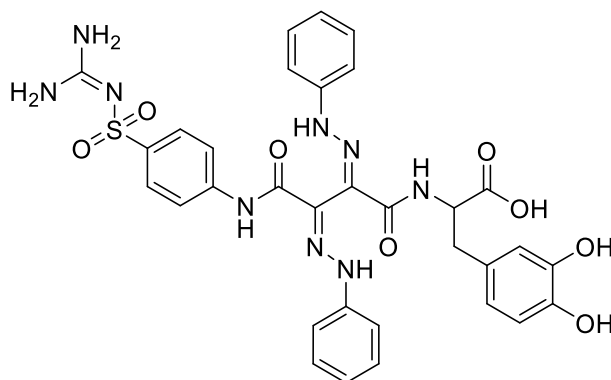
The general procedure as described above in the section 4.8.2 was followed using Leucine (0.03 g, 0.27 mmol, 1 eq.), and sulfamethoxazole (0.06 g, 0.27 mmol, 1 eq.) to afford ((2*E*,3*E*)-4-(4-(*N*-(5-methylisoxazol-3-yl) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono)butanoyl leucine **148b** as a brown powder (0.05 g, 30% yields). m.p. 189-203°C, (HRMS: *m/z* calculated for C₃₂H₃₄N₈O₇S (MH⁺) 674.2227, found: 675.2229; *U*_{max}./cm⁻¹ 3320 (N-H), 2680 (OH carboxylic acid), 3230 (C=N), and 1677 (amide N-C=O); CHN calculated C(56.96%), H(5.08%), N(16.61%), O(16.60%), S(4.75%); found: C(56.77%), H(5.54%), N(16.81%), O(16.53%), S(4.38%); δ_{H} (300 MHz, CDCl₃) 0.93 (dd, *J* = 15.0, 6.9 Hz, 6H, 2x CH₃), 1.50 (m, 1H, CHCHCH₂), 1.84 (m, 2H, CHCH₂), 2.29 (s, 3H, CH₃ sulfamethizole), 5.86 (s, 1H, NH), 6.09 (s, 1H, ArH), 6.48 (d, *J* = 9.4 Hz, 1H, NH), 7.01 (m, 2H, ArH), 7.29 (m, 11H, ArH and CDCl₃), 7.70 (m, 5H, ArH), 9.75 (s, 1H, NH), 10.7 (s, 1H, CO₂H); δ_{C} (75 MHz, CDCl₃) 12.6 (ArCCH₃), 22.5 (CH(CH₂)₂), 24.8 (CH(CH₂)₂), 41.5 (CHCH₂CH), 55.9 (CHCH₂CH), 95.6 (ArCH), 113.8 (4x ArCH), 118.3 (2x ArCH), 121.8 (2x ArCH), 128.7 (4x ArCH), 129.7 (2x ArCH), 134.3 and 134.7 (C=N), 135.5 (ArCS), 141.8 (ArCNH), 143.5 (2x ArCNH), 151.3 (ArC(N₂)(CH)), 159.9 and 161.4 (NC=O), 169.4 (ArC(CH₃)), 174.6 (C=O).

4.8.2.27. 3-(3,4-dihydroxyphenyl)-2-((2*E*,3*E*)-4-oxo-2,3-bis(2-phenylhydrazono)-4-((4-(*N*-(pyrimidin-2-yl) sulfamoyl)phenyl) amino) butanamido)propanoic acid 149b



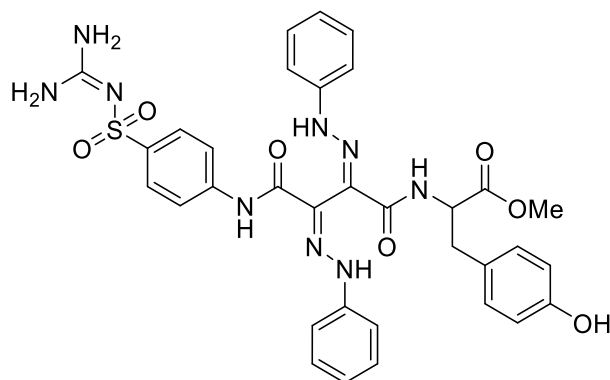
The general procedure as described above in the section 4.8.2 was followed using sulfadiazine (0.06 g, 0.27 mmol, 1 eq), and DOPA (0.05 g, 0.27 mmol, 1 eq.) to afford 3-(3,4-dihydroxyphenyl)-2-((2E,3E)-4-oxo-2,3-bis(2-phenylhydrazono)-4-((N-(pyrimidin-2-yl) sulfamoyl) phenyl) amino) butanamido) propanoic acid **149b** as a grey gel which solidified on standing (0.10 g, 52% yields). m.p. 299-304°C, C₃₅H₃₁N₉O₈S (MH⁺) 705.2295, found: 706.2299; max./cm⁻¹ 3478 (OH), 3034 (N-H), 1740 (OH carboxylic acid), 2720 (C=N), and 1653 (amide N-C=O); CHN calculated C(56.98%), H(4.24%), N(17.09%), O(17.35%), S(4.35%); found: C(56.80%), H(4.38%), N(17.80%), O(17.56%), S(4.45%); δ_H (300 MHz, CDCl₃) 2.98 (m, 2H, CHCH₂), 4.96 (dt, *J* = 9.4, 7.8 Hz, 1H CHCH₂), 7.06 (m, 3H, ArH), 7.22 (dt, *J* = 2.1, 1.0 Hz, 1H, ArH), 7.34 (m, 12H, ArH), 7.73 (m, 2H, ArH), 7.80 (d, *J* = 0.5 Hz, 2H, ArH), 7.96 (m, 3H, ArH), 8.73 (d, *J* = 3.4 Hz, 2H, NH), 9.75 (s, 1H, NH), 10.2 (s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 36.8 (CHCH₂), 59.4 (CHCH₂), 112.5 (4x ArCH), 113.8, 115.0, 115.5, 116.7, 118.3 (2x ArCH), 121.8 (2x ArCH), 122.7, 128.7 (4x ArCH), 129.7 (2x ArCH), 130.5, 134.3 and 134.7 (C=N), 135.3, 141.8 (ArCNH), 143.5 (2x ArCNH), 144.4 and 145.6 (ArC(OH)), 157.6 (2x ArCH), 159.6 and 161.9 (NC=O), 169.7 (ArC(N₃)), 174.8 (C=O).

4.8.2.28. 2-((2E,3E)-4-((4-(N-(diaminomethylene) sulfamoyl)phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanamido)-3-(3,4-dihydroxyphenyl)propanoic acid 150b



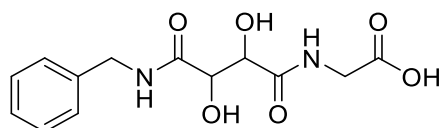
The general procedure as described above in the section 4.8.2 was followed using DOPA (0.05 g, 0.27 mmol, 1 eq.), and sulfaguanidine (0.05 g, 0.27 mmol, 1 eq.) to afford *2-((2E,3E)-4-((4-(N-(diaminomethylene)sulfamoyl)phenyl)amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanamido)-3-(3,4-dihydroxyphenyl)propanoic acid* **150b** as a grey powder (0.03 g, 19% yields). m.p. 192-195°C, (HRMS: m/z calculated for $C_{32}H_{31}N_9O_8S$ (MH⁺) 701.2016, found: 702.2018; $U_{max.}/cm^{-1}$ 3360 (N-H), 2280 (OH carboxylic acid), 3300 (C=N), and 1679 (amide N-C=O); CHN calculated C(54.77%), H(4.45%), N(17.96%), O(18.24%), S(4.57%); found: C(54.86%), H(4.55%), N(17.14%), O(18.44%), S(4.19%); δ_H (300 MHz, $CDCl_3$) 3.26 (m, 2H, CHCH₂), 4.95 (dt, $J = 9.3, 7.7$ Hz, 1H CHCH₂), 6.02 (s, 1H, NH), 6.25 (d, $J = 9.4$ Hz, 1H, NH), 6.73 (s, 4H, ArH), 7.08 (m, 3H, ArH), 7.31 (m, 11H, $CDCl_3$ and ArH), 7.77 (s, 4H, NH), 8.50 (s, 1H, NH), 9.75 (s, 1H, CO₂H); δ_C (75 MHz, $CDCl_3$) 36.8 (CHCH₂), 59.4 (CHCH₂), 113.8 (4x ArCH), 115.5, 116.7, 118.3 (2x ArCH), 121.8 (2x ArCH), 122.7, 126.8 (2x ArCH), 129.7 (4x ArCH), 130.3, 134.3 and 134.7 (C=N), 139.5, 142.4, 143.5 (2x ArCNH), 144.4 and 145.6 (ArC(OH)), 158.8 (C(N₃)), 159.6 and 161.9 (NC=O), 174.7 (C=O).

4.8.2.29. Methyl ((2E,3E)-4-((4-(N-(diaminomethylene) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tyrosinate 151b



The general procedure as described above in the section 4.8.2 was followed using sulfaguanidine (0.05 g, 0.27 mmol, 1 eq.), and tyrosine methyl ester (0.05 g, 0.27 mmol, 1 eq.) to afford methyl ((2*E*,3*E*)-4-((4-(*N*-(diaminomethylene) sulfamoyl) phenyl) amino)-4-oxo-2,3-bis(2-phenylhydrazono) butanoyl) tyrosinate **151b** as a grey powder (0.03 g, 17% yields). m.p. 192-195°C, (HRMS: *m/z* calculated for C₃₃H₃₃N₉O₇S (MH⁺) 699.2224, found: 700.2221; *U*_{max}./cm⁻¹ 3290 (N-H), 3000 (OH carboxylic acid), 3298 (C=N), and 1668 (amide N-C=O); CHN calculated C(56.64%), H(4.75%), N(18.02%), O(16.00%), S(4.58%); found: C(56.71%), H(4.87%), N(18.19%), O(16.21%), S(4.49%); δ_{H} (300 MHz, CDCl₃) 3.30 (m, 2H, CHCH₂), 3.70 (s, 3H, OCH₃), 4.87 (dt, *J* = 9.4, 7.8 Hz, 1H, CHCH₂), 6.15 (d, *J* = 9.3 Hz, 1H, NH), 6.67 (s, 1H, NH), 6.73 (s, 4H, ArH), 7.04 (m, 5H, ArH), 7.33 (m, 11H, ArH), 7.83 (m, 4H, NH), 9.75 (s, 1H, NH); δ_{C} (75 MHz, CDCl₃) 36.8 (CHCH₂), 51.5 (OCH₃), 57.4 (CHCH₂), 113.8 (4x ArCH), 115.9 (2x ArCH), 118.6 (2x ArCH), 122.5 (2x ArCH), 126.8 (2x ArCH), 129.5, 129.7 (4x ArCH), 130.3 (2x ArCH), 134.3 and 134.7 (C=N), 139.6, 142.4, 143.5 (2x ArCNH), 155.9 (ArC(OH)), 158.8 (C(N₃)), 159.6 and 161.9 (NC=O), 171.3 (C=O).

4.8.2.30. (4-(benzylamino)-2,3-dihydroxy-4-oxobutanoyl)glycine **152a**



The general procedure as described above in the section 4.8.2 was followed using benzylamine (0.03 g, 0.27 mmol, 1 eq.), and glycine (0.02 g, 0.27 mmol, 1 eq.) to afford (4-(benzylamino)-2,3-dihydroxy-4-oxobutanoyl)glycine **152a** as a yellowish powder (0.07 g, 92% yields). m.p. 84-87°C, *U*_{max}./cm⁻¹ 3310 (N-H), 3440 (OH

carboxylic acid), and 1632 (amide N-C=O); CHN calculated C(52.70%), H(5.44%), N(9.46%), O(32.40%); found: C(52.48%), H(5.30%), N(9.38%), O(32.60%); δ_H (300 MHz CDCl₃) 1.92 (s, 2H, OH), 4.29 – 3.96 (m, 2H, HNCH₂CO₂H), 4.36 – 4.61 (m, 2H, (HNCH₂Ph)), 4.95 (d, J = 5.9 Hz, 1H, (CH(OH)), 5.23 (d, J = 5.9 Hz, 1H, (CH(OH))), 7.19 – 7.36 (m, 7H, ArH), 7.77 (t, J = 5.7 Hz, 1H, HNCH₂CO₂), 7.93 (t, J = 6.2 Hz, 1H, HNCH₂Ph), and 10.2 (s, 1H, CO₂H); δ_C (75 MHz, CDCl₃) 43.2 (HNCH₂CO₂H), 43.8 (HNCH₂Ph), 73.1 and 73.9 (CH(OH)), 126.0 (ArC), 2x 126.7 (ArC), 2x 128.5 (ArC), 137.5 (ArC), 162.0 (NC=O), 163.7 (NC=O), and 174.2 (C=O).

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Appendix

HIV PRO Assay

The HIV protease assay was performed using the flourogenic substrate Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg (Sigma Aldrich) as previously described by Lam *et al.* (2000).¹ The substrate was dissolved in normal DMSO to make a 500 μ M stock. Test compounds were diluted to desired concentrations in the reaction buffer (0.1 M sodium acetate, 1 M NaCl, 1 mM EDTA, 1 mM DTT and 0.1 % BSA, 5% DMSO, pH 4.7) in a separate plate before being added to a fluorescence assay plate at 50 μ l per well. Substrate (25 μ l) to the final concentration of 8 μ M and enzyme (25 μ l) to the final concentration of 50 ng/ μ l were added. The mixture was incubated at 37 °C for 40 minutes after which fluorescence was read at an excitation wavelength 340 nm and emission wavelength 485 nm using a Synergy MX (BioTek®) plate reader. Ritonavir was used as the standard inhibitor. Flourescence values were converted to % enzyme activity relative to the readings obtained from control wells (enzyme without inhibitor).

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