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Cyclic Sulfamide HIV-1 Protease Inhibitors

Design, Synthesis and Modelling

ANNA AX

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Abstract
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Ten years ago, the first protease inhibitor targeting the human immunodeficiency virus (HIV) was approved for clinical use. Highly active antiretroviral therapy (HAART), which combined protease and reverse transcriptase inhibitors, quickly became the standard therapy for treating patients infected with HIV and Acquired Immune Deficiency Syndrome (AIDS). Nevertheless, last year the AIDS pandemic reached its highest level ever. Many infected patients, mainly in the developing countries, are still without treatment. Among those patients who receive treatment, an increase in drug resistance and new-infection with drug-resistant strains are seen. To come to terms with these problems, new drugs that are efficient against resistant strains and can be produced at low cost are needed.

In this study, we have focused our research efforts on cyclic sulfamides active as HIV-1 protease inhibitors. Distinctive to this compound class, as compared to the inhibitors so far approved for clinical use, was the incorporation of a water mimic that displaces the structural water (W301) observed in the X-ray crystal co-complexes. The first part of the study was aimed at understanding the rationale behind the nonsymmetric binding mode that the inhibitor adopted when bound to the enzyme. Symmetric and nonsymmetric inhibitors were synthesized and the structure-activity relationships and preferable binding modes were rationalized with the help of Comparative Molecular Field Analysis (CoMFA).

In the second part of the study, an attempt was made to reduce the size of these inhibitors. As a result, the traditional P1/P1' substituents were removed, while the P2/P2' substituents were elongated in an attempt to reach between the binding sites. The design hypothesis was shown to be successful and inhibitors possessing nanomolar activity were identified.

Keywords: AIDS, HIV, protease inhibitor, aspartic protease, molecular modelling, 3D-QSAR, CoMFA

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List of Papers

This thesis is based on the following papers:

I Schaal, W.; Karlsson, A.; Ahlsén, G.; Lindberg, J.; Andersson, H. O.; Danielson U. H.; Classon, B.; Unge, T.; Samuelsson, B.; Hultén, J.; Hallberg, A.; Karlén A. Synthesis and Comparative Molecular Field Analysis (CoMFA) of Symmetric and Nonsymmetric Cyclic Sulfamide HIV-1 Protease Inhibitors. *J. Med. Chem*. **2001**, 44, 155-169.

II Ax, A.; Schaal, W.; Vrang, L.; Samuelsson, B.; Hallberg, A.; Karlén A. Cyclic Sulfamide HIV-1 Protease Inhibitors, with Sidechains Spanning from P2/P2' to P1/P1'. *J. Bioorg. Med. Chem.* **2005**, 13, 755-764.

III Ax, A.; Schaal, W.; Vrang, L.; Samuelsson, B.; Hallberg, A.; Karlén A. Nonsymmetric Cyclic Sulfamide HIV-1 Protease Inhibitors with Sidechains Spanning from P2/P2' to P1/P1'. *Manuscript in preparation*, **2005**.

IV Gold, H.; Ax, A.; Vrang, L.; Samuelsson, B.; Karlén, A.; Hallberg, A.; Larhed, M. Fast and Selective Synthesis of Novel Cyclic Sulfamide HIV-1 Protease Inhibitors under Controlled Microwave Heating. *Manuscript in preparation,* **2005**.

Contents

Abbreviations and Definitions

AIDS	Acquired Immune Deficiency Syndrome
CA	capsid protein
CD4	receptor found on surface of certain immune cells, Cluster of Differentiation antigen 4
$CD4^+T$	T helper lymphocytes
CoMFA	Comparative Molecular Field Analysis
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEAD	diethyl azodicarboxylate
DIEA	*N,N*-diisopropylethylamine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DNA	2'-deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
DPPF	1,1'-bis(diphenylphosphino)ferrocene
FDA	U.S. Food and Drug Administration
gp41, gp120	glycoproteins 41 and 120
HAART	Highly Active Anti-Retroviral Therapy
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate
Hermann's catalyst	trans-Di(mu-acetato)bis[o-(di-o-tolylphosphino)benzyl]-dipalladium (II)
HIV	Human Immunodeficiency Virus
HTLV	Human adult T-cell Leukemia Virus
IC_{50}	The inhibitor concentration that results in a 50% inhibition of the enzyme function
IN	integrase
K_i	inhibitory constant
MA	matrix protein
MIF	Molecular Interaction Field
M-MuLV	Moloney Murine Leukemia Virus
MW	Microwave heating
NC	nucleocapsid protein
NMP	1-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTIs	Nucleoside Reverse Transcriptase Inhibitors

o-	ortho
p6	protein 6
PC	Principal Component
PCA	Principal Component Analysis
PDB	Protein Data Bank
$Pd(dba)_2$	Bis(dibenzyldieneacetone)palladium
PLS	Partial Least-Squares Projection to Latent Structures
PR	protease
q^2	"cross-validated r^2", internal predictability of the model
QSAR	Quantitative Structure-Activity Relationship
r^2	explained variance; a quantitative measure of the model's ability to explain the variance in the data
r^2_{pred}	external predictability of the model
RMSD	Root-Mean-Square-Distance
RNA	ribonucleic acid
RSV	Rous Sarcoma Virus
RT	reverese transcriptase
SAR	structure-activity relationship
SIV	Simian Immunodeficiency Virus
TB	Tuberculosis
THF	tetrahydrofuran
TsCl	4-toulenesulfonyl chloride
TsOH	4-toulenesulfonic acid
UNAIDS	Joint United Nations Program on HIV and AIDS
WHO	World Health Organization

1 Introduction

1.1 Acquired Immune Deficiency Syndrome (AIDS)

It all began in the early 1980s when an increased incidence of unusual opportunistic infections, such as *Pnemocystis carinii* pneumonia and Kaposi's cancer, was reported.[1] Previously, these rare diseases were usually seen in patients on immunosuppressant therapy but now previously healthy homosexual men, intravenous drug abusers, hemophiliacs, infants and blood transfusion recipients were the main groups affected.[2] The syndrome became known as Acquired Immune Deficiency Syndrome (AIDS).[3] In 1983, a retrovirus, later proven to be the causative agent, was isolated from AIDS-infected patients.[4,5] The virus had been given several different names: lymphadenopathy-associated virus (LAV),[6] human T-cell lymphotropic virus type III (HTLV-III),[7] and AIDS-associated retrovirus (ARV).[8] The International Committee on the Taxonomy of Viruses proposed the name now used: Human Immunodeficiency Virus (HIV).[9]

The primary HIV infection occurs years before the patients present symptomatic opportunistic infections.[10] A clinical illness, characterized by flu-like symptoms (fevers, sweats, headaches, sore throat and rashes) that lasts from 3 to 14 days, may be associated with the primary infection.[11,12] As with most acute infections, the levels of viremia are extremely high during this period.[13,14] When the immune response sets in, a rapid fall in viremia level is seen. However, the level of viremia soon reaches a steady state that can persist for months or years.[15] The virus is still highly active, replicating and infecting new lymphocytes and macrophages,[16,17] but this activity is unnoticed due to the rapid turnover of virions and cells.[18,19] An HIV-infected cell has an estimated life span of 2 days, compared with 0.3 days for a virion.[20] Finally, the number of lymphocytes decreases and the viral load increases past a critical level. The infection has progressed to AIDS.[21,22]

Today, AIDS is recognized as a long lasting pandemic (Figure 1). The World Health Organization (WHO) and Joint United Nations Program on HIV and AIDS (UNAIDS) has estimated that 39 million men, women and children were infected by HIV in 2004.[23] The pandemic has reached its highest level ever.

Figure 1. Global estimates of HIV/AIDS infection as of December 2003.[24]

1.2 Human Immunodeficiency Virus (HIV)

The key to understanding the origin of HIV was the discovery of a closely related virus, the Simian Immunodeficiency Virus (SIV), present in African primates.[25] The SIV virus has been transferred between species (zoonosis),[26,27] probably when hunting or preparing primate bushmeat, often sold in African markets.[28] In fact, HIV exists in two subtypes: HIV-1, the type first discovered, which is most closely related to SIVcpz found in chimpanzees,[29] and HIV-2, isolated from patients in 1986,[30] which is most closely related to SIVsm found in sooty mangabey monkeys.[26]

The short life span of the virions is compensated for by the high reproduction rate. It has been estimated that a total of 10 billion HIV-1 virions is produced in a single day.[20] Any attempt to eliminate or diminish the number of infectious virions has to be directed to the virus replication phase. This is a difficult task since the virus uses some of the host's own natural processes for reproduction. To diminish side effects affecting the host, potential drugs are therefore required to target enzymes or events associated only with the virus.

1.3 Replication Cycle of HIV and Current Drug Targets

A great deal of research has been focused on understanding the replication cycle of HIV,[31-35] in search of new drug targets and agents.[36-41] There is still a need for improvements in the selection of currently available anti-retroviral therapy: drugs of higher potency and those with better tolerability (to encourage adherence to the treatment regimen) and better resistance profiles are sought.

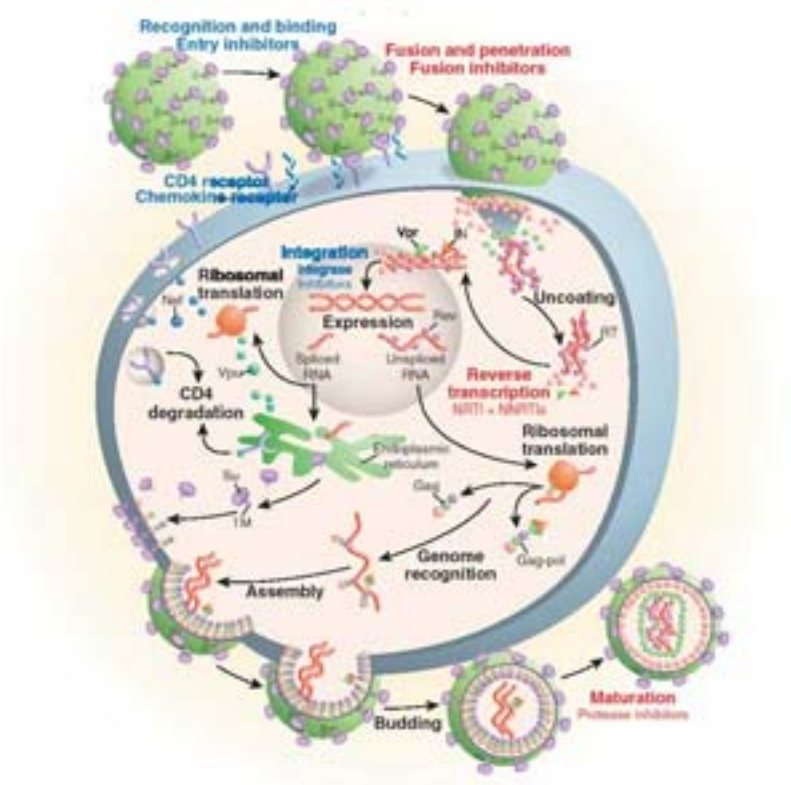


Figure 2. HIV-1 life cycle. Text in red refers to targets inhibited by existing agents approved by the FDA. Text in blue refers to targets that are inhibited by agents that are likely to be available in the near future. Reprinted with permission from the authors and the publisher (graphic by Katie Ris).[34]

1.3.1 Binding and Fusion

HIV targets cells that carry the CD4 receptor on their surface;[42] corresponding to a subset of T helper lymphocytes (CD4+ T) and monocyte-macrophages.[31]

Fusion is initiated by the formation of a high-affinity bond between the CD4 receptor and the viral surface glycoprotein gp120 (Figure 2). This contact induces a conformational change in gp120 that allows further interac-

tions between the virus and co-receptors, such as chemokine CXCR4 or CCR5, found on the surface of the target cell. This permits the insertion of the N-terminal of the viral glycoprotein gp41 directly into the target cell lipid bilayer. Here it forms a hairpin intermediate that helps to bring the cell membranes into close proximity, allowing fusion and viral entry.[31,32,35] It has been proposed that fusion takes place in specific membrane microdomains, that are rich in sphingolipids and cholesterol and are organized together in lipid rafts. These lipid rafts are thought to support the insertion of gp41 into the target cell and/or destabilize the bilayer architecture to facilitate fusion.[43]

Enfuvirtide,[44] approved by the U.S. Food and Drug Administration (FDA) in 2003, is currently the only registered fusion inhibitor. Enfuvirtide is a 36-amino acid peptide corresponding to a sequence found in gp41. This synthetic peptide binds to gp41, thus preventing the conformational change that would lead to the hairpin formation and, as a result, preventing virus fusion and entry.[45]

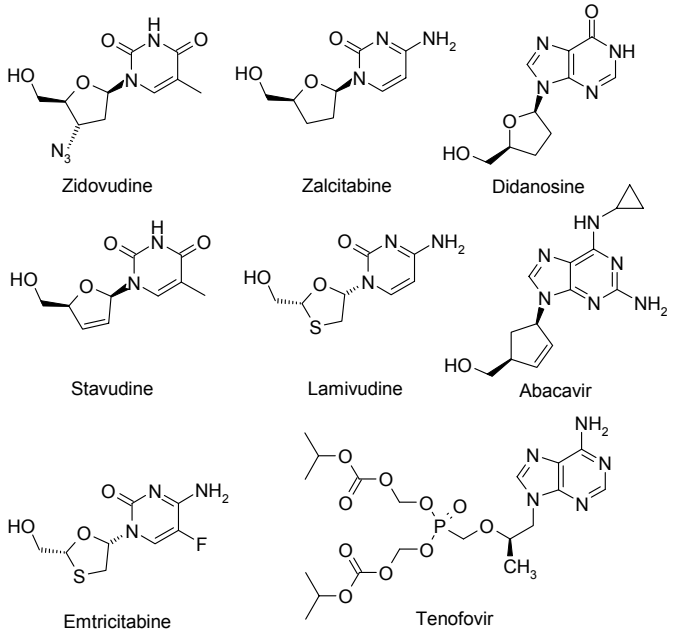


Figure 3. NRTIs licensed for clinical use.[46]

1.3.2 Reverse Transcription

The HIV virus is dependent on the host cell for its reproduction. The viral RNA must first be transcribed into DNA before the genetic information can be integrated into the host's genome.[34] The reverse transcription is catalyzed by the reverse transcriptase enzyme (RT). Each virion carries two copies of its RNA genome, and since reverse transcription involves "jumps" from one template to another, the enzyme-RNA affinity is relatively low. This can

result in unwanted template switches, yielding RNA molecules that differ from the parent RNA molecule. RT makes approximately five to ten errors per genome per round of replication.[47,48] In the end, a novel recombinant DNA genome is formed, giving rise to viral quasi species within the infected patient.[35]

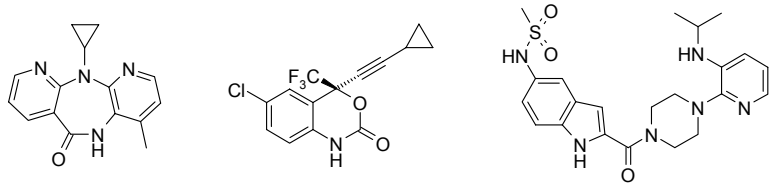


Figure 4. NNRTIs licensed for clinical use.[46]

There are two classes of drugs that target the RT enzyme: nucleoside reverse transcriptase inhibitors (NRTIs) (Figure 3) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Figure 4). In 1987, the first anti-HIV drug was approved by the FDA: the NRTI 3'-azido-3'-deoxythymidine (AZT) or zidovudine.[49] Today there are eight FDA-approved NRTIs: zidovudine (1987), didanosine (1991), zalcitabine (1992), stavudine (1994), lamivudine (1995), abacavir (1998), tenofovir (2001) and emtricitabine (2003). The NRTIs compete with the natural nucleosides for incorporation into the growing DNA molecule. Once incorporated, the NRTI causes chain termination, since it lacks a handle for further chain elongation.[34] The NNRTIs target the RT enzyme itself. These inhibitors bind to an allosteric site in the enzyme, thus altering the conformation of the active site, causing inhibition of the enzyme.[34] Three NNRTIs have been approved to date: nevirapine (1996), delavirdine mesylate (1997) and efavirenz (1998).

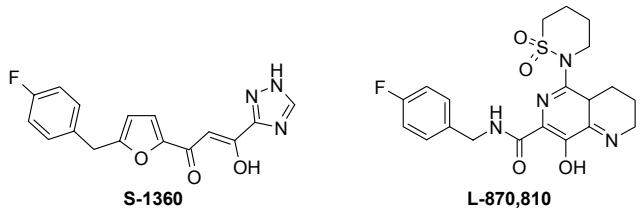


Figure 5. Integrase inhibitors in clinical trials.[41]

1.3.3 Integration

The viral enzyme integrase (IN) is transported into the cell nucleus with the double stranded viral DNA (see Figure 2). IN catalyzes the formation of covalent bonds between the C-termini of each viral DNA strand and the host DNA. Host cell enzymes then repair the small gaps remaining in the DNA (previously N-termini of the viral DNA).[50] The integrated viral DNA, the

provirus,[35] serves as a template for synthesis of viral RNAs that encode the structural, regulatory and accessory proteins needed to form new viral particles. At present there are no approved anti-retroviral drugs targeting this stage of replication; however, two compounds (Figure 5), S-1360 and L-870,810, have entered clinical trials.[41,50]

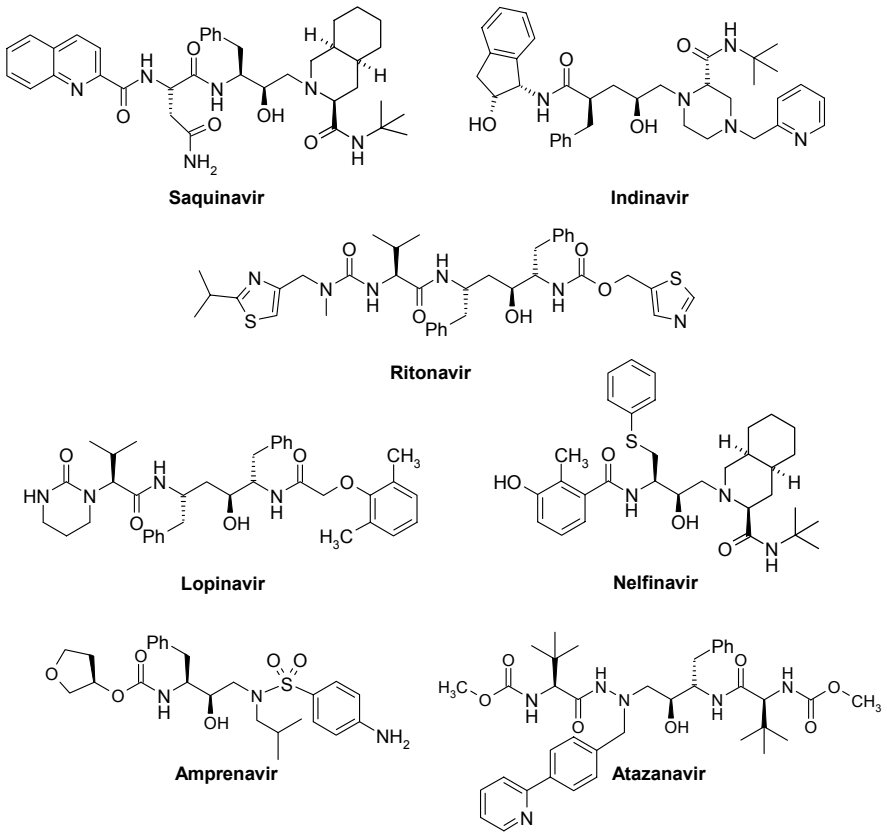


Figure 6. PR inhibitors approved for clinical use.

1.3.4 Budding and Polyprotein Processing

After the DNA has been transcribed and the *gag* and *gagpol* polyprotein precursors have been produced, an assembly process begins. The polyprotein precursors and the viral RNA gather at the surface of the infected cell, where they are encapsulated by the cell membrane and released as new virions (see Figure 2). It has been suggested that budding takes place through lipid rafts, whereby host cell cholesterol and sphingolipids are incorporated into the viral envelope.[51] Shortly after budding from the cell, the viral protease (PR) cleaves the *gag* and *gagpol* polyprotein precursors into functional enzymes.

The new virion doesn't become infectious until the mature proteins are generated.[35]

PR inhibitor drugs bind to the active site of the viral PR, thus preventing polyprotein processing and consequently hampering maturation to infectious virions. Seven PR inhibitors (Figure 6) have been approved by the FDA: saquinavir[52] (1995), ritonavir[53] (1996), indinavir[54] (1996), nelfinavir (1997), amprenavir (1999), lopinavir (2000) and atazanavir (2003). The structure and function of the PR and the design of PR inhibitors will be discussed in greater detail below (see Chapter 3).

1.4 Anti-HIV Chemotherapy and Drug Resistance

Prior to the introduction of highly active anti-retroviral therapy (HAART), an HIV diagnosis corresponded to a death sentence. HAART treatment significantly prolonged the lives of HIV-infected patients, and death rates fell to one-fifth.[55] However, problems such as lack of long-term adherence to drug regimens, drug-related toxicity and drug resistance continue to emerge.

1.4.1 Highly Active Anti-Retroviral Therapy (HAART)

At its approval in 1987, zidovudine was the recommended initial therapy for HIV infection. Within a few years, however, HIV strains with reduced sensitivity to zidovudine were isolated from patients receiving prolonged therapy.[56] In 1993, the first cases of patients primarily infected with zidovudine-resistant strains were reported.[57] As new NRTIs were approved for clinical use, combination therapy with two NRTIs was introduced, but strains resistant to this form of therapy soon emerged.[58]

When the first PR inhibitors, saquinavir and indinavir, appeared on the market, triple therapy with zidovudine and zalcitabine[59] or lamivudine[58] was suggested. Combination therapy including PR inhibitors made it possible to target two steps in the HIV life cycle. It was hypothesized that a triple combination would prove more effective than single or dual anti-retroviral therapy. The hypothesis was proven correct, the levels of HIV RNA were significantly reduced[58,59] in patients receiving this highly active form of anti-retroviral therapy, and thus HAART was introduced. In some patients, HIV RNA could not be seen after therapy: the number of copies was below the level of detection.[58] Mortality was reduced among patients on anti-retroviral therapy, particularly if the therapy included a protease inhibitor.[55] It was speculated that the virus could be eliminated from the body if HAART was introduced early in the infection and was thereafter used long enough for infected cells, for instance in lymphoid tissue, to die off.[21,60]

However, the half-life of CD4+ T cells was found to be 44 months; this would mean over 70 years of treatment before virus reservoirs were eradi-

cated.[61] Furthermore, CD4+ T cells can revert from an active to a resting state. When cells become infected and then revert into a latent state, the virus will exist simply as integrated DNA, unaffected by anti-retroviral drugs. The brain can also serve as a sanctuary site, where the virus can exist beyond the reach of anti-retroviral drugs. [60,62,63]

There is no simple answer to the question of when to start HAART treatment. The benefits of introducing HAART early include preservation of the immune system, a decreased risk of HIV transmission, and earlier suppression of viral replication. The associated risks include potential for lack of adherence to therapy and adverse side effects.[64] The most common side effects are: nausea,[65] diarrhea,[65] the lipodystrophy syndrome[66,67] (lipodystrophy and hyperlipidaemia leads to redistribution of fat and insulin resistance; that is, an overall reduction of insulin action, probably due to the actions of adiponectin[67]), hyperlactatemia (high blood levels of lactate due to mitochondrial dysfunction)[68], and coronary heart disease[69] (probably as a consequence of lipodystrophy). In addition, if therapy is delayed, future treatment options are preserved (new, more powerful regimens may be introduced) and drug resistance cannot develop, but there is an increased risk of irreversible damage to the immune system and also a risk of virus transmission.[64]

1.4.2 Drug Resistance

The main reasons for the development of HIV drug resistance are the high level of virus production and the high error rate in reverse transcriptase activity. If a mutant virus has some advantage over other viruses, such as a decreased sensitivity to antiviral dugs, it will be favored and produced to a larger extent, according to Darwinism.[70] However, it is not simply a case of "the survival of the fittest"; HIV infection results in survival of all the major forms that have ever been generated within a patient, and replication favors the form that is fittest under the current conditions. If conditions change, archived variants can re-emerge.[61,71]

In patients who receive HAART as the first line of anti-retroviral therapy, emergence of viral resistance is possible only if HIV continues to replicate in the presence of drug treatment, i.e. if the drug concentration is insufficient to block viral replication but sufficient to exert a positive selective pressure for variants with decreased drug susceptibility. Under these conditions, viruses resistant to all the components of the regimen will gradually emerge.[71]

Reduced plasma levels of one or more anti-retroviral drugs are not necessarily the result of poor compliance; they can also result from other factors. Tuberculosis (TB) is one of the main opportunistic infections among HIV-positive patients in the world, especially in sub-Saharan Africa and Asia. Unfortunately, major drug-drug interactions can occur in patients taking TB and HIV therapy, especially between rifamycins, which are the first choice drugs for TB therapy, and HIV PR inhibitors or NNRTIs.[72] Administration

of rifamycins in combination with PR inhibitors (saquinavir, indinavir or ritonavir) or NNRTIs (nevirapine or delavirdine) will greatly reduce the plasma levels of the anti-retroviral agent used.[72]

New drugs, which appear to be active against strains resistant to many older drugs, are becoming available. These drugs are either new members of existing structural classes, exhibiting increased potency and improved pharmacokinetic properties, or are members of new structural classes that are as yet not susceptible to cross-resistance.[71] The new drugs, used in triple class combinations, are saved for later use as salvation therapies, to be introduced in cases of drug resistance or if treatment is first started at a very advanced stage of infection.[73]

1.4.3 Anti-retroviral Therapy in Developing Countries

Demands for the introduction of anti-retroviral treatment in developing countries have been debated in the media over recent years. The drug industry is being pressured to provide drugs at a low cost or to allow the drugs to be produced locally.[74] The fact that developing countries often lack the functioning healthcare systems required to adequately distribute and monitor the anti-retroviral treatment is seldom discussed.[75,76]

Nonetheless, access to treatment may make infected patients more willing to undergo voluntary counseling and testing and also to disclose their HIV status. Patients will be able to return to work, and parents will live longer, resulting in smaller numbers of children growing up as orphans.[75] New easy-to-use techniques, such as viral load and resistance determination kits which will help in this process, are being developed.[77,78]

Management of HIV in Brazil has been called a success story: Brazil is a developing country in which the HIV/AIDS epidemic has been brought under control, and where all AIDS-infected patients receive the full range of anti-retrovirals, completely cost free.[79] The question is, can others do it? Brazil has several advantages over other developing countries: (*i*) Strong social mobilization, involving the formation of non-governmental organizations working for HIV/AIDS prevention and care, occurred early in this country.[79] This early response was not seen, for example, in South Africa, where the government authorities have only recently abandoned complete denial of an HIV/AIDS problem[80]. (*ii*) Human rights protection, with legislation against discrimination of people living with HIV/AIDS, helps to encourage individuals to acknowledge their disease.[79] (*iii*) Brazil has a functioning healthcare system with a good infrastructure, including laboratories for CD4+ counting, viral load counting and anti-retroviral resistance genotyping.[79]

WHO has taken “The 3 by 5 Initiative”, where the goal is to provide HIV/AIDS treatment to three million people by the end of 2005.[81] Uganda is one of the first countries to launch the program and has now started to dis-

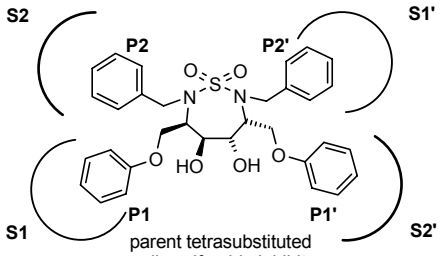
tribute anti-retroviral drugs to patients in need. Patients will be linked with one of 26 hospitals where they receive their monthly doses of anti-retroviral drugs. The daily dose will be taken under the observation of a community volunteer.[82] Although this WHO initiative is welcomed, it may give rise to new ethical dilemmas. For example, if there is not enough money or medicine to treat all, who will be chosen for treatment?[83]

2 Aims of the Thesis

The study undertaken in this thesis was part of an ongoing research project, which has the overall aim of identifying new approaches to unique HIV-1 PR inhibitors. The specific objectives of the thesis were:

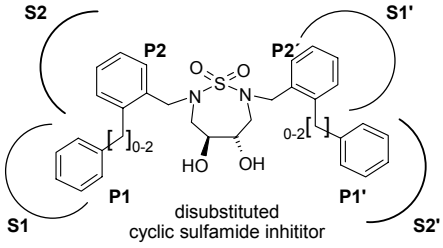
- To design and synthesize nonsymmetric tetrasubstituted transition state HIV-1 PR inhibitors from the cyclic sulfamide class, focusing on their ability to bind nonsymmetrically to HIV-1 PR.

- To evaluate and rationalize the mode by which the nonsymmetric tetrasubstituted cyclic sulfamide compounds bind to HIV-1 PR.



parent tetrasubstituted
cyclic sulfamide inhibitor

- To investigate the possibility of elongating the P2/P2' side chains of cyclic sulfamide compounds to reach from S2 to S1 and from S1' to S2'. Thereby, removing the traditional P1/P1' substituents, to produce a disubstituted compound.



disubstituted
cyclic sulfamide inhititor

3 Design of HIV Protease Inhibitors

3.1 HIV Protease

As virions bud from an infected cell, the HIV PR is responsible for the polyprotein processing, which is necessary for the virions to mature and become infectious.

3.1.1 Discovery, Structure and Function

Most HIV drug research was initially focused on RT. After the introduction of the RT inhibitor zidovidine,[49] new drug targets were sought. What was thought at the time to be a second HIV enzyme encoded by the *pol*-gene had recently been identified. Investigations into the related Moloney Murine Leukemia Virus (M-MuLV) showed that deletions or mutations in this part of the gene would block the proteolytic processing of the *pol*-encoded protein precursors, producing immature and noninfectious virus particles.[84,85] The proteolytic effect was later also proven for the HIV *pol*-gene, by expressing the *gag*- and *pol*-gene in yeast cells.[86]

Comparison of the amino acid sequences for the *pol*-gene of several retroviruses, for example Rous Sarcoma Virus (RSV), Human adult T-cell Leukemia Virus (HTLV), M-MuLV and HIV, revealed a conserved Asp-Thr-Gly sequence, homologous to the sequence found in aspartic proteases.[87,88] Aspartic proteases, found in mammalian species, usually consist of about 300 amino acids. Two catalytic aspartic acid residues are centrally placed at the base of the active site. In the short sequence encoding the retroviral proteases (99 amino acids for HIV PR), there is only one catalytic triad, and a dimeric structure was thus proposed for the functional enzyme.[87]

Shortly thereafter, the hypothesis of the catalytic activity of HIV PR was confirmed through: (*i*) Site-directed mutagenesis (Asp to Asn) led to the complete loss of proteolytic activity.[89] (*ii*) Addition of pepstatin A, a known aspartic protease inhibitor, inhibited the proteolytic activity.[90] In 1989, the crystal structure of the HIV PR was elucidated and the dimeric structure was confirmed.[91-93]

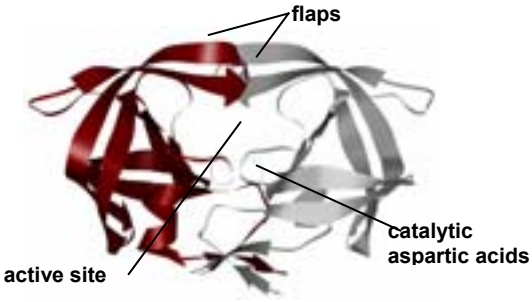


Figure 7. X-ray crystal structure revealing the dimeric form of HIV PR. The molecular graphics image was produced using the program UCSF Chimera.[94]

The catalytic aspartic acids contributed by each dimer, Asp25 and Asp25', are located towards the shallow bottom of the active site (Figure 7). The two flaps close over the active site like a lid to form a tunnel through the enzyme. Using standard nomenclature,[95] the S1 and S1' (S2 and S2' *etc*.) subsites are structurally equivalent, because of the enzyme's dimeric C_2-symmetic structure (Figure 8). The subsites are hydrophobic except for Asp-29, Asp29', Asp30 and Asp30' located in the S2/S2'-subsites. The S3/S3'-subsite is adjacent to the S1/S1'-subsite.[96]

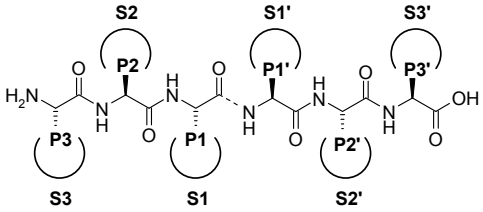


Figure 8. Nomenclature of the peptide sidechains and enzyme subsites designated according to the convention of Schechter and Berger.[95] Numbering of the residue positions (Pn, Pn') and the corresponding subsites (Sn, Sn') in the enzyme starts from the scissile peptide bond (dashed) in the ligand. The non-prime side is located towards the N-terminal and the prime side towards the C-terminal.

3.1.2 Mechanism of Cleavage

The most widely accepted cleavage-mechanism for aspartic proteases is the general acid-base mechanism. The Asp closest to the catalytic water molecule carries a negative charge and thus activates the water molecule through hydrogen bonding. The water molecule attacks the substrates at the carbonyl in the amide bond to be cleaved (the scissile bond), to generate an oxyanion tetrahedral intermediate. Protonation of the amide nitrogen and subsequent rearrangement results in the breakdown of the tetrahedral intermediate to yield the hydrolysis products.[97-99]

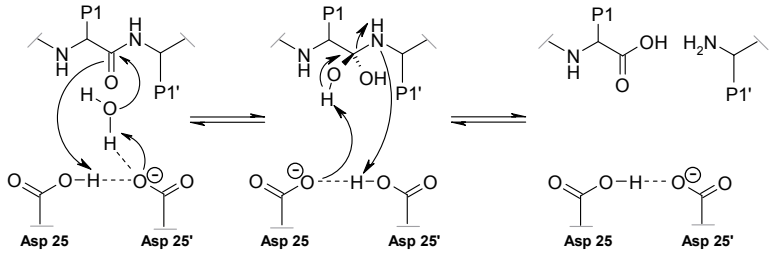


Figure 9. Schematic representation of the catalytic mechanism proposed for aspartic proteases.[97-99]

3.1.3 Protonation State

When undertaking molecular modelling, it is important to try to assign the correct protonation state for the enzyme-ligand complex. It has been suggested that the protonation state of the two aspartic groups in HIV PR depend on whether or not a substrate is bound to the enzyme.[100] If it is bound, the protonation state will depend on the structure of the inhibitor. This has been widely studied using pH rate studies,[101,102] molecular dynamics calculations, usually in combination with X-ray crystallography,[103] and nuclear magnetic resonance (NMR) studies.[100,104-106] Harte et al. argues that differences in protonation state depend on the ability of the inhibitor to stabilize the charge. For example, a hydroxyethylene isostere (see Figure 11) contains no ionizable group and it would therefore be plausible that both aspartic acids would remain protonated. On the other hand, if the transition state is mimicked using a secondary amine, with the possibility of carrying a positive charge, one of the aspartic groups will be deprotonated.[103]

In this study, all compounds were modeled with the aspartic groups protonated, as suggested for the dihydroxyethylenes.[104]

3.2 Substrate-Based Design

Any inhibitor designed for blocking the catalytic activity of the HIV PR has to compete with the natural substrates for the active PR site. Several design strategies can be used to design potential inhibitors.[107,108] One possible way of creating an inhibitor would be to use substrate-based design. This method uses the same peptide sequence as that in one of the natural substrates, but the scissile bond is exchanged for a noncleavable isostere. Several isosteres have been developed for use in inhibitors of the aspartic PR renin. [The renin-angiotensin system is involved in blood pressure regulation in humans.][109] In the first X-ray crystal structure of an HIV PR — inhibitor complex (MNT-101), a reduced amide was used as isostere for the scissile peptide bond.[110] The X-ray crystal structure indicates that the reduced amide

function (Figure 11) is not an optimal isostere, since it is positioned too far from the catalytic aspartic acids for direct hydrogen bonding. The inhibitory potency was determined to 0.79 μM.[110,111]

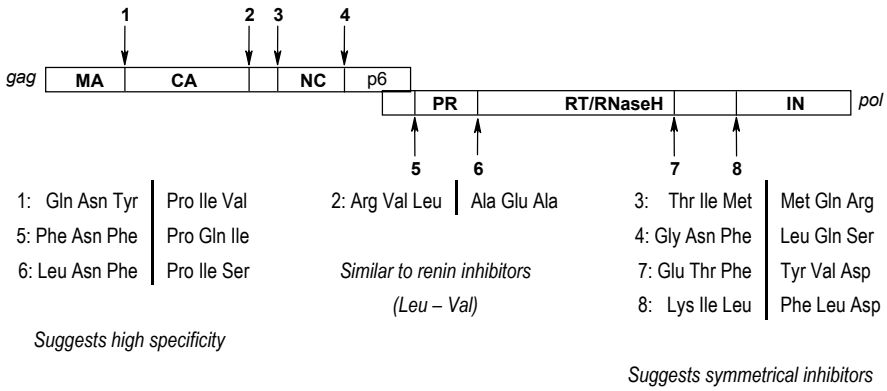


Figure 10. Summary of sites cleaved by HIV PR within the *gag* and *pol* polyproteins.[109,112]

Pepstatin A has been shown to mimic the tetrahedral intermediate formed in the peptide bond cleavage sequence,[113] and this statine isostere has potential to produce effective inhibitors.[114] The potencies of pepstatin A and acetyl-pepstatin were determined to 1.4 μM[115] and 35 nM,[116] respectively. The X-ray structure of acetyl-pepstatin co-crystallized with HIV PR revealed hydrogen bond interactions with both of the catalytic aspartic acids.[117] However, the statine isostere replaced two amino acids, leaving the inhibitor without a P1'-substituent.

The hydroxyethylene isostere, a homolog of statine with an extra methylene in the backbone, allowed for the introduction of a correctly placed P1'-substituent.[111] Replacement of the scissile bond for the hydroxymethylene led to the first highly potent inhibitors, with sub-nanomolar activities.[115] Further examples of isosteres that have been widely used in HIV PR inhibitors are depicted in Figure 11.[99,109,118]

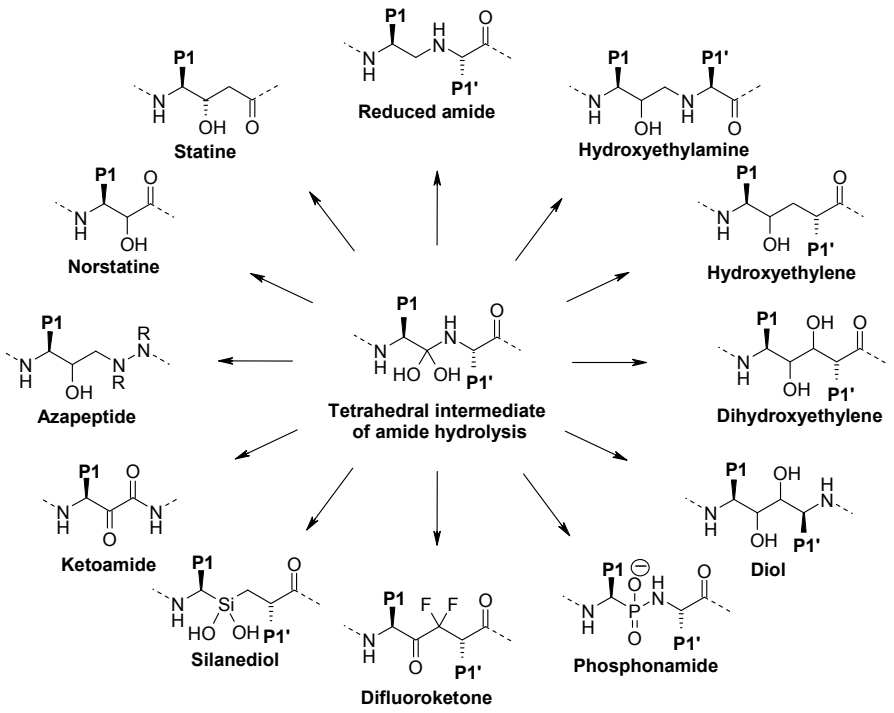


Figure 11. Noncleavable isosteres of the tetrahedral intermediate in peptide bond cleavage that have been developed for use in producing HIV PR inhibitors.[99,109,118]

3.2.1 A Case Study — Saquinavir

The design of saquinavir (Ro-31,8959), the first HIV PR inhibitor to enter the market (in 1995), was based on a substrate sequence with proline (Pro) in the P1' position (see Figure 10, cleavage site 6). Since no mammalian endopeptidases are able to cleave Pro-peptide bonds, such inhibitors are likely to be highly selective for the viral enzyme.[52] The first inhibitor tested was the truncated substrate **I** (Figure 12), where the scissile bond was replaced by a hydroxyethylamine isostere. The minimum size needed for inhibition was investigated, resulting in the shortened inhibitor **II**. To further improve the potency, each residue was optimized individually to give the final compound saquinavir.[52]

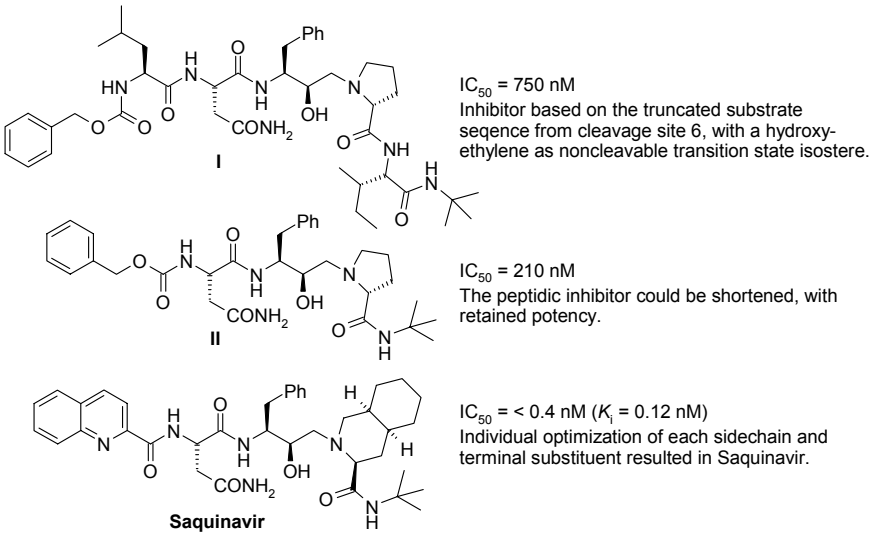


Figure 12. The design strategy for the development of saquinavir (Ro-31,8959).

3.3 Structure-Based Design

3.3.1 Symmetry-Based Design

As soon as the crystal structure of HIV PR (apo and in complex) were known, a structure-based approach was applied to facilitate the design of potential drugs.[119] One approach was to take the C_2-symmetry of the PR into account and apply the same symmetry to the inhibitors by C- or N-terminal duplication.[107,120,121] The advantage with this line of design was the specificity gained towards the viral PR.[122] Aspartic PRs are common in the human body, but none are symmetric dimers. Furthermore, it was envisioned that the symmetric inhibitors would be less peptidic in character, thus avoiding poor absorption and rapid metabolism, well known problems for peptidic compounds.

Depending on where in the inhibitor the C_2-axis is placed (at the alcohol or midpoint of the scissile bond), two types of symmetric compounds are possible. The research group at Abbot laboratories synthesized both of these (see **III** and **IV** in Figure 13). The diol-compounds turned out to be the most potent, because of the favorable hydrogen bond pattern between the P1 and P1' amides and the backbone of the enzyme.

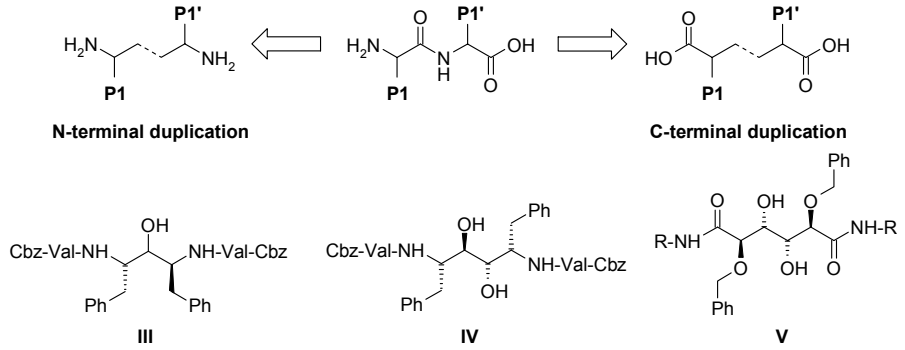
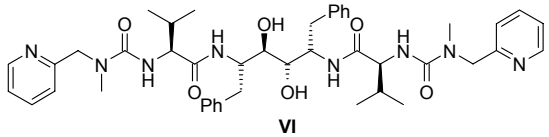


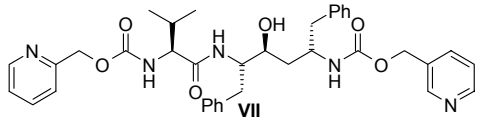
Figure 13. The N- and C-terminal duplication strategy. The N-terminal duplication inhibitors **III** (A-74704)[122] and **IV** (A-756214)[123] were designed by Abbot and the C-terminal duplication inhibitor **V**[124,125] was designed by our group.

3.3.2 A Case Study — Ritonavir

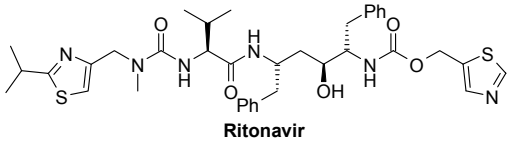
Structure-activity studies, based on analogs of **IV** (Figure 13), led to the identification of **VI** (Figure 14)**,** which possessed adequate anti-HIV potency and aqueous solubility for intravenous use.[126] Further investigation revealed that only one of the alcohols was optimally placed between the catalytic aspartic acids and thus needed for activity.[127,128] The removal of one hydroxyl and reduction in size (by truncation of the prime-side) resulted in **VII**, which possessed significant oral bioavailability.[53,128] Unfortunately, **VII** was rapidly metabolized by N-oxidation of one or both of the pyridyl end groups.[126] Several analogs of **VII** were explored, which led to the discovery of Ritonavir (ABT-538), approved for clinical use in 1996.[53]



K_i = 0.084 nM
VI possesses aqueous solubility for intravenous use.



K_i = 0.25 nM
VII possesses oral bioavailability but is unfortunately rapidly metabolized.



K_i = 0.01 nM
Ritonavir possesses enhanced antiviral activity and exceptional oral pharmacokinetics.

Figure 14. The design strategy for the development of ritonavir (ABT-538), via the analogs **VI** (A-77003) and **VII** (A-80987).

3.3.3 3D-Screening of Compound Libraries

The number of available X-ray crystal structures of HIV PR – inhibitor complexes encouraged research into non-peptidic inhibitors. The centrally placed structural water molecule (W301) was common to all the complexes published to this point. The water molecule was tetrahedrally coordinated through hydrogen bonds to carbonyl groups within the inhibitor and amide groups in the backbone of the enzyme flaps (Ile50 and Ile50'). Erickson et al. concluded that the water molecule must have a role in the energetics of inhibitor binding, and that its functional replacement would afford a logical and challenging target for future inhibitor design.[122] It was also thought that a conformationally restricted cyclic structure might provide a positive entropic effect.[129]

3D-pharmacophores (built from specific hydrogen bond donor/acceptor and/or hydrophobic interaction sites) were used to search compound libraries in the race for new nonpeptidic lead structures. Several structurally different lead compounds were identified including haloperidol (which is a known neuroleptic drug).[130]

The seven-membered cyclic urea inhibitor, designed by DuPont-Merck Pharmaceutical Company, was also a result of pharmacophore search, followed by structure-aided design. The cyclic urea was the first published inhibitor to evidently displace the structural water W301[129] (the design strategy is outlined in Section 3.3.4).

X-ray crystallography of 4-hydroxycoumarin derivatives (warfarin analogs found in biological screenings of compound libraries) in complex with HIV PR showed that also the lactone functionality could act as a water mimic, and displace the structural water.[131,132]

3.3.4 A Case Study — Cyclic Ureas

The history behind the discovery of the cyclic urea inhibitors started with a database search based on symmetric diol inhibitors, previously synthesized within the company and docked into the HIV PR (Figure 15). A 3D-pharmacophore model was derived and used in the 3D-database search.[129] One of the hits from the search not only fulfilled the pharmacophore criteria, it also included a methoxy group that mimicked the structural water, **VIII**. The central phenyl ring was abandoned for a cyclohexanone ring, which was able to position the substituents in a more appropriate orientation. Further modelling led to compound **IX**, where a diol function was incorporated to increase the interactions with the catalytic aspartic acids. In addition, the carbonyl functionality was exchanged for a urea group to strengthen the hydrogen bond interactions with the flaps and also to simplify the synthesis.[129] Closely related structures were identified and investigated by other research groups, using similar methodology: six- and eight-membered rings,[133,134] related water mimics[134-137] and different transition state groups.[138,139]

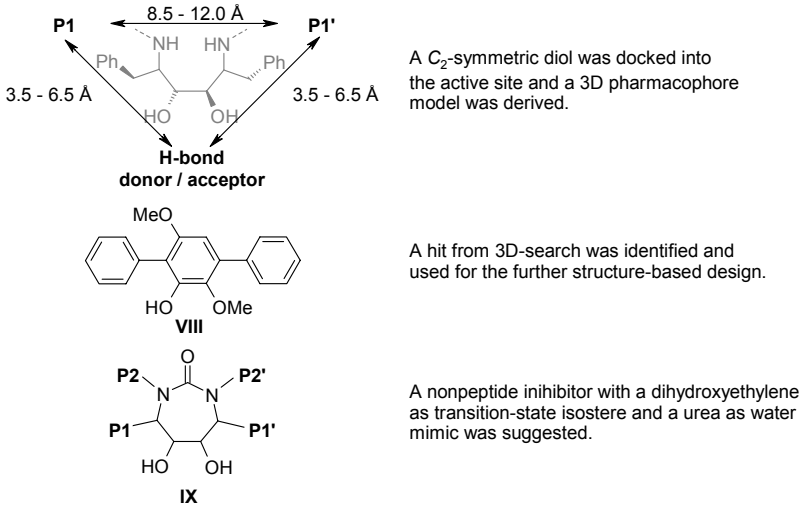


Figure 15. The design strategy for the development of the cyclic ureas.[129]

4 Computational Chemistry

It is impossible to make all the potential analogs of a compound class; a selection of which analogs to synthesize has somehow to be made. For the best results, this selection would comprise a wide range of compounds with diverse characteristics, thus covering property space.[140]

4.1 Diversity in Drug Design

The selection of compounds that are to be synthesized can be made based on the structure-activity relationships of compounds that have already been synthesized or systematically, using statistical design. A set of statistically designed compounds is well balanced and is thus able to provide information from most parts of property space.[141] The selection can be based on the final products or on the building blocks (reagents) used. It has been suggested that there is no significant difference in efficiency between a compound selection in building block space as compared to product space.[140]

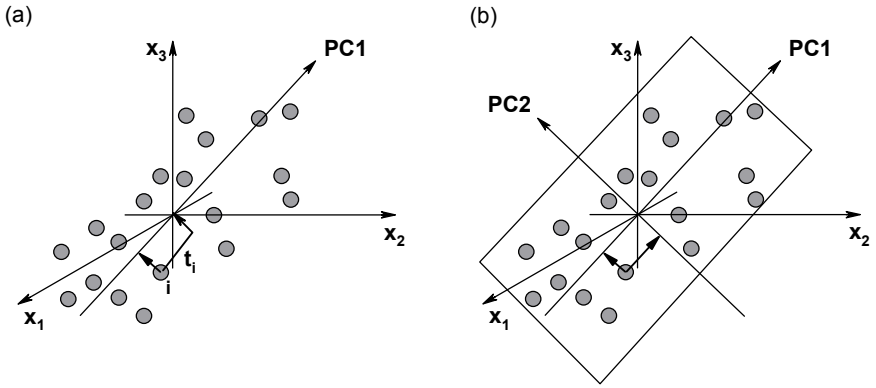


Figure 16. (a) The first principal component (PC1) is the line that best accounts for the largest variation in the data (the shape of the point swarm). Observation **i** is projected onto PC1 and is given the score value $\mathbf{t_i}$ (equal to its distance from the origin). (b) The second component (PC2) accounts for the second largest variation in the data. PC1 and PC2 form a plane in space onto which the observations are projected.[141]

Compounds may be described using a number of descriptors such as molecular weight, electronegativity, volume, surface area, hydrogen bond acceptors and/or donors etc. To graphically plot these properties would demand a graph with as many dimensions as descriptors, which would be very hard to manage and interpret. A principal component analysis (PCA) can be used to simplify the interpretation of such diverse data. PCA strives to give an overview of multivariate data using a smaller number of new, uncorrelated variables. Each data point is projected onto principal components (PC), which in turn make up lines, planes or hyper-planes (Figure 16).[141]

4.2 Molecular Mechanics

Historically, the visualization of molecular structure was greatly assisted by hand-held 3-dimensional models. The introduction of computational molecular modelling and computational chemistry programs has further increased the ease of generating and comparing several conformations of the same molecule or the structures of different molecules.[142]

Molecular mechanics can be used to perform calculations on systems (molecules) that contain thousands of atoms. In these methods, the energy of a system is considered as a function of the nuclear positions only; electronic motion is not taken into account.[143]

The energy within a system is described in terms of stretching bonds, opening and closing angles, rotation about single bonds and non-bonded (van der Waals and electrostatic) interactions. Deviations from the reference bond length or angle, etc. are associated with an energetic penalty.[143] The reference bond lengths, angles and atom types are all parameters that are defined in the force field. For example a carbon atom can have various hybridizations and also be part of several different functional groups (e.g., single or double bond, carbonyl or cyano group). As a consequence, the carbon atom will acquire different properties and the atom type can be assigned accordingly.[144]

There are a variety of force fields; some are for general use and others are developed for specific systems (proteins, nucleic acids, metals etc.).[143] Each force field has its own unique set of parameters.

4.3 Docking Studies

It is important for drug optimization that the processes involved in the binding of a ligand to the target enzyme are understood. Advances in molecular biology have made it possible to produce natural or modified proteins suitable for rapid, iterative crystallographic studies.[145] However, it is still highly improbable that access to X-ray crystal depiction of all the synthesized

ligands would be possible. A conformational search in the enzyme's active site can provide insight into the possible mode by which the inhibitor binds to the enzyme. The low-energy conformations found in this way should, at least hypothetically, come close to the actual binding conformation. Several methods and programs have been developed for this task.

4.3.1 Monte Carlo Search in the Active Site

A Monte Carlo conformational search generates conformations of a molecule by randomly changing its internal coordinates (e.g., torsion angles and the positions of the constituent molecules). However, the search is usually not carried out completely randomly, but is normally focused on the areas of conformational space where novel low energy conformations are most likely to be found.

In each Monte Carlo step, a new starting geometry is produced by randomly changing one or more torsion angles and, possibly, translating and rotating the molecule. The molecule is then minimized and the new conformation is compared with conformations found earlier. The new conformation is saved if it is unique and the relative energy of the structure is within a predefined range of the lowest energy conformation found to that point. The Monte Carlo step is then completed and a new cycle begun.[146]

The search can be terminated on reaching a pre-determined number of Monte Carlo steps or when each minimum within the energetic window has been found a predetermined number of times. Flexible molecules have many rotatable bonds; to randomly vary all of them would require a lot of computer time. In order to perform the search in a reasonable time-frame, parts of the molecule can be frozen, i.e., not allowed to be changed.

4.4 Quantitative Structure-Activity Relationships

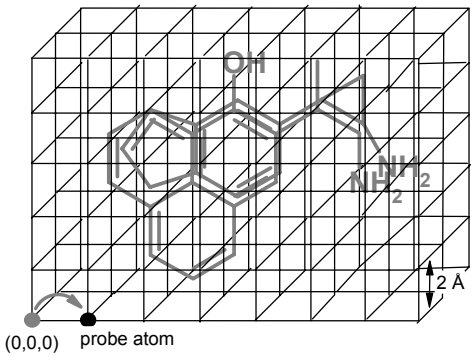
Medicinal chemists are constantly striving to find ways of correlating chemical structure with biological function or response, in order to create structure-activity relationships (SAR). The main goal is to predict the behavior of new compounds using relationships derived from analysis of previously tested compounds. The concept of quantitative structure-activity relationships (QSAR) is based on the idea that an alteration in the chemical structure of a compound will give rise to a change in the biological response. In 1868, Crum-Brown and Fraser formulated a function that recognized the "physiological activity" Φ of a molecule, as a function of chemical structure C.[147]

$$\Phi = f(C) \qquad (4.1)$$

In 1869, Richardson performed what was possibly the first QSAR study, in which he correlated the narcotic effect of a series of alcohols with their molecular weights.[148] Almost a century later, Hansch et al. advanced the technique; they combined several physicochemical descriptors and correlated them with biological activity.[149,150] The descriptors were limited to those that were easily determined, such as molecular weight, log P (the logarithm of the *n*-octanol/water partition coefficient), density and so on. The development of computational molecular modelling methods allowed access to calculated descriptors that describe changes in molecular shape and geometry (3D-QSAR).[151]

4.4.1 3D-QSAR

Molecular shape and geometry are essential characteristics of drugs that exert their biological effects by interaction with a receptor, enzyme, ion-channel or other macromolecule. The better the fit of the macromolecule–drug surface, the higher the affinity and, most likely, the better the biological activity.[152]



		Steric Energy						Electrostatic Energy				
	Activity pK_i	Col 1 (0,0,0)	Col 2 (2,0,0)	Col 3 (4,0,0)	…	Col N (N,N,N)		Col 1 (0,0,0)	Col 2 (2,0,0)	Col 3 (4,0,0)	…	Col N (N,N,N)
Compound 1												
Compound 2												
...												
Compound N												

Figure 17. Molecules aligned in a 3D grid. Steric and electrostatic interactions between each compound and a "probe atom" placed at the various intersecting grid points are calculated and used as descriptors.[153]

The interactions of a molecule with its surroundings can be described in terms of steric and electrostatic forces. Steric and electrostatic energies can be used to produce an energy contour surface or molecular interaction field

(MIF) where energy and shape can be considered simultaneously.[154] In Comparative Molecular Field Analysis (CoMFA),[153] the calculated field values are used as spatial descriptors for generating a 3D-QSAR. The molecules to be analyzed are placed in a three-dimensional grid (Figure 17). The field values are generated by calculating steric and electrostatic interactions between each compound and a "probe atom" (placed at the various intersecting grid points).[153] The probe atom is generally an sp^3-carbon with a charge of +1, but other atom types can also be used. The grid point values generated can be used as is, or can be transformed by employing parabolic, indicator or H-bonding fields.

4.4.2 Alignment

In all cases, the compounds used to construct the 3D-QSAR model, using CoMFA, have to be aligned according to some predetermined alignment rule to reflect the manner of binding to the target.[151] Defining the alignment has been recognized as one of most important and difficult steps in CoMFA studies.[155,156] Attempts to try to avoid this issue, lead to the development of alignment-free descriptor systems.[157]

If the structure of the target is unknown, the active analog approach can be used.[158] Functionalities (hydrogen bond donor/acceptor, hydrophobic/hydrophilic and ionic groups) thought to be important for biological activity are identified. With the help of different modelling techniques, the equivalent functionalities in all compounds are compared and correlated and a pharmacophore model is constructed.[151,159]

When an X-ray crystal structure of the target enzyme is available, it can be used to align the compounds. If an X-ray crystal structure of the target enzyme — inhibitor complex is available, the ligand can be extracted and used as template for building the inhibitors that are to be investigated. The newly built inhibitors are either minimized as is,[160] subjected to a conformational search,[155,160-163] or docked[164-166] into the active site of the enzyme crystal structure.

4.4.3 Deriving and Validating the Model

The calculated spatial descriptors are used to derive a function, which relates the descriptors of each molecule to its biological properties.[151] Since the number of descriptors (*variables*) often greatly exceeds the number of compounds (*observations*), a multivariate statistical analysis, e.g., partial least-squares (PLS), is used.[141,167]

PLS, an extension of PCA (see Section 4.1), is used to connect the observations (compounds) to some kind of response (biological activity) in order to find the maximum correlation (r^2).[141] It is important to evaluate the model for self-consistency and predictability using either internal (q^2, cross-

validated r^2) or external (r^2_{pred}, predicted r^2) validation methods. For internal validation, the leave-one-out cross-validation method is often used. In this case, all the available compounds are used to build the model and the PLS analysis is performed iteratively, each time leaving out one compound. The activity of that compound is then predicted and compared with the measured activity to yield an overall q^2 for the model. The probability of a chance correlation for an average-sized data set is negligible if the model yields a q^2 greater than 0.3.[168]

If an external validation method is used, the compounds are divided into training and test sets. The training set is used to construct the model (which is normally evaluated by internal validation) and the model is then used to predict the activity of the compounds of the test set. The predicted values of the test set are compared with the actual values, to yield an r^2_{pred} for the model.

5 Cyclic Sulfamide-Based HIV-1 PR Inhibitors

Inspired by the elegant design of cyclic ureas as HIV-1 PR inhibitors that were reported by Lam et al. in 1994[129], we embarked on a study of related ureas and sulfamides. The ureas reported by Lam et al. were synthesized from D-Phenylalanin via a pinacol type coupling.[169,170] The synthetic procedure did not allow full control over the stereochemistry and a mixture of three diastereomers was obtained. We developed a synthetic route, where carbohydrates (D-mannitol and L-mannonic-γ-lactone) were used as chiral precursors for the cyclic scaffolds. The stereochemistry of the transition state diol-isostere, and the stereochemistry and length of the P1/P1' sidechains, were investigated for the urea-based compounds. The optimal combination derived was then used in the synthesis of cyclic sulfamides.[137]

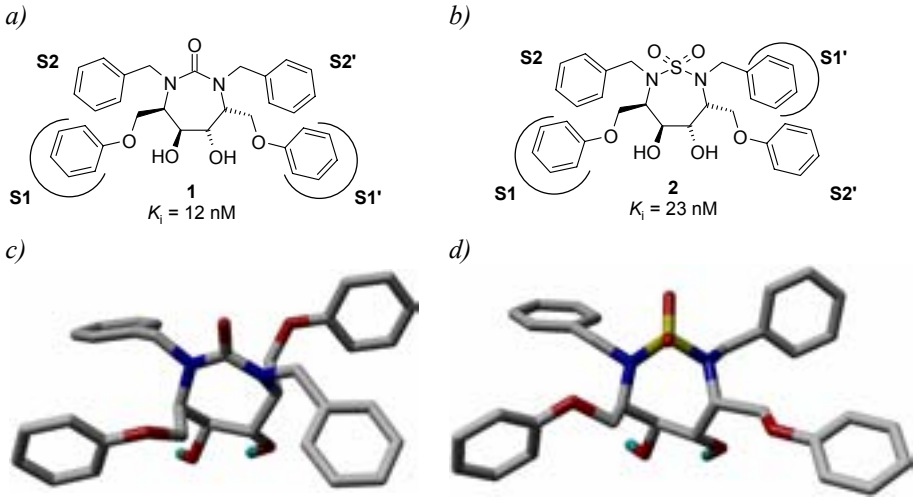


Figure 18. a) A cyclic urea inhibitor, **1**, and b) a cyclic sulfamide inhibitor, **2**, both C_2-symmetric. c) The urea adopts a C_2-symmetric conformation, thus binding symmetrically to the C_2-symmetric enzyme. d) The sulfamide, on the other hand, adopts a nonsymmetric ring conformation, which in turn results in a nonsymmetric binding to the enzyme. The molecular graphics image was produced using the program UCSF Chimera.[94]

X-ray crystallographic analysis of cyclic sulfamide inhibitors in complex with HIV-1 PR revealed an unexpected nonsymmetric binding mode.[171] The

P1' and P2' sidechains were transposed relative to the expected C_2-symmetric binding mode observed for the cyclic ureas (Figure 18). Attempts were made to induce a symmetric binding mode in the sufamides by introducing P2/P2' substituents foreseen to bind preferentially to the S2/S2' binding sites.[172] As could be deduced from X-ray crystallography, the nonsymmetric binding mode persisted, even though the *meta*-methyl ketoxime substituent, known form the cyclic urea class to have high affinity, was incorporated.[172] Thus, although the cyclic sulfamide compounds are C_2-symmetric, they seem to bind to the enzyme in a nonsymmetric fashion. *Ab initio* calculations performed on the truncated sulfamides (all sidechains reduced to methyls) indicated that the nonsymmetric sulfamide ring conformation was 2.4 kcal/mol lower in energy than the symmetric conformation.[172]

5.1 Investigation of the Nonsymmetric Binding Mode (Paper I)

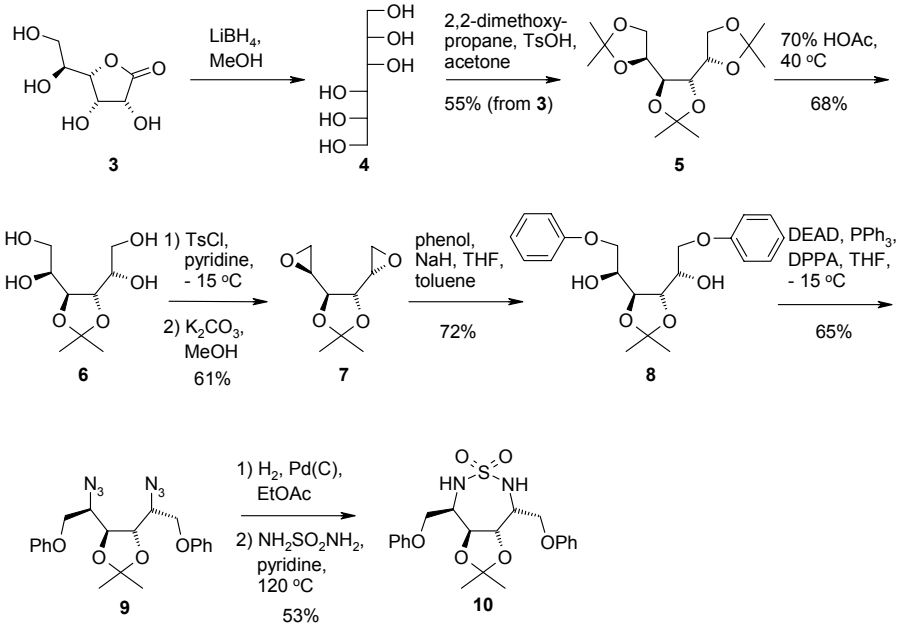
To further explore the SAR of the cyclic sulfamides, a set of symmetric and nonsymmetric compounds with sidechains of varying size, polarity and hydrogen bonding capacity were synthesized. The S1/S1' and S2/S2' subsites are essentially lipophilic in nature. However, the S2/S2' subsites are slightly more prone to participate in hydrogen bonding. It was therefore anticipated that the nonsymmetric sulfamide inhibitors, with P2/P2' side chains modified individually for the S1' and S2 subsites, should be more potent than the corresponding symmetric analogs. Some of the substituents had previously been used in other classes of HIV-1 PR inhibitors with good results.[173] The SAR and binding mode for the compounds were rationalized via conformational analysis and CoMFA.[174,175]

5.1.1 Synthesis and Inhibitory Potencies

The Cyclic Sulfamide Scaffold

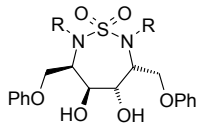
The cyclic sulfamide scaffold was synthesized in nine steps from L-mannonic-γ-lactone **3**, a precursor for the more expensive L-mannitol, **4** (Scheme 1). The four-step synthesis of the diepoxide compound **7** was performed essentially as described by Le Merrer et al..[176]

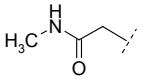
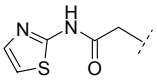
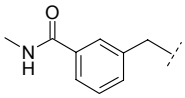
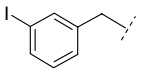
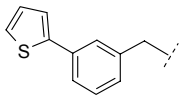
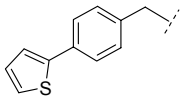
Scheme 1.



The L-mannonic-γ-lactone was reduced to L-mannitol with the use of lithium borohydride[169,177] and the alcohols were protected, two by two, as acetals using 2,2-dimethoxypropane, to afford compound **5**. The terminal acetals were selectively hydrolyzed with 70% acetic acid at 40 °C, to give **6**. The primary hydroxyls were selectively tosylated and the crude product was converted into diepoxide **7**, using potassium carbonate. The epoxides were subjected to a nucleophilic ring opening with phenol to give compound **8**.[178,179] A Mitsunobu reaction[180] with diphenylphosphoryl azide (DPPA) inverted the stereochemistry, and produced compound **9**. The azides were reduced to amines in a palladium-catalysed hydrogenation, at atmospheric pressure. The cyclization was facilitated using sulfamide in pyridine to afford the cyclic sulfamide scaffold **10**.[134]

Table 1. Inhibitory Potencies of Symmetric Inhibitors.



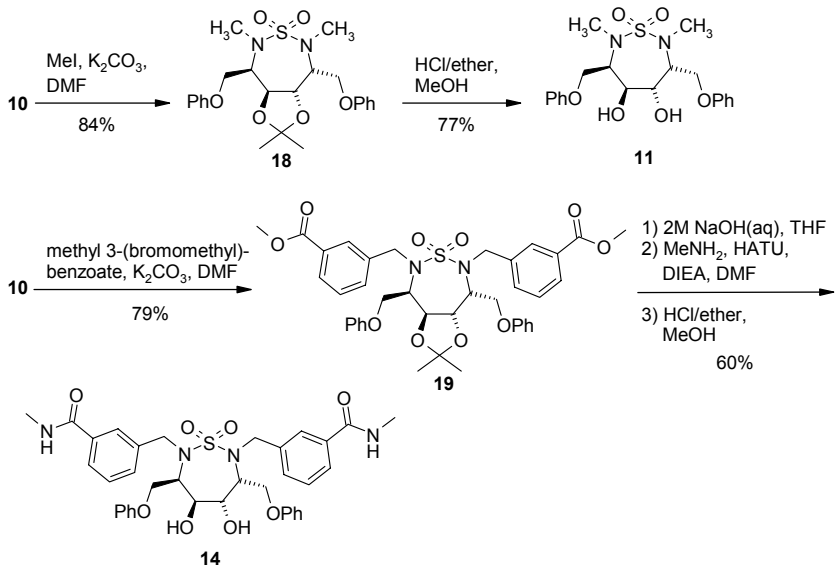
compd	R	K_i (nM)	pK_i [a] obsd	pK_i [a] calcd [b]
11		710	6.15	6.04
12		25 000	4.60	4.73
13		2 200	5.66	5.93
14		39	7.41	7.43
15		93	7.03	7.27
16		2 200	5.66	5.72
17		920	6.04	6.14

[a] pK_i = -log K_i. [b] The calculated pK_i values are based on the CoMFA model of all 25 inhibitors (see Section 5.1.2 CoMFA).

Symmetric Inhibitors

The *N*,*N*'-dimethylated compound, **11**, was synthesized from **10** via alkylation with methyl iodide followed by deprotection (Scheme 2). Compound **14** was synthesized via alkylation of **10** with methyl 3-(bromomethyl)benzoate to produce compound **19**. The methylester was hydrolyzed, an excess of methylamine and the coupling reagent HATU produced the methylamide, and subsequent deprotection afforded compound **14**. In total, seven symmetric inhibitors were synthesized (Table 1). For details of the synthesis of the individual compounds see Paper I. Compound **14** was the most active of the symmetric compounds synthesized, with a K_i of 39 nM. Compound **15** also showed good activity for the PR (93 nM). The activity of the smaller, more polar, compound **12** was much lower than that of all the other compounds.

Scheme 2.

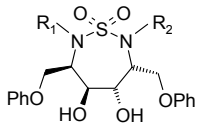


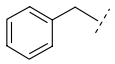
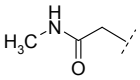
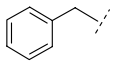
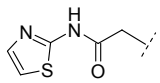
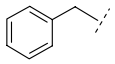
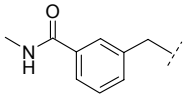
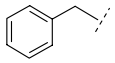
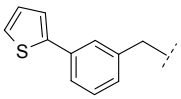
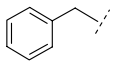
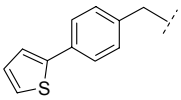
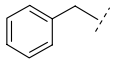
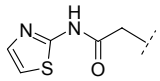
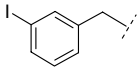
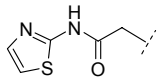
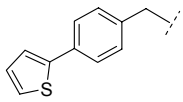
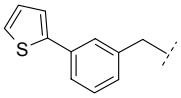
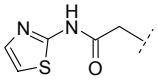
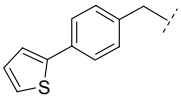
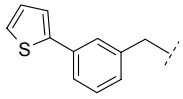
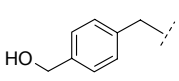
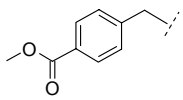
Nonsymmetric Inhibitors

Nonsymmetric sulfamide inhibitors were synthesized via mono-alkylation (Scheme 3). High dilution and slow addition of the alkylating agent gave the best result, with a turnover of 80% in a 1:3:1 ratio of starting material, mono-, and di-alkylated product, respectively. The three components were easily separated by column chromatography.

Mono-alkylation using benzyl bromide afforded compound **31** (Scheme 3). Compound **20** was synthesized via a Mitsunobu reaction with methanol, and subsequent deprotection. Compound **23**, the nonsymmetric analog of **14,** was prepared according to the procedure outlined above. In total, eleven nonsymmetric inhibitors were synthesized (Table 2). For details of the synthesis of the individual compounds, see Paper I. Generally, the nonsymmetric compounds **21**-**23** and **26**-**28**, which contain a polar group intended for the P2 position, were more active than their symmetric analogs. Only compounds **23** and **30** had a higher inhibitory potency than the parent compound **2**.

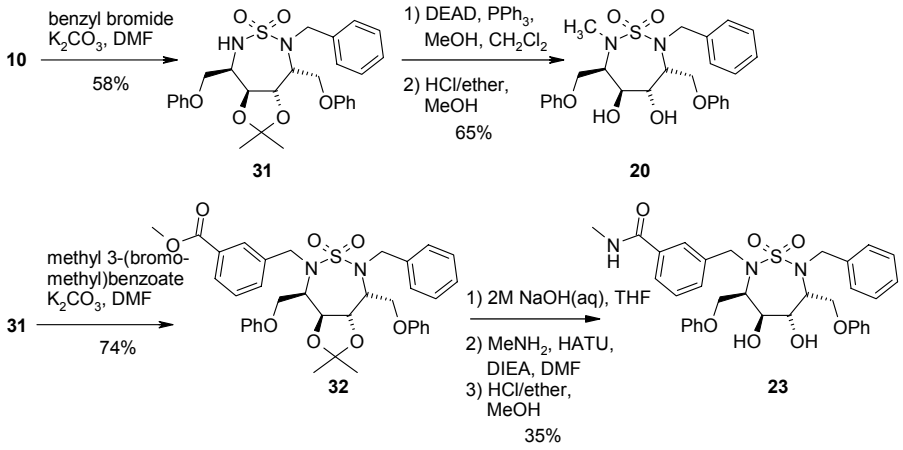
Table 2. Inhibitory Potency of Nonsymmetric Inhibitors.



compd	R_1 (P2) [c]	R_2 (P1') [c]	K_i (nM)	pK_i [a] obsd	pK_i [a] calcd [b]
20			510	6.29	6.40
21			1 400	5.85	5.56
22			350	6.46	6.65
23			11	7.96	8.11
24			86	7.07	7.43
25			63	7.20	6.94
26			510	6.29	6.09
27			1 800	5.75	5.85
28			140	6.85	6.89
29			6 000	5.22	5.18
30			7.3	8.14	8.04

[a] pK_i = -log K_i. [b] The calculated pK_i values are based on the CoMFA model of all 25 inhibitors. [c] Assignment of the R1 and R2 groups was based on the alignment derived from the CoMFA model (see Section 5.1.2 CoMFA).

Scheme 3.



5.1.2 CoMFA

CoMFA was used to rationalize the binding mode of the nonsymmetric compounds. The eighteen PR inhibitors under study and seven related compounds from earlier studies[137,172] were used to construct the model. A nonsymmetric ring conformation was assumed for all inhibitors. The X-ray crystal structure of **23** (in complex with HIV-1 PR, PDB code 1g2k) served as a template for the construction of the inhibitors, which were built to fit the conformation of the central ring and the P1/P1' groups. The positions of the P2/P2' substituents were investigated using a conformational search in the active site of the PR. Uncertainty regarding the binding mode of the 11 nonsymmetrical inhibitors, i.e., which of the P2/P2' substituents interact with S1/S1' subsite, prompted us to generate CoMFA models for each of the 2048 possibilities. In addition, the 10 combinations of fields available in the *Advanced CoMFA module* of Sybyl[181] (standard, indicator and parabolic fields, the steric or electrostatic components thereof, and hydrogen bonding) were explored, resulting in 20480 models. The CoMFA models were sorted according to the lowest error of prediction, and the most promising) few models were chosen for further investigation. The second best model from this list appeared most appropriate to explain both the SAR and the manner in which the nonsymmetric compounds were bound to PR. This model had a q^2 of 0.68 and an r^2 of 0.95; the optimal number of components was three (for further details see Paper I).

5.1.3 Summary and Discussion

When the lipophilic interactions with the enzyme were reduced by exchanging one of the benzyl groups of the parent compound **2** (K_i 23 nM) for a

methyl group, the expected loss in activity was seen (the K_i for compound **20** was 510 nM). However, the relative potency of inhibitor **11**, which had two methyl group substitutions, was surprisingly high (K_i 710 nM), despite the substantial reduction in favorable hydrophobic interactions.

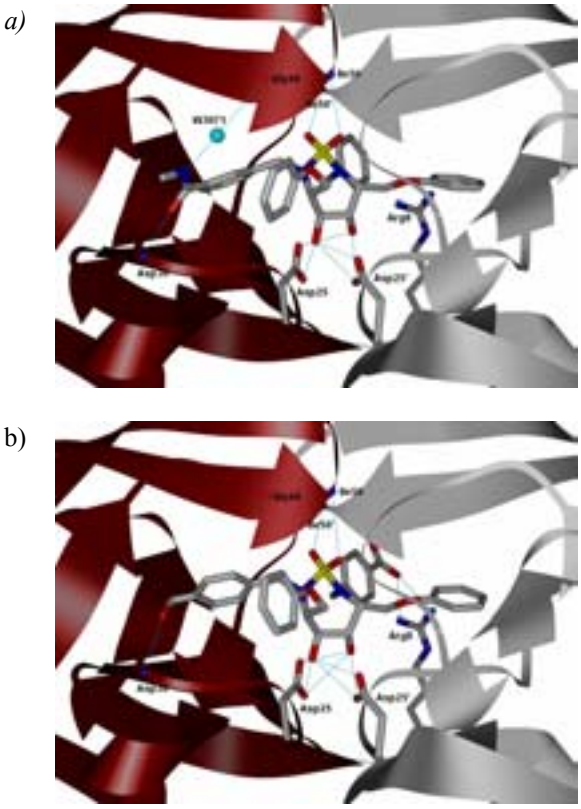


Figure 19. a) X-ray structure of compound **23** in the HIV-1 protease active site (monomer A colored red and monomer B gray). b) X-ray structure of compound **30** in the HIV-1 protease active site (monomer A colored red and monomer B gray).

It was anticipated that compound **14,** in which one of the methylamide substituents in the *meta*-position of the P2/P2' benzyl substituents, would interact favorably with the Asp30 in the S2/S2' subsites. Because this interaction involved nonsymmetric binding, the inhibitory potency of this symmetric compound decreased slightly (K_i 39 nM) compared with compound **2**. Since compound **14** is symmetric, one of the methylamides will end up in the lipophilic S1' subsite where no favorable hydrogen bond interactions with the enzyme seem likely. Interestingly, the inhibitory potency of the nonsymmetric compound **23** increased (K_i 11 nM), which is in agreement with the hypothesis of nonsymmetric binding.

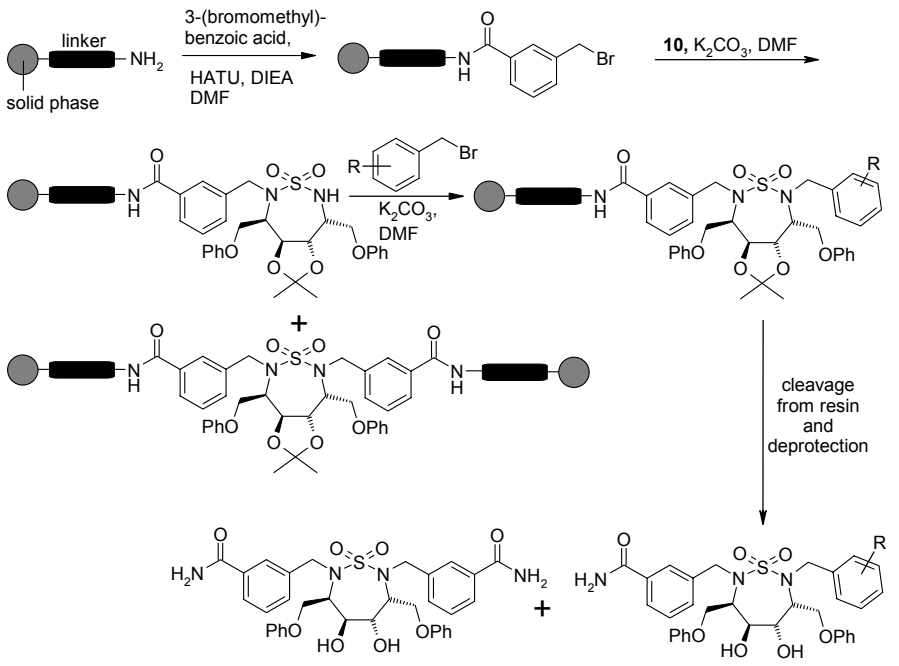
The two most active compounds of the series, **23** and **30**, were both nonsymmetric and contained polar substitution aimed for the S2/S2' subsite.

Fortunately, we were able to co-crystallize both compounds with HIV-1 PR (PDB codes 1g2k and 1g35). The crystal structures confirmed our hypothesis. Nonsymmetric binding to the enzyme and hydrogen bonds to Asp30 at the S2 subsite were demonstrated for both compounds (Figure 19). The methyl amide nitrogen of compound **23** coordinated with a structural water molecule, which in turn was associated via hydrogen bonding with the backbone carbonyl of Gly48. The carbonyl oxygen of the methylester in compound **30** formed a hydrogen bond with Arg8 at the S1'/S3' subsite.

5.2 Solid Phase Synthesis of Cyclic Sulfamides

Solid phase synthesis was initially explored in an attempt to facilitate the synthesis of nonsymmetric sulfamide inhibitors since it can be used to simulate high dilution reaction conditions[182,183] and therefore should be ideal for improving the mono-alkylation step. In this process, the first building block (the alkylating agent) is coupled to the solid phase; the sulfamide scaffold can then be connected to this, leaving the second sulfamide nitrogen exposed for further coupling (Scheme 4). The reagents can be used in excess since they are easily washed from the solid phase and, if expensive, they can be collected and re-used.

Scheme 4.



To our surprise, we obtained two products when cleaving the inhibitor from the solid phase. The byproduct was a symmetric inhibitor, which was formed as a result of cross-coupling. When the sulfamide scaffold was reacted with the solid phase, both nitrogens underwent coupling, not one as intended. The conditions [the concentration of the sulfamide scaffold and the choice of solid support (TentaGel, Polystyrene and ArgoPore)] were then varied, but without success. It almost seemed as if the second sulfamide nitrogen became activated when the first was coupled to the solid phase. The solid phase strategy was therefore abandoned.

5.3 Elongation of P2 and P2' in an Attempt to Reach the S1 and S2' Binding Sites (Paper II)

Encouraged by the relatively high potency of the small sulfamide inhibitors **11** and **20** (Paper I, Section 5.1.1), we explored this design avenue further. Unfortunately, the P1/P1' substituents are introduced early in this cumbersome synthetic route, which made the process of varying these substituents tedious. Because of the nonsymmetric conformation of the sulfamides, one of the traditional P1/P1' substituents will in fact interact with one of the S2/S2' subsites. Thus, moving the substituents from the carbons to the nitrogens in the sulfamide ring would yield the same interactions with the enzyme (Figure 20).

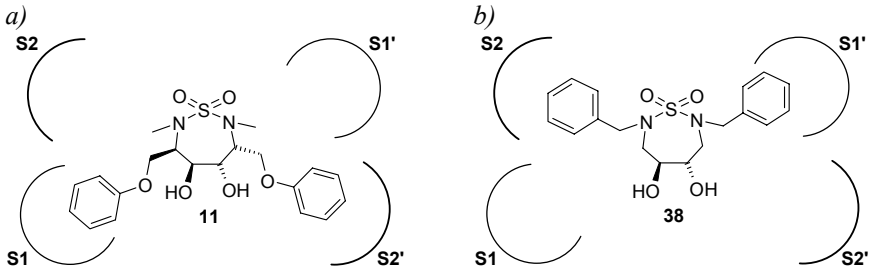


Figure 20. a) Compound **11** is nonsymmetrically bound to HIV-1 PR. This allows transpositioning of the sidechains from the ring carbons to the ring nitrogens, **38**, while still allowing interactions with one S1/S1' and one S2/S2' binding site (see b).

Previous studies of HIV-1 PR inhibitors have shown that it is possible to elongate the P1/P1' substituents to reach the S3/S3' binding sites.[184] In analogy, we therefore hypothesized that it might be possible to design a compound that reached between the S1/S1' and S2/S2' binding sites. Molecular modelling suggested that this could be achieved with appropriate *ortho* substitution on the P2/P2' benzyl groups (Figure 21).[185]

5.3.1 Modelling

We decided to explore *ortho* substituents with spacers of different lengths. The methano, ethano and etheno bridges (compounds **40**, **41** and **42**, respectively) were investigated along with the biphenyl (**39**) and the non-substituted benzyl (**38**) compounds (Scheme 5 and Table 3). The compounds were subjected to a Monte Carlo conformational search in the active site of the HIV-1 PR, using MacroModel.[186] The conformation of the sulfamide ring was not explored. The ring conformation found (by X-ray) for the tetra-substituted sulfamides was assumed. The lowest energy conformation from each complex was compared with the X-ray crystal structure of the parent compound **2** (Figure 21).

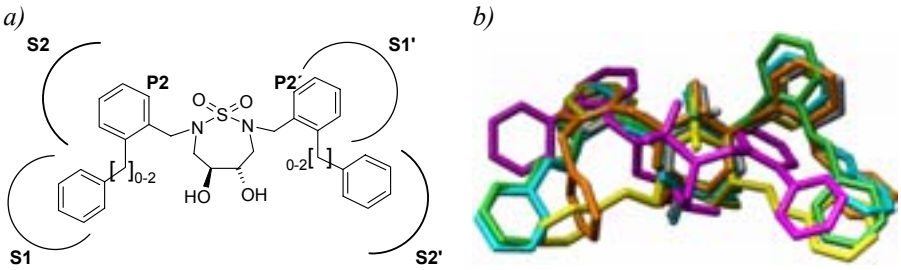


Figure 21. a) Generic structures of the *ortho*-substituted compounds intended to span from the S2 to S1 and from the S1' to S2' subsites, and the anticipated non-symmetric binding to the HIV-1 protease. b) Results from the Monte Carlo conformational search. The molecular graphics image was produced using the UCSF program Chimera.[94]

All compounds, except the biphenyl, adopted binding modes similar to that observed for **2**. The biphenyl compound and the compound lacking ortho substituents seemed to only occupy two binding sites and it was therefore predicted that they would have relatively lower inhibitory potency. However, to aquire as much information as possible for this new class of inhibitors, we decided to synthesize all the compounds modelled.

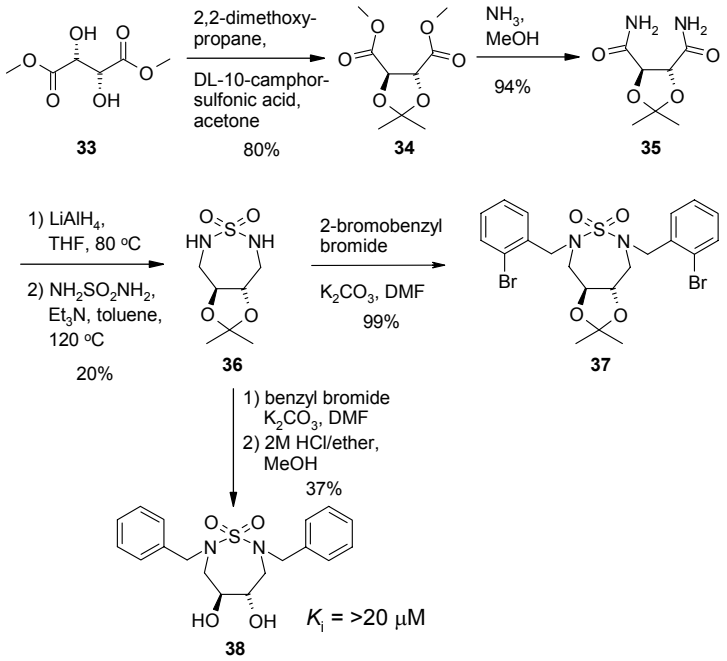
5.3.2 Synthesis and Inhibitory Potencies

Several synthetic routes to cyclic ureas and sulfamides have recently been published.[187-190] However, these approaches are associated with insufficient control of the stereochemistry, especially when introducing the hydroxyl groups which are essential for binding to the active site of the PR. A new short, efficient synthetic route, including adequate stereochemical control, was therefore developed.

The Cyclic Sulfamide Scaffold Lacking P1/P1' Sidechains

Dimethyl L-tartrate served as the chiral starting material (Scheme 5). The first two steps, protection of the alcohols and reaction with ammonia to form amides, were performed essentially as described in the literature.[191-193] Reduction of the amides, using lithium aluminium hydride,[194,195] and subsequent cyclization with sulfamide furnished the seven-membered cyclic scaffold. Alkylation with 2-bromobenzyl bromide yielded the intermediate **37,** which was used to synthesize the *ortho*-elongated compounds, and an alkylation with benzyl bromide afforded compound **38**.

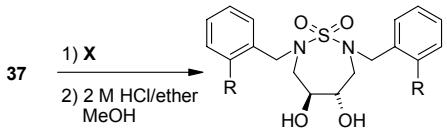
Scheme 5.



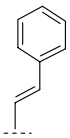
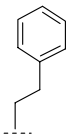
Elongated *O*-Benzyl P2/P2' Sidechains

The elongated compounds were obtained via palladium-catalyzed cross couplings. A Suzuki-Miyaura coupling[196,197] afforded the biphenyl compound **39**, a Negishi coupling[198] gave the methano-elongated compound **40,** and a Heck coupling[199,200] yielded the etheno-elongated compound **41**. Reduction of the double bond in **41**, using palladium on charcoal and hydrogen gas, afforded the ethano bridge in compound **42** (Table 3). Subsequent deprotection of the alcohols followed all cross-couplings.

Table 3. Reagents and Conditions for Synthesis of the Elongated Biaryl Compounds, and Their Inhibitory Potency.

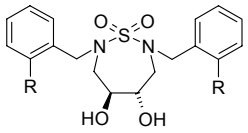


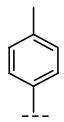
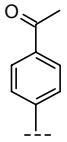
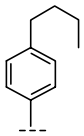
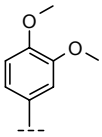
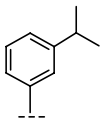
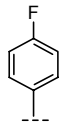
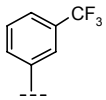
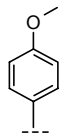
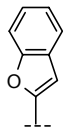
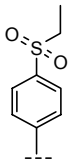
39- 42

compd	R	yield	K_i (μM)	Type of Reaction	X
39		53	2.5	**Suzuki-Miyaura-coupling**	Phenylboronic acid $Pd(OAc)_2$, PPh_3,, EtOH, H_2O, DME, Na_2CO_3, **MW: 150 °C, 20 min**
40		51	2.5	**Negishi-coupling**	Benzylzinc bromide, $PdCl_2$, DPPF, THF **80 °C, 76 h**
41		53	2.0	**Heck-coupling**	Styrene, $Pd(OAc)_2$, DIEA, DMF, H_2O **MW: 150 °C, 20 min**
42		28	3.0	**Heck-coupling & hydrogenation**	1. As for **39** 2. H_2/Pd(C), EtOAc **r.t, over night**

Elongation of the P2/P2' sidechains yielded four inhibitors with equivalent potency (K_i 2.0-3.0 μM). The potency of compound **39** was unexpected, since it was predicted (by modelling) to occupy only two of four subsites. To further explore this result, with the anticipation of improving the potency, we synthesized a series of 15 substituted biaryl compounds (Table 4). The boronic acids used in the coupling reactions were selected from those available in house (see Section 5.3.3 Diversity of Reactants). The same reaction conditions as those developed for **39** were used. We required a fast, efficient method for work-up and purification to facilitate the synthesis of a large number of compounds. An extraction procedure was developed that resulted in pure products, according to NMR and elemental analysis, for all except one compound.

Table 4. Inhibitory Potency for Biphenyl Analogs.[a]



compd	R	yield (%)	K_i (μM)	compd	R	yield (%)	K_i (μM)
43		60	1.5	**51**		65	5.3
44		63	2.7	**52**		60	7.4
45		60	1.9	**53**		57	9.4
46		56	3.2	**54**		54	5.1
47		62	5.3	**55**		68	1.1
48		52	2.4	**56**		45	2.7
49		50	9.7	**57**		31	0.53
50		26	5.2				

[a] Compounds **43**-**57** were prepared as described for **39** (Table 3), except for **54** and **55,** where slight modifications of time and temperature were required (140 °C, 20 min).

5.3.3 Diversity of Reactants

PCA analysis was used to ensure that the selected reactants represented the desired regions of chemical space. The Available Chemicals Directory (ACD)[201] was searched for boronic acids and esters. Seven physicochemical descriptors were calculated for each reactant. A two-component model, representing 71% of the variance, was derived (Figure 22). Inspection of the score and loading plots show that the selected boronic acids were spread throughout the desired chemical space. Among the reactants used, a preference for lipophilic compounds with low molecular weight can be seen, which also corresponds to our design criterion.

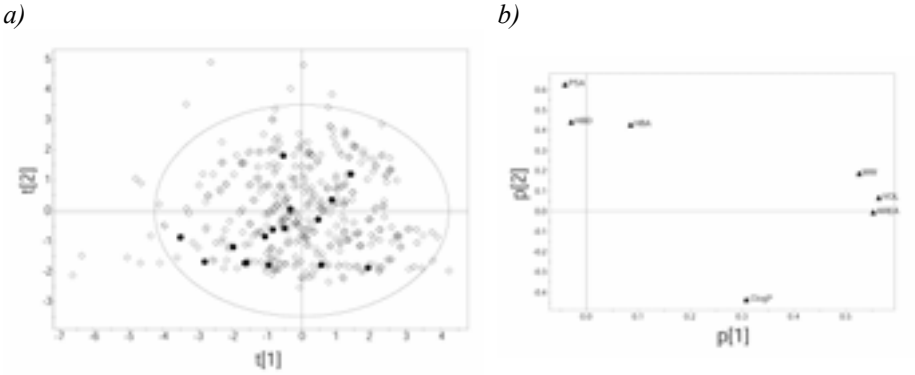


Figure 22. Results from the principal component analysis (PCA) of the selected boronic acids and esters. In the score plot (a), the building blocks used are depicted (filled circles) among the commercially available building blocks (open diamonds). Analysis of the loading plot (b) demonstrated a preference for lipophilic reactants with low molecular weight, which corresponds to our design criterion.

5.3.4 Summary and Discussion

It appears to be possible to design inhibitors with sidechains that span from P2/P2' to P1/P1'. Compounds with different bridging groups were explored, resulting in compounds **39-42**, which were equally potent with K_i values of 2.0-3.0 μM. The compound lacking *ortho* substituents, **38**, was also synthesized and evaluated for inhibitory potency. As anticipated, it was inactive.

Unexpectedly, the biphenyl compound **39** was as active as the elongated compounds. Computational modelling had indicated that it would occupy only two of the four possible binding sites. This called into question the validity of the model. However, assuming that the model was reliable, we reasoned that substitution of the phenyl ring would facilitate interactions with the unfilled binding sites and thus improve the potency.

A series of biaryl derivatives was synthesized by means of Suzuki-Miyaura coupling reactions. Inspection of the scores and loading plots obtained from PCA of available boronic acids and esters unveiled an over-representation of small lipophilic compounds, which was in agreement with our design criterion.

The biaryl compounds with lipophilic substituents in the *para* position had improved activity (Table 4). Substitution in the *meta* position was also tolerated but did not improve activity over that of **39**. Polar substituents in the *meta* and/or *para* positions resulted in poor activity. Only compound **57**, substituted with an *o*-benzofuran, showed a significant improvement in potency, with a K_i of 0.53 μM. Modelling suggested that the benzofuran oxygen might favorably interact with the backbone NH of Gly48. The S3 subsite also seemed to be better filled by the benzofuran compound than by the parent biphenyl compound. A lead compound for further optimization was thus identified.

5.4 Investigation of Nonsymmetric Substitution (Paper III)

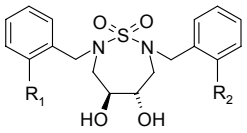
The investigation of *ortho* substituents with bridging groups of different lengths (performed in Paper II) suggested a difference in the occupancy of the S1 and S1' subsites. We therefore hypothesized that nonsymmetric compounds might be more potent inhibitors of the HIV-1 PR than symmetric compounds. The sidechains used in Paper II were combined to produce nonsymmetric compounds. The synthesis of the nonsymmetric compounds was made possible as a result of the mono-alkylation procedure outlined in Paper IV.

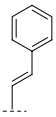
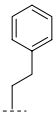
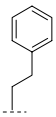
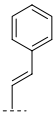
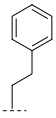
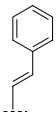
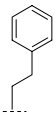
5.4.1 Modelling and CoMFA

The conformational preference of the compounds was explored with a conformational search in the active site of the HIV-1 PR. The search was performed as outlined in Paper II. Both binding modes were explored for each inhibitor (see Table 5).

The inhibitory potency of the compounds was predicted with the help of CoMFA. The CoMFA model from Paper I served as the starting point. The symmetric compounds investigated in Paper II were added to the model, since they define the new type of binding mode. The compounds were aligned using the enzyme backbone as template, and a new model was derived. This model had a q^2 of 0.650 and an r^2 of 0.970; the optimal number of components was five (for further details see Paper III).

Table 5. Calculated and Observed Inhibitory Potencies for the *ortho*-Elongated Nonsymmetric Compounds.



compd	R_1	R_2	K_i (μM)	pK_i [a]	
				obsd	calcd [b,c]
58			>20	4.70	4.53 / 5.70
59			>20	4.70	5.44 / 6.71
60			>20	4.70	5.29 / 5.07
61			>20	4.70	4.47 / 5.25
62			6.3	5.20	4.90 / 5.17
63			9.3	5.03	5.84 / 5.54
64			4.9	5.31	5.10 / 5.80
65			7.5	5.12	5.12 / 6.39
66			>20	4.70	5.95 / 6.57

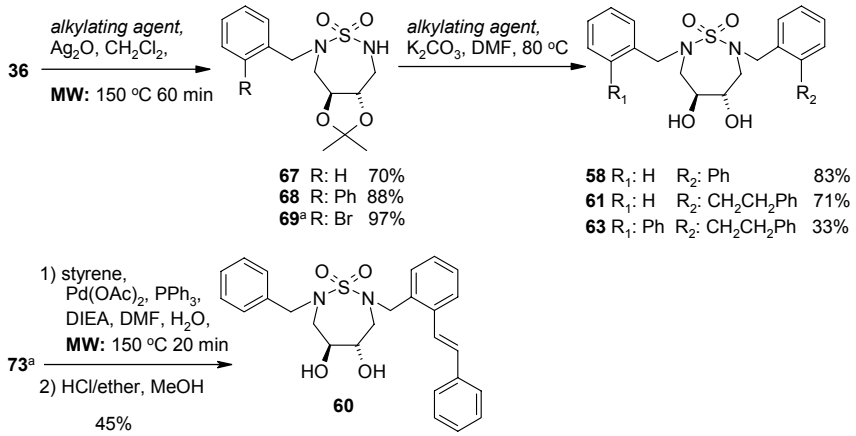
[a] pKi = -log *K*i. [b] pK_i values were calculated for both possible binding modes, based on the CoMFA model described in Section 5.4.1 Modelling and CoMFA. [c] Left values correspond to R_1→S1 and R_2→S2' while the right values correspond to R_1→S2' and R_2→S1.

5.4.2 Synthesis

The nonsymmetric compounds containing combinations of *ortho* elongations were synthesized using alkylation and palladium-catalyzed Negishi and Heck coupling conditions, derived in Paper II (see also Section 5.3.2).

The 2-phenylbenzyl bromide was synthesized as described by Lombardino et al.[202] Mono-alkylation of **36** with either benzylbromide, 2-phenylbenzyl bromide or 2-bromobenzyl bromide resulted in compounds **67, 68** and **69** (Scheme 6). A second alkylation step using either 2-phenylbenzyl bromide or 2-phenethylbenzyl bromide afforded compounds **58**, **61** and **63**. Heck coupling of **73** with styrene and subsequent deprotection afforded compound **60** (the synthesis of **73** is described in section 5.5.1 and Paper IV).

Scheme 6.

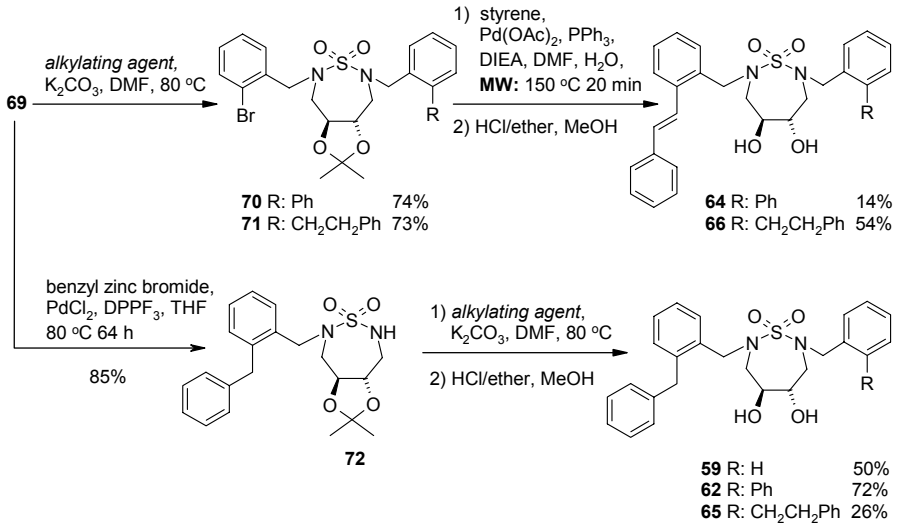


Alkylating agent; benzyl bromide for **67**, 2-phenylbenzyl bromide for **58** and **68**, 2-bromobenzyl bromide for **69**, and 2-phenethylbenzyl bromide for **61** and **63**. [a] For details on the synthesis of compounds **69** and **73** see Section 5.5.1.

Compound **69** served as the key intermediate in the synthesis of the remaining compounds (Scheme 7). Alkylation using either 2-phenylbenzyl bromide or 2-phenethylbenzyl bromide afforded compounds **70** and **71**. Heck coupling and subsequent deprotection afforded compounds **64** and **66**.

Negishi coupling of **69,** using benzylzinc bromide, produced intermediate **72**. Alkylations of **72** with the appropriate alkylating agents, and subsequent deprotection, produced compounds **59**, **62** and **65**.

Scheme 7.



Alkylating agent; benzyl bromide for **59**, 2-phenylbenzyl bromide for **62** and **70**, and 2-phenethylbenzyl bromide for **65** and **71**.

The inhibitory potencies were determined. None of the nonsymmetric compounds synthesized had a higher potency for inhibition of the HIV-1 PR than their parent symmetric compounds.

5.4.3 Summary and Discussion

The activities of compounds **58-66** were reasonably well predicted by the derived CoMFA model. The lower potencies predicted for the two possible alignments correlated best with the measured K_i values. These compounds were hypothesized to occupy the subsites more effectively than their parent symmetric analogs. Unfortunately, no improvement in inhibitory potency was seen over that of the symmetric analogs. All the small compounds (**58-61)**, which contained only one elongated substituent, were inactive (K_i >20 μM). This lack of potency may imply that the inhibitors adopt a different binding mode from that suggested by modelling.

5.5 Exploring Amides as Possible Bridging Groups (Paper IV)

The *o*-benzofuran lead compound identified in Paper II served as a starting point for further investigation. Modelling and isosteric replacement sug-

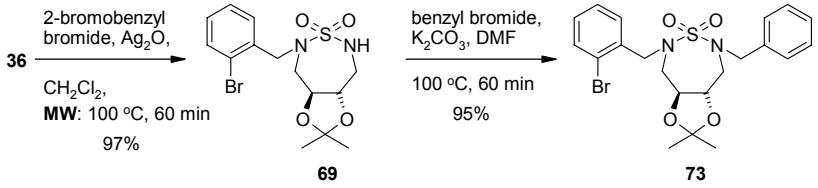
gested that an amide function, mimicking the furan ring, could be incorporated as a bridge to the *ortho* substituent (Figure 23). The reagents used were selected to provide variation in the flexibility and size of the *ortho* substituents. Both possible orientations for the amide group were investigated. In order to further reduce the size of the inhibitors, nonsymmetric compounds with *ortho* substitution on only one of the benzyls were investigated.

Figure 23. Superposition of *o*-benzofuran (**57**) and *o*-anilide (**74**) substituted *N,N'*-dibenzylic seven-membered sulfamide structures. The molecular graphics image was produced using the UCSF program Chimera.[94]

5.5.1 Synthesis and Inhibitory Potencies

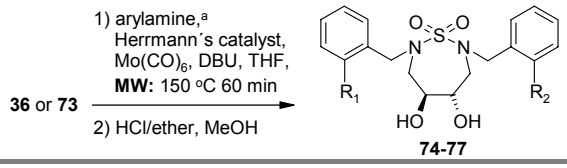
The symmetric compounds were synthesized from compound **36** (see Section 5.3.2). A new protocol was derived to facilitate the mono-alkylations. Addition of 1.5 equiv of Ag_2O and subsequent microwave-assisted benzylation afforded pure mono-alkylated product with excellent selectivity (di/mono: 1/99).

Scheme 8.

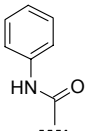
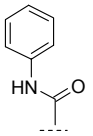
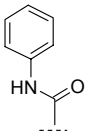
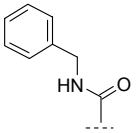
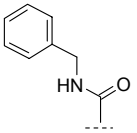
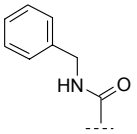


The inhibitors were synthesized via microwave-mediated palladium-catalyzed aminocarbonylations and *N*-amide arylations. The aminocarbonylations were performed using molybdenum hexacarbonyl ($Mo(CO)_6$) as a source of carbon monoxide.[203] Aniline and benzylamine were used to synthesize both the symmetric (starting from **36**) and the nonsymmetric (starting from **73**) analogs in good yields (Table 6). However, the inhibitory potencies were not as anticipated. Only the symmetric compound **76** showed some potency (K_i 8.6 μM).

Table 6. Inhibitory Potencies of the Inhibitors Produced via Aminocarbonylation.



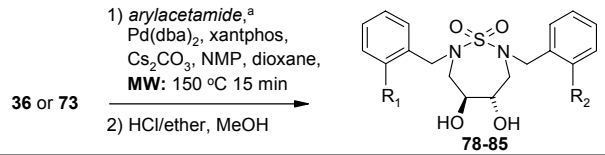
74-77

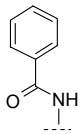
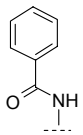
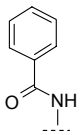
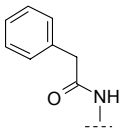
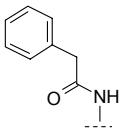
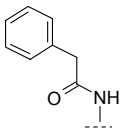
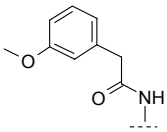
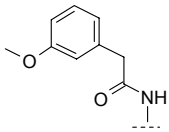
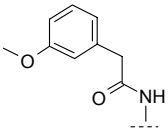
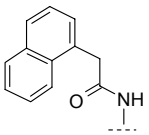
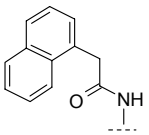
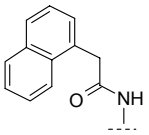
compd	R_1	R_2	yield (%)	K_i (μM)
74			59	>20
75			77	>20
76			80	8.6
77			74	>20

[a] Arylamine = aniline for **74** and **75** and benzylamine for **76** and **77**.

The *N*-amide arylations afforded the reverse amide analogs in good yields (Table 7). Unexpectedly, compound **80** appeared to be more potent than compound **76**. Substitution with a methoxy group in the *meta* position of the phenylacetamide (**82**) did not change the potency of the symmetric compound from that of **80**. However, for the nonsymmetric compound (**83**), the methoxy group was required for inhibitory activity. The most potent compounds were attained by substitution with 2-naphthylacetamide (compounds **84** and **85**).

Table 7. Inhibitory Potencies of the Inhibitors Produced via *N*-amide arylation.



compd	R_1	R_2	yield (%)	K_i (μM)
78			72	>20
79			77	>20
80			53	1.2
81			51	>20
82			54	1.3
83			68	7.7
84			57	0.02
85			66	0.14

[a] Arylacetamide = benzamide for **78** and **79**, 2-phenylacetamide for **80** and **81**, 2-(3-methoxyphenyl)acetamide for **82** and **83** and 2-(2-naphthyl)acetamide for **84** and **85**.

5.5.2 Summary and Discussion

As hypothesized, amide functionalities work well as bridging groups for symmetric compounds. However, some extra flexibility, introduced as an amidobenzyl or phenylacetamide group, was needed to attain inhibitory potency (compounds **76**, **80**, **82** and **84**). The direction of the amide function (CONH or NHCO) seemed to be important, since a difference in potency was seen for compounds **76** and **80** (K_i values of 8.6 and 1.2 μM, respectively). The most active compound, **84** (K_i 20 nM), was obtained when the larger, more lipophilic naphthylacetamide was introduced.

To our satisfaction, the smaller nonsymmetric compounds **83** and **85** were also active. For the nonsymmetric compounds, the extra flexibility and an extra substitution of the phenylacetamide were needed. The naphthylamide substituent was also used to produce the most active nonsymmetric compound, **85** (K_i 140 nM).

6 Concluding Remarks

- A number of tetrasubstituted nonsymmetric cyclic sulfamides, incorporating sidechains chosen to take advantage of the nonsymmetric binding mode, were synthesized. The symmetric analogs were also synthesized for comparison. Two potent nonsymmetric inhibitors were identified and crystallized in complex with HIV-1 protease.

- A CoMFA model, able to rationalize the alignment of the tetrasubstituted nonsymmetric inhibitors and improve understanding of the structure-activity relationship of the compounds, was derived.

- A number of symmetric disubstituted cyclic sulfamides were synthesized to investigate the elongations of the P2/P2' substituents using sidechains reaching from S2 to S1 and from S1' to S2'. Symmetric bridging groups (methano, ethano, etheno) were investigated along with the benzyl and biphenyl groups. The biphenyl analog was surprisingly potent, despite indications from computer modelling that it only occupied two of the four possible binding sites. A set of 14 biphenyl analogs was synthesized to further examine the structure-activity relationships of the novel compounds. A biphenyl analog possessing a benzofuran was identified as a lead structure.

- Elongated nonsymmetric compounds were synthesized in an attempt to optimize activity, since modelling suggested a difference in the occupation of the S1 and S2' sites for symmetric compounds. Unfortunately, no improvement in potency was seen.

- Symmetric and nonsymmetric isosteres of the benzofuran lead-compound were synthesized using amides as bridging groups. This led to the identification of two highly potent inhibitors possessing nanomolar affinity for the enzyme.

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