Design and Synthesis of Novel HIV-1 Protease Inhibitors Comprising a Tertiary Alcohol in the Transition-State Mimic

JENNY EKEGREN
Abstract


HIV-1 protease inhibitors are important in the most frequently used regimen for the treatment of HIV/AIDS, the highly active antiretroviral therapy (HAART). For patients with access to this treatment, an HIV infection is no longer lethal, but rather a manageable, chronic infection. However, the HIV-1 protease inhibitors are generally associated with serious shortcomings such as adverse events, development of drug resistance and poor pharmacokinetic properties. Most of the approved inhibitors suffer from high protein binding, rapid metabolism and/or low membrane permeability.

In this project, novel HIV-1 protease inhibitors comprising a rarely used tertiary alcohol in the transition-state mimic were designed, synthesized and evaluated. The rationale behind the design was to achieve 'masking' of the tertiary alcohol by for example, intramolecular hydrogen bonding, which was believed could enhance transcellular transport.

A reliable synthetic protocol was developed and a series of highly potent inhibitors was obtained exhibiting excellent membrane permeation properties in a Caco-2 cell assay. However, the cellular antiviral potencies of these compounds were low. In an attempt to improve the anti-HIV activity, microwave-accelerated, palladium-catalyzed cross-coupling reactions and aminocarbonylation of aryl bromide precursors were employed to produce P1'-extended test compounds. Inhibitors demonstrating up to six times higher antiviral effect were obtained, the best derivatives having para 3- or 4-pyridyl elongations in P1'.

Fast metabolic degradation was observed in liver microsome homogenate, which is believed, at least partly, to be attributable to benzylic oxidation of the indanol P2 group of the inhibitors. To enable facile variation of the P2 side chain a new synthetic route was developed using an enantiomerically pure, benzyl-substituted epoxy carboxylic acid as the key intermediate. Cyclic and amino-acid-residue-derived P2 groups were evaluated, and inhibitors equipotent to the series containing an indanol moiety were produced.

Keywords: HIV/AIDS, HIV-1 protease inhibitor, transition-state mimic, tertiary alcohol, palladium, cross-coupling, aminocarbonylation, microwave, molybdenum hexacarbonyl

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Till Henrik
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<th>Definition</th>
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<tbody>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2'-azobis(2-methylpropionitrile)</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
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<tr>
<td>aq.</td>
<td>aqueous</td>
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<td>Ar</td>
<td>aryl</td>
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</tr>
<tr>
<td>Asp</td>
<td>L-aspartic acid</td>
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<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>CA</td>
<td>capsid protein</td>
</tr>
<tr>
<td>Cbz</td>
<td>benzoxycarbonyl</td>
</tr>
<tr>
<td>CC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>inhibitor concentration causing a 50% decrease in cell proliferation</td>
</tr>
<tr>
<td>Cmpd</td>
<td>compound</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome P-450</td>
</tr>
<tr>
<td>DIEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>inhibitor concentration that reduces the cytopathic effect of the virus by 50%</td>
</tr>
<tr>
<td>EDC</td>
<td>N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride</td>
</tr>
<tr>
<td>equiv</td>
<td>equivalent(s)</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
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<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
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<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>Gly</td>
<td>glycine</td>
</tr>
<tr>
<td>HAART</td>
<td>highly active antiretroviral therapy</td>
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<tr>
<td>HATU</td>
<td>O-(7-azabenzotriazole-1-yl)-N,N,N',N'*-tetramethyluronium hexafluorophosphate</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<tr>
<td>HOBT</td>
<td>1-hydroxybenzotriazole</td>
</tr>
<tr>
<td>IN</td>
<td>integrase</td>
</tr>
<tr>
<td>Kᵢ</td>
<td>inhibition constant, $Kᵢ = [E][I]/[EI]$</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
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<tr>
<td>L</td>
<td>ligand</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
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<tr>
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<td>metal</td>
</tr>
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<td>matrix protein</td>
</tr>
<tr>
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<td>3-chloroperoxybenzoic acid</td>
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<tr>
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<td>methyl</td>
</tr>
<tr>
<td>MS</td>
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</tr>
<tr>
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</tr>
<tr>
<td>n.d.</td>
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<tr>
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</tr>
<tr>
<td>NMM</td>
<td>4-methylmorpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NNRTI</td>
<td>non-nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NRTI</td>
<td>nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NtRTI</td>
<td>nucleotide reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein Data Bank</td>
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<tr>
<td>Ph</td>
<td>phenyl</td>
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<tr>
<td>PI</td>
<td>protease inhibitor</td>
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<td>Pr</td>
<td>propyl</td>
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<tr>
<td>PR</td>
<td>protease</td>
</tr>
<tr>
<td>PyBOP</td>
<td>(benzotriazol-1-yl oxy) tripyrrolidinophosphonium hexafluorophosphate</td>
</tr>
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<td>quantitative</td>
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<tr>
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</tr>
<tr>
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<td>room temperature</td>
</tr>
<tr>
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<td>reverse transcriptase</td>
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<tr>
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<td>simian immunodeficiency virus</td>
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<tr>
<td>Sta</td>
<td>statine</td>
</tr>
<tr>
<td>SU</td>
<td>surface glycoprotein</td>
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<tr>
<td>temp</td>
<td>temperature</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<tr>
<td>Thr</td>
<td>L-threonine</td>
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<tr>
<td>TM</td>
<td>transmembrane glycoprotein</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>The Joint United Nations Programme on HIV/AIDS</td>
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</tbody>
</table>
1 Introduction

1.1 HIV/AIDS

1.1.1 Background
In the early 1980s, several cases of death in the US among patients with unusual opportunistic infections (e.g. Pneumocystis carinii pneumonia) and rare cancer (Kaposi’s sarcoma) gained the world’s attention. Physicians tried all the available treatments for the different conditions but the patients still died. An urgent search for the cause of these deaths began.

In 1983, two research groups, led by R. C. Gallo and L. Montagnier, succeeded in isolating a new human retrovirus from patients with symptoms often preceding the fatal illness, called the acquired immunodeficiency syndrome (AIDS). The virus was cloned and its genomic sequence was published in 1985. Different names were suggested for this previously unknown retrovirus, but today it is known as the human immunodeficiency virus type 1 (HIV-1). Establishment of HIV-1 as the cause of AIDS initiated intense research on therapies targeting it and the first anti-HIV regimen to be approved by the US Food and Drug Administration (FDA) was zidovudine (AZT) in 1987. However, the limited effects of AZT monotherapy and the rapid spread of the disease caused fear and frustration.

In the following decade, several anti-HIV drugs acting through different mechanisms reached the market (see Section 1.1.3). The introduction of protease inhibitors and the subsequent use of combination therapy made the prognosis for HIV patients much more optimistic (see Section 1.1.5). With access to new drug combinations, the progress of an HIV infection to AIDS could now be prevented, making HIV a chronic but manageable condition, and mortality rates among HIV patients decreased markedly. Unfortunately, new problems arose. As a consequence of long-term use of the antiretroviral therapy, a high prevalence of side effects in patients was recorded, and it was difficult for patients to continue the prescribed regimen. Numerous drug-resistant strains of HIV were also identified and, as a result, resistance testing became standard in the late 1990s.

At present, we face a situation where HIV/AIDS has developed into one of the most devastating pandemics the world has ever seen. At the end of 2005, estimations by the Joint United Nations Programme on HIV/AIDS
(UNAIDS) indicated that 40 million people were living with HIV worldwide (Figure 1). During the same year, five million people became infected and more than 3 million lives were claimed by AIDS. The pandemic has an enormous impact not only on the afflicted families but also on entire countries. As young adults die, orphans and elderly family members are left behind, and the lack of a healthy workforce severely disturb economics and politics in the worst-affected regions.

Figure 1. Global estimates of the number of people living with HIV/AIDS in 2005

1.1.2 The HIV-1 Virus

Origin
Following the isolation of HIV-1 in 1983, the closely related HIV-2 was reported a few years later. Both viruses are characterized by extensive genetic diversity and both cause AIDS, although HIV-1 is the most widely spread. It has been established that HIV-1 and HIV-2 originate from a group of viruses called the simian immunodeficiency viruses (SIVs) present in African primates. SIVs were probably transferred to humans via handling of butchered primates, whose meat was sold in African markets. This is suggested to have occurred around 1930 ± 15 years.

Structure
HIV-1 is a retrovirus of the lentiviridae genus and, as in all retroviruses, RNA encodes the viral genome. The RNA is transcribed into DNA by a viral
reverse transcriptase. The virion is spherical and about 100 nM in diameter (Figure 2). A lipid bilayer membrane, transversed by surface glycoproteins (SU, gp120), envelops the viral particle. The glycoproteins are anchored to the virus via transmembrane proteins (TM, gp41), and beneath the membrane a matrix shell protein (MA, p17) lines the inner surface of the particle. Two copies of the viral RNA, stabilized by nucleocapsid proteins (NC, p7) and the three viral enzymes, reverse transcriptase (RT, p66, p51), integrase (IN, p31) and protease (PR, p11), are encapsulated by a cone-shaped structure consisting of capsid proteins (CA, p24). HIV-1 carries most of its genetic information in three genes; gag, pol and env. These genes are expressed as large polyproteins, which are processed into the functional proteins by either the viral PR (gag and pol) or host cellular enzymes (env). The gag gene encodes a polyprotein precursor that is cleaved to CA, MA and NC, the pol gene encodes the viral enzymes PR, RT and IN, and env processing results in the formation of functional SU and TM proteins.

![Figure 2. Schematic representation of the HIV-1 morphology](image)

The HIV-1 Replication Cycle

HIV-1 infects cells expressing a surface protein called CD4. The viral surface glycoprotein gp120 has high affinity for the CD4 molecule, which acts as a viral receptor (Figure 3). Primarily, helper T-lymphocytes carry CD4, but it is also present, to a lesser extent, on for example dendritic cells and macrophages. Upon binding, gp120 undergoes conformational changes that result in an increased affinity of gp120 for the CXCR4 or CCR5 chemokine coreceptors, also present on the target cells. The CD4-gp120-coreceptor complex which is formed triggers conformational changes in the transmembrane protein gp41 and ultimately viral-host membrane fusion. The viral core enters the host cell and uncoating leads to the release of its contents into the cytoplasm. RT starts to synthesize a double-stranded DNA copy of the viral RNA genome, a process termed ‘reverse transcrip-
However, the transcription by RT is notoriously prone to errors, resulting in a large number of mutations for each generation of virus produced (see Section 1.1.5). The DNA copy is transported into the cell nucleus as part of a preintegration complex, also including IN, PR, MA and accessory proteins. IN incorporates the viral DNA into the host chromosome forming a ‘provirus’. The acute HIV infection is characterized by high viral replication often causing influenza-like symptoms in patients. After this, an immune response is initiated resulting in a decrease in viral load and usually a stage of latency ensues, which can last for several years. When an infected, resting CD4 helper T-lymphocyte is activated, the proviral DNA is transcribed to mRNA (Figure 3). The mRNA is transported to the cytoplasm of the cell, where it is translated into Env precursor polyproteins (in the endoplasmic reticulum) and Gag and Gag-Pol protein precursors (in the ribosomes). Cellular enzymes process the Env protein into SU and TM, which are transported to the plasma membrane. After synthesis of the Gag and Gag-Pol polyproteins, the Gag-precursor recruits two copies of single-stranded viral RNA which are transported to the cell membrane together with the Gag-Pol proteins. A curvature is induced in the cell membrane leading to the formation of a bud. The Env glycoproteins are incorporated into the budding particle and an immature virion is released from the host cell. After budding the viral PR cleaves the Gag and Gag-Pol polyproteins to generate a mature, infectious virus particle capable of initiating a new replication cycle.

**Figure 3. The HIV-1 replication cycle (Illustration by Johan Wannberg)**
1.1.3 Antiretroviral Therapy

In the early days of the AIDS epidemic, therapy was restricted to the treatment of the plethora of opportunistic infections developed by patients. Today a number of drugs, divided into four classes with respect to their therapeutic target in the HIV-1 replication cycle, are available and these will be briefly outlined in the following sections. Anti-HIV agents acting on several new targets are also under investigation.

**Nucleoside Reverse Transcriptase Inhibitors**

As mentioned above, the first approved HIV/AIDS drug was AZT, a nucleoside reverse transcriptase inhibitor (NRTI). This class of drugs is phosphorylated in the body and then acts as an alternative substrate for RT. When incorporated into the growing DNA chain, the phosphorylated NRTIs prevent further nucleosides from being added and viral transcription is terminated. Eight NRTIs are currently available on the market, including the pro-drug tenofovir disoproxil, a nucleotide reverse transcriptase inhibitor, NtRTI (Figure 4).

![Figure 4. Examples of nucleoside reverse transcriptase inhibitors](image)

**Non-Nucleoside Reverse Transcriptase Inhibitors**

During the period 1996-98, three non-nucleoside reverse transcriptase inhibitors (NNRTIs) were introduced (Figure 5). These are structurally diverse compounds targeting RT by a different mechanism from the nucleoside analogs. The NNRTIs bind to an allosteric site on RT resulting in conformational changes at the active site of the enzyme, thereby inhibiting its activity.

![Figure 5. Approved non-nucleoside reverse transcriptase inhibitors](image)
Protease Inhibitors
Together with a greater understanding of the virus and its life cycle new drug targets, in addition to the reverse transcriptase, were recognized. In 1995, the first protease inhibitor (PI) reached the market (saquinavir, Figure 9). This class of drugs inhibits the viral PR, preventing the new virions from maturing and as a consequence they remain in a non-infectious state. The HIV-1 protease and its inhibitors will be discussed in greater detail in Sections 1.1.4 and 1.1.5.

Fusion Inhibitors
Enfuvirtide, a linear 36-amino-acid peptide corresponding to residues 127-162 of the viral glycoprotein gp41, was approved by the FDA in 2003 as the first, and so far only, therapeutically used fusion inhibitor.31,32 This peptide is administered by subcutaneous injection and inhibits the conformational change in gp41 necessary for the fusion of viral and host cell membranes. As a result, HIV is prevented from entering the target cell.

Highly Active Antiretroviral Therapy
Shortly after the introduction of AZT, the limited effects of monotherapy on HIV became clear. However, when the first PIs were launched in the mid 1990s, a new treatment strategy including combination therapy with drugs from both these classes of anti-HIV agents was evaluated. Several reports indicated efficient suppression of viral loads and reduced mortality among HIV patients receiving the combination regimen.33-36 Initially, two NRTIs and one PI were used in this highly active antiretroviral therapy (HAART) and today several drug combinations are available, including NNRTIs.37 Although HAART does not completely eradicate HIV,38 the efficient suppression of viral plasma loads and increased CD4 cell counts offer prolonged life expectancy and better quality of life to HIV patients. In addition, the risk of viral mutations and the development of drug resistance is reduced (see Section 1.1.5).

1.1.4 The HIV-1 Protease
Aspartic proteases, the group of enzymes to which HIV-1 protease belongs, are characterized by their ability to cleave peptidic substrates with the aid of two catalytically active aspartic acid residues. These enzymes are involved in a number of biological processes and apart from HIV/AIDS, aspartic proteases have also been considered as viable therapeutic targets for the treatment of hypertension, Alzheimer’s disease, malaria and candidal infections for example.39-41
Structure
The HIV-1 PR was structurally characterized by X-ray crystallography for the first time in 1989\textsuperscript{42-47} and a large number of different crystal structures are presently available. This dimeric enzyme consists of two identical non-covalently associated subunits with 99 amino acid residues in each monomer.\textsuperscript{15,48,49} Formation of the active protease dimer is a reversible process and high concentrations of monomer are needed for this to occur, which is the case in the newly budded virion. The monomer concentration inside the host cell is too low, preventing the active protease from forming and degradation of cellular proteins is avoided. The $C_2$-symmetric active site is located at the dimer interface and each subunit contributes one catalytic aspartic acid residue present in a tripeptide sequence, Asp-Thr-Gly. Two hairpin turn structures, made up of the amino acid residues 47-52 in both monomers, are known as the ‘flaps’ and cover the cylindrically shaped active site. The flaps play an important role in substrate/inhibitor binding and release as they can undergo large conformational changes during these events. In X-ray crystal structures of PR-inhibitor complexes a structural water molecule is found to be part of a hydrogen bond network with Ile50 and Ile50’ in the flap region and the carbonyl groups of the P2 and P1’ inhibitor residues (see below).

The active site contains a number of well-defined subsites where protruding substrate or inhibitor side chains can be accommodated upon binding. According to standard nomenclature, the amino acid residues of the substrate are termed P1, P2…Pn and P1’, P2’…Pn’ counted from the bond which is cleaved during hydrolysis, called the ‘scissile’ bond (Figure 6).\textsuperscript{50} The enzyme subsites occupied by these amino acid residues have the numbering beginning with the letter S; S1, S2…Sn and S1’, S2’…Sn’. Hydrophobic amino acid residues account for most of the PR subsites, and the S1/S1’ pockets are adjacent to S3/S3’. As a consequence of the symmetric nature of the HIV-1 PR the S1 and S1’ subsites are identical, as are the S2 and S2’ subsites, and so on.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure.png}
\caption{Standard nomenclature used to denote the substrate amino acid residues and enzyme subsites\textsuperscript{50}}
\end{figure}
Catalytic Mechanism

The Gag and Gag-Pol polyproteins are cleaved by PR according to a general acid-base mechanism (Figure 7).\textsuperscript{49} The protonation states for the catalytic aspartic acids have been investigated using different methods.\textsuperscript{51-57} A water molecule, activated by one of the aspartic acids, attacks the carbonyl carbon of the scissile bond, generating a tetrahedral intermediate. Protonation of the amide nitrogen followed by collapse of the tetrahedral intermediate results in the carboxylic acid and amine hydrolysis products.

![Proposed catalytic mechanism of HIV-1 PR](Image)

**Figure 7.** Proposed catalytic mechanism of HIV-1 PR\textsuperscript{49}

1.1.5 HIV-1 Protease Inhibitors

The first PIs were developed with the structure of the natural substrate hydrolysis sites and the amino acid residues surrounding these sites as starting points.\textsuperscript{58-61} The PR has eight specific cleavage sites and, in particular, the phenylalanine-proline site, specific to retroviral proteases, has been used as a basis for the design of PIs. The inhibitors generally have a peptide-like structure enclosing a central hydroxyl structural element, which is termed a transition-state analog or mimic. The nomenclature is derived from the fact that the non-scissile hydroxy unit mimics the tetrahedral intermediate formed during peptide hydrolysis (Figure 7). The transition-state analog binds to one or both of the catalytically active aspartic acids. Therapeutically useful PIs bind to the active site of the enzyme with an affinity several orders of magnitude greater than the natural peptide substrate. This is largely accomplished due to their structural similarity to the tetrahedral intermediate. A number of transition-state analogs have been developed and the hydroxyethylamine scaffold has been extensively used in HIV-1 PIs (Figure 8).

Seven inhibitors are available on the market and in addition, the non-peptide-like drug tipranavir\textsuperscript{62,63} has been granted accelerated approval by the FDA (Figure 9). Although very successful in HAART, the use of PIs is associated with certain disadvantages. The most apparent are adverse events (e.g. gastrointestinal side effects, hypersensitivity and metabolic complications),\textsuperscript{64-66} poor pharmacokinetic properties and resistance to PIs, of which the latter two will be discussed in more detail below.
Drug Resistance
Development of drug resistance limits the long-term effectiveness of the therapeutically used reverse transcriptase and protease inhibitors. The first cases of decreased response to antiretroviral therapy were reported in 1989 based on isolates taken from patients receiving AZT. Since then, a vast number of mutated HIV strains have been detected and the virus constantly creates new variants. Two factors are crucial for understanding the underlying principles of drug resistance. First, HIV has a very high reproduction rate, producing up to 10 billion new virions per day. Secondly, the high error rate of the RT when transcribing viral RNA to DNA results in a large number of mutated forms of the virus being produced. For PIs, sequence analysis of drug-resistant clones has shown mutations within the enzyme itself and at several of the substrate polyprotein cleavage sites. Mutations associated with PI resistance have been observed in the PR active site, the flap and other sites of the enzyme.
Pharmacokinetic Properties of HIV-1 Protease Inhibitors

Most of the initial PIs suffered from shortcomings in pharmacokinetic properties such as poor aqueous solubility, high protein binding, rapid metabolism and low membrane permeability. In the treatment of HIV infection it is necessary to maintain high drug concentrations in the blood and infected tissues. One of the major obstacles to achieving this has been poor adherence to treatment by the patients due to the high frequency of dosing, including high pill burdens and multiple daily dosing. Therefore, compounds with improved pharmacokinetics that allow more convenient dosing should improve patient compliance. Atazanavir was the first PI that showed excellent...
antiviral activity combined with high oral bioavailability and is the only inhibitor administered once daily.73-75

The antiviral potency of PIs can be reduced by binding to serum proteins, in particular albumin and $\alpha_1$-acid glycoprotein (AGP). Protein binding causes a reduction in the amount of free drug in plasma and is most pronounced with ritonavir, saquinavir and nelfinavir, all being bound to 98-99%.59

For an orally given drug, the rate and extent of absorption are closely related to the water solubility of the compound. However, many PIs are lipophilic and poorly soluble. Structural modifications involving the incorporation of polar and ionizable groups have been used to improve the aqueous solubility of these drugs.74,76,77 Furthermore, the solubility of weak acids and bases can be increased markedly by using a salt in the clinical formulation.78

Cytochrome P-450 (CYP) enzymes metabolize HIV-1 PIs, and strongly influence the oral bioavailability of these drugs.72 PIs have also been found to act as efficient CYP inhibitors and especially ritonavir exhibits a high inhibitory potency against several CYP isoforms. This has been used to ‘boost’ the activity of one PI by subsequent administration of ritonavir as a CYP inhibitor, leading to increased drug exposure and less frequent dosing.79

PIs must be able to pass across the lipid bilayer of epithelial cell membranes to be potent antivirals. Lipophilicity is often used as a molecular descriptor to predict passive diffusion through membranes. However, in studies on the effect of lipophilicity on the transport of small peptides across Caco-2 cell monolayers, this descriptor did not correlate well with membrane permeation properties.80,81 Hydrogen bonding potential has been suggested as an alternative property to describe membrane permeation characteristics.80-87 Reduced hydrogen bonding potential, for example as a result of intramolecular hydrogen bonds, decreases the desolvation energy needed for a solute to enter into the lipophilic membrane phase from the aqueous phase. Several reports have described this ‘masking’ of polar groups as an important factor in achieving enhanced membrane permeability.84-87 As mentioned above, all therapeutically useful HIV-1 PIs contain a polar, secondary alcohol as part of the transition-state mimicking unit. Thus, if the crucial hydroxyl group in these compounds could be masked via internal hydrogen bonding in an appropriate chemical environment, improved membrane permeability might be attained.
1.2 Tools in Medicinal Chemistry

1.2.1 Palladium(0)-Catalyzed Reactions

In 1803, a new transition metal was isolated and named palladium (Pd) after the recently discovered asteroid Pallas. However, the understanding of the unique properties of this metal and the development of palladium-catalyzed chemistry progressed slowly until the mid 20th century. The Wacker process (1959) represents a milestone in the use of palladium in organic synthesis.\(^88,89\) It is used for the production of acetaldehyde from ethylene and oxygen by Pd(II) catalysis. Many types of Pd-catalyzed reactions have been developed since then, and the versatility, selectivity and overall synthetic usefulness of this transition metal are now widely appreciated.\(^90,91\)

Pd is characterized by its tendency to form d\(^{10}\) and d\(^8\) complexes favoring the 0 and +2 oxidation states. Generally, Pd has a high affinity for nonpolar \(\pi\)-compounds such as alkynes and alkenes and also forms \(\sigma\)-bonds to non-bonding electron donors present in compounds containing N, P, S and O. Another important property of Pd is its relatively pronounced electronegativity leading to low reactivity towards polar functional groups such as the carbonyl, hydroxyl and nitro groups. In turn, high chemoselectivity in reactions with compounds containing multiple functional groups can be achieved.

In Pd(0)-catalyzed reactions, a few fundamental reactions or ‘micro-steps’ provide the basis for understanding the course of the reactions, and these are briefly outlined in this section.\(^92\) Oxidative addition is the term used to describe the addition of a molecule (R\(\text{X}\)) to a Pd(0) species accompanied by cleavage of one covalent bond in the substrate and the formation of two new bonds to Pd (Figures 10 and 11).\(^93,94\) Generally, ligands that increase the electron density at Pd (e.g. R\(3P\)) facilitate oxidative addition. Compounds containing a C(sp\(^2\))–X bond, such as alkynyl and aryl halides or triflates are often used for addition to Pd(0), but alkynyl, allyl and benzyl substrates have also been used. As a result of oxidative addition, a stable trans-\(\sigma\)-palladium(II) complex is formed. The relative reactivity of compounds with different X-groups in oxidative additions decreases in the order I > OTf > Br >>Cl.

Transmetallation occurs when organometallic compounds (M–R’) or hydrides (M–H) of the main group metals, such as Mg, Zn, B, Al, Sn, Si and Hg, react with the R–Pd(II)L\(_n\)–X complex formed by oxidative addition (Figure 10).\(^95,96\) The R’-group or hydride is transferred to Pd, and X acts as a leaving group forming the by-product MX. The driving force for this reaction is the difference in electronegativity of the two metals, M being the most electropositive. Transmetallation is a reversible process and the nature of both M and X may be important for the course of the reaction. The reac-
tion rate can often be enhanced by the addition of appropriate counterions, X, facilitating the formation of a more stable MX salt.

Insertion reactions occur when an unsaturated ligand, such as CO, an olefin or alkyne, is inserted into an adjacent Pd–C σ-bond (Figure 11). The insertion of carbon monoxide, a 1,1-insertion, is an important process in organic chemistry as it permits the direct introduction of CO into organic molecules producing different carbonyl compounds. In CO insertions, an R-group on Pd migrates to a cis-bound CO ligand forming an acylpalladium complex. The acyl complex, which can be viewed as an activated carboxylic acid derivative, is readily attacked by nucleophiles rendering the different products (more about carbonylation below).

Reductive elimination is usually the last step in Pd(0)-catalyzed reactions. This is the reverse reaction of oxidative addition and involves the loss of two, one-electron ligands in cis-configuration from Pd (Figure 10). Anything that reduces the electron density on Pd, for example the dissociation of a ligand by the application of heat or light, promotes reductive elimination. The two ligands are combined with a σ-bond into a single product molecule accompanied by the formal reduction of Pd(II) to Pd(0). The Pd(0) species generated is then available to enter a new catalytic cycle.

Stable, commercially available Pd(II) salts are often used as catalyst precursors in Pd(0)-catalyzed reactions. Reduction of Pd(II) to Pd(0) is usually performed in situ promoted by either an organometallic reagent, a phosphine ligand,97-99 a base,100 the solvent101 or CO in the reaction mixture. Numerous protocols have been developed utilizing Pd(0) as a catalyst, and four of them fall within the scope of this thesis. First, the Stille, Suzuki and Sonogashira reactions, all examples of carbon-carbon cross-coupling reactions, will be discussed, and then the concept of carbonylation chemistry is described.

Carbon-Carbon Cross-Coupling Reactions
Cross-coupling reactions between organic electrophiles (R–X) and organometallic compounds (M–R’) rank amongst the most straightforward and general reactions for the formation of carbon-carbon bonds. The general mechanism for these reactions involves three of the fundamental reaction steps described above: oxidative addition of R–X to a Pd(0) species, transmetallation with the organometallic compound M–R’ and finally reductive elimination rendering the R–R’ product and regeneration of Pd(0) (Figure 10).
When organotin compounds are used as the organometallic counterpart the protocol is referred to as the Stille reaction after J. K. Stille, who performed extensive synthetic and mechanistic studies on these transformations after initial reports by the groups of Eaborn and Kosugi. The organostannane reagents offer mild reaction conditions and have a high tolerance to different functionalities in the reactants. High stability and easy preparation further characterize these reagents, and a large number of organostannanes are commercially available. However, the stannanes are relatively toxic and should be handled with special care. Tributyltin derivatives, \( \text{R} \&\text{SnBu}_3 \) (R' being the group transferred to palladium in the transmetallation step) are usually preferred above the more volatile trimethyltin analogs. The transfer of alkyl groups from tin to \( \text{Pd(II)} \) is much slower than for aryl or alkenyl groups, making the transfer of a reagent butyl or methyl group less likely. In addition, only one of the four groups on the stannane is transferred as the tin halide formed after the first transfer is less reactive. Copper additives (usually \( \text{Cu(I)} \) salts) have been thoroughly investigated in the Stille reaction. Additionally, \( \text{CuO} \) has been reported to improve reaction rates and yields in the reactions between pyridyltributyltin and different aryl halides.

The Suzuki, or Suzuki-Miyaura cross-coupling, was invented by Akira Suzuki and Norio Miyaura and refers to the \( \text{Pd(0)} \)-catalyzed reaction of organoboron compounds with organic electrophiles. The reactions take place in the presence of a base and an organoboronic acid or ester. The remarkable interest in the Suzuki protocol, both in small-scale laboratories and in industry, can partly be attributed to the organoboron reagents generally being thermally stable and insensitive to air and water. Compared to the corresponding organostannanes they are also significantly less toxic. Negatively charged bases, such as sodium and potassium carbonates, phosphates, hydroxides and alkoxides, have been used in the Suzuki reactions. The role of the base is probably to form a complex with the organoboron reagent.

Figure 10. General catalytic cycle for cross-coupling reactions
thereby increasing the nucleophilicity of the organic group on boron. Alkenyl and aryl boronic acids/esters readily undergo cross-coupling with organic halides and related electrophiles, but alkyl boron reagents usually fail to give the cross-coupling products.

The Sonogashira reaction was named after Kenkichi Sonogashira and is closely related to the Stille and Suzuki cross-coupling reactions, but terminal acetylenes are used to couple with C(sp²) halides.¹¹⁰,¹¹¹ The Sonogashira protocol usually includes Pd(0) and PPh₃ sources, an alkylamine as base, the terminal acetylene, an aryl or vinyl halide and CuI as cocatalyst. Cu(I) is claimed to activate the acetylene by formation of a copperacetylide, which undergoes transmetallation with the arylpalladium halide formed by oxidative addition (Figure 10). The conjugated acetylenic products formed in this type of reaction have been used, for example, in natural product synthesis¹¹²,¹¹³ and pharmaceuticals.¹¹⁴

Carbonylation Reactions

Palladium(0)-catalyzed carbonylation reactions have been extensively used to obtain various carbonyl compounds.¹¹⁵-¹¹⁷ Generally, aryl halides undergo carbonylation in the presence of a carbon monoxide source, a nucleophile and catalytic amounts of Pd(0). In these reactions, carbon monoxide coordinates to an available site on Pd, followed by insertion into the C(sp²)–Pd bond formed by oxidative addition of R–X (Figure 11). The nucleophile attacks the aroylpalladium complex formed producing the corresponding carbonyl product. Different nucleophiles provide carboxylic acids (water), esters (alcohols), ketones (organometallic reagents) and amides (amines).

![Figure 11. General catalytic cycle for carbonylation of R–X substrates](image-url)
Gaseous carbon monoxide has been the most commonly used CO source despite its high toxicity\textsuperscript{118} and the cumbersome handling of the pressurized containers. Strategies circumventing the problems associated with CO gas have been given a great deal of attention.\textsuperscript{119} Applications including, for example, formic acid (and derivatives thereof) or aldehydes as alternative CO sources have been developed. Solid metal carbonyls are other alternatives for the \textit{in situ} generation of carbon monoxide. For example, solid molybdenum hexacarbonyl (Mo(CO)\textsubscript{6}) has been successfully applied as a condensed CO source in carbonylation reactions.\textsuperscript{120-123}

1.2.2 Microwave-Accelerated Organic Synthesis

When Robert Bunsen introduced his burner in 1855 it was the first tool to heat reaction vessels using fire in a controlled manner. Since then, the development of, for example, the isomantle and the oil bath, standard equipment in almost every small-scale and industrial laboratory today, has further simplified heating procedures. The use of microwave heating in synthesis was reported for the first time in 1986.\textsuperscript{124,125} At that time, difficulties due to poor understanding of the basics of microwave-accelerated heating as well as lack of control and reproducibility of the reactions limited the widespread use of this technology. Today the situation has changed drastically and microwave-induced heating performed in dedicated instruments has proven in many cases to reduce process time, increase yield and improve the reproducibility of chemical reactions.\textsuperscript{126-132}

Heating of materials as a result of microwave irradiation can occur by two major mechanisms: dipolar polarization and ionic conduction. In dipolar polarization, dipoles in the sample (for example, solvent or reagent molecules) try to align with the electric field component of the electromagnetic irradiation applied. As the field oscillates, the dipoles continuously attempt to realign and heat is generated as a result of molecular friction. The amount of heat generated by the dipolar polarization mechanism is related to the ability of the dipoles to align themselves with the frequency of the applied field. At high frequencies the components do not have time to realign and at low frequencies they reorient too quickly and as a consequence, no heating will occur. All domestic microwave ovens and reactors for chemical synthesis operate at a frequency of 2.45 GHz, which lies in between these two extremes. Ionic conduction occurs when dissolved charged particles in the sample oscillate in the applied electromagnetic field and collide with surrounding molecules or ions.

The ability of a material to transform microwave electromagnetic energy into heat is dependent on the dielectric properties of the material, which are given by the loss tangent, $\tan \delta$. The loss tangent is expressed as $\tan \delta = \varepsilon''/\varepsilon'$ where $\varepsilon''$ is the dielectric loss describing the efficiency with which electromagnetic irradiation is converted into heat and $\varepsilon'$ is the dielectric constant,
indicative of the polarizability of the molecules in the electric field. Generally, a high value of tan δ is required for a material to efficiently convert microwave irradiation into thermal energy. Data for some common solvents are listed in Table 1. Solvent molecules without a permanent dipole moment, for example benzene and CCl₄, are almost microwave transparent. However, these solvents can be used in microwave-accelerated synthesis since the added reagents and/or catalysts are often dipoles/ions and the dielectric/ionic properties of the whole sample may provide sufficient heat generation.

Table 1. Loss Tangent (tan δ) Values for Common Solvents at Room Temperature

<table>
<thead>
<tr>
<th>Solvent</th>
<th>tan δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene glycol</td>
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</tr>
<tr>
<td>Ethanol</td>
<td>0.941</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.825</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.659</td>
</tr>
<tr>
<td>1,2-dichlorobenzene</td>
<td>0.280</td>
</tr>
<tr>
<td>DMF</td>
<td>0.161</td>
</tr>
<tr>
<td>Water</td>
<td>0.123</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.062</td>
</tr>
<tr>
<td>THF</td>
<td>0.047</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.042</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.040</td>
</tr>
</tbody>
</table>

*2.45 GHz, 20 °C.*

Microwave-accelerated heating of reaction mixtures is often more energy efficient than classic heating procedures. The reactors used for microwave-induced heating in chemical synthesis are available as either multimode or monomode (single-mode) instruments. In the multimode version, the walls of the relatively large reactor space reflect the irradiation, spreading it throughout the entire microwave cavity. In the single-mode instruments the irradiation is focused as a standing wave directly on the reaction mixture. As the energy is directed towards the sample itself, no prior heating of the container walls is needed. Uneven heat distribution within the sample as well as so-called wall effects, common with conventional heating equipment, are also avoided. Both open and sealed vessel techniques are available; with sealed vessels superheating of solvents, often to temperatures well above the actual boiling point, is advantageous. This is safely performed as all commercially available instruments feature on-line temperature and pressure control by regulation of the microwave power output.

Water is not always appropriate as a solvent in organic synthesis due to its relatively high polarity compared to standard organic solvents, although a number of examples have been reported. Interestingly, at the elevated
temperatures enabled by sealed vessels and microwave-induced heating, the
dielectric constant, $\varepsilon'$, of water decreases, facilitating the use of water as a
pseudo-organic solvent (Figure 12).\textsuperscript{126,141}

A plethora of different organic transformations have been performed using
microwave irradiation.\textsuperscript{130} The advantages of microwave-accelerated
heating were realized early in the field of homogeneous palladium catalysis.
Results indicating high reaction rates and yields, in addition to minimal oc-
currence of competing side reactions in, for example, carbon-carbon bond-
forming reactions, carbon-heteroatom coupling and asymmetric applications,
have been reported.\textsuperscript{128,142}

![Figure 12. Dielectric constants of various solvents as a function of temperature (data from reference 126)]
2 Aims of the Present Study

The rapidly growing HIV/AIDS pandemic, the severe adverse effects associated with treatment, the increasing numbers of drug resistant HIV strains and the poor pharmacokinetic properties of most approved HIV-1 protease inhibitors have created a need for new, unique chemical entities in antiretroviral therapy.

The work described in this thesis is part of a research project aimed at the identification of novel and bioavailable HIV-1 protease inhibitors.

The specific objectives of this study were:

- To establish reliable synthetic procedures offering potential HIV-1 protease inhibitors comprising a novel, tertiary-alcohol-containing transition-state mimic.

- To develop microwave-accelerated, palladium-catalyzed reactions for elongation of the inhibitor’s P1’ side chain. The aim of this was to improve the anti-HIV activity of the inhibitors in cell assays.

- To derive a general aminocarbonylation protocol for aryl iodides, aryl bromides and aryl chlorides using water as an environmentally friendly solvent and to apply this method to the new class of inhibitors.
3 Evaluation of a New Transition-State Mimic and Prime-Side Variations (Paper I)

Tertiary alcohols as parts of transition-state analogs in protease inhibitors have been reported in a few cases. As a rule, less potent inhibitors were obtained than with the corresponding secondary alcohol derivatives. In studies of aspartyl protease inhibitors (not including HIV-1 PIs), pepstatine was compared with the corresponding \((3R)-\text{Me}^3\text{Sta}\) derivative with a tertiary alcohol in the transition-state analog. Interestingly, the \((3R)-\text{Me}^3\text{Sta}\) compound, having the unnatural \(3R\) absolute configuration was a potent inhibitor, implying that this peptide must inhibit the enzyme by a different mechanism. I was interested in further investigating tertiary-alcohol-containing transition-state mimics in HIV-1 protease inhibitors. In an appropriate structural environment, ‘masking’ of this hydroxy unit by, for example, internal hydrogen bonding, could improve transcellular transport, which is a step towards enhanced oral bioavailability. The concept of masking polar groups to enhance membrane permeation has been successfully applied in several cases, recently in the development of NK\(_1\) receptor antagonists. Preliminary modeling indicated that a tertiary alcohol in the \(\alpha\)-position to a carbonyl, linked with the ethylhydrazine group successfully used in atazanavir, could provide potent inhibitors of the HIV-1 PR (Figure 13). Decoration of this core structure with the \((1S,2R)-1\)-amino-2-indanol \(\text{P2}\) group, also present in indinavir, benzyl \(\text{P1}\) and \(\text{P1}'\) residues and amino-acid-derived \(\text{P2}'/\text{P3}'\) side chains became the starting point for the work presented in this thesis.

![Figure 13. General structure of the new class of HIV-1 protease inhibitors](image-url)
3.1 Chemistry

A number of procedures are available for the synthesis of quaternary carbon centers from various starting materials. In this work, ring-opening reactions of 2,2-disubstituted epoxides were employed to yield chiral quaternary carbon atoms bearing the sought-after tertiary alcohol. A straightforward approach to these intermediate oxiranes was the epoxidation of substituted α,β-unsaturated carbonyl compounds (Figure 14). Epoxide 3, a synthetic precursor of the inhibitor P1 benzyl and P2 (1S,2R)-1-amino-2-indanol group, was synthesized in three steps from commercially available benzylmalonic acid. Treatment of this acid with Et₂NH and formaldehyde rendered 2-benzylacrylic acid (1) via a Mannich reaction followed by in situ decarboxylation, as described in the literature. Amide coupling of 1 and (1S,2R)-1-amino-2-indanol was performed using EDC, NMM and HOBT. Epoxidation of α,β-unsaturated amide 2 by mCPBA produced a 50:50 mixture of diastereomers (S)-3 and (R)-3 (S and R refer to the absolute configuration of the chiral center in the epoxide). A large difference in Rₜ values of (S)-3 and (R)-3, 0.58 and 0.13 respectively, (silica, EtOAc/pentane 50:50) allowed excellent separation of the two diastereomers using flash chromatography. An attempt to further improve the epoxidation reaction was made by changing the solvent to CH₂Cl₂ and heating to 60 °C overnight with a catalytic amount of AIBN. This led to a 32% isolated yield of (S)-3 but also additional by-products and more complicated purification.

Figure 14. Synthesis of epoxide 3

The central hydrazide unit in the inhibitors, connecting the P1' and P2' groups, was synthesized by coupling alkyl hydrazines to N-derivatized L-valine or L-tert-leucine (Figure 15). Benzylhydrazine and 4-bromo-benzylhydrazine were used to provide two different P1' side chains. The latter was prepared according to a slightly modified literature procedure from 4-bromo-benzyl bromide and hydrazine monohydrate. The N-derivatized L-valine and L-tert-leucine compounds were synthesized under basic conditions from the free amino acids and methyl chloroformate, methanesulfonyl chloride or benzyl isocyanate. The crude products were coupled to the alkyl hydrazines giving hydrazides 6–10 (Figure 15). Com-
commercially available Cbz-protected L-valine and L-tert-leucine were reacted with the hydrazines yielding the corresponding benzoyloxycarbonyl compounds 4, 5 and 11 (Figure 15). All coupling reactions were performed using EDC, HOBT and NMM in EtOAc producing 4–11 at isolated yields of 50–79%. Urea derivative 9 was unstable and after filtration through a short plug of silica this compound was used in the inhibitor synthesis without further purification.

**Figure 15. Synthesis of hydrazides (yields are calculated from the N-derivatized amino acid intermediates)**

Hydrazides 4–11 were used to regioselectively ring open epoxide (S)-3 (8 examples) and (R)-3 (2 examples) yielding ten potential HIV-1 protease inhibitors (Table 2). Initially, a protocol in which the reactants were heated to 80 °C in i-PrOH was used to synthesize target compounds 12–19 and 21 (Table 2). Later, this sluggish process was replaced by a Ti(Oi-Pr)₄-catalyzed system in THF which resulted in complete conversion of epoxide 3 in only 3 h (20, Table 2). For most compounds, preparative reverse-phase LC-MS, alone or in combination with flash chromatography, was needed to obtain pure products.
3.2 Biological Evaluation

Compounds 12–21 were evaluated in an HIV-1 protease assay and for *in vitro* anti-HIV activity in MT4 cells. The results are summarized as $K_i$ and EC$_{50}$ values in Table 2. Data obtained from the diastereomers 12/13 and 16/17 revealed that the tertiary alcohol with (S)-configuration gave the more potent enzyme inhibitors with at least 40 times higher $K_i$ values (Table 2). The $N$-methoxycarbonyl derivatives afforded more potent inhibitors ($K_i = 2.4–9.0$ nM, Table 2) than the $N$-benzoxycarbonyl and benzylurea analogs ($K_i = 17–23$ nM, Table 2). This could be attributed to negative interference between the relatively large P3' benzyl groups and the P1' side chain via the continuous S1'–S3' spanning enzyme pocket. Compared to the $N$-methoxycarbonyl compound, the methyl sulfonamide derivative 18, which was similar in size, was ten times less potent. The para-bromo compound 20 was 2.5 times more potent in the enzyme assay than analog 16, lacking a P1' substituent.

The antiviral activity in HIV-1 infected MT-4 cells exceeded 10 $\mu$M for all compounds except inhibitors 15, 16 and 20 which contained $N$-methoxycarbonyl P3' groups (Table 2). A minor difference was observed between the inhibitors with L-valine and L-tert-leucine derived P2' groups, as deduced from the EC$_{50}$ values. Compound 16 with a tert-butyl P2' group was twice as active as the iso-propyl analog, 15. The advantage of an L-tert-leucine residue in HIV-1 PIs has previously been reported in the atazanavir series. The overall best compound was the bromo-substituted inhibitor 20, with a $K_i$ value of 2.4 nM and an EC$_{50}$ value of 1.1 $\mu$M.

Inhibitors 16 and 20 were evaluated *in vitro* with two selected ritonavir or a symmetric diol-based inhibitor resistant HIV-1 strains. Both 16 and 20 were found to be as potent to these mutant viruses as to the wild type (Table 2). Furthermore, the membrane permeation properties of 16 and 20 in a Caco-2 cell assay and intrinsic clearance in liver microsomes were assessed. Excellent permeability through the Caco-2 cell layers was observed for both 16 and 20, $P_{app} = 35\cdot10^{-6}$ and $42\cdot10^{-6}$ cm/s, respectively. However, the high membrane penetration rates are somewhat contradictory to the poor cellular antiviral activities obtained for these compounds (at best EC$_{50} = 1.1$ $\mu$M). One explanation could be high protein binding of the inhibitors in the MT4 cell assay. Compounds containing the (1S,2R)-1-amino-2-indanol unit are known to be rapidly degraded by metabolic enzymes. Unfortunately, this was also the case for compounds 16 and 20 ($Cl_{int} = 527$ and 266 $\mu$L/min/mg, respectively).
Table 2. Synthesis of Inhibitors 12-21 and Biological Data

(S)- or (R)-3 \[\text{4-11} \rightarrow \text{12-21}\]

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<tr>
<th>Cmpd</th>
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<th>Yield (%)</th>
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<th>EC$_{50}$ (µM)</th>
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<tr>
<td>21</td>
<td>13</td>
<td>2</td>
<td>17</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>

* Reaction conditions: i-PrOH, 80 °C, 3–7 days (compounds 12–19 and 21) or Ti(Oi-Pr)4, THF, room temp to 40 °C, 3 h (compound 20). * Purification methods: 1 = column chromatography, silica; 2 = RPLC-MS. * Indinavir: $K_i = 0.52 \text{nM}$; atazanavir: $K_i = 2.66 \text{nM}$. * Indinavir: EC$_{50} = 0.041 \mu$M; atazanavir: EC$_{50} = 0.0039 \mu$M. * $P_{app}$ (Caco-2) = 35·10$^{-6}$ cm/s and Cl$_{int} = 527$ μL/min/mg. * Mutations in MT4/HIV-1 protease: V32I, M46I, V82A. * Mutations in MT4/HIV-1 protease: V32I, M46I, A71V, V82A. * $P_{app}$ (Caco-2) = 42·10$^{-6}$ cm/s and Cl$_{int} = 266$ μL/min/mg.

3.3 X-ray Crystallography

X-ray diffraction data collected from compound 15 cocrystallized with HIV-1 protease provided a 3D structure at 1.8 Å resolution (PDB code 2bqv, Figure 16). This structure confirmed the (S)-configuration at the quaternary carbon atom in the transition-state mimic. The tertiary alcohol in 15 was hydrogen bonded to one of the catalytically active aspartic acid residues in the active site of the enzyme (Asp125, Figure 16). In addition, the hydrazide β-nitrogen was hydrogen bonded to the other aspartic acid, Asp25. A structural water molecule bridged the non-prime-side amide carbonyl and the prime-side hydrazide carbonyl via hydrogen bonds.

Figure 16. X-ray structure of inhibitor 15 cocrystallized with HIV-1 protease. Hydrogen bonds are indicated by green dotted lines.
By comparing the data obtained for the 15-HIV-1 protease complex and an X-ray structure of indinavir (PDB code 1hsg) it became clear that whereas the P2 and P2' groups in the two inhibitors overlapped well, the positions of the P1 and P1' side chains differed substantially (Figure 17). In the complex with 15, the P1 and P1' groups penetrated less deeply (1.4 and 4.5 Å, respectively) into the corresponding S1 and S1' pockets of the enzyme than in indinavir. The angle at which the P1' side chain was directed into the S1' subsite also differed substantially. Regarding the indinavir X-ray structure, the secondary alcohol in the transition-state mimic was in close contact with all four oxygen atoms of the catalytic aspartic acids. In the complex with 15, the tertiary alcohol and the hydrazide β-nitrogen were symmetrically positioned over Asp25 and Asp125. This led to a weaker hydrogen bond between the hydroxyl group and Asp125 and the additional hydrogen bond between the hydrazide β-nitrogen and Asp25, as mentioned above. The tertiary alcohol was also found to be hydrogen bonded to Gly27, a feature not observed in the secondary alcohol in indinavir.

Figure 17. A: Superimposition of the crystal structures of inhibitor 15 (gray) and indinavir (orange) in the active site of HIV-1 PR. B: Comparison of central hydrogen-bonding interactions (green dotted lines) between the enzyme and 15 (left) and indinavir (right).
4 Microwave-Accelerated Palladium(0)-Catalyzed P1’ Extensions

Although potent enzyme inhibitors were obtained, all compounds within the first series exhibited poor antiviral activity in cell culture (Section 3.2). As deduced from the crystal structure of the 15-HIV-1 PR complex, the P1’ side chain in 15 did not penetrate as deeply into the enzyme S1’ pocket as indinavir, indicating that the pocket was not completely filled (Figure 17). This position was therefore suitable for optimization of the scaffold aimed at both increased enzyme inhibition and improved cellular anti-HIV activity.

4.1 Stille, Suzuki and Sonogashira Cross-Coupling
(Paper II)

4.1.1 Chemistry

The most potent inhibitor in the first series, para-bromo compound 20 (Table 2), served as a convenient starting point for microwave-accelerated, Pd(0)-catalyzed cross-coupling reactions to obtain P1’-elongated inhibitors (Table 3). The meta-bromo analog 23 was synthesized according the same protocol as 20, from 3-bromo-benzylhydrazine, N-(methoxycarbonyl)-L-tert-leucine\textsuperscript{74} and epoxide (S)-3 to yield an arylpalladium precursor for the production of the corresponding meta series (Figure 18).

A large number of boronic acids, tin reagents and acetylenes are commercially available for use in the Stille, Suzuki and Sonogashira cross-coupling.

\textbf{Figure 18.} Synthesis of meta-bromo arylpalladium precursor 23
reactions. Primarily, the phenyl group was interesting to compare with heteroaromatic substituents as P1’ extensions (Table 3). These polar aromatic groups are found in many drugs and are often used as a more hydrophilic alternative to a phenyl substituent.\textsuperscript{74,76,77} The tolerance of the enzyme to even longer P1’ groups was investigated by synthesis of the two-carbon-extended, phenyl- or pyridyl-substituted alkenyl and acetylene derivatives. In all cross-coupling reactions 5–10 mol % Pd(PPh\textsubscript{3})\textsubscript{2}Cl\textsubscript{2} was used as precatalyst, and microwave irradiation accelerated the transformations reducing the reaction times to a maximum of 1 h.

Stille reactions were performed using 4 equiv of the tributyltin reagent and 1 equiv of CuO in DMF at 130 °C for 20 min in the microwave cavity. The resulting products, 25, 26, 33, 37 and 38 were isolated at low yields (17–27%, Table 3). The Suzuki-type of reactions with aryl bromide precursors 20 or 23 and different boronic acids generally afforded higher isolated yields of the P1’-extended inhibitors than with the Stille protocol (Table 3). Mild reaction conditions with Na\textsubscript{2}CO\textsubscript{3} as base and 5 equiv of boronic acid, heated to 120 °C for 30 min in a DME/EtOH mixture\textsuperscript{169} gave compounds 24, 27, 28, 32, 34, 35, 36, 39 and 40 in low to good yields (26–62%, Table 3), with the exception of the sluggish pyridine-4-boronic acid yielding para derivative 27 at only 17%. Acetylenes 29–31 and 41–43 were synthesized according to the Sonogashira protocol from phenylacetylene or ethynylpyridines in the presence of catalytic amounts of CuI (Table 3). The use of diethylamine as base and 30–40 min of microwave heating at 140 °C resulted in partial decomposition of the starting materials, and inhibitors 41–43 were isolated at yields of only 19–27% (Table 3). Changing the conditions to triethylamine at 130 °C for 60 min gave slightly higher yields of acetylenes 29–31 (22–45%, Table 3). The pure target compounds were obtained by rigorous purification of the reaction mixtures by reverse-phase LC-MS. Some of the structures partly formed salts with formic acid in the LC gradient, as evidenced by NMR and elemental analysis. In these cases, additional HRMS analysis was performed to further establish sample identity.

4.1.2 Biological Evaluation

The \( K_i \) and EC\textsubscript{50} values for compounds 20, 23–43 are given in Table 3. All the P1’-elongated derivatives evaluated were found to be good PIs with \( K_i \) values ranging from 2.1 to 23 nM, except meta compound 39, which exhibited a \( K_i \) value of only 93 nM (Table 3). Generally, the inhibitory potencies obtained by the meta series were slightly lower than those of the para compounds. One explanation could be that the angle of approaching the S1’ pocket of the meta-substituted P1’ side chains was unfavorable.

High substituent dependence was observed in the cellular antiviral activities (Table 3). As opposed to the first generation of inhibitors, almost all compounds in the extended para series were active in the cell assay. How-
ever, structures 28, 29 and 35, with phenyl groups attached at a two-carbon distance from the parent P1’ benzyl group exhibited EC<sub>50</sub> values above 10 μM. The high lipophilicity of these compounds, resulting from the large hydrophobic P1’ side chains, probably accounts for this effect. The para-phenyl derivative 24, 2-pyridyl 25, acetylenes 30 and 31 and heterocycles 32–34 exhibited cell-based antiviral potencies comparable to that of the bromo precursor 20 (EC<sub>50</sub> = 0.70–1.1 μM, Table 3). On the other hand, the 3-pyridyl and 4-pyridyl para analogs, 26 and 27, were substantially more active, with EC<sub>50</sub> values of 0.18 and 0.20 μM, respectively (Table 3). Only two of the meta compounds were active in cell culture at low potencies (23 and 39, EC<sub>50</sub> = 3.3 and 2.0 μM, Table 3). The anti-HIV activity of 39 was somewhat surprising as this compound was also the least potent enzyme inhibitor within this series. A rough idea of the inhibitor cytotoxicity is given by the CC<sub>50</sub> value, which is the inhibitor concentration causing a 50% decrease in cell proliferation. Only four compounds (30, 33, 37 and 38, Table 3) displayed signs of cytotoxicity below 10 μM. The 3-pyridyl extended inhibitors in each series, 26 and 38, were evaluated regarding membrane permeability in a Caco-2 assay and stability in liver microsomes (Table 3). Para compound 26 afforded an excellent P<sub>app</sub> value of 3.3·10<sup>-6</sup> cm/s, whereas the meta analog 38 was more slowly transported across the Caco-2 membrane (P<sub>app</sub> = 11·10<sup>-6</sup> cm/s, Table 3). Compared to the first generation of inhibitors, somewhat lower intrinsic clearance values were obtained from 26 and 38; Cl<sub>int</sub> = 154 and 190 μL/min/mg, respectively.
Table 3. Synthesis of PI' Elongated Inhibitors 24–43 and Biological Data

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<th>Reaction type</th>
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<th>EC$_{50}$ (μM)</th>
<th>CC$_{50}$ (μM)</th>
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Microwave conditions: Suzuki reactions: R’R’(OH)2, Pd(PPh3)2Cl2, Na2CO3 (aq.), EtOH, DME, 120 °C, 30 min; Stille reactions: R”Sn(n-Bu)3, Pd(PPh3)2Cl2, CuO, DMF, 130 °C, 20 min; Sonogashira reactions: 29–31 R”H, Pd(PPh3)2Cl2, Et3N, CuI, DMF, 130 °C, 60 min; 41–43 R”H, Pd(PPh3)2Cl2, Et2NH, CuI, DMF, 140 °C, 30–40 min.

4.1.3 X-ray Crystallography

X-ray crystallographic data were obtained from para compounds 20, 25 and 26 cocrystallized with the HIV-1 PR (PDB codes 2cej, 2cem and 2cen, respectively, Figure 19). The tertiary alcohols in 20, 25 and 26 were found to be hydrogen bonded to one of the catalytically active aspartic acid residues, which was also the case in the 15-enzyme complex (Figure 16). Hydrogen bonds linked the hydrazide nitrogen atoms and Gly27. The P1’ side chains of the inhibitors were embedded in the S1’–S3’ spanning pocket formed by amino acid residues 179–183. The positioning of the P1’ groups was largely

Figure 19. X-ray crystal structures of inhibitors 20, 25 and 26 in complex with HIV-1 protease. Hydrogen bonds are indicated by green dotted lines.
governed by Arg108. In the complex with 20, the electronegative bromine was close-packed against the positively charged Arg side chain. In contrast, the pyridyl groups in 25 and 26 were hydrogen bound to Arg108 via a bridging water molecule forcing the P1’ arms to be ‘lifted up’ and away from Arg108. This, in turn, caused the position of Pro181 to vary up to 1.5 Å between the three complexes.

4.2 Aminocarbonylation in Water (Paper III)

4.2.1 Method Development

The demand for environmentally friendly alternatives to volatile and toxic organic solvents has increased during the recent years. Water is probably one of the most ‘green’ solvents available, although the low solubility of most organic compounds and its reactivity towards, for example, organometallics, have limited its widespread use in organic chemistry. In a study of microwave-accelerated aminocarbonylation of aryl bromides using Mo(CO)₆ in water, the reactions were found to be quite general. The expected hydroxycarbonylation was efficiently suppressed and the benzamide products were isolated at good to excellent yields. In this work, an extended study, including the aminocarbonylation of aryl iodides and aryl chlorides, is presented. Mild reaction conditions and methods in which both the aryl halide and amine could be used as yield-determining reactants were important, to enable the use of the new protocol in medicinal chemistry applications with highly functionalized molecules as starting materials. All reactions were conducted in sealed reaction vessels under air in the presence of a Pd source, Mo(CO)₆, Na₂CO₃ and neat water, heated by thermo-controlled microwave irradiation (Figure 20).

Figure 20. General procedure for the microwave-accelerated aminocarbonylation of aryl iodides, bromides and chlorides in water
Aryl Iodides as Coupling Partners

Aryl iodides are usually good substrates for oxidative addition to Pd(0) species. Accordingly, a phosphine-free protocol with Pd(OAc)$_2$ as pre-catalyst was developed for the aminocarbonylation of different aryl iodides (Table 4). In a general protocol, Pd(OAc)$_2$ (5.0 mol %), Na$_2$CO$_3$ (3.0 equiv) and Mo(CO)$_6$ (0.50 equiv) in 2 mL of water were allowed to react in the presence of various amines and aryl iodides at 110 °C during only 10 min of microwave-induced heating. Different ratios of 1-iodonaphthalene and $n$-butylamine were evaluated and a 1:5 ratio was found to be the best combination, although only a slightly lower yield was recorded with the reversed 5:1 stoichiometry (entries 1–6, Table 4). High isolated yields (above 80%) were obtained with both electron-rich and electron-deficient aryl iodides in reactions with $n$-butylamine, except with the sterically hindered 2-tolyl iodide (entries 7–11, Table 4). Most of the amines evaluated were found to compete favorably with water as nucleophiles producing the corresponding amides at good to excellent yields (entries 12–19, Table 4). Morpholine and the bulky tert-butylamine were the only exceptions, with 15–43% isolated yields of the benzamide products (entries 14, 15 and 19, Table 4).

Aryl Bromides as Coupling Partners

Increased reaction temperatures (170–180 °C) were required in the aminocarbonylation of aryl bromides in order to keep the reaction times short (10–15 min) (Table 5). The thermo-stable palladacycle developed by Wolfgang Herrman$^{176-178}$ was therefore a convenient choice of Pd(0) source. At these increased temperatures, the hydroxycarbonylation became more pronounced and the reaction with 1-bromonaphthalene in the absence of amine yielded 67% 1-naphthyl carboxylic acid (entry 1, Table 5). However, in the presence of an amine, good isolated yields of the benzamide product were obtained (entries 2 and 3, Table 5). The influence of stoichiometry was evaluated using different concentrations of $n$-butylamine and 1-bromonaphthalene with microwave irradiation at 170 °C for 10 min (entries 2–6, Table 5). A five-fold excess of aryl bromide gave the highest isolated yield of the amide product.

One aim of this project was to conduct the aminocarbonylation reaction with a bromo-substituted HIV-1 protease inhibitor, and therefore a more extensive study using both the amine and the aryl bromide as the yield-limiting reactant was undertaken. In the reactions with $n$-butylamine and various bromides slightly higher isolated yields were encountered with the aryl bromide in excess. However, productive yields, 55–80%, were obtained with the bromide as the yield-determining reactant (entries 7, 11, 13 and 15, Table 5).
### Table 4. Aminocarbonylation of Aryl Iodides

<table>
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<tr>
<th>Entry</th>
<th>Iodide (44)</th>
<th>Ar group</th>
<th>Amine (47)</th>
<th>Equiv 44/47</th>
<th>Product (48)</th>
<th>Yield (%)$^b$</th>
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$^a$ Conditions: 0.50 equiv of Mo(CO)$_6$, 3.0 equiv of Na$_2$CO$_3$, 5.0 mol % Pd(OAc)$_2$, 2 mL of H$_2$O, microwave irradiation at 110 °C for 10 min, >95% conversion of yield-limiting reactant according to GC-MS. $^b$ >95% purity by GC-MS, yield based on the limiting reactant. $^c$ Incomplete conversion of 1-iodonaphthalene, isolated yield of 1-naphthyl carboxylic acid 6%.
### Table 5. Aminocarbonylation of Aryl Bromides$^a$

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$^a$Conditions: 0.50 equiv of Mo(CO)$_6$, 3.0 equiv of Na$_2$CO$_3$, 5.0 mol % palladacycle, 2 mL of H$_2$O, >95% conversion of yield-limiting reactant according to GC-MS. $^b$>95% purity by GC-MS. $^c$Isolated yield of 1-naphthyl carboxylic acid 67%. $^d$With 0.50 equiv of imidazole. $^e$With 10 mol % ([t-Bu$_3$PH]BF$_4$).
Attempts to lower the reaction temperature to 140 °C gave promising results when 0.50 equiv of imidazole was present as a nucleophilic catalyst (entry 8, Table 5). An even higher isolated yield was obtained with 10 mol % of the \([\text{(t-Bu)}_3\text{PH}]\text{BF}_4\) salt, which was developed by Fu\(^{180}\) after initial publications by Koie\(^{181,182}\) and which acts as a convenient precursor of air-sensitive \((\text{t-Bu})_3\text{P}\) (entry 9, Table 5).

In analogy with the iodides, both electron-rich and electron-poor aryl bromides were reactive in aminocarbonylation with \(n\)-butylamine as nucleophile giving 54–86% isolated yields of the product amides (entries 7–16, Table 5). Furthermore, in the reactions between 4-tolyl bromide and various amines the bulkiness of the amines seemed to have the greatest influence on the outcome of the reactions (entries 17–25, Table 5). Primary, unhindered amines performed satisfactorily, while more bulky, primary or secondary derivatives reacted sluggishly. Even the reluctant aniline was coupled to 4-tolyl bromide at an isolated yield of 70% (entry 22, Table 5).

**Aryl Chlorides as Coupling Partners**

Aryl chlorides are attractive substrates for carbon-carbon cross-coupling reactions from the point of view of cost and availability.\(^{183,184}\) However, aryl chlorides have shown poor reactivity in aminocarbonylation reactions.\(^{116,117,183}\) A limited number of successful examples of carbonylation with amines as nucleophiles using either electron-poor or metal-carbonyl activated aryl chlorides have been reported.\(^{185-192}\) The first general aminocarbonylation protocol including both electron-poor and electron-rich aryl chlorides in THF was recently published.\(^{193}\)

In the present work, the aminocarbonylation reactions between aryl chlorides and various amines in water, aided by the \([\text{(t-Bu)}_3\text{PH}]\text{BF}_4\) salt in combination with microwave-accelerated heating, were investigated (Table 6). The reactions between 1-chloronaphthalene and \(n\)-butylamine at 170 °C for 10 min resulted in the benzamide at 79–82% isolated yield, regardless of whether the amine or aryl chloride was used in excess (entries 3–5, Table 6). In the absence of \([\text{(t-Bu)}_3\text{PH}]\text{BF}_4\), only trace amounts of the product were detected (entry 2, Table 6). Prolonging the reaction time from 10 to 30 min at 170 °C for sluggish, electron-rich aryl chlorides such as the 4-tolyl and 2-tolyl chlorides, used in a 1:5 ratio with \(n\)-butylamine increased the yield (entries 6–9, Table 6). High product yields were also obtained with other aryl chlorides except the 3-thionyl derivative, which gave only a 64% isolated yield of benzamide (entries 10–12, Table 6). 4-Tolyl chloride was further investigated with different primary, secondary and aromatic amines (entries 13–22, Table 6). Generally, yields were improved when the aryl chloride:amine ratio was changed from 1:5 to 5:1 or 2:1 and the corresponding benzamides were isolated at yields up to 99%.  

46
Table 6. Aminocarbonylation of Aryl Chlorides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Chloride (46) Ar group</th>
<th>Amine (47)</th>
<th>Equiv 46:47</th>
<th>Product (48)</th>
<th>Temp/time (°C/min)</th>
<th>Yield (%)</th>
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<td>1/5</td>
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a Conditions: 0.50 equiv of Mo(CO)6, 3.0 equiv of Na2CO3, 5.0 mol % palladacycle, 10 mol % [(t-Bu)3PH]BF4, 2 mL of H2O. b Incomplete conversion of 1-chloronaphthalene, isolated yield of 1-naphthyl carboxylic acid 56%. c Without [(t-Bu)3PH]BF4. d >95% conversion of yield limiting-reactant according to GC-MS.
4.2.2 Synthesis of an Amide-Elongated HIV-1 Protease Inhibitor

Arylpalladium precursor 50 was synthesized according to the procedure described for aryl bromides 20 and 23 (Sections 3.1 and 4.1.1) from 4-bromo-benzylhydrazine, (2S)-2-methanesulfonylamino-3,3-dimethyl-butryric acid and epoxide (S)-3 (Figure 21). Attempts were made to use 20 in the aminocarbonylation reaction, but extensive degradation of the P3’ carbamate group was encountered and therefore the more stable methyl sulfonamide derivative 50 was employed. Aminocarbonylation was performed using the conditions described above for aryl bromides at 130 °C or 140 °C in the presence of either 2.5 equiv of imidazole or 10 mol % [(t-Bu)3PH]BF4 with 10 equiv of n-butyamine and 5 mol % of Herrman’s palladacycle. The reaction with imidazole yielded a complex product mixture, which was difficult to purify by reverse-phase LC-MS. With [(t-Bu)3PH]BF4 the product pattern was less complicated and benzamide 51 was isolated at a yield of 34% in addition to 15% of the corresponding benzoic acid. In the HIV-1 protease assay, 51 exhibited a $K_i$ value of only 588 nM, and was considerably less potent than the corresponding P1’-elongated PIs described in Section 4.1. This could be due to the high flexibility of the n-butyl substituent, allowing unfavorable interactions to occur with the enzyme amino acid residues.

**Figure 21.** Synthesis of para P1’-extended N-butyl benzamide inhibitor 51
5 Variations of the P2 Group (Paper IV)

Different substituents on the prime side of the tertiary-alcohol-based inhibitors produced compounds that were highly potent in the enzyme assays. However, the results obtained from the inhibitors evaluated in a liver microsome homogenate, indicated rapid metabolism of these compounds, with intrinsic clearance values between 154 and 527 µL/min/mg (Tables 2 and 3). The \( (1S,2R) \)-1-amino-2-indanol P2 group is known from related compounds to be metabolized by benzylic oxidation.\(^{78,166}\) I was therefore interested in developing a modified synthetic route which, in a straightforward manner, could facilitate the incorporation of alternative P2 side chains. Cyclic P2 substituents are found, for example, in the approved inhibitors saquinavir, nelfinavir and amprenavir, (Figure 9). In the atazanavir series, amino acid residues, primarily L-valine and L-\textit{tert}-leucine make up the P2/P3 and P2'/P3' side chains of the inhibitors. Bearing this in mind, nine new groups were chosen as potential P2 substituents for evaluation in the tertiary alcohol containing inhibitors (Figure 23). In the previously used protocol, the chiral \( (1S,2R) \)-1-amino-2-indanol group enabled efficient chromatographic separation of epoxides \( (S) \)-3 and \( (R) \)-3 on silica (Figure 14). For the P2-varied inhibitors both chiral and achiral substituents were of interest and another approach to producing the enantiomerically pure \( (S) \)-epoxide intermediates had to be developed. One solution to this end was offered by key compound \( (S) \)-54 which, after amide coupling with different amides and ring opening by a hydrazide, allowed the formation of a new series of potential PIs including diverse P2 groups (Figure 22).

5.1 Chemistry

A short screening study revealed that inexpensive ethyl-(\(S\))-lactate could serve as a covalently bound resolving group in place of \( (1S,2R) \)-1-amino-2-indanol (Figure 22). Using basically the same synthetic strategy but replacing the indanol by the lactate yielded the corresponding ester-linked epoxide diastereomers. Another advantage of using the lactate was the ester bond to the epoxide part, which could be cleaved under relatively mild conditions.
The synthesis of key intermediate (S)-54, which could conveniently be performed on a multi-gram scale, started with 2-benzylacrylic acid (1). The carboxylic acid was activated by SOCl₂, and ester formation with ethyl-(S)-lactate in the presence of DMAP then gave alkene 52 at 75% yield (Figure 22). Epoxidation with mCPBA resulted in a 50:50 mixture of (S)-53 and (R)-53. Two subsequent flash chromatography purification steps separated the two diastereomers with a small remaining fraction of S-R mixture (S:R:mix ratio 4:5:1, \( R_f = 0.47 \) for (S)-53 and \( R_f = 0.58 \) for (R)-53 using silica and EtOAc/pentane 20:80). Hydrolysis of (S)-53 was performed using 2 equiv of NaOH in THF for 1 h at room temperature and produced the free acid at quantitative yield. Epoxide (S)-54 was used without further purification in the amide coupling reactions. The absolute configuration at the quaternary carbon in (S)-54 was assigned after coupling with (1S,2R)-1-amino-2-indanol. Optical rotations and NMR data for the resulting product ((S)-53, Figure 14) were thereafter compared with the reported values (Paper I).

Reagents and conditions:
- a, b, f, h: P2-NH₂, EDC, HOBT, NMM, EtOAc, rt
- c, d: P2-NH₂, PyBOP, (i-Pr)₂NH, CH₂Cl₂, rt
- e: P2-NH₂, HATU, DIPEA, CH₂Cl₂, rt.
Coupling reactions with (S)-54 and various amines were initially performed using EDC, HOBT and NMM (Figure 23). The reactions were performed at room temperature and opening of the epoxide ring by the added amines was not observed. However, low isolated yields were obtained and aromatic amines could not be coupled in this system. Instead, PyBOP and (i-Pr)_2NH were used to provide aromatic amides 55c and d (Figure 23). HATU and DIEA gave the best result for the formation of product 55e (60%). Minor amounts of d-amino acid residues in intermediates 55e–h, as a result of partial racemization, were detected by NMR. These impurities were removed by reverse-phase LC-MS of the final products.

Figure 24. Synthesis of epoxide 57

Incorporation of L-tert-leucine-methylamide as P2 substituent was performed by amide coupling to 1 followed by mCPBA epoxidation (Figure 24). Separation of (S)-57 and (R)-57 by flash chromatography gave the pure diastereomers at 76% total yield. The absolute configuration of the quaternary ring carbon in (S)-57 and (R)-57 was determined after collection of X-ray data from inhibitor 66, obtained by epoxide opening of (S)-57 (Figure 25).

Figure 25. X-ray crystal structure of inhibitor 66
Ring opening of epoxides 55a-h and 57 was performed with bromohydrazide 10 (Table 7). Attempts to use Ti(Oi-Pr)_4 as a catalyst caused the formation of by-products and the highly increased reaction rates observed in the previous series were not achieved. Instead, heating in i-PrOH at 80 °C was used providing clean products although at a slower rate. Target compounds 58–67 were isolated at yields of 35–73%, except for the reactions with aromatic amides 55c and d, for which the isolated yields were very poor (7–11%, Table 7).

5.2 Biological Evaluation

The $K_i$ and EC$_{50}$ values for compounds 20 and 58–67 are included in Table 7. From the inhibition data it was clear that the enzyme S2 pocket was very sensitive to variation in size of the P2 substituents. The cyclic P2 groups in 58–61 gave compounds with low to moderate inhibitory potencies (Table 7). A methylene spacer between the amide bond and an aromatic ring in the P2 position led to a 5 times more potent inhibitor (cf. 58 with 60, Table 7). For the amino-acid-derived P2 substituents in compounds 62–67 only the iso-propyl group, present in compounds 62 and 63 yielded highly potent inhibitors with $K_i = 7.4$ and 5.1 nM, respectively (Table 7). The iso-butyl, tert-butyl and benzyl P2 side chains gave many times less potent inhibitors. Inhibitors 62 and 63 were also the only compounds that exhibited anti-HIV activity in the cell-based assay (EC$_{50}$ values down to 3.1 μM, Table 7). The non-prime-side amino acid residues in compounds 62–67 exhibit potential to interact with both the enzyme S2 and S3 sub sites, a feature less probable with the P2 groups in compounds 58–61. The high potencies of inhibitors 62 and 63 might be accounted for an appropriate size of the iso-propyl group matching the S2 pocket in addition to favorable S3 interactions.

The fact that L-tert-leucine compound 66 was inactive on a cellular level was somewhat surprising. This structural element as a similar P2/P3 group in the approved inhibitor atazanavir afforded excellent potency in cell culture. As expected, compound 67, exhibiting a tertiary alcohol with (R)-configuration, was inactive in the enzyme assay.
Table 7. Synthesis of Inhibitors 58–67 and Biological Data\textsuperscript{a}

![Chemical Structures]

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<tr>
<th>Cmpd</th>
<th>( \text{P}2 ) group</th>
<th>From epoxide</th>
<th>Yield (%)</th>
<th>( K_i ) (nM)</th>
<th>EC\textsubscript{50} (( \mu )M)</th>
</tr>
</thead>
<tbody>
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<td>-</td>
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<tr>
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<td>&gt;10</td>
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</tr>
<tr>
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<td>69</td>
<td>&gt;5000</td>
<td>&gt;10</td>
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</table>

\textsuperscript{a} Conditions: \( \text{-PrOH}, 80^\circ \text{C}, 4–8 \text{ days.} \) \textsuperscript{b} Based on amount of converted epoxide after isolation of the unreacted starting material.
6 Concluding Remarks

The present study has contributed to the field of HIV-1 protease inhibitor design by providing knowledge about a new type of transition-state mimic, not previously evaluated in potential HIV-1 protease inhibitors. The main conclusions are presented below.

- A reliable protocol, without the use of protecting groups, for the synthesis of potential HIV-1 protease inhibitors containing the new, tertiary-alcohol-based transition-state mimic has been developed. The protocol enables easy variation of the inhibitor P1', P2' and P3' groups. Inhibitors with $K_i$ values down to 2.4 nM were obtained.

\[
\text{The new tertiary-alcohol-containing transition-state mimic}
\]

- The binding mode to the enzyme and the absolute stereochemistry of the tertiary alcohol in the most potent inhibitors were determined by X-ray crystallography.

- Microwave-accelerated, palladium-catalyzed cross-coupling reactions were used to elongate the scaffold in the P1' position yielding meta- and para-extended inhibitors. A 3- or 4-pyridyl substituent in the para position resulted in compounds with notably higher anti-HIV activity in cell culture with a lowest EC$_{50}$ value of 0.18 μM.

\[
\text{EC}_{50} = 0.18 \mu M
\]
Methods were developed to synthesize various benzanides via palladium-catalyzed and microwave-accelerated aminocarbonylation reactions of aryl iodides, aryl bromides and aryl chlorides in water. A large number of benzamide products were synthesized in short reaction times using either the aryl halide or the amine in excess. A medicinal chemistry application of the aminocarbonylation protocol was demonstrated by the synthesis of a P1'-elongated HIV-1 protease inhibitor.

A modified synthetic strategy to the tertiary-alcohol-containing inhibitors with the option to vary the P2 group was derived. An enantiomerically pure epoxide that could be synthesized on a large scale, was identified as a key intermediate.
I would like to express my sincere gratitude to the following people, without whom the work described in this thesis would have been much less enjoyable or, in some cases, not even possible.

Prof. Anders Hallberg, my supervisor, for believing in me in the first place, and for giving me the opportunity to perform this work. Thank you for sharing your vast knowledge in medicinal chemistry and for creating a pleasant atmosphere at the Department; this is truly a wonderful place to work at.

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My parents

Fia and Lina, the BEST of sisters, I miss you constantly.

Kajsa, the weirdest dog I have ever met, but maybe it is true what they say: “Sådan matte, sådan hund…”

Henrik, this is where words are not enough…for your never-ending support and love, in good times and bad times, I am so privileged to have you by my side, I love you.

Jenny

Uppsala, March 2006
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