Antiviral drugs acting against RNA viruses: HIV

Structure and life cycle of HIV

- HIV is an example of a group of viruses known as the retroviruses .
- At present, most clinically useful antiviral drugs act against two targets: the viral enzymes reverse transcriptase and protease .
- there is a need to develop effective drugs against a third target and a good knowledge of the life cycle of HIV is essential in identifying suitable targets .

- HIV is an RNA virus which contains two identical strands of (+) ssRNA within its capsid. Also present are the viral enzymes reverse transcriptase and integrase , as well as other proteins called p6 and p7.
- the capsid is made up of protein units known as p24 ; surrounding the capsid there is a layer of matrix protein (p17), then a membranous envelope which originates from host cells and which contains the viral glycoproteins gp120 and gp41 . Both of these proteins are crucial to the processes of adsorption and penetration.

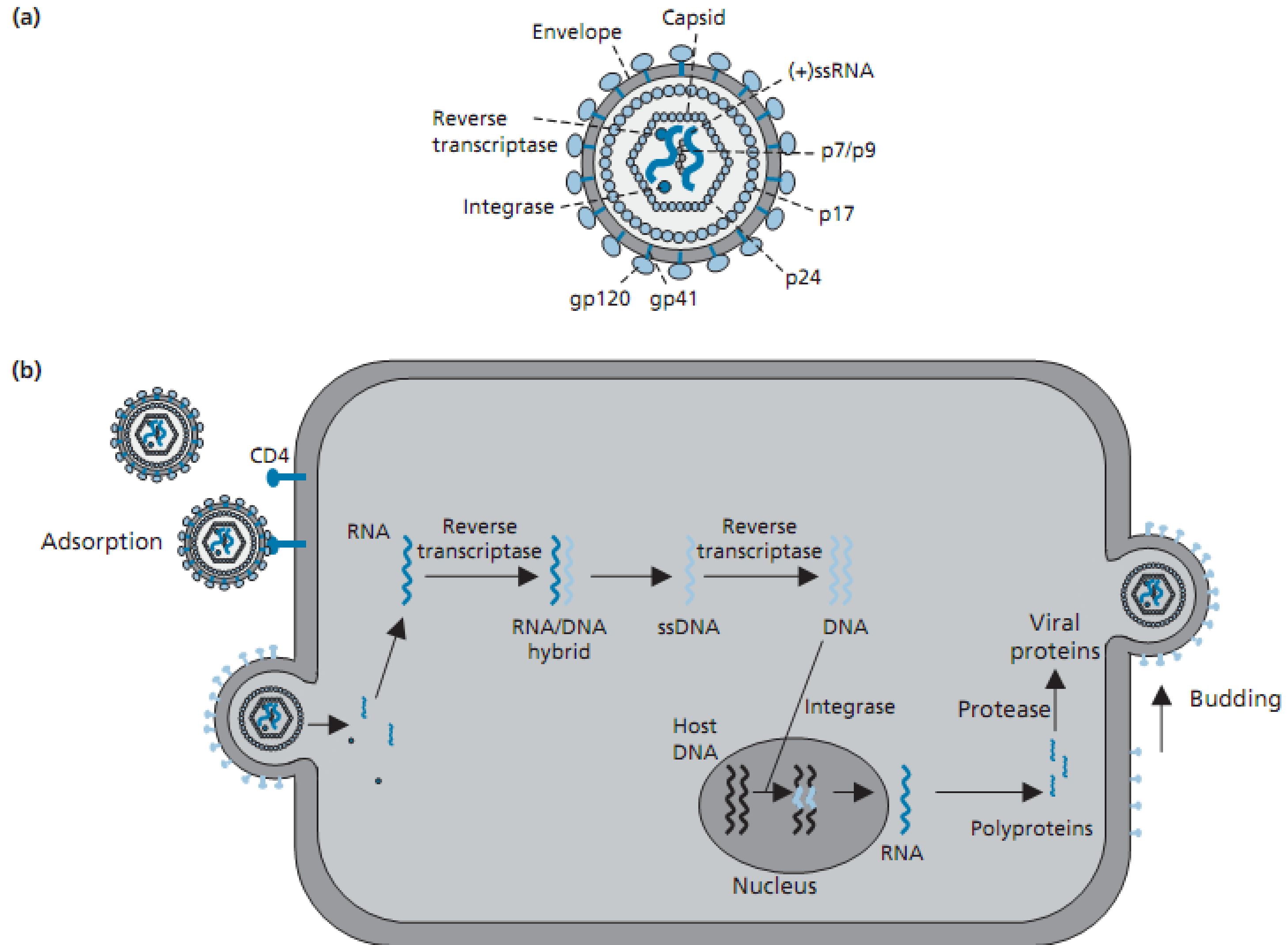


FIGURE 20.11 (**a**)Structure of HIV particle (p = protein; gp = glycoprotein) and (**b**) life cycle of HIV in a host T-cell.

- When the virus approaches the host cell, gp120 interacts and binds with a trans-membrane protein called CD4 , which is present on host T-cells. the gp120 proteins then undergo a conformational change which allows them to bind simultaneously to chemokine receptors (CCR5 and CXCR4) on the host cell (not shown).
- Further conformational changes peel away the gp120 protein allowing the viral protein gp41 to reach the surface of the host cell and anchor the virus to the surface. The gp41 then undergoes a conformational change and pulls the virus and the cell together so that their membranes can fuse.

- Once fusion has taken place, the HIV nucleocapsid enters the cell. Disintegration of the protein capsid then takes place, probably aided by the action of a viral enzyme called protease. Viral RNA and viral enzymes are then released into the cell cytoplasm. The released viral RNA is not capable of coding directly for viral proteins or of self-replication. Instead, it is converted into DNA and incorporated into the host cell DNA. The conversion of RNA into DNA is not a process that occurs in human cells, so there are no host enzymes to catalyse the process.
- HIV carries its own enzyme— **reverse transcriptase** —to do this. This enzyme is a member of a family of enzymes known as the DNA polymerases, but is unusual in that it can use a RNA strand as a template. The enzyme first catalyses the synthesis of a DNA strand

using viral RNA as a template. This leads to a (+)RNA–(−)

DNA hybrid. Reverse transcriptase catalyses the degradation of the RNA strand then uses the remaining DNA strand as a template to catalyse the synthesis of dsDNA (proviral DNA).

- **Proviral DNA** is now spliced into the host cell's DNA—a process catalysed by the viral protein integrase . Once the proviral DNA has been incorporated into host DNA, it is called the provirus and can remain dormant in host cell DNA until activated by cellular processes.
- When that occurs, transcription of the viral genes **env, gag , and pol** takes place to produce viral RNA, some of which will be incorporated into new virions, and the rest of which is used in translation to produce three large, non-functional polyproteins.

- **Budding then takes place to produce an immature**, membrane-bound virus particle. During the budding process a viral enzyme called protease is released from the gag–pol polypeptide.

- Once released, **the protease enzyme dimerizes** and cleaves the remaining polypeptide chains to **release reverse transcriptase, integrase, and viral structural proteins**.
- **The capsid proteins** now self-assemble to form new **nucleocapsids** containing viral RNA, reverse transcriptase, and integrase.
- It has been observed that a **viral protein called Vpu** has an important part to play in the budding process.

Antiviral therapy against HIV

Antiviral therapy against HIV

- Until 1987, no anti-HIV drug was available, but an understanding of the life cycle of HIV has led to the identification of several possible drug targets.
- At present, most drugs that have been developed act against the viral enzymes reverse transcriptase and protease.
- However, a serious problem with the treatment of HIV is the fact that the virus undergoes mutation extremely easily. This results in rapid resistance to antiviral drugs.

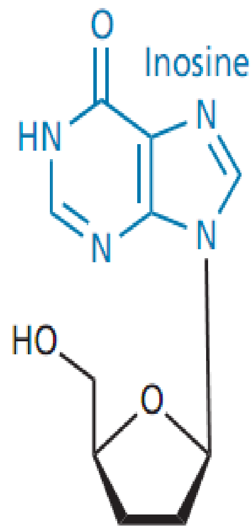
Antiviral therapy against HIV

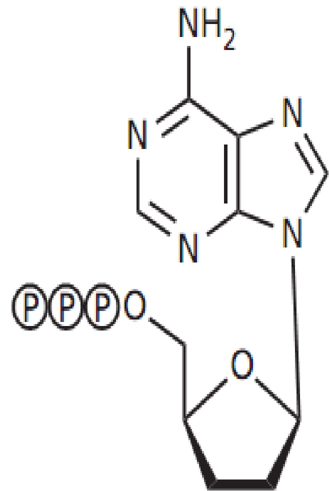
Inhibitors of viral reverse transcriptase Nucleoside reverse transcriptase inhibitors

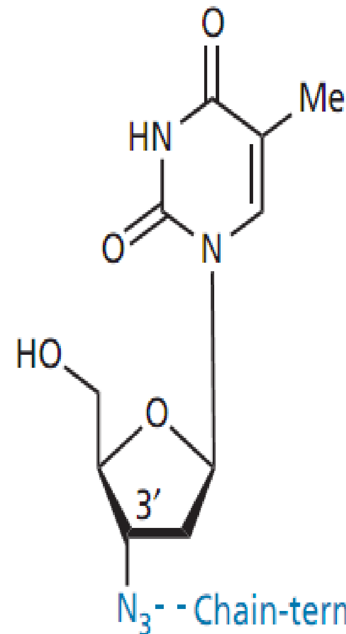
- As the enzyme **reverse transcriptase** is unique to HIV, it serves as an ideal drug target.
- Various nucleoside-like structures have proved as antiviral agents.
- The vast majority of these are not active themselves, but are phosphorylated by three cellular enzymes to form an active nucleotide triphosphate. cellular enzymes are required to catalyse all three phosphorylations because HIV does not produce a

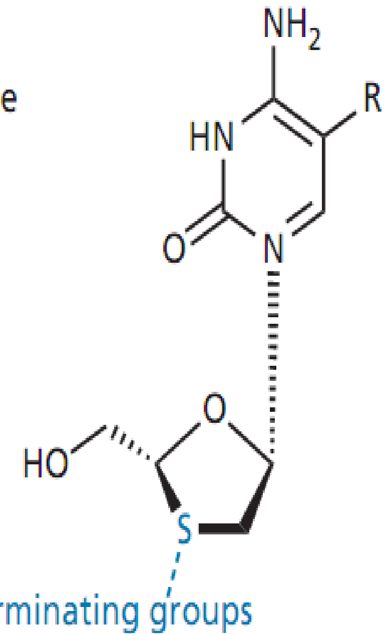
Zidovudine

- was developed originally as an anticancer agent but was the first drug to be approved for use in the treatment of AIDS.
- It is an analogue of deoxythymidine where the sugar 3′-hydroxyl group has been replaced by an azido group.
- On conversion to the triphosphate, it inhibits reverse transcriptase. Furthermore, the triphosphate is attached to the growing DNA chain. Since the sugar unit has an azide substituent at the 3′ position of the sugar ring, the nucleic acid chain cannot be extended any further









Didanosine

2′,3′-Dideoxyadenosine triphosphate

Zidovudine or azidothymidine (AZT)

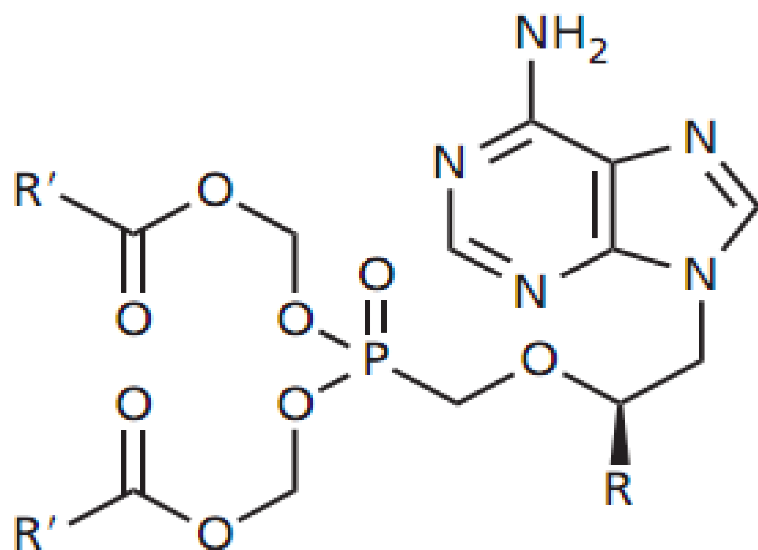
Lamivudine (R=H)
Emtricitabine (R=F)

FIGURE 20.12 Inhibitors of viral reverse transcriptase. Ⓟ = phosphate.

- **Didanosine**
- was the second anti-HIV drug approved for use in the USA (1988). Its activity was unexpected as the nucleic acid base present is **(inosine— a base)** which is not incorporated naturally into DNA. However, a series of enzyme reactions converts this compound into 2′,3′-**dideoxyadenosine triphosphate** which is the active drug.

- Studies of the target enzyme's active site led to the development of **lamivudine** and **emtricitabine** (analogues of deoxycytidine where the 3′ carbon has been replaced by sulphur).

- **Tenofovir disoproxil** and **adefovir dipivoxil** are prodrugs of modified nucleosides. Both structures contain a monophosphate group protected by two extended esters. Hydrolysis *in vivo* reveals the phosphate group which can then be phosphorylated to the triphosphate as described previously.



Adefovir dipivoxil (R=H, R′ = CMe_3 (^tBu))
Tenofovir disoproxil (R=Me, R′=$OCHMe_2$)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

- They include first-generation NNRTIs, such as **nevirapine** and **delavirdine**,
- as well as second-generation drugs, such as **efavirenz**, **etravirine**, and **rilpivirine**
- The NNRTIs (Fig. 20.14) are generally hydrophobic molecules that bind to an allosteric binding site which is hydrophobic in nature.
- the NNRTIs are non-competitive, reversible inhibitors.
- Binding of a NNRTI to the allosteric site results in an induced fit which locks the neighbouring substrate-binding site into an inactive conformation.

- Unfortunately, rapid resistance emerges as a result of mutations in the binding site of NNRTI the most common being the replacement of **Lys-103 with asparagine**. This mutation is called **K103N** and is defined as a **pan-class resistance mutation**.
- The resistance problem can be countered by combining an NNRTI with an NRTI from the start of treatment.

- **Nevirapine**
- It has a rigid butterfly-like conformation that makes it chiral. One 'wing' interacts through hydrophobic and van der Waals interactions with aromatic residues in the binding site, while the other wing interacts with aliphatic residues.

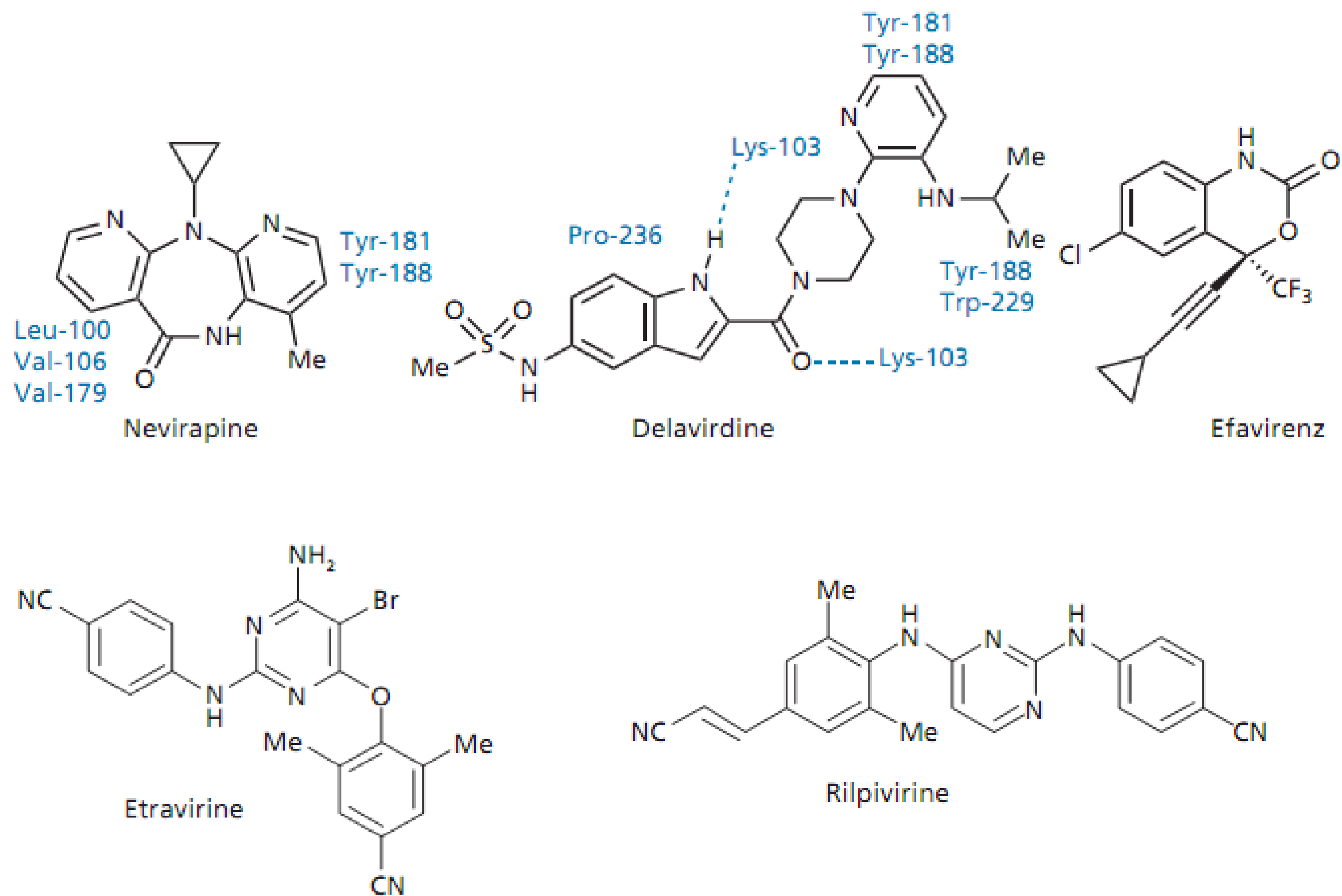


FIGURE 20.14 Non-nucleoside reverse transcriptase inhibitors in clinical use (interactions with amino acids in the binding site are shown in blue).

Delavirdine

- It is larger than other NNRTIs and extends beyond the normal pocket such that it projects into surrounding solvent.
- The pyridine region and iso- propylamine groups are the most deeply buried parts of the molecule and interact with tyrosine and tryptophan residues. There are also extensive hydrophobic contacts.
- Unlike other first-generation NNRTIs, there is hydro-gen bonding to the main peptide chain next to Lys-103.
- The indole ring of delavirdine interacts with Pro-236, and mutations involving Pro-236 lead to resistance.
- Analogues having a pyrrole ring in place of indole may avoid this problem.

- Second-generation NNRTIs **efavirenz**, **etravirine**, and **rilpivirine** were developed specifically to find agents that were active against resistant variants as well as wild-type viruses.
- **Efavirenz** is a benzoxazinone structure which has activity against many mutated variants but has less activity against the mutated variant K103N.
- Nevertheless, activity drops less than for nevirapine and a study of X-ray structures of each complex revealed that the cyclopropyl group of efavirenz has fewer interactions with Tyr-181 and Tyr-188 than does nevirapine

- Consequently, mutations of these amino acids have a lesser effect on efavirenz than they do on nevirapine.
- Efavirenz is also a smaller structure and can shift its binding position when K103N mutation occurs, allowing it to form hydrogen bonds to the main peptide chain of the binding site.
- A relatively small bulk and the ability to form hydrogen bonds to the main peptide chain are important as they allow compounds to change their binding mode when mutations occur. The most recent NNRTIs to be approved are etravirine (2008) and rilpivirine (2011).

Protease inhibitors

Protease inhibitors

- In the mid 1990s, the use of X-ray crystallography and molecular modelling led to the structure-based design of a series of inhibitors which act on the viral enzyme HIV protease.
- Unlike the reverse transcriptase inhibitors, the PIs are not prodrugs and do not need to be activated. Therefore, it is possible to use *in vitro* assays involving virally infected cells in order to test their antiviral activity.
- Like the reverse transcriptase inhibitors, protease inhibitors (PIs) have a short-term benefit when they are used alone, but resistance soon develops.

- Another complication is the requirement for anti-HIV drugs to have a good oral bioavailability (i.e. to be orally active). This is a particular problem with the PIs.
- Most PIs are designed from peptide lead compounds.
- Peptides are well known to have :

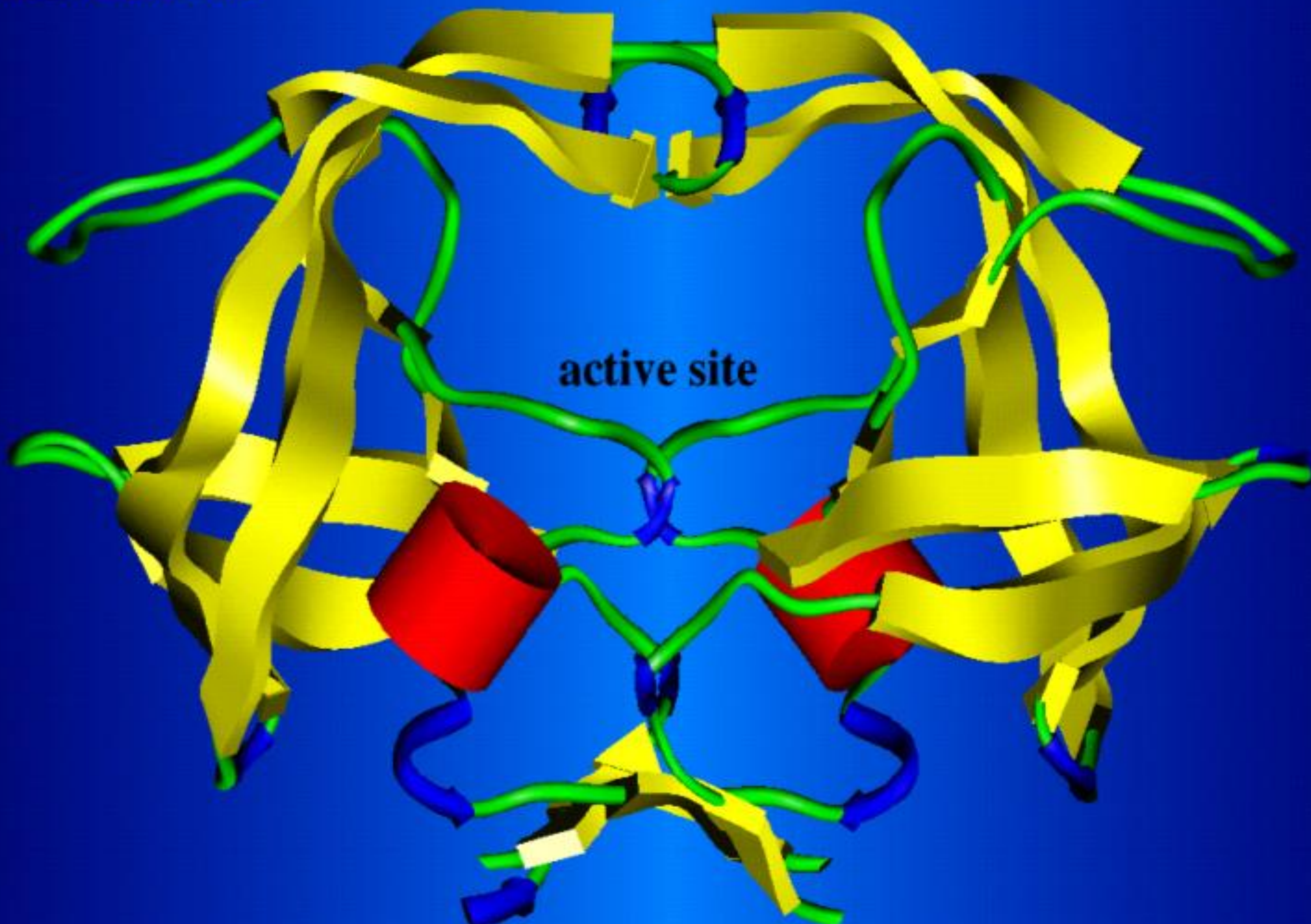
1. poor pharmacokinetic properties (i.e. poor absorption,
2. metabolic susceptibility,
3. rapid excretion,
4. limited access to the central nervous system,
5. and high plasma protein binding).

- This is mainly due to high molecular weight, poor water solubility, and susceptible peptide linkages.

- Subsequent work was then needed to reduce the peptide character of these compounds in order to retain high activity, whilst gaining acceptable levels of oral bioavailability and half-life.
- Clinically useful PIs are generally less well absorbed from the gastrointestinal tract than reverse transcriptase inhibitors, and are also susceptible to first pass metabolic reactions involving the cytochrome P450 isozyme (CYP3A4). This metabolism can result in drug–drug interactions with many of the other drugs given to AIDS patients to combat opportunistic diseases (e.g. rifabutin, ketoconazole, rifampin, and astemizole).

The HIV protease enzyme

- The HIV protease enzyme is an example of an enzyme family called the **aspartyl proteases—enzymes** which contain an aspartic acid in the active site that is crucial to the catalytic mechanism and which catalyse the cleavage of peptide bonds .
- The enzyme is relatively small and can be obtained by synthesis.
- The enzyme is crystallized with or without an inhibitor bound to the active site, making it an ideal target for structure-based drug design where X-ray crystallographic studies of enzyme–inhibitor complexes allow the design of novel inhibitors.


HIV- Protease
flaps
active site
dimerization domain

- The HIV protease enzyme is a symmetrical dimer made up of two identical protein units, each consisting of 99 amino acids.
- The active site is at the interface between the protein units and is also symmetrical with twofold rotational (C2) symmetry.
- The enzyme has a broad substrate specificity and can cleave a variety of peptide bonds in viral polypeptides, but, crucially, it can cleave bonds between a proline residue and an aromatic residue (phenylalanine or tyrosine) .
- The cleavage of a peptide bond next to proline is unusual and does not occur with mammalian proteases such as renin, pepsin, or cathepsin D, and so the chances of achieving selectivity against HIV protease over mammalian proteases are good.

- Moreover, the symmetrical nature of the viral enzyme and its active site is not present in mammalian proteases, again suggesting the possibility of drug selectivity.

Binding subsites:

- There are eight binding subsites in the enzyme—four on each protein unit, located on either side of the catalytic region (Fig. 20.16). These subsites accept the amino acid side chains of the substrate and are numbered **S1–S4** on one side and **S1′–S4′** on the other side.
- The relevant side chains on the substrate are numbered **P1–P4** and **P1′–P4′** (Fig. 20.17).
- Peptide bonds in the substrate are also involved in hydrogen bonding interactions with the active site.

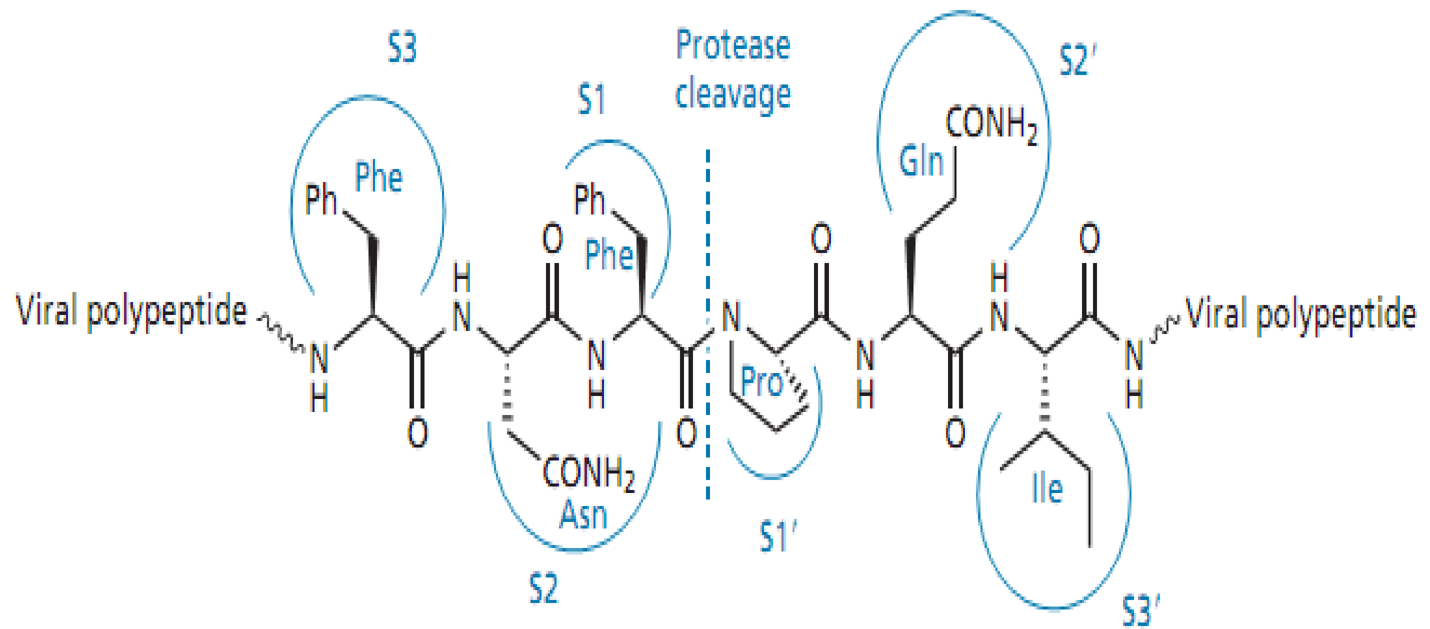


FIGURE 20.16 The aromatic–proline peptide bond that is cleaved by HIV protease (six of the eight binding subsites are shown).

Design of HIV protease inhibitors (PIs)

- **A similar hydrolytic mechanism to that shown in Fig. 20.18 takes place for a mammalian aspartyl protease called renin.**
- **This enzyme (renin) was studied extensively before the discovery of HIV protease, and a variety of renin inhibitors were designed as antihypertensive agents These agents act as transition-state inhibitors and many of the strategies resulting from the development of renin inhibitors were adapted to the design of HIV PIs.**
- **Transition-state inhibitors are designed to mimic the transition state of an enzyme-catalysed reaction.**
- **The advantage of this approach is that the transition state is likely to be bound to the active site more strongly than either the substrate or product. Therefore, inhibitors resembling the transition state are also likely to be bound more strongly.**
- **The transition state (of the HIV protease-catalysed reaction) resembles the tetrahedral intermediate shown in Fig. 20.18. and such structures are inherently unstable .**

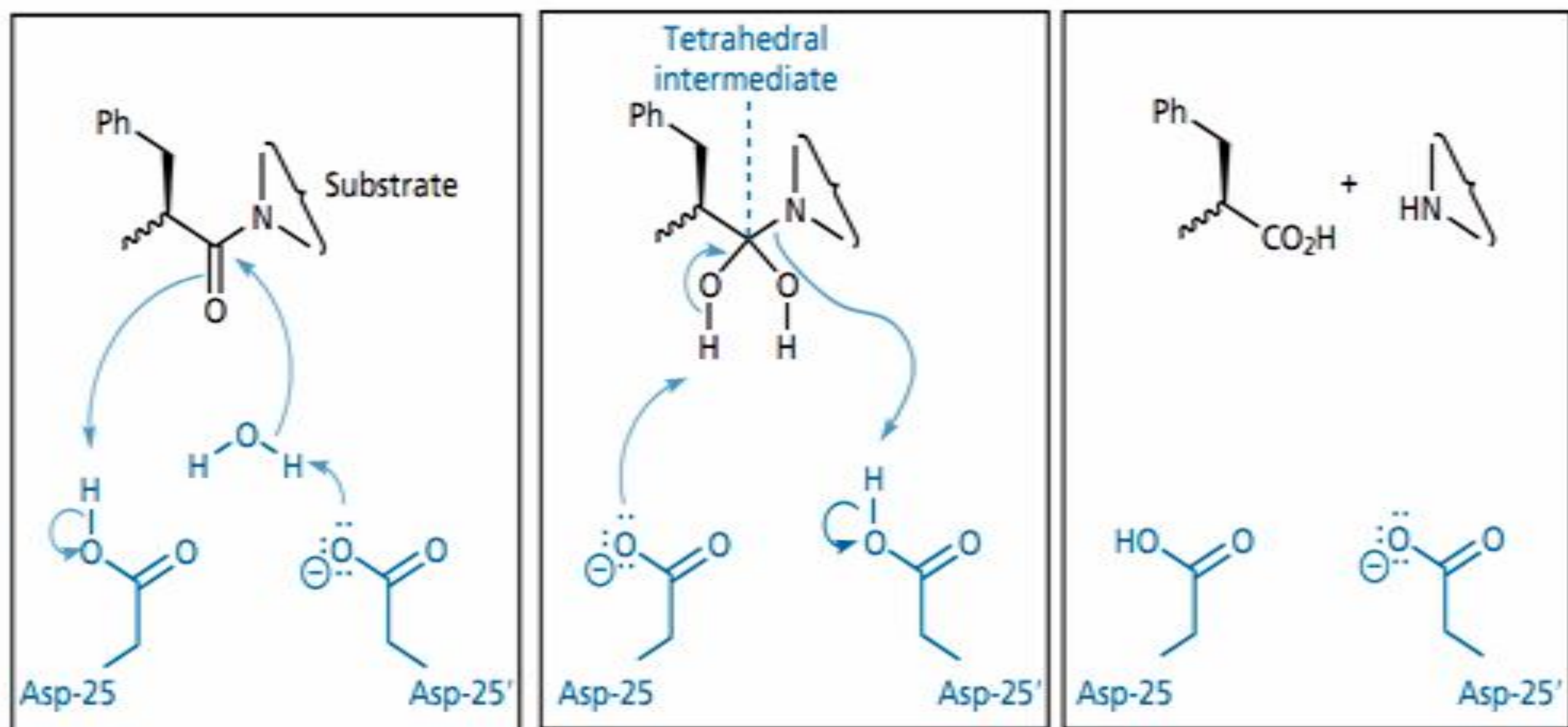
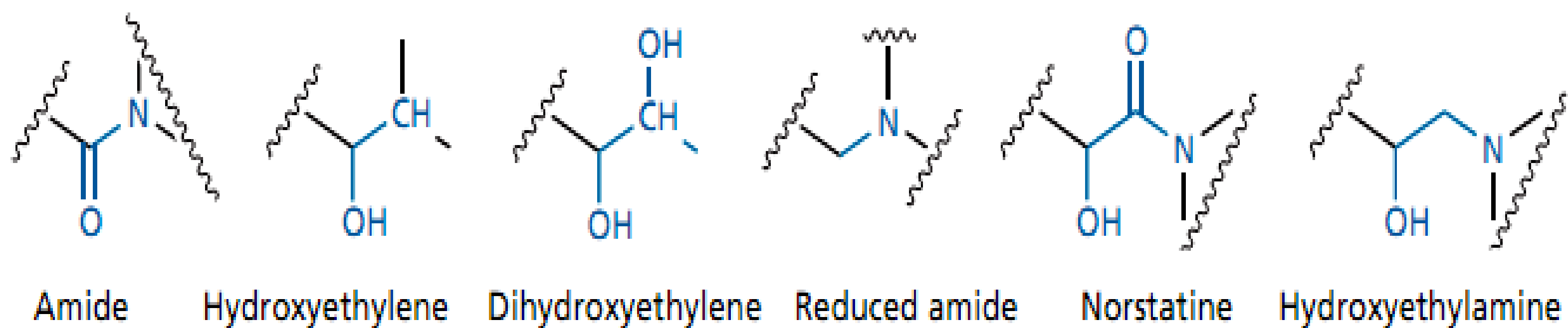
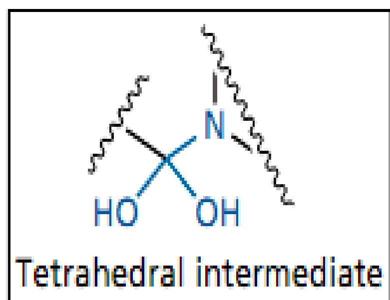
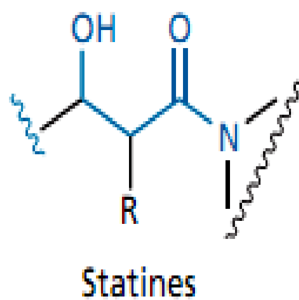


FIGURE 20.18 Mechanism of the reaction catalysed by HIV protease.

- It is necessary to design an inhibitor which contains a **transition-state isostere** , such an isostere would have a tetrahedral centre to mimic the tetrahedral centre of the transition state, yet be stable to hydrolysis.
- Fortunately, several such isosteres had already been developed in the design of renin inhibitors (Fig. 20.19). Thus, a large number of structures were synthesized incorporating these isosteres, with the hydroxyethylamine isostere proving particularly effective .







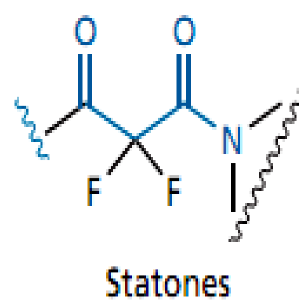


FIGURE 20.19 Transition-state isosteres.

- **This isostere** has a hydroxyl group which mimics one of the hydroxyl groups of the tetrahedral intermediate and binds to the aspartate residues in the active site.
- Having identified suitable **transition-state isosteres,** inhibitors were designed based on the enzyme's natural peptide substrates, as these contain amino acid residues which fit the eight subsites and allow a good binding interaction between the substrate and the enzyme.

- In theory, it might make sense to design inhibitors such that all eight subsites are filled to allow stronger interactions. However, this leads to structures with a high molecular weight and, consequently, poor oral bioavailability.
- Early inhibitors, such as saquinavir (Fig. 20.21), have amino acid side chains that bind to most of the subsites .
- Unfortunately, these compounds have a large molecular weight and a high peptide character leading to poor pharmacokinetic properties.
- More recent inhibitors have been designed with increased aqueous solubility and oral bioavailability by reducing the molecular weight and peptide character of the compounds.

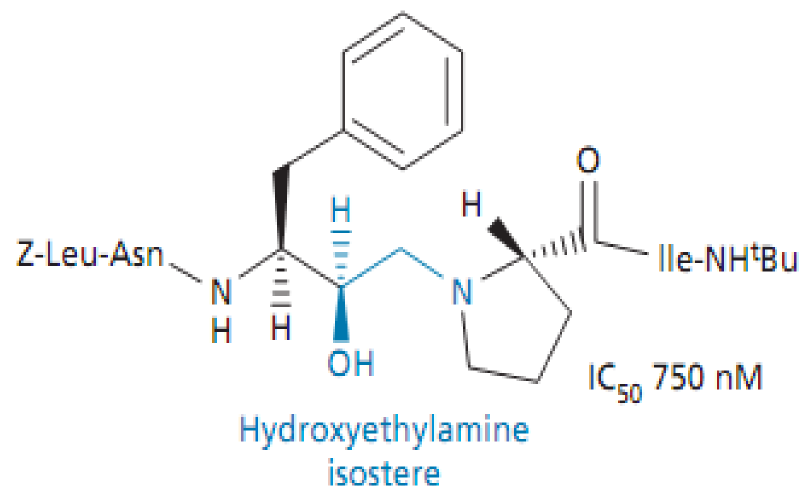
- The S2 and S2′ subsites of the protease enzyme appear to contain both polar (Asp-29, Asp-30) and hydrophobic (Val-32, Ile-50, Ile-84) amino acids, allowing the design of drugs that contain hydrophobic P2 groups which are also capable of hydrogen bonding.
- It has also been possible to design a P1 group that can span both the S1 and S3 subsites, allowing the removal of a P3 moiety, thus lowering the molecular weight.
- So P2 will bind with S2 and S2′ .

Saquinavir

Saquinavir

- **Saquinavir** was developed by Roche and, as the first PI to reach the market, it serves as the benchmark for all other PIs.
- The design of saquinavir started by considering a viral polypeptide substrate and identifying a region of the polypeptide which contains a phenylalanine–proline peptide link.
- **A pentapeptide** sequence Leu– Asn–Phe–Pro–Ile was identified and served as the basis for inhibitor design. The peptide link normally hydrolysed in this sequence is between Phe and Pro, and so this link was replaced by **a hydroxyethylamine transition-state isostere** to give a structure which successfully inhibited the enzyme(Fig. 20.20).

- This structure bind to the five subsites S3–S2′.
- enzyme inhibition is relatively weak.
- The compound also has high molecular weight and peptide-like character, both of which are detrimental to oral bioavailability



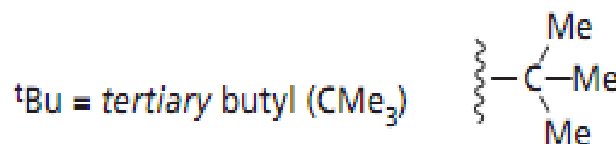


FIGURE 20.20 Pentapeptide analogue incorporating a hydroxyethylamine transition-state isostere.

- Consequently, the Roche team set out to identify a smaller inhibitor, starting from the simplest possible substrate for the enzyme—the dipeptide Phe–Pro.
- The peptide link was replaced by the hydroxylamine transition-state isostere and the resulting *N*- and *C*-protected structure (I) was tested and found to have weak inhibitory activity. The inclusion of an asparagine group (structure II) to occupy the S2 subsite resulted in a 40-fold increase in activity, which meant that structure II was more active than the pentapeptide analogue .This might seem an unexpected result as the latter occupies more binding subsites.
- However, it has been found that the crucial interaction of inhibitors is in the core region S2–S2′.

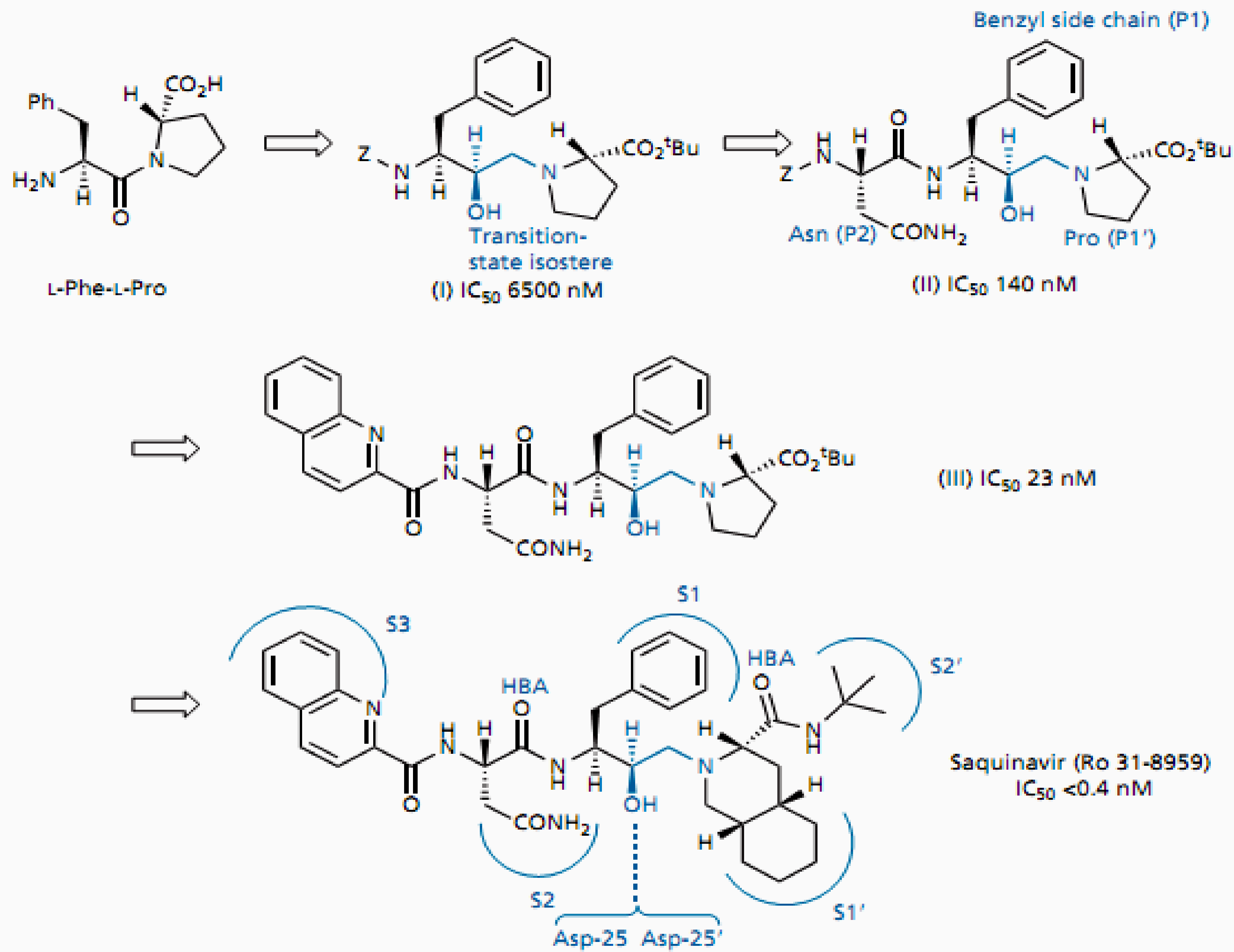


FIGURE 20.21 Development of saquinavir ($Z = PhCH_2OCO$).

- Structure II was adopted as the new lead compound and the residues P1 and P2 were varied to find the optimum groups for the S1 and S2 subsites.
- the benzyl group and the asparagine side chain were already the optimum groups.
- An X-ray crystallographic study of the enzyme–inhibitor complex revealed that the protecting group (Z) occupied the S3 subsite, which proved to be a large hydrophobic pocket.
- Therefore, the protecting group was replaced with a larger quinoline ring system which could occupy the subsite more fully. This led to a six fold increase in activity (structure III)

- Proline fits into the S1′ pocket, but it was possible to replace it with a bulkier **decahydroisoquinoline** ring system.
- The *t*-butyl ester protecting group was found to occupy the S2′ subsite and could be replaced by a *t*-butylamide group which proved more stable in animal studies.
- The resulting structure (saquinavir) showed a further 60-fold increase in activity.
- The *R*-stereochemistry of the transition-state hydroxyl group is essential. If the configuration is *S*, all activity is lost.

➢ **X-ray crystallography of the enzyme–saquinavir complex demonstrated the following**:

1. the substituents on the drug occupy the five subsites S3-S2′ (S3,S2,S1,S′,S2′);
2. the *t*-butylamine nitrogen is positioned in such a way that further *N*-substituents would be incapable of reaching the S3′ subsite;
3. there are hydrogen bonding interactions between the hydroxyl group of the hydroxyethylamine moiety and the catalytic aspartates (Asp-25 and Asp-25′);

- Saquinivir is still used clinically but suffers from
- poor oral bioavailability and
- susceptibility to drug resistance.

- Various efforts have been made to design simpler analogues of saquinavir which have lower molecular weight, less peptide character, and, consequently, better oral bioavailability.

Thank You