Beta-Strand Mimetics

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1. Introduction

1.1 Beta-Strands

The simplest peptide structural element is the peptide β-strand. The β-strand is a linear or saw-toothed arrangement of amino acids with amide bonds being almost coplanar, side chains alternating above and below the plane of the peptide backbone (Figure 1), and there are no intramolecular hydrogen bonds between component amino acid residues. Ideally, when in an antiparallel arrangement, the torsional angles of β-strands ϕ, ψ, and τ would be -139, 135, and $-177°$, respectively, and $d = 8.0$ Å.[1] The dipole moments of the peptide bonds alternate along the strands, which imparts stability to the formation of β-sheets from β-strands.

β-Strands are usually found hydrogen bonded in at least pairs, forming β-sheet structures in proteins. Isolated β-strands are not common. Normally considered a random structure rather than a discrete element of protein secondary structure, the peptide β-strand is now known to be the crucial structural element recognized on its own by, for example, proteolytic enzymes,[2,3] major histocompatibility complex (MHC) proteins (Figure 2),[4] and transferases.[5] The β-strand must therefore now be viewed as a fundamental structural element that is specifically recognized by biomolecular receptors.

Proteases account for a significant component (~2%) of the human genome and 1−5% of the genomes of infectious organisms. They are involved in the synthesis and turnover of all proteins, are associated with most diseased states, and their selective inhibitors show very promising therapeutic uses.[6] Proteolytic enzymes (aspartic, serine, metallo, and cysteine proteases) bind their inhibitors/substrates using the same extended β-strand peptide backbone conformation or equivalent non-peptide structure.[2,3,7] In immune defense, the major histocompatability complex (MHC) proteins[4] selectively bind the extended β-strand conformation of peptides derived from intracellular processing of viral, bacterial, and endogenous proteins,[8] and present them at the cell surface for recognition and immunological destruction.[9] This type of strand recognition is implicated in leukemia, and inflammatory and neurological diseases.[10] Other enzymes such as farnesyl transferases[5] and SRC kinases[11] also recognize peptide β-strands and are implicated in the development of cancers.

For these reasons alone, small molecules that mimic β-strands could be very useful enzyme inhibitors and antagonists with important potential applications in medicine. It is only in the last 10 years or so that small molecule β-strand mimetics have been reported.[12−15] While there are relatively few examples of monomeric β-strand mimetics identified as such,[12] probably due to their high tendency to aggregate,[16] this field is growing with the realization of the importance of β-strands. Illustrating this, there are now examples of human therapeutics that are β-strand mimetics, notably, inhibitors of proteases.[6,17,18]

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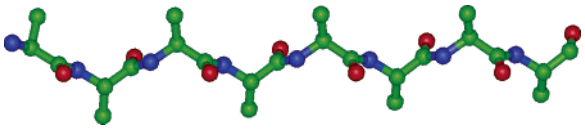


Figure 1. Idealized β-strand composed of Ala residues.

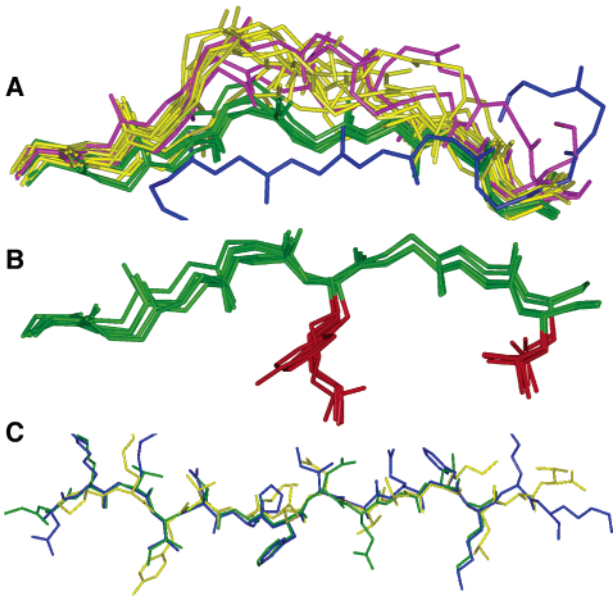


Figure 2. Superimposition of (A) 22 MHC class I protein-bound peptides; 8-residue peptides (green), 9- and 10-residue peptides (yellow and pink, respectively), and an N-formylated peptide bound to class Ib (dark blue). (B) backbones of seven octameric protein-bound peptides (green), with their anchor residues (red) displayed at P5 and P8. (C) Superimposition of three MHC class II protein bound peptides.

1.2 Beta-Sheets

In addition to such specific recognition of discrete β-strands, combinations of two or more strands to form β-sheets not only act as important scaffolding

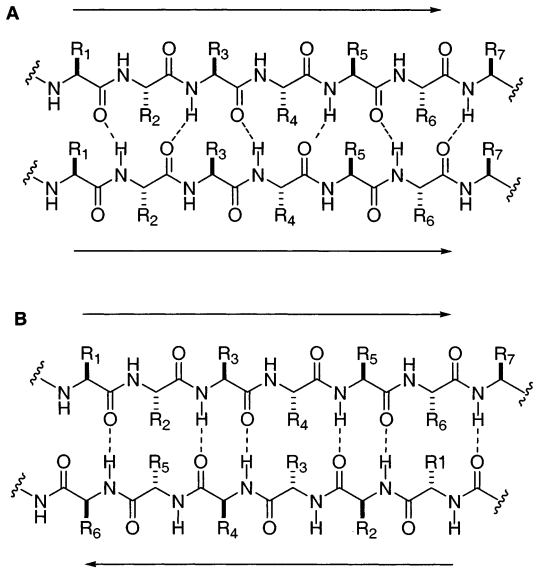


Figure 3. Schematic representation of (A) parallel β-sheet and (B) antiparallel β-sheet. Amino acid side chains are represented by R. Hydrogen bonds are indicated by dashed lines. Arrows indicate direction from N- to C-terminus.

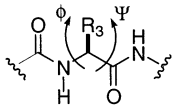


Figure 4. Phi (Φ) and psi (Ψ) angles for a β-strand and β-sheets.

elements to stabilize protein structure, but are sometimes key recognition motifs that bind to other proteins or DNA. The β-sheet secondary structure accounts for over 30% of all protein structure. It consists of two or more paired β-strands arranged in either parallel, antiparallel, or mixed alignments held together through interstrand hydrogen bonds (Figure 3).

Parallel β-sheets contain strands that run in the same direction and are characterized by a series of 12-membered hydrogen-bonded rings (Figure 3A). Antiparallel β-sheets contain strands that run in opposite directions and are characterized by an alternating series of 10- and 14-membered hydrogen-bonded rings (Figure 3B). Mixed β-sheets contain mixtures of both patterns. The term "pleated" sheet refers to optimal hydrogen-bonding interactions between strands, forcing contiguous side chains to opposite sides of the peptide chain. The dihedral angles commonly found in parallel ($\Phi = -119°$, $\Psi = 113°$) and antiparallel ($\Phi = -139°$, $\Psi = 135°$) β-sheets are the closest to those in the fully extended single strand conformation ($\Phi = \Psi = \pm 180°$). The dihedral angles for amino acid residues in β-strands fall in the upper left-hand quadrant of a Ramachandran plot. Ideal phi (Φ) and psi (Ψ) angles (Figure 4) for a β-strand and β-sheets are listed in Table 1.[1,19]

Although the β-sheet plays mainly a scaffolding role in protein architecture, it is also a key recognition motif in some protein−protein and protein−DNA interactions that are important in many biological processes and in some diseases. For example, normal gene regulation by the *met* repressor requires its dimerization through β-sheet domains with the resulting sheet being recognized by the major groove of DNA.[20] Figure 5A,B shows the complex formed between two Arc represser protein dimers and the 21-base-pair DNA duplex.[21,22] The antiparallel β-sheet

Table 1. Ideal Phi (Φ) and Psi (Ψ) Angles of Some Secondary Structures

secondary structure[a]	Φ^o	Ψ^o
parallel β-sheet	−119	113
antiparallel β-sheet	−139	135
α-helix	−58	−47
β-turn Type I	−60	−30

[a] Refs 1 and 19.

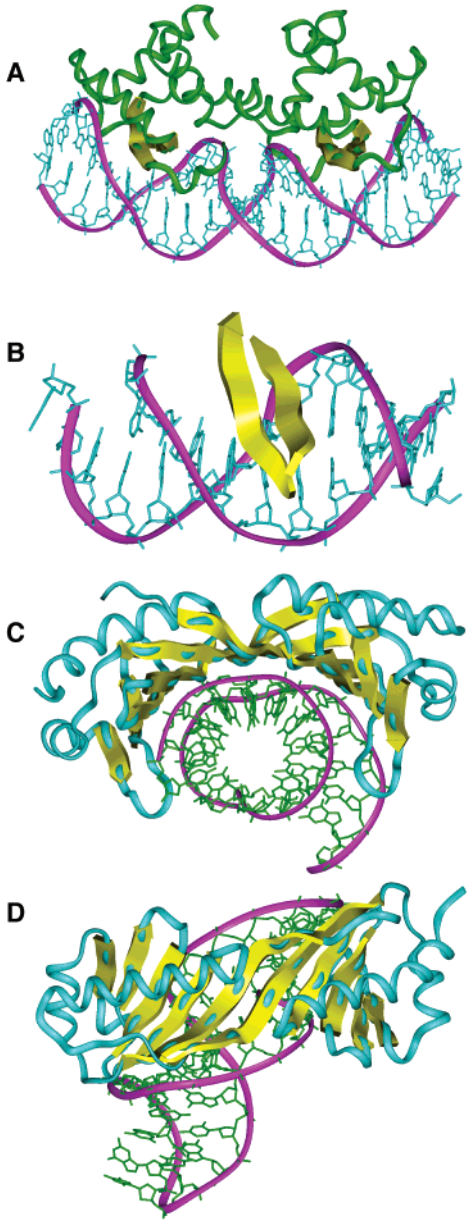


Figure 5. DNA recognition of β-sheets. Complex of two arc repressor protein dimers with DNA illustrating (A) the overall interactions, and (B) the β-sheet interaction (flat arrows, PDB entry: 1par). Adapted from ref 21. The second complex is that of the TATA box-binding protein (TBP) and DNA where (C) shows the protein straddling the DNA strand, and (D) displays the positioning of the β-sheet over the DNA strand (PDB entry: 1ytb). Adapted from refs 23−25.

is formed from a single strand from each monomer. The β-sheet binds to the center of the major groove of DNA. The residues Gln9, Ala11, Arg13, Gln9′, Ala11′, and Arg13′ make extensive base contacts. Figure 5C,D shows more extensive β-sheet interactions between the TATA box-binding protein (TBP) and DNA.[23−25] Hydrogen bonds, van der Waals interactions, and phenylalanine base stacking interactions are formed between the entire under surface of the saddle region of TBP and the minor groove of DNA.[23,24] Clustering of membrane ion channel PDZ domains also involves β-sheets,[26] as does the binding interaction between lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1).[27]

Aggregation of some proteins to form insoluble β-sheet structures is thought to be responsible for a number of neurological disorders. For example, there are over 30 diseases that are characterized by amyloid fibrils composed of proteins that have "misfolded" into β-sheets that aggregate into insoluble polymers.[28−30] Examples include Alzheimer's disease[31−34] due to aggregation of beta amyloid protein, spongiform encephalopathies such as Creutzfeldt-Jakob disease (humans), scrapie (sheep), bovine spongiform encephalopathy or mad cow disease due to prion proteins, familial polyneuropathy due to transthyretin, Huntington's disease due to huntingtin, Parkinson's disease due to α-synuclein, type 2 diabetes mellitus due to amylin or islet amyloid peptide, and systemic amyloidosis due to lysozyme or transthyretin.[35] Silk and spider webs are also examples of naturally occurring β-sheets.[36,37] Infection of human immune cells by the HIV virus requires a β-strand mediated interaction of viral gp-120 with CD4 receptors located on the surface of target cells.[38]

Although β-sheet mimetics are more prevalent than structurally validated β-strand mimetics, with a variety of structurally diverse examples having been reported,[39,40] there are currently no human therapeutics involving β-sheet mimicry. Most β-sheet mimetics are unikely to be therapeutically useful, but can provide useful information for the design of improved β-strand mimetics. β-Strand peptidomimetics could thus find important roles as competitive ligands for receptors/enzymes that typically bind to β-sheets, or as inhibitors of β-sheet formation when β-sheets themselves are undesirable.

1.3 Differences in Strand/Sheet/Turn/Helix Recognition

To design and develop molecules that selectively mimic the β-strand, one also needs to consider differences between size, shape, and composition of a β-strand versus sheets, turns, and helices. The extended β-strand presents contiguous residues on alternating sides of the strand, so that their side chains are separated by the maximum possible distance. For example the i and i+4 residues in an extended β-strand are 14.5 Å apart (Figure 6A). This separation minimizes steric clashes between side chains, which also have the maximum possible exposure to solvent (or receptor) in this structure. A single β-strand permits maximum exposure of main chain atoms for hydrogen bonding to a receptor.

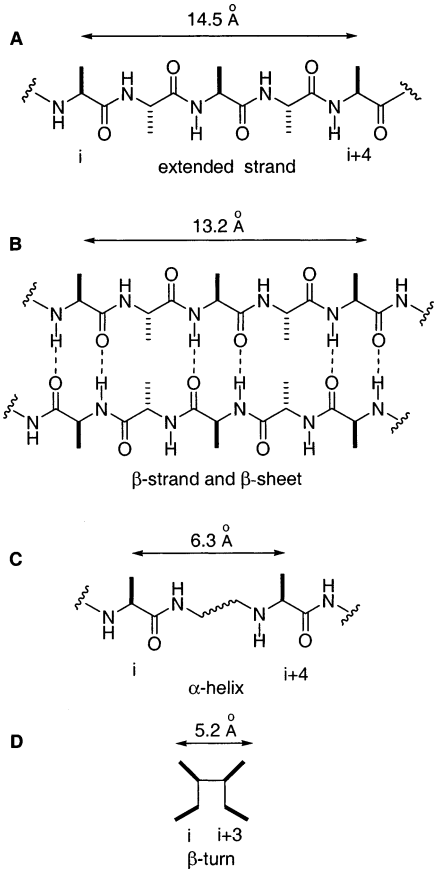


Figure 6. Comparative sizes Å for (A) extended β-strand (Φ = Ψ = 180°), (B) antiparallel β-sheet (Φ = −139°, Ψ = −135°), (C) α-helix (αCi − αC(i+4) distance), and (D) β-turn (βC(i+1) − βC(i+2)).

A β-strand within a β-sheet has i and i+4 residues 13.2 Å apart, but the extensive interstrand hydrogen-bonding network also protects all main chain atoms from solvent/ligand interactions (Figure 6B). Parallel and antiparallel β-sheet arrangements (Figure 3) present side chains in two opposing directions. The strands on the edges of the sheets still have half of their main chain atoms available for hydrogen bonding to a receptor or solvent. The side chains are exposed for potential enzyme interactions on both the top and bottom surfaces. This presents a more three-dimensional recognition site, with i and i+4 residues being 13.2 Å apart on the same strand and the αC−αC interstrand distance of adjacent residues on adjacent strands being approximately 4.5−5.5 Å apart (distances were measured from a DNA binding antiparallel β-pleated sheet complex).[23] The corresponding hydrogen-bonding distance from amide N to carbonyl O is approximately 3.0 Å. The strand thus offers three different recognition sites: either via the top or bottom faces or via hydrogen bonding with main amide atoms on each side of the sheet.

On the other hand, an α-helix has the i and i+4 residues only ∼6.3 Å apart (Figure 6C). The helix is a compressed peptide chain, all main chain oxygen and NH atoms being involved in intramolecular hydrogen bonds. Consequently, main chain atoms are protected from intermolecular hydrogen bonding with a receptor. Thus, only the side chains are available for intermolecular interactions.

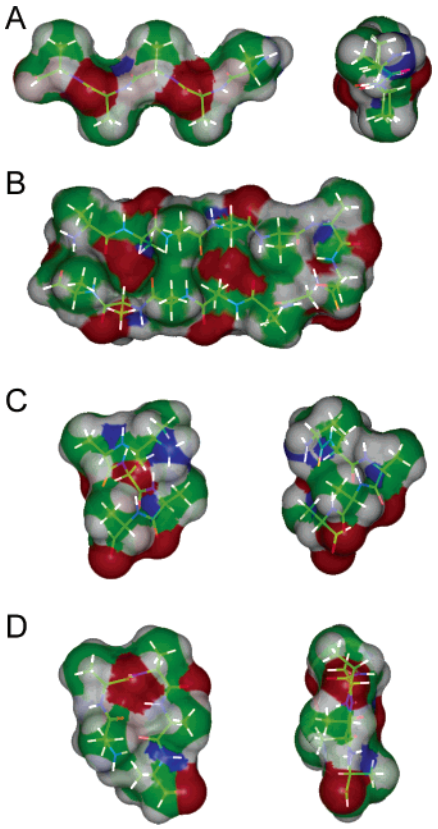


Figure 7. Rendered Connolly surfaces of secondary structural motifs; (A) β-strand, (B) β-sheet, (C) α-helix, and (D) β-turn.

The β-turn conformation provides maximum two-dimensional exposure of its side chains to either solvent or a receptor, but limits the exposure of main chain atoms to hydrogen bonding. The average $\beta C_{(i+1)}-\beta C_{(i+2)}$ distance is 5.2 Å (Figure 6D), which is similar to the distance between two adjacent side chains of an α helix, but is distinguished by the presentation of the side chains in two dimensions for a turn compared with three dimensions for an α helix.

Simplistically, the peptide backbone for each of the four secondary structural motifs (β-strand, antiparallel β-sheet, α-helix, and β-turn) can be represented by geometric surface area shapes, such as a rectangular prism, a rectangular prism excluding one face, the surface of a cylinder excluding the ends, and a three-dimensional horseshoe, respectively. The β-strand rectangular prism presents all surfaces to solvent or receptor, whereas a β-strand within a two-stranded sheet loses one surface through interstrand hydrogen bonding. The α-helix appears similar to a β-strand in terms of volume; however, the surface accessible area is limited to only the curved surface. The solvent accessible Connolly surface areas (Figure 7) for the five-residue peptide pentaalanine in each of the ideal secondary structures, β-strand ($\Phi = -120°$ and $\Psi = 120°$), antiparallel β-sheet, α-helix, and type II β-turn varies from 646, 393, 522, and 516 Å^2, respectively (see footnote in Table 2).

1.4 Toward Beta-Strand Mimetics

Mimicking β-strands to antagonize β-sheet formation or recognition may represent a viable therapeutic strategy toward the prevention or treatment of diseases associated with β-sheet structures. Drug design often involves the examination of protein−protein interactions associated with disease, followed by the design of small molecules that can mimic or bind to one of the interacting proteins.[6,17,18,41−44] Often the bioactivity of proteins stems from only a small localized region of a protein surface created by secondary structural elements (such as α-helices, β- or γ- turns, or β-strands/sheets), so small molecules based on these structures are at least in principle feasible as antagonists.

Table 2. Recognition Motifs of Secondary Structures

secondary structure	hydrogen bonding	side chain interactions	surface area (Å^2)[a] of Ala_5
extended β-strand	max	max	646.1
β-sheet	intermediate	max	393.3[b]
α-helix	min	max	522.4
β-turn	intermediate	intermediate	516.0

[a] Surfaces were calculated using molmol. [b] Surface area calculated from the DNA binding antiparallel β-pleated sheet of the TFIIB−TBP−TATA−element complex (residues 70−82). Residues 70−75 and 78−82 were replaced with alanines and the surface area for the individual residues (i to i+4) summed.

The simplest approach to interfering with β-strand/sheet recognition would be to use short peptides corresponding to strand/sheet regions of proteins recognized by other proteins or DNA. However, short peptides suffer from a number of disadvantages that compromise their use as drugs.[18,45,46] They are conformationally flexible, existing mainly as random structures in aqueous solution.[44,47−49] Peptides are also susceptible to degradation through peptide bond cleavage by peptidases, have low bioavailability, and exhibit poor pharmacological profiles due to a combination of these factors, other forms of metabolism, rapid clearance rates, and poor membrane permeability. Thus, while biomolecular interactions with peptides provide very useful clues for drug design, changes need to be made to create more pharmacologically acceptable drug candidates.

With respect to conformational stability, the arrangement of peptides into β-strands for recognition by biomolecular targets is either a chance event in which the receptor captures the small percentage of peptide present in a β-strand conformation, or else the receptor plays an active role in contorting the peptide into the preferred strand shape. A central principle in medicinal chemistry is that molecules, which are conformationally preorganized or fixed into a shape that is recognized by a receptor, can have higher affinity for that receptor due to the reduced entropy penalty for adopting the receptor-binding shape. It is therefore surprising that, unlike the case for turns and helices, there are relatively few reported conformationally restricted, surrogates for the β-strand.

One minimalist approach to conformational stability involves restricting peptide freedom though cyclization,[50] either via side chain to side chain, side chain to main chain, or main chain to main chain linkages, to form macrocycles. Nature frequently uses cyclization to force peptides into bioactive conforma-

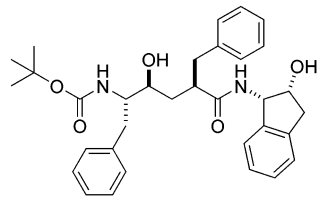
tions.[42] Cyclic peptides also have the advantages over linear peptides of being more resistant to amide bond cleavage by proteolytic enzymes and of being more conformationally restrained or less flexible. Examples are given in the next section of synthetic strand peptidomimetics created through macrocyclization (generally $C_i^{\alpha} \rightarrow$ (C_{i+1}^{α}, C_{i+2}^{α}, or C_{i+3}^{α}), inter- or intraresidue cyclization, and ring fusion.

Alternatively, conformational constraints can be incorporated in peptide sequences. Nature frequently molds peptides into turn shapes by replacing amino acid components with a wide variety of constraints such as disulfides, double bonds, *N*-methyl amino acids, D-amino acids, aromatic and heterocyclic rings, often in conjunction with cyclization.[42,44] Similar conformational constraints could be used to synthetically mimic the peptide β-strand. For example, one or more amino acid residues could be replaced in a peptide sequence by one or more rigid organic units. Such replacements can produce peptidomimetics with drug-like components, examples of which are presented ahead.

Conformationally and metabolically stable peptidomimetics or nonpeptidic mimetics are the goals of β-strand mimicry. While significant advances have been made in the synthesis of conformationally constrained β-sheet mimetics,[51−54] there is a pressing need for small molecules that mimic the β-strand and for generic rigid scaffolds as components of strands and libraries of strands. Recent progress made toward nonpeptidic β-strand scaffolds warrants this overview, which takes into account reports between 1990−2003 and includes a few examples from broader reviews on peptide secondary structure.[39,55−58] In particular, we focus on inhibitors reported in crystallographic structures of protease−ligand complexes, this information being available in the protein data bank for >1500 structures. The Protein Data Bank[59] entry codes (PDB entry: 1abc) are reported in the text where applicable. Scaffolds that were structurally validated in solution by NMR spectroscopy also are included. Secondary structure motifs (extended strand, β-strand, and α-helix) were generated (default values) using the biopolymer module of the InsightII modeling package (Accelrys).[60]

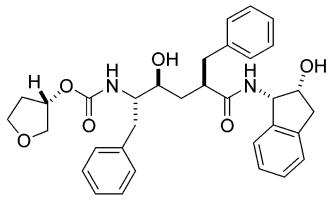
An integrated approach has often been adopted in the design of β-strand mimetics; thus, this review focuses on a structure-based coverage of the principal strand mimetic types rather than being divided into categories based on biological targets (such as protease inhibitors of thrombin and HIV, which are reviewed elsewhere, for example, refs 57 and 61−67), with key themes incorporated in the discussion. Approaches to categorizing the general classes of β-strand mimetics are potentially variable. Historically mimicry of the β-strand conformation in a compound has been pursued for fundamental purposes, and subsequently it has been stimulated by the pursuit of inhibitors of enzymes such as HIV-protease, thrombin, and caspases.

In terms of features of the β-strand that are being mimicked, primarily these are replacements for the main chain hydrogen bonding, side chain interactions or both. Typically, this has been achieved using compounds where the scaffold binds a specific enzyme subsite(s) to help fix the β-strand conformation, or the scaffold merely projects pendant substituents to binding locations, or a combination of both. Scaffolds can be classified by amino acid surrogate components or by heterocycles or in other ways. Illustrative of this are 1(*S*)-amino-2-(*R*)-hydroxyindanyl and 3(*S*)-hydroxytetrahydrofuran ligands employed in HIV-1 protease inhibitors **1**[68] and **2**,[69] respectively.



1

IC_{50} (HIV-1 protease) = 0.3 nM



2

IC_{50} (HIV-1 protease) = <0.03 nM

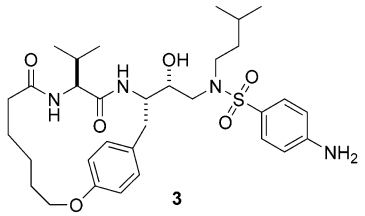
In this article, scaffolds will be classified principally on the basis of their heterocyclic subunits, even though they may be perceived as amino acid surrogates elsewhere. For acyclic molecules, the development of irreversible inhibitors of enzymes such as HIV protease, cathepsin B, caspases, and other proteases has often involved design and optimization of dipeptide isosteres flanked by peptidic or nonpeptidic appendages. Isostere units have included carbon−carbon double bonds and azapeptides (section 3), and heterocyclic units such as aziridines (section 5) and epoxides (section 6). An encyclopedia-style organization has been adopted for this review, according to compound class, to facilitate finding compounds, to identify structural similarities and differences, and particularly to compare different chemical types of β-strand mimetics.

2. Macrocyclic Peptidomimetics

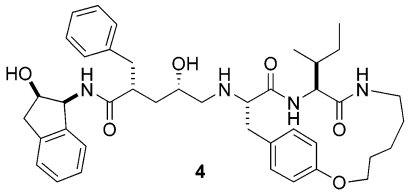
Macrocycles formed through condensing peptide side chains to the main chain have been shown to be highly constrained structural mimics of tri- and tetrapeptide components of a linear peptide substrate or inhibitor. These peptidomimetics fix the receptor bound conformation of bioactive peptides in an extended conformation, which results in functional mimicry. This has been demonstrated for example by crystal structures that reveal macrocycle−protease interactions with protein residues in active sites of proteases.[50,70−73] Some of those macrocycles possess two trans amide bonds and a para substituted aromatic ring within 15−17 membered rings, which can replace tri- or tetrapeptide segments within peptidic substrates and inhibitors.

Figure 8. Comparison of the HIV-1 protease active site binding conformation of the macrocyclic inhibitor **3** (PDB entry: 1d4l) (orange) and the linear peptidic substrate (PDB entry: 1mt7) (yellow). Images were generated using Insight II.

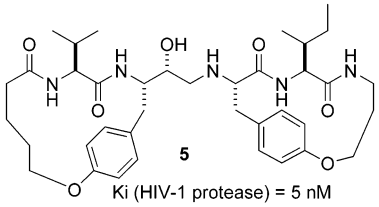
These constraints prevent intramolecular hydrogen bonding and preorganize the macrocycle in an extended conformation. Each macrocycle is restrained to a preorganized β-strand conformation for protease binding.[71] This approach, together with side chain to side chain cyclization, has produced a variety of macrocyclic peptidomimetics.[50,70−72,74−77] For example, **3**[71] (PDB entry: 1d4l), **4**[72] (PDB entry: 1d4k) and **5**[74] are potent and selective inhibitors of HIV proteases. This minimalist approach retains all the amide bonds and associated hydrogen-bonding interactions made between peptide and receptor (Figure 8). Other analogues of **3**, such as **6**,[12] have been designed to be effective scaffolds for appending N- and C-terminal nonpeptidic substituents.



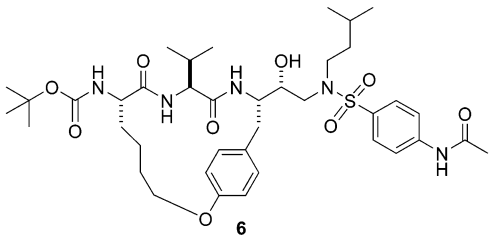
3
Ki (HIV-1 protease) = 1.7 nM
IC_{50} (HIV-1 infected MT2 cells) = 90 nM



4
Ki (HIV-1 protease) = 0.6 nM
IC_{50} (HIV-1 infected MT2 cells) = 60 nM

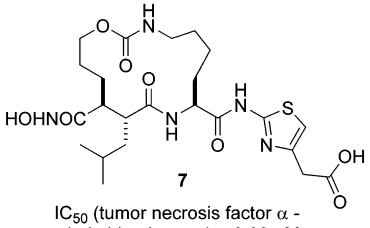


5
Ki (HIV-1 protease) = 5 nM

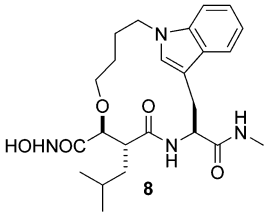


6
IC_{50} (HIV-1 infected MT2 cells) = 177 nM
Ki (HIV-1 protease) = 2.0 nM

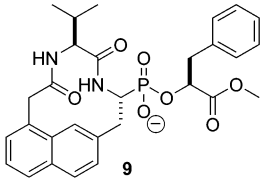
Other macrocyclic peptidomimetics, such as **7**,[78] **8**,[78] **9**,[79] and the 14-membered **10**,[78] have been designed and constructed as potent inhibitors of other aspartic, serine and metallo proteases.[42,73,78,79] In the case of the macrocycle **9**, the naphthalene bridge was used to constrain the peptide backbone to the conformation adopted by the binding site of penicillopepsin.



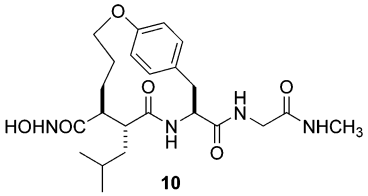
7
IC_{50} (tumor necrosis factor α - whole blood assay) = 0.08 μM



8
Ki (MMP-3) = 2.9 nM



9
Ki(penicillopepsin) = 0.8 μM

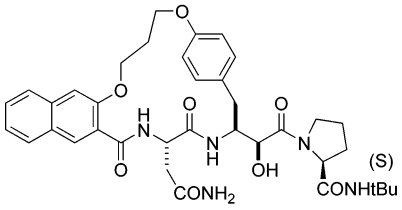


10
IC_{50} (tumor necrosis factor α - whole blood assay) = 0.22 μM

Constricting peptide sequences into macrocycles in such a way makes the amide bonds less recognizable to other proteases. Thus, such macrocycles are not only resistant to peptidases of the gut, bloodstream, or cells, but can also penetrate cell membranes and exhibit potent antiviral activity in cell culture.[72,75,80] Illustrative of this is macrocycle **11**,[75,76] which provides ~17-fold less HIV-1 protease inhibition than its acyclic counterpart, but displays more potent antiviral activity. This suggests that the macrocyclic inhibitor **11** may have improved cell permeability and or resistance to cellular enzymes.

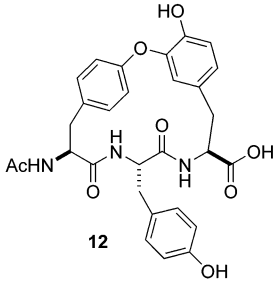
There are also a number of macrocyclic natural products that were originally thought to be turn mimetics but are now known[42,73] to present short extended peptide strands to proteases. Among these are K-13 (**12**)[81,82] and OF4949−IV (**13**)[83,84] (and other analogues of OF4949 analogues[85−87]), which are

inhibitors of the metalloproteases angiotensin converting enzyme (ACE) and aminopeptidase B. They lack the metal binding functionality normally associated with inhibitors of metalloenzymes, suggesting an alternate mechanism of interaction. They also contain the novel amino acid isodityrosine. When such a side chain-linked amino acid is built into a peptide, the peptide becomes more conformationally homogeneous. NMR and molecular mechanics calculations on the thioether analogue of **12** suggested an extended conformation for the peptide.[88] This is now supported by NMR and crystal structure analyses of synthetic analogues where, for solution and solid state conformations, the 17-membered cyclic biphenyl ether peptide system is a mimetic of a β-strand.[89]

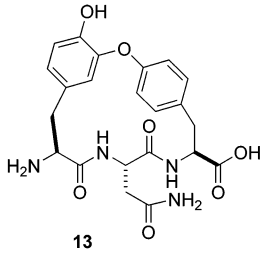


11

IC_{50} (HIV-1 protease) = 19 nM
EC_{50} (HIV-1 infected MT2 cells) = 76 nM

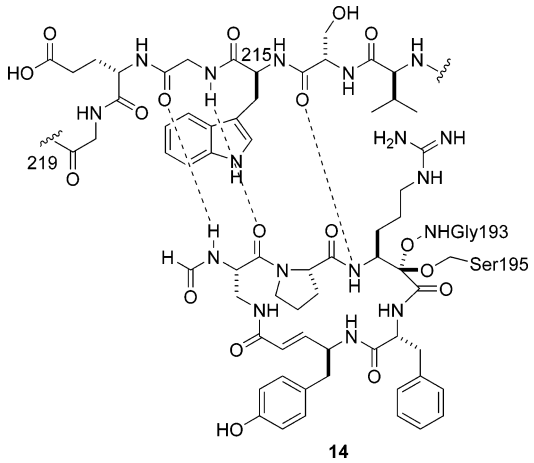


12



13

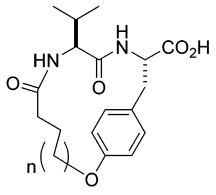
Another example is the 19-membered macrocyclic peptide cyclotheonamide A (**14**), isolated from the marine sponge *Theonella* sp.[90] Cyclotheonamide A **14** moderately inhibits the serine protease thrombin (K_i = 0.18 μM), but is a more potent inhibitor of the serine proteases trypsin (K_i = 0.023 μM) and streptokinase (K_i = 0.035 μM). Originally thought to be presenting turns to the protease, crystal structure studies of complexes of **14** with these serine proteases (trypsin[91] PDB entry: 1tyn, thrombin[90] PDB entry: 1tmb) have since confirmed an extended strand conformation for the protease-binding segment of this macrocycle (Figure 9).[92] The NH−CHR−C(O)−Pro−Arg segment forms a hydrogen-bonded two-strand antiparallel β-sheet with Ser214/Trp215/Gly216. Moreover, solution NMR studies of cyclotheonamide A **14** in aqueous media reveal that the conformation of the protease-binding segment D-Phe-Arg-Pro is almost identical to that found in the solid state,[93] indicating that cyclotheonamide preorganizes this segment in the extended conformation needed for protease binding. Subsequent studies of simplified cyclotheonamide analogues, with an aliphatic tether, realized a number of potent thrombin inhibitors, which were shown to maintain the tripeptide array in the extended conformation.[94]



14

Figure 9. Schematic representation of the interaction between the β-strands of cyclotheonamide A (**14**) and thrombin. Adapted from ref 92.

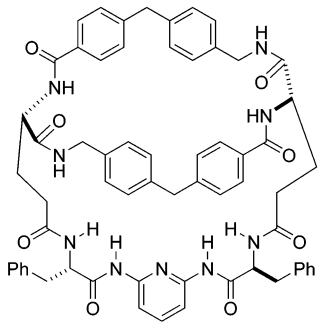
Although crystal structures have been reported for many macrocycle−enzyme complexes,[71,72,79,95] until recently it was not known the extent of preorganization of a macrocycle as a β-strand mimetic away from the enzyme.[96] Short peptide segments (3−10 amino acids) rarely adopt defined structures in solution, and the β-strand peptide conformation is not a stable structure in water.[47,97] This issue was examined in 15−22-membered macrocyclic analogues of peptides[96] (for example, **15** and **16**), each constrained by two trans amide bonds and a planar aromatic ring. The macrocycles were shown from NMR spectra, and structure calculation for the smallest and most constrained (15-membered) analogue **15**, to adopt a well-defined β-strand structure in water. Such macrocycles that are sufficiently constrained to adopt a β-strand mimicking structure in water may be useful as preorganized scaffolds for the design of compounds that interfere with β-strand recognition in proteins.



15 n = 1
16 n = 8

Synthetic macrocyclic receptors incorporating a carboxylic acid binding site specifically for peptides with a free carboxylic acid terminus have been developed.[98] One such macrocyclic structure, **17**, has a diamidopyridine unit at the base of an open, bowl-shaped cavity. Additional amide functionality around the rim of the bowl-shaped structure provides hydrogen-bonding sources to interact with peptide guest molecules. NMR spectroscopic studies concluded that macrocycle **17** was a flexible receptor, able to bind Cbz-β-alanyl-D-alanine substrate within the macrocyclic cavity via a series of well-defined hydrogen

bonds making the peptidic substrate an extended conformation.[98] In addition, an acyclic variant of **17** was reported by the same group.[99]

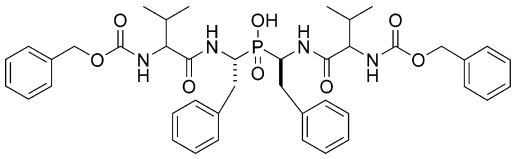


17

3. Alicyclic Compounds

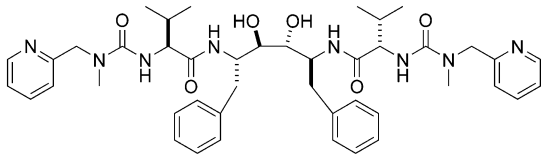
Acyclic β-strand molecules typically have involved design and optimization of dipeptide isosteres flanked by peptidic or nonpeptidic appendages. HIV-1 protease is a homodimer with a 2-fold axis of symmetry extending through the active site. Compounds that take advantage of this C_2 symmetry and project substituents into the S1/S1′ and S2/S2′ binding sites of the protease have been reviewed elsewhere.[100−104] For example, in compounds **18** and **19**, the symmetry-related P1 phenyl and P2 isopropyl side chains are embedded in hydrophobic side-pockets. In acyclic protease inhibitors, the central linking unit between side chains (typically phenyl) has been varied to include hydroxyethylene,[105−116] dihydroxyethylene,[106,110−113,116−124] bis-(hydroxyethylene),[125] hydroxyethylamine[18] ethylenediamine,[126] and phosphinate moieties[127] linked with numerous functionalities (such as furfuran,[108] penicillin,[128] and peptidic side chains).

Illustrating these features, crystallographic analysis showed the highly potent, C_2-symmetric phosphinate inhibitor **18** of HIV-1 protease showed **18** to be



18

IC_{50} (HIV-1 protease) = 2.8 nM

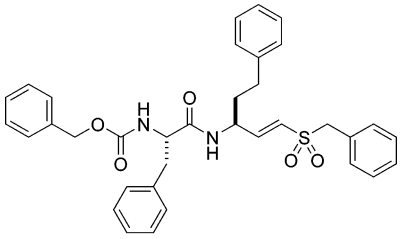


19

IC_{50} (HIV-1 protease) = <1 nM

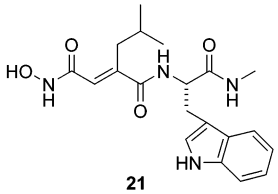
held by a set of hydrophobic and hydrogen-bonding interactions in the active site of the enzyme resulting in an extended conformation of the inhibitor.[127] In related work, structure activity relationships for two series of novel, symmetry-based inhibitors of HIV-1 protease, such as diol-based inhibitor **19**, have been described.[116] These inhibitors were designed with an arbitrary deletion of the C-terminus of the substrate, 2-fold rotation of the remaining N-terminus, a C_2 axis on the tetrahedral intermediate for cleavage of a peptide substrate, and chain-extension of the inhibitors. Examples of C_2-symmetric and pseudo-symmetric inhibitors that contain a cyclic or heterocyclic central moiety are incorporated into subsequent sections.

Crystal structures of various inhibitors with cysteine proteases show that binding occurs along the active site cleft in an extended backbone conformation. The strategies that have been pursued in the design of cysteine protease inhibitors have been reviewed elsewhere.[129−132] One approach has been the replacement of the carbonyl group with a suitable nucleophilic trapping moiety such as a carbon−carbon double bond, while still allowing maximum preservation of structural features for binding in an extended conformation. Using this concept, vinylogous amino acid esters,[133] vinyl sulfonate esters,[134] vinyl sulfones,[135] pseudopeptide enol lactones,[136] fluoroolefins,[137] as protease inhibitors or matrix metalloproteinase inhibitors,[138] have been reported. Recent examples are **20**, an inhibitor of cruzain,[134] and **21**, an inhibitor of human fibroblast collengenase (HFC).[138] Efforts to fully probe the structural requirements for inhibitors containing a carbon−carbon double are still in progress.



20

k_{ass} (cruzain) = 1.956 x 10^6 $s^{-1}M^{-1}$

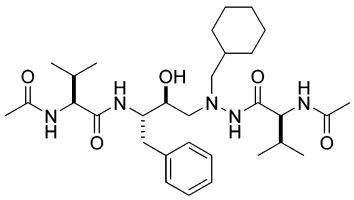


21

Ki (MMP-1) = 0.13 μM

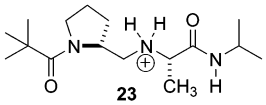
A useful class of peptidiomimetics are azapeptides, in which the α-CH group of one or more amino acid residues in the peptide chain is replaced by a nitrogen atom. Azapeptide-derived inhibitors were originally designed as substrate analogues of papain and cathepsin B,[139−141] where the bound substrate lies in an extended conformation in the enzyme. The later azapeptides have been designed as inhibitors of HIV-1 protease,[142,143] such as **22**[142] and other proteases.[144−148] The α-CH/N replacement forms an azapeptide in a different peptide conformation. Illustrative of this are crystal structures for unbound azapeptide **23** and reduced dipeptide **24**.[149] Azapep-

tide **23** has a rather extended conformation in which the C-terminal NH is hydrogen bonded to the central N nitrogen.

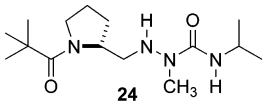


22

IC_{50} (HIV-1 protease) = 8.5 nM



23



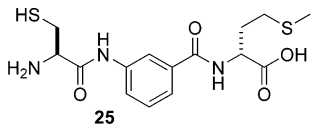
24

On the other hand, reduced dipeptide **24** is folded by an intramolecular hydrogen bond in a similar way to a β-turn structure. Aza-amino acids confer unique conformational properties to the peptide backbone. However, there are examples in which the aza-analogue residue appears to be a strong β-turn-inducing motif (such as aza-alanine, aza-asparagine)[150,151] or a bent conformation (such as aza-glycine)[152] within a peptide chain. The conformational effects of other variations, such as the incorporation of an aza group within β-lactams[153] and aza-peptide Michael acceptors[154] have not yet been fully explored.

4. Aliphatic and Aromatic Carbocycles

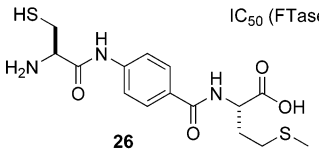
Planar aromatic spacers also have been incorporated into inhibitor design as extended dipeptide mimics in, for example, inhibitors of Ras farnesylation. Such inhibitors can slow the development of cancers in which oncogenic Ras proteins are active.[155,156] A series of tetrapeptides CA1A2X (A = aliphatic amino acid, X = Met or Ser) were potent inhibitors of FTase *in vitro*, but were inactive in cell-based assays.[157−160] Replacing the central A1A2 residues with rigid hydrophobic scaffolds to enforce an extended conformation led to potent inhibitors that were less peptidic in nature.[5,161,162] This is illustrated by compounds **25**[5] and **26**[5] (*benzene* scaffold), **27**[161] (1,5- or 1,6-*naphthalene* scaffold) and **28**[163] (*biphenyl* scaffold).

Inhibitors designed as β-strand tetrapeptide mimetics, such as **25** and **28**, are able to block Ras processing in a range of ras-transformed tumor cell lines and inhibit tumor growth in nude mice.[5,163−165] In related work, incorporation of the conformationally constrained (*L*)-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (Tic) as the A2 residue in the CA1A2X sequence resulted in a significant increase in potency that directly correlated with the proportion of extended conformation present.[162]



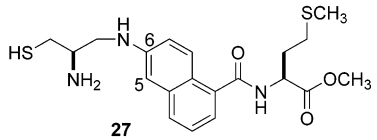
25

IC_{50} (FTase) = 6500 nM



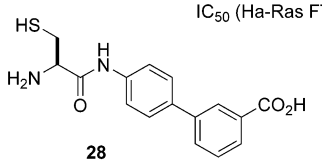
26

IC_{50} (FTase) = 50 nM



27

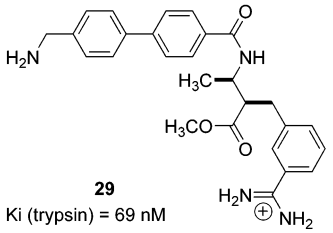
IC_{50} (Ha-Ras FTase) = 80 nM



28

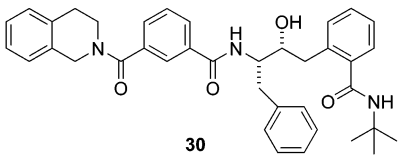
IC_{50} (FTase) = 114 nM

In another example, the crystal structure of the complex of **29** with trypsin (PDB entry: 1f0u) showed a rigid extended inhibitor containing the biphenyl scaffold,[166] the benzamidine substituent located in the S1 pocket and the aminomethyl biphenyl group in the S4 pocket. Compounds such as **30**, which incorporate substituted isophthalic acid as asparagine replacements, inhibit HIV-1 protease. In the crystal structure of **30** bound to HIV-1 protease, the inhibitor is in an extended conformation and interacts with the S3 to S2′ enzyme residues.[167]



29

Ki (trypsin) = 69 nM

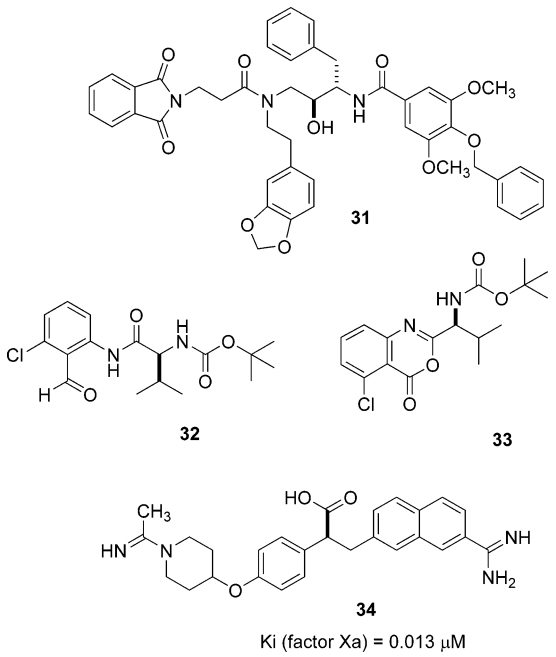


30

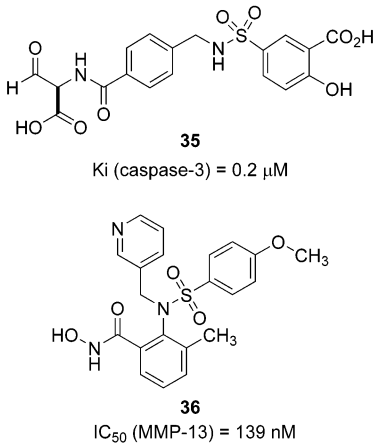
IC_{50} (HIV-1 protease) = 50 nM

Progressive shortening of the amino acid side chain linked to the aromatic spacer led to other β-strand mimetics. Carbocycles **31**[168] (PDB entry: 1lf3) and **32**[169] (PDB entry: 1inc) bind in a β-strand conformation to Plasmepsin II (an aspartic protease from *Plasmodium falciparium*) and pancreatic elastase, respectively. In the latter example, the carbocycle scaffold of **32** arises from *in situ* ring opening of the benzoxazine-2-yl ring in **33**. In another example, a crystal structure shows **34** bound to trypsin (PDB entry: 1mts)[170] in an extended conformation. The aromatic group acts as a scaffold, projecting the bulky naphthamidine moiety to the S1 site, filling it almost

completely, while the piperidine ring exhibits a chair conformation and projects into the S3 site. Compound **34** is a specific inhibitor of factor Xa, but also binds trypsin in an extended conformation.

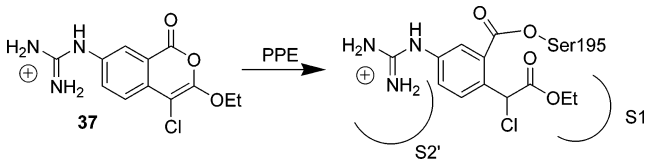


Some mimetics bear a sulfonamide moiety that orients part of the molecule away from the aromatic spacer and is not part of the β-strand mimicry. A crystal structure has demonstrated this for the inhibitor **35**[171] (PDB entry: 1nms) bound to caspase-3, the benzyl group being the scaffold. In another example, a crystal structure shows inhibitor **36**[172] (PDB entry: 1fls) bound to MMP-1 (matrix metalloproteinase-1) in an extended conformation, the inhibitor forming a β-sheet with the β-strand IV of MMP-13.



An interesting variation to the design of an aromatic scaffold was observed in the crystal structure of isocoumarin **37**[173] (PDB entry: 8est) bound to porcine pancreatic elastase (PPE). The coumarin ring opens *in situ* to provide a substituted carbocycle covalently bound to Ser195, and acting as a scaffold to position the side chains into the S1 and S2′ enzyme pockets (Scheme 1).

Scheme 1



Appropriate substitution of the aromatic spacer has led to 5-amino-2-methoxy benzamide and 5-amino-2-methoxybenzoic hydrazides (**38**)[174] being incorporated into novel β-strand mimetics to induce artificial β-sheet formation (Figure 10). These mimetics duplicate the hydrogen bonding functionality of a dipeptide and tripeptide, respectively. The specific use of this scaffold in chemical models of protein β-sheets has been described in detail elsewhere.[175] In summary, the β-strand mimetic has been located either along one edge (upper or lower)[176−180] or on the edge[181] or in the middle of a three-stranded artificial β-sheet[174] and mixed artificial β-sheets.[182] Further aggregation to antiparallel β-sheet dimers also has been observed.[177,178,183] In mixed three-stranded artificial β-sheets, the β-strand mimetic acts in conjugation with a tris-urea scaffold and forms a "corner bracket" that stabilizes the β-sheet structure. The β-strand mimetic **39** adopts an extended conformation, helps to organize the middle peptide strand, and in turn organizes the third strand in a slightly less ordered β-strand conformation[182] (Figure 11).

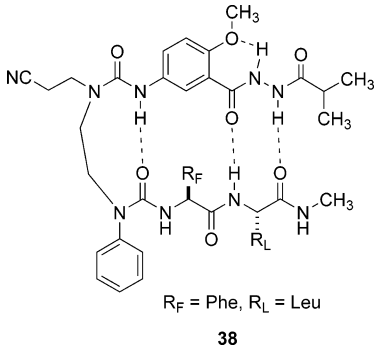


Figure 10. Schematic representation of hydrogen bonding in **38**. Hydrogen bonds are indicated by dashed lines. Adapted from ref 174.

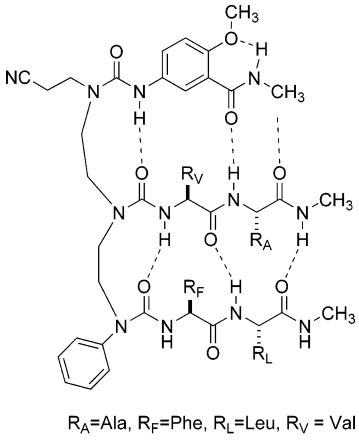
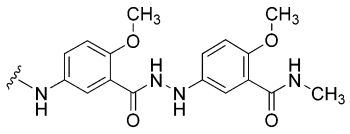


Figure 11. Schematic representation of hydrogen bonding in **39**. Hydrogen bonds are indicated by dashed lines. Adapted from ref 182.

Modification of the 5-amino-2-methoxybenzoic acid unit led to development of β-stand mimetics **40**, **41**, and **42**.[184] Mimetics **40**−**42** adopt hydrogen-bonded antiparallel β-sheet conformations and were viable β-strand scaffolds.[184] The folding of compounds similar to **38** was studied in competitive solvents. The artificial β-sheet was partially folded in methanol and aqueous methanol solutions, even though these solvents competitively bind to the hydrogen-bonding groups within the molecule.[185] This is significant as proteins fold in water, but β-sheets and β-strands typically experience a nonpolar environment in the interior of a protein. The evidenced sheet formation by these scaffolds confirms the extended nature of the scaffold[174,176,177,184,186] and suggests new approaches to β-strand mimetics with high potency. This has recently been realized for β-strand mimetic **43**,[187] which inhibits dimerization of HIV-1 protease.



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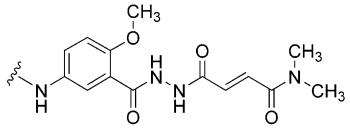

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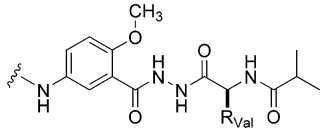

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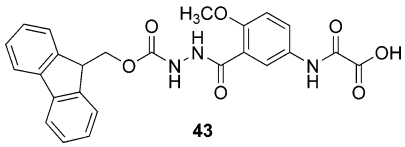
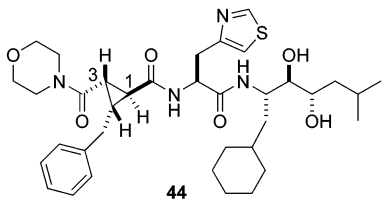


43

IC_{50} (HIV-1 protease) = 30 μM

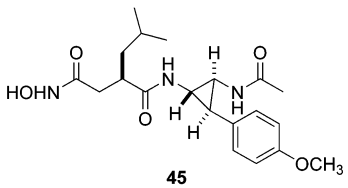
Other carbocyclic conformational constraints, which restrict the ϕ-angle to approximate the idealized β-strand, include 1,2,3-trisubstituted *cyclopropanes*, where the side chain is rigidly fixed to χ-angles corresponding to ±60°.[15,188] The cyclopropane is a novel isosteric replacement that enforces an extended β-strand conformation and projects the amino acid side chains in a specific orientation. A trans arrangement at C1 and C3 of the cyclopropyl group was required for an extended β-strand conformation, whereas a cis arrangement induced a turn in the backbone. Potent inhibitors of the aspartic protease renin (**44**)[15] and MMP-1 (**45**)[189] have been realized using this constraint.

Another constrained system has been observed in the polycyclic cage, which contains *cyclobutane,* of novel nonpeptidic HIV-1 protease inhibitor **46**.[190] The crystal structure of **46** complexed with HIV-1 protease revealed the central polycyclic cyclobutane cage and the hydroxymethylene substituents approximating the C_2-symmetry of the enzyme.[191]



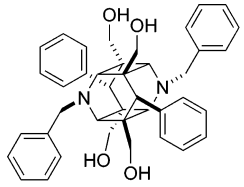
44

IC_{50} (renin) = 10 nM



45

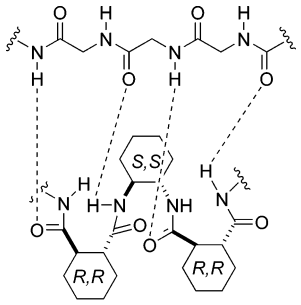
IC_{50} (MMP-1 HFC) = 0.054 μM



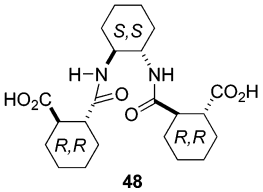
46

Ki (HIV-1 protease) = 7.8 μM

In a less constrained aliphatic carbocycle, *cyclohexane* has been used to construct a novel receptor motif with complementarity for a tripeptide in an extended conformation. Oligomers were constructed from monomers of cycloalkyl-1,2-trans-diamines of one stereochemistry alternated with cycloalkyl-1,2-trans-dicarboxylic acids of the opposite stereochemistry, which adopt a flat extended conformation. The dipole−dipole directed alignment of the amide linkages presents an alternating array of hydrogen-bond donors and acceptors above and below the plane defined by the cyclohexane rings, reminiscent of the antibiotic vancomycin, which binds the peptide backbone in an extended conformation (**47**, Figure 12). The fragment is large enough to span a tripeptide sequence. The conformational preference for the



47

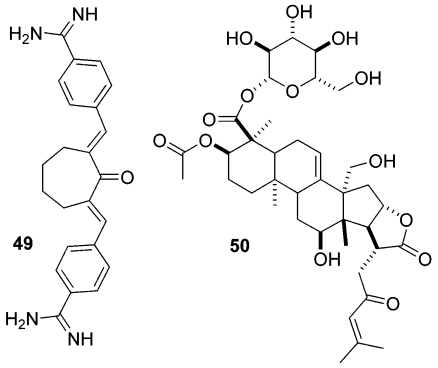


48

Figure 12. Schematic representation of **48** and of hydrogen bonding in **47**. Hydrogen bonds are indicated by dashed lines. Adapted from ref 192.

unbound receptor motif **48** was verified from crystallographic studies.[192]

In another aliphatic carbocycle, a crystal structure of *heptanone* **49**[193] (PDB entry: 1a5h) bound to human tissue-type plasminogen activator showed the cycloheptanone ring spacer projecting two benzamidines in an extended conformation by virtue of the trans−trans conformation of its exo double bonds. This simple, symmetrical structure (**49**) inhibited factor Xa. The different carbocycle, of tripene **50**,[194]



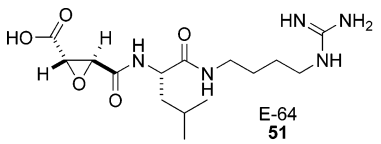
49 **50**

isolated from the leaves of the plant *Lantana camara*, was bound to human α-thrombin (PDB entry: 1awf), with the 5,5-trans lactone ring opened in the complex and a covalent bond between Ser195 and the carbonyl group of the lactone formed to anchor **50**. The enone side chain occupies the S1 pocket allowing the *steroid* ring to participate in a β-strand conformation.

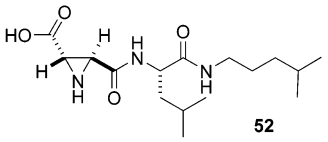
5. Ligands Containing One Ring with One Heteroatom (N)

Whereas carbocycles, particularly aromatic scaffolds, feature in interesting examples of rigid β-strand mimetics, ligands with one ring and one heteroatom (nitrogen) represent the largest general class of β-strand mimetics. One of the smallest scaffolds in this general class is the three-membered ring of aziridine. Development of irreversible and selective cysteine protease inhibitors has been based on the discovery of E-64 (**51**),[195,196] a potent inhibitor that binds in the nonprime subsites of the enzyme. The aziridine ring is closely related to the epoxide, similarly susceptible to ring opening by nucleophiles, and can be derivatized at its heteroatom.

Aziridine analogues of **51**, such as **52**[197] and N-acylated **53** and **54**,[198] revealed an interesting difference in inhibition and selectivity dependent on the position of aziridine within the peptide chain, and whether the aziridine was in the protonated ring form. For example, **54** is a much more selective and potent cathepsin L inhibitor. The results were explained by the different binding modes with respect to their orientation in the S and S′ binding sites of the enzymes. In docking experiments of **53** with papain, the phenylalanine residue binds as part of an extended backbone conformation in the hydrophobic S2-pocket with the Boc group located in the S3 subsite.[198] The recent isolation of miraziridine, a cathepsin B inhibitor, may also provide a lead for developing other analogues.[199]

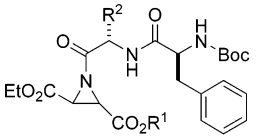


E-64
51



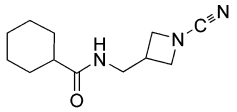
52

k_{inac}/K_i (papain at pH 6.5) = 1.8 (10^3 $M^{-1}s^{-1}$)

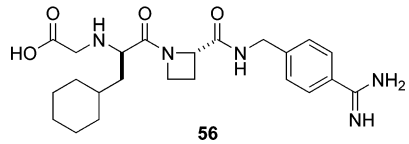


53 R^1 = Et, R^2 = CH_3
K_i (papain at pH 6.5) = 0.020 mM
54 R^1 = Bzl, R^2 = H
K_i (papain at pH 6.5) = 0.069 mM

Analogues of four-membered ring of 1-cyano*azetidine* inhibit human cathepsins K, L, and B, **55** being a potent inhibitor. A one-step binding mechanism of the cyanoazetidine inhibitors to the enzyme was reported in conjunction with NMR studies on a related cyanopyrrolidine compound, suggesting that a covalent enzyme−inhibitor isothiourea ester bond forms, allowing the inhibitor to extend from the active site.[200] Another example of an azetidine ring is contained within **56**, its crystal structure[201] (PDB entry: 1k1p) showing the azetidine ring occupying the S2 pocket of trypsin to form β-sheet hydrogen bonding.



55
IC_{50} (Cathepsin K) = 0.005 μM



56

The next examples belong to the five-membered ring of proline. Peptidomimetic *proline*-containing antagonists of human leukocyte elastase (HLE), a serine protease implicated in adult respiratory distress syndrome (ARDS) and many other human inflammatory conditions, have been developed from crystallographic studies of the peptidic trifluoromethyl ketone **57** bound to the closely related enzyme porcine pancreatic elastase (PPE)[202,203] (PDB entry: 4est). Critical hydrogen-bonding interactions between the β-sheet region of Val216 and the inhibitor prompted the incorporation of a P3 to P2 conformational restraint, as seen in compound **58** (Figure 13).[204]

Two examples of a *pyrrolidine* scaffold were identified from the PDB. A crystal structure shows inhibi-

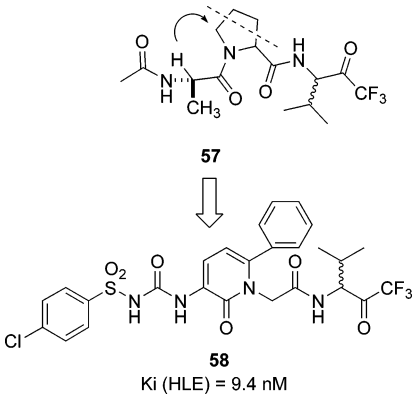
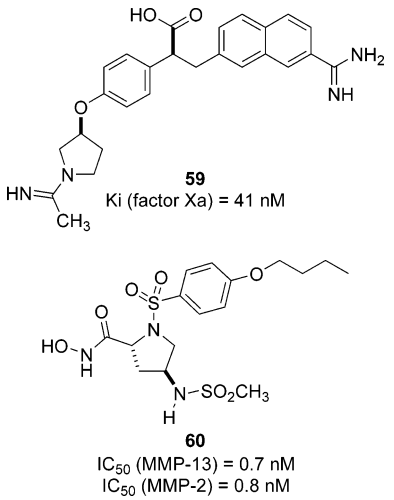


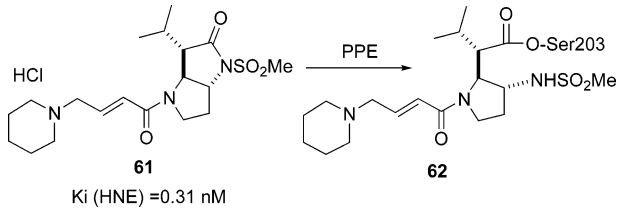
Figure 13. Schematic representation of the design of inhibitor **58** from the peptide **57**. Adapted from ref 204.

tor **59**[205] bound in β-strand conformation of the active site of human coagulation factor Xa (PDB entry: 1fax), the pyrrolidine ring occupying S4 of factor Xa. Similarly, in the crystal structure of MMP-3 (stromelysin) with inhibitor **60**[206] (PDB entry: 1g49), a β-sheet hydrogen-bonding pattern is observed between the enzyme and the hydroxamic acid and sulfonamide moieties, a consequence of the positioning of these groups by the pyrrolidine scaffold.

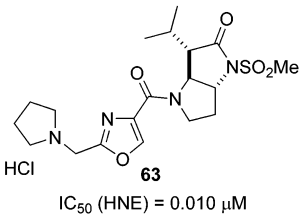


In another variation, the trans-lactam **61**[207] undergoes *in situ* ring opening to a pyrrolidine analogue **62** being substituted at the N2 and 3 positions, which covalently binds to Ser203 of PPE (Scheme 2). In both

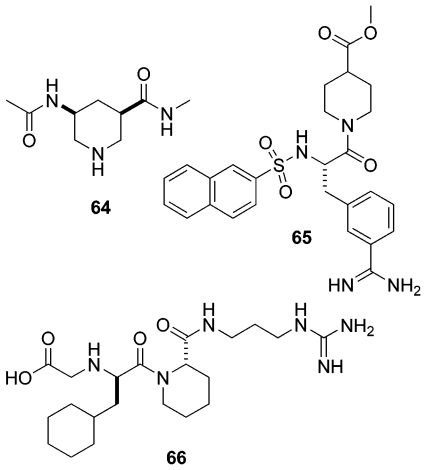
Scheme 2



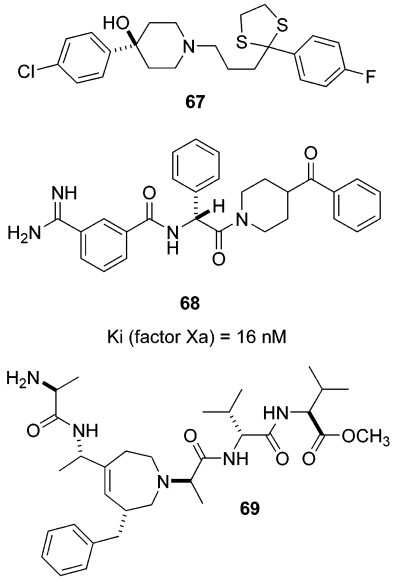
crystal structures of **61** with PPE[207] (PDB entry: 1hv7) and **63** with HNE[208] (PDB entry: 1h1b) the pyrrolidine N-side chain lies in an extended conformation with good shape complementarity for the active site.



3,5-Disubstituted *piperidine* rings such as **64**[209] represent a conformationally constrained β,γ-diamino acid derivative that mimics a hydrogen-bonding pattern β-strand. When the piperidine ring has a 1,4-substitution pattern, β-sheet hydrogen bonding has been observed, such as in the crystal structure (PDB entry: 1k1j) of **65** with trypsin.[201] Similarly, the piperidine ring of **66**[201] (PDB entry: 1k1o) occupied the S2 pocket, with a short β-ladder formed via two hydrogen bonds between the adjacent amino and carbonyl groups of cyclohexylalanyl/cyclohexylglycyl, respectively, and Gly216 CO and Gly216 NH of trypsin.

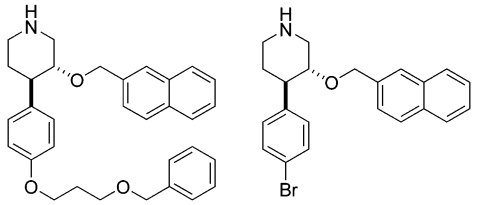


Crystal structures of **67**[210] with HIV-1 protease (PDB entry: 2aid), **68**[211] with trypsin (PDB entry: 1eb2), and a seven-membered analogue of piperidine **69**[212] with HIV-1 protease (PDB entry: 1hbv) simi-



larly show binding to respective proteases in an extended conformation.

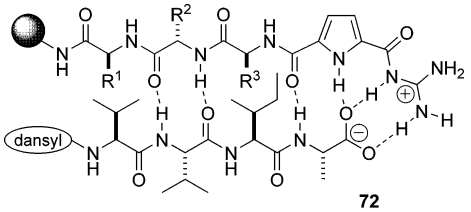
Piperidine scaffolds sometimes display unexpected binding modes. In the crystal structures for the aspartic protease renin, complexed with **70**[213] (PDB entry: 1uhq) and **71**[214] (PDB entry: 1pr7), the protonated nitrogen of the piperidine ring is positioned between two catalytic aspartates and forms strong hydrogen bonds with the enzyme. Induced fit adaptation of the piperidine substituents leads to the hydrophobic 2-naphthyl-methoxy group occupying the large hydrophobic and contiguous subsites S1 and S3. The 4-phenyl ring of the inhibitor **70** occupies a position close to that formerly occupied by the side chain of Tyr75, and disrupts the hydrogen bonding at this location. Further substitution of the piperidine ring at the 5-position may extend the β-strand into the S2′ pocket of renin.[215]



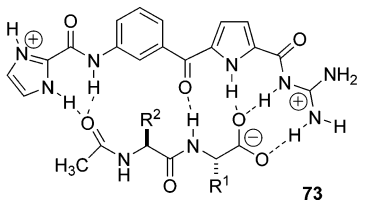
70 IC_{50} (renin) = 8.0 nM

71 IC_{50} (renin) = 5.0 μM

A recent new class of a *de novo* designed one-armed tripeptide-based guanidinocarbonyl *pyrrole* receptors has been reported.[216−218] The cationic guanidinocarbonyl pyrrole group acts as a carboxylate binding site, tethering the tripeptide substrate. The tripeptide unit of the receptor **72** provides binding sites for the formation of hydrogen-bonded antiparallel β-sheet and, in doing so, induces a β-strand conformation in the backbone of the tetrapeptide substrate of **72**. A combinatorial approach to the generation of such receptors, and binding studies established that complex formation is controlled by a balanced interplay of hydrophobic and electrostatic interactions.[218] Combinatorial approaches to synthetic receptors have been reviewed further elsewhere.[219] In a further variation, the receptor **73** stabilizes a complex with a dipeptide backbone, through hydrogen bonds with the carbonyl, amide, and imidazole NH groups. NMR spectroscopy studies of **73** showed a clear change in coupling constants upon binding of the dipeptide with **73**, and thus were in good agreement with the binding mode depicted in Figure 14.[216,220]



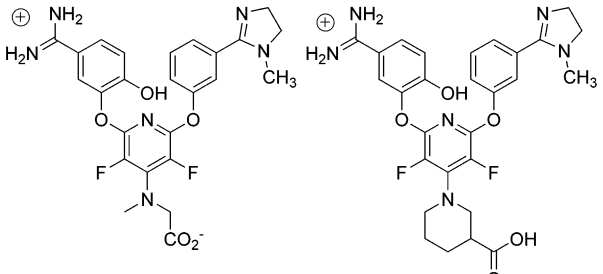
72



73

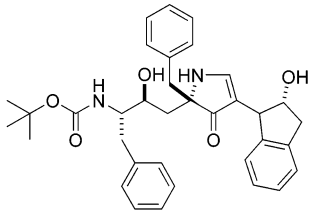
Figure 14. Schematic representation of hydrogen bonding in **72** and **73**. Hydrogen bonds are indicated by dashed lines. Adapted from refs 216 and 220.

The rigidity, planarity, and solubility of *pyridines* have made them useful as scaffolds to position side chains in a β-strand conformation. In **74** and **75**, the benzamidine group fits into the S1 pocket and is tethered to the 2,4-substituted pyridine scaffold, and the second basic/hydrophobic moiety, the methyl imidazoline fits snugly in the S4 pocket of the enzyme. Thus, in the crystal structure of **74**[221] with factor Xa (PDB entry: 1fjs), and **75**[222] with trypsin (PDB entry: 1qb1), the inhibitors have an extended structure.

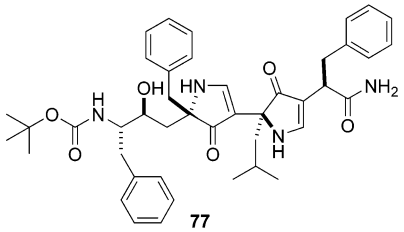


74 Ki (factor Xa) = 0.11nM

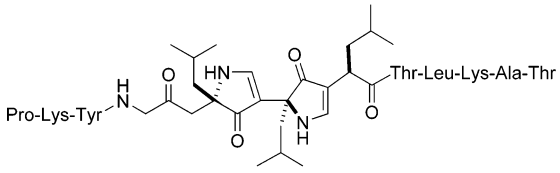
75



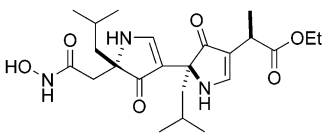
76 IC_{50} (HIV-1 protease) = 12 nM



77 IC_{50} (HIV-1 protease) = 10 nM



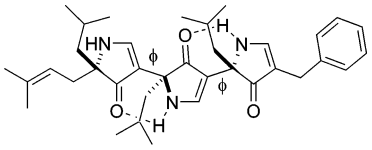
78 IC_{50} (MHC HLA-DR1) = 137 nM



79
Ki (MMP-2) = 2.9 μM
Ki (MMP-7) = 6.4 μM
Ki (MMP-15) = 6.8 μM

Incorporation of a ketone into the ring, such as *pyrrolinones* (five-membered) and *pyrrolidinones*, has a dramatic impact on the hydrogen-bonding capacity of the scaffold. Strand mimetics have been developed using *pyrrolinones* (**76**,[223] **77**,[46] **78**,[224] **79**[225]) within novel scaffolds that replace the hydrolyzable backbone of bioactive peptides. These mimetics are stable to proteases,[223] and have an extended conformation both in solution and solid states.[14,226,227]

Pyrrolinones, such as **80**,[228] closely mimic the positions and orientations of the backbone carbonyls and side chains,[229] maintain key side chain interactions and some of the intermolecular hydrogen bonds made with the enzyme,[46,226,230] and are stabilized by intramolecular hydrogen bonding between adjacent rings, with dihedral angles between rings (ϕ angles) of about 205°.[14,226] Two different pyrrolinone systems have been developed, the 3,5- and 2,5-linked scaffolds. The 3,5-system has an all carbon backbone (**76**−**79**), while the 2,5-system has backbone NHs. It is envisaged that the 3,5-system would be employed when hydrogen bonding to enzyme amide NHs is most important, while the 2,5-system would be more useful when hydrogen-bond donors are required.

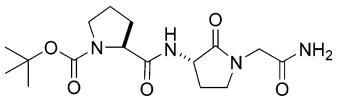


80

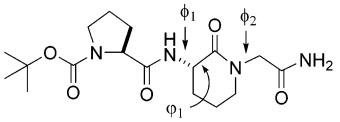
The 3,5-linked pyrrolinone scaffold has been successfully incorporated in inhibitors of HIV-1 protease,[46,223,231] matrix metalloproteases (MMPs),[225] and HLA-DR1.[224,232,233] High potencies for enzyme inhibition are observed, in addition to enhanced cellular uptake and bioavailability.[46,223] The binding of peptides to class II MHC can be inhibited by oligopyrrolinone-peptide hybrids,[232] which suggests that constrained β-strand mimetics in general warranted further investigation as potential drugs. Where X-ray crystallography, combined with model-fitting of the pyrrolinone inhibitor to the enzyme, has been possible, remarkably good mimicry of peptides has been observed.[232] Further work on the pyrrolinone scaffold has included a second-generation asymmetric synthesis of poly-pyrrolinones using scalemic α-amino lactones.[225,234] In addition, a three-step iterative synthesis of poly-pyrrolinones on solid-support has been reported, suggesting that the construction of a wide variety of libraries based on the pyrrolinone scaffold should be possible.[228]

In *pyrrolidinones* such as **81**, the ring is incorporated into the peptidic backbone. In related strategies toward conformational restriction of peptides, incorporating the backbone into a Freidinger lactam structure has proven useful in the design of inhibitors.[235] Cyclization ($C_i^{\alpha} \rightarrow N_{i+1}$) to form Freidinger lactams, loosely defined to include γ-, δ-, and ϵ-lactams, produces a peptidic backbone constraint that maintains trans amide bond configurations, significantly limiting attainable ψ angles ($-125 \pm 10°$),[236] and in turn biases neighboring ϕ_1 and ϕ_2 torsional angles. Generally considered a type II′ β-turn mimetic, conformational analyses[235,237] of lactams in some systems by NMR, X-ray, and modeling indicate a discrete extended motif in the lactam backbone ($C_i^{\alpha} - C_i^{CO} - N_{i+1}$).

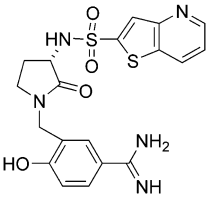
Crystallographic studies[238] of this constraint in lactam derivatives of **81** and **82** have clearly identified an extended conformation. In a five-membered ring, the lactam **83** also binds in an extended conformation to trypsin (PDB entry: 1f0t).[166] The carbonyl oxygen of the lactam (pyrrolidinone) ring forms a hydrogen bond with Gly219 of trypsin. The lactam scaffold projects the thienopyridine ring into the S4 pocket and the hydroxybenzamidine establishes a salt bridge in the S1 pocket.[166]



81

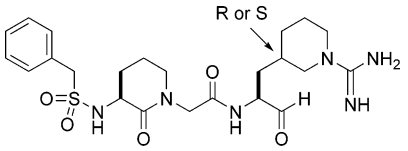


82



83 Ki (factor Xa) = 072 nM

In the six-membered piperidinone, the crystal structures of the selective thrombin inhibitors **84** (PDB entry: 1zzz)[239] and **85** (PDB entry: 1yyy)[239] bound to trypsin show that the main chains make three antiparallel β-strand hydrogen bonds with the Ser214−Gly216 residues of trypsin. The hydrogen bonds of the β-strand are reflected in tight binding.[239]



84 R Ki (thrombin) = 4.4 nM; Ki (trypsin) = 7.3 μM
85 S Ki (thrombin) = 0.32 nM; Ki (trypsin) = 8.1 μM

Similar observations[240] were made on the seven-membered lactams **86** and **87** (Figure 15). The cis diastereomer **87** in this series was observed by crystallography to have an extended conformation, while the trans isomer **86** formed the expected β-turn. More interestingly, NMR solution studies of the cis isomer **87** in noncoordinating solvents indicated head-to-tail self-association. This type of interaction was only weakly apparent for the trans isomer **86**.[240] These observations suggest that cyclic constraints of this type, although generally associated with turn structures, are also able to access extended conformations.

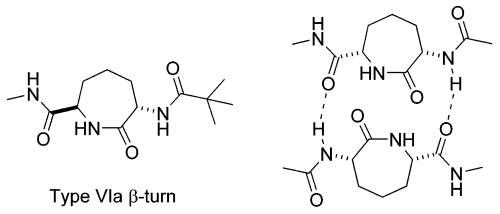
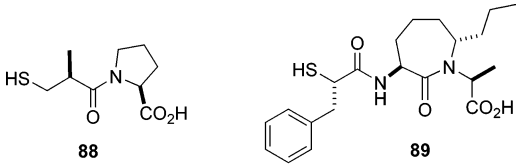
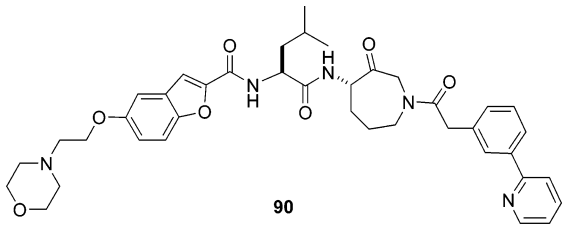


Figure 15. Lactams **86** and **87**; shown by crystal structures to form an extended conformation. Hydrogen bonds are indicated by dashed lines.

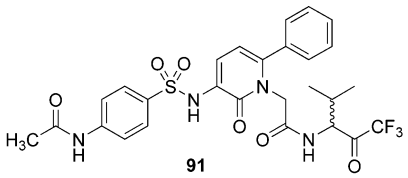
Appropriately functionalized lactams also have been found to be potent inhibitors of the metalloprotease ACE,[241−244] which has been proposed to select for a conformationally extended pharmacophore.[245] The use of these lactams as potential ACE inhibitors originated from attempts to improve the activity of captopril **88**, the first orally active ACE inhibitor, by restricting both the available ψ angles and the configuration of the amide bond.[246,247] Subsequent work identified a number of potent lactam-based inhibitors of both ACE and the related metalloprotease neutral endopeptidase (NEP) **89**.[248] Discussion of lactams incorporated within two ring systems is reserved for sections 9 and 10.

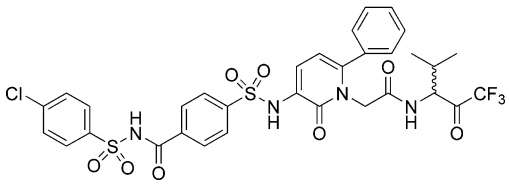


In a related structure (PDB entry: 1nl6), the *hexahydroazepinone* inhibitor **90** has been cocrystallized within the active site of the cysteine protease cathepsin K.[249] The inhibitor **90** spans the S3−S3′ binding region in an extended conformation where the azepanone ring places the side chains appropriately for β-sheet hydrogen bonding with Gly66 and Trp184 of cathepsin K.[249]

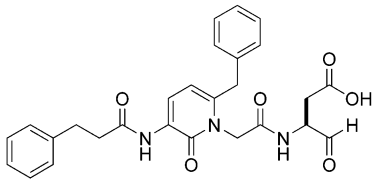


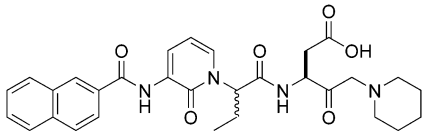
Incorporation of unsaturation within a lactam ring increases the rigidity of the scaffold. The *pyridone* ring is an example of such a scaffold, and acts as a P3 to P2 conformational restraint. A number of potent inhibitors of HLE based on this scaffold, such as **91**[250] and **92**,[251] have been developed.[251,252] Subsequent crystallography studies of **91** bound to PPE[250] (PDB entry: 1eas) demonstrated the successful retention of this hydrogen-bonding pattern,[253] via β-sheet hydrogen bonds between the pyridone carbonyl oxygen and the pyridone 3-amino nitrogen with PPE.

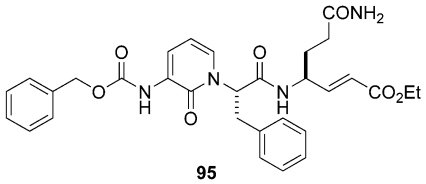




This structural constraint was subsequently adopted in the design of antagonists of interleukin-1β converting enzyme (ICE),[148,254] a cysteine protease that cleaves prointerleukin-1β to generate biologically active mature IL-1β,[255] a cytokine that elicits inflammatory responses *in vivo*.[254] A mimetic was sought to replace the P3−P2 Val−Ala dipeptide segment of the native substrate but maintain the P3 carbonyl and NH, to ensure integrity of this β-sheet hydrogen-bonding motif.[148,256−258] These compounds were optimized by manipulation of peripheral alkyl and aryl substituents to produce inhibitors of HLE such as **92**, of ICE such as **93**[148,257] and **94**,[258] and inhibitors of human rhinovirus 3C protease (HRV 3CP) such as **95**,[259] with extended side chains providing two further sites of hydrogen bonding.

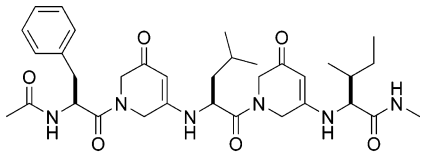






A further variation has included the use of the 1,2-dihydro-3(6H)-*pyridinone* unit (or azacyclohexenone unit and termed Ach or @) as a β-strand mimetic.[260−262] Alternating oligomers that contain the cyclic amino acid replacement were termed @-tides. NMR studies

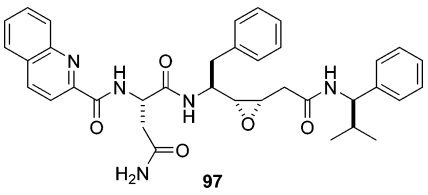
of the penta-@-tide **96**[261] supported a β-sheet model of dimerization that indicated the ability of **96** to mimic a β-strand. The synthesis of the @-tide unit and solution and solid-phase methods for its incorporation into an oligomer alternating with peptide units has been devised.[261]



96
@-tide

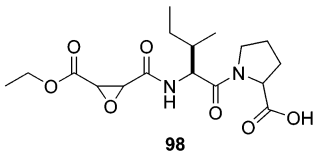
6. Ligands Containing One or Multiple Rings with One Heteroatom (O, S)

One dipeptide isostere in irreversible inhibitors of HIV-1 protease is the cis-epoxide. The *epoxide* is used as an amide isostere for the scissile peptide bond[263] and is contained within pseudo C_2-symmetric inhibitors.[264] NMR studies of inhibitors containing a cis-epoxide, such as **97**, have identified the conformational preference of the inhibitors in solution.[265] Epoxide **97** adopts an extended conformation similar to the β-strand. The epoxide plays a key role in stabilizing extended conformations, as the torsion angles about the bonds attached to the epoxide are greater than 130°. Epoxides have been used as electrophiles for nucleophilic attack by cysteine proteases leading to alkylation and inhibition of the enzyme.[266] As found in the crystal structure of cathepsin B inhibited with epoxide **98** (PDB entry: 1csb),[267] upon binding the epoxide is ring opened, and the chain of **98** lies in an extended conformation. The isoleucine-proline segment mimicks the substrate P1′ and P2′ residues, and the ethyl ester group occupies the S2 site.

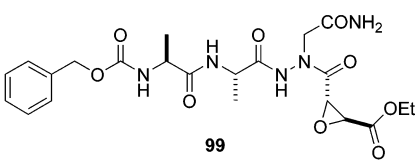


97

IC_{50} (HIV-1 protease) = 10-50 nM



98



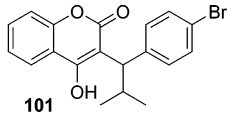
99

k/[I] (caspase-3) = <10 $M^{-1}s^{-1}$

Recently, *aza-peptide epoxides*, a class of irreversible protease inhibitors specific for the clan CD cysteine proteases, have been reported.[154,268,269] In most caspase inhibitor complexes, an antiparallel β-sheet interaction forms between the peptide chain of the inhibitor and active site residues of the caspase. Notably, aza-peptide epoxides, such as **99**,[268] bind in such a manner but in the same direction as the normal peptide substrate. A *S*,*S*-stereochemistry of the epoxide moiety gave more potent inhibitors than the *R*,*R*-isomer. From a synthetic perspective, aza-epoxides offer potential as inhibitors as they are easily extended in the P′ direction, allowing interactions with the S′ subsites of the enzyme.

In larger rings containing an oxygen, an interesting concept in the design of inhibitors of HIV-1 protease and HIV integrase has been the use of a scaffold to project hydrophobic substituents into the appropriate P2, P1, P1′, and P2′ positions for interaction with the enzyme. Although these compounds do not mimic all the protease-binding hydrogen-bond donors and acceptors of peptide strands, they can still be considered as constrained peptidomimetic scaffolds in which the ring substitutents do occupy the same spaces within the protease active site as amino acid side chains of linear peptide β-strands. The idea is embodied in *coumarin* (**100**) derived inhibitors[270−273] of HIV integrase and HIV-1 protease, the cycle serving as a central scaffold upon which substituents can be mounted to mimic the side chains of peptides.

100

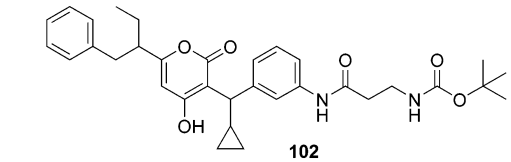


101

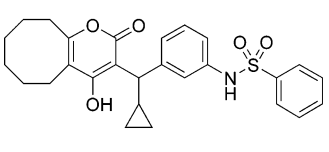
In an example, the crystal structure of **101** in a complex with HIV-1 protease (PDB entry: 1upj) revealed a binding mode in which the lactone oxygen atoms hydrogen bond to the enzyme and the inhibitor lies in an extended conformation spanning S1′ to S2 of the binding site.[271] This structural information provided the rational basis for structure-based design of active analogues.[272] Further modifications include placement of substituents at the meta carbon of the phenyl ring in **101**, and the design and synthesis of inhibitors such as **102**−**106**.

Support for the mode of binding and extended structures was derived from the crystal structures with HIV-1 protease of **102**[271] (PDB entry: 2upj), **103**[274] (PDB entry: 7upj), **105**[275] (PDB entry: 1d4s) and **106**.[276] In the study associated with the identification of **104**[272] as an effective inhibitor of HIV-1 protease,[273] it was recognized that the 5,6-dihydro-4-hydroxy-2-pyrone scaffold incorporated side chains at the C-6 position that appropriately extended into the S1′ and S2′ subsites of the enzyme.

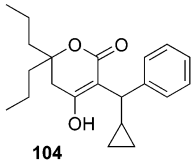
In the crystal structure of **105** with HIV-1 protease, nearly symmetrical hydrogen bonding between the 4-hydroxyl group and the catalytic aspartic acids was observed.[275] This anchored the scaffold with the phenethyl and propyl groups at C-6, projecting into the S1′ and S2′ subsites respectively, and with the ethyl and phenyl groups from C3 occupying the S1 and S2 subsites respectively. In related work, the general arrangement of two aromatic units separated by a central linker has emerged as a common motif



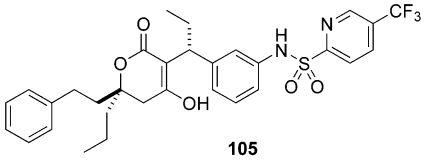
102
Ki (HIV-1protease) = 0.041 μM



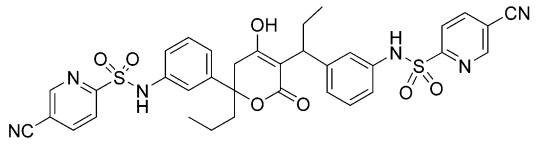
103
Ki (HIV-1 protease) = 3.2 nM



104
Ki (HIV-1 protease) = 15 nM
IC_{50} (HIV-1_{IIIB} infected MT4 cells) = 5 μM



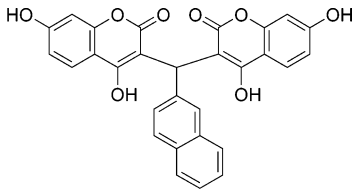
105
Ki (HIV-1 protease) = 0.008 nM



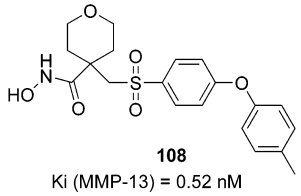
106
Ki (HIV-1 protease) = 0.1 nM

for a large number of HIV−integrase inhibitors. The use of a coumarin-based structure, in which the bottom half places simple aryl rings, such as **107**,[270] supports β-stranded hydrogen bonding across two coumarin rings.

Another example of a heterocycle with one oxygen atom is the *pyran* ring scaffold of inhibitor **108**. Its crystal structure in complex with MMP-13 (metallomatrix protein human collengenase-3)[277] (PDB entry: 966c) shows the sulfone oxygen as a β-strand hydrogen-bond acceptor, with the diphenyl ether substituent in an extended conformation deep within the S1′ pocket of MMP-13.

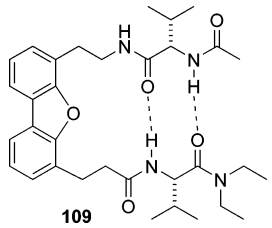


107
IC_{50} (HIV-I integrase) 3′-processing 4.2 μM



108
Ki (MMP-13) = 0.52 nM

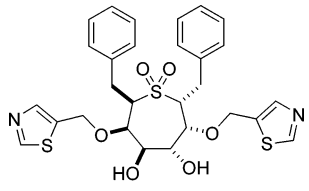
One aspect of β-strand mimicry is that of β-sheet nucleation. Although not in the scope of this review, scaffolds that nucleate β-sheet formation via formation of a β-strand hydrogen-bonded network should be mentioned. Various scaffolds that nucleate β-sheet formation have been reported,[278−282] and an example illustrating this concept is that of the dibenzofuran **109** (Figure 16). In the hexapeptide analogues of **109** the dibenzofuran scaffold was necessary, but not sufficient, to stabilize the β-sheet structure. A side chain sequence that facilitated intrastrand hydrophobic interactions was also important,[283,284] a feature demonstrated for aminomethoxybenzamides (section 4).



109

Figure 16. Schematic representation of the hydrogen-bonding pattern of **109**. Hydrogen bonds are indicated by dashed lines. Adapted from refs 283 and 284.

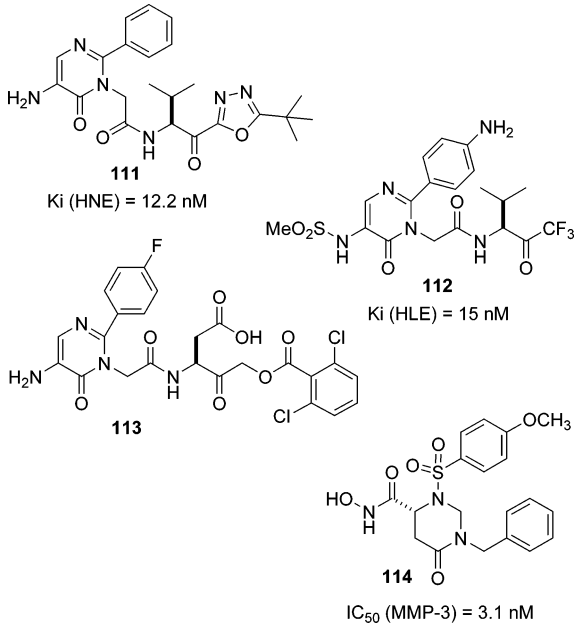
An example of a sulfur-containing one ring system can be found in the nonpeptidic *cyclic sulfone* inhibitors of HIV-1 protease.[285] A crystal structure showed that inhibitor **110** binds to HIV-1 protease in a highly symmetric fashion. The sulfonyl group and the two hydroxyl groups provided anchoring hydrogen bonds, while hydrophobic interactions between the substrate S1, S2, S1′, and S2′ enzyme residues and inhibitor side chains were observed.



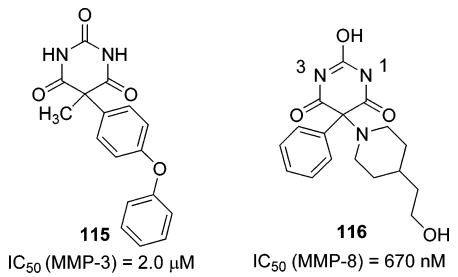
110
IC_{50} (HIV-1 protease) = 0.3 nM

7. Ligands Containing One Ring with Two Heteroatoms (N, N)

A scaffold related to pyridones (section 5), the *pyrimidone* displays a similar hydrogen-bonding pattern, in which the extra N atom in the ring is not involved in hydrogen bonding. Crystal structures of PPE bound to **111**[286] (PDB entry: 1fzz) and to **112**[253] (PDB entry: 1eat) demonstrated retention of a β-sheet format in the enzyme. For **111**, the side chain of P1 (Val) was inserted into the S1 pocket of PPE.[286] In related work, examples of peptidomimetic inhibitors of ICE, such as **113**,[254] also demonstrated that the pyrimidone ring was an effective P3−P2 (Val−Ala) strand mimetic. Saturation of the heterocyclic ring still provides a scaffold capable of projecting substituents into the S1′ and S2′ binding sites. From the crystal structure of the inhibitor **114** with MMP-3[287] (PDB entry: 1d8m, 1g05), the alkyl group attached to the distal nitrogen of the *hexahydropyrimidine* ring was found to extend toward the S2′ pocket.

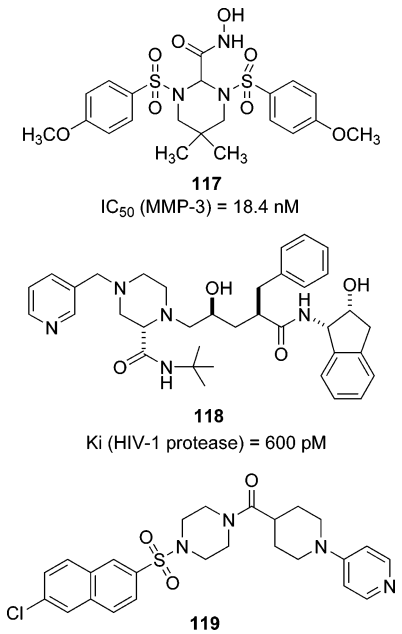


Introduction of further sites of oxidation into the heterocyclic ring led to *pyrimidine triones*. These scaffolds mimic substrates in forming hydrogen bonds to key residues in the active site, providing opportunities for placing appropriately chosen groups in the S1′ specificity pocket of MMPs. The crystal structure of inhibitor **115** with MMP-3[288] (PDB entry: 1g4k) clearly demonstrated these design features. Pyrimidine triones are able to form tautomers. In a related example, as shown in the crystal structure of **116** with MMP-8 (human neutrophil collengenase)[289] (PDB entry: 1jj9), inhibitor **116** chelates zinc through the N3 and OH of the barbiturate ring and rigidly orientates the two cyclic substituents into the S1′ and S2′ substrate-binding sites.



Removal of all sites of oxidation in the heterocyclic scaffold led to the use of hexahydropyrimidines (1,3-piperazines and 1,4-piperazines). In the 1,3-piperazines, a plane of symmetry of the inhibitor was maintained and thus substitution was on both N atoms in the ring. The change in substitution pattern provided inhibitors of MMP-1 and MMP-3 with an extra, unique interaction to those observed previously for inhibitors **114**.

The crystal structure of inhibitor **117** with MMP-3[290] (PDB entry: 1bqo), the second alkoxy aryl sulfonyl group binds to the S1 and S2 pocket, whereas the 1,4-piperazine in **118** orientates the *tert*-butyl substituent to bind in the S2 pocket with the benzyl and indanyl groups in the S1′ and S2′ pockets, respectively. This was demonstrated by the crystal structure of inhibitor **118** with HIV-1 protease[291] (PDB entry: 1hsg), where hydrogen bonds also contributed to the tight formation of the enzyme−inhibitor complex. The 1,4-piperazine **118** is a potent inhibitor of HIV-1 protease and is currently used to treat humans infected by HIV.[292] Another 1,4-piperazine **119** inhibits factor Xa, which, when complexed with bovine trypsin, induced a factor Xa-like conformation in trypsin, as shown by a crystal structure[293] (PDB entry: 1ql7).



Unlike the six-membered heterocyclic scaffolds described so far, *aminopyrazole* is a singular example of a five-membered scaffold. Several rigid 3-aminopyrazole derivatives, such as **120**,[294] have been reported. The 3-amino pyrazole unit is effectively linear, and is capable of stabilizing the β-sheet conformation of N/C protected dipeptides and tripeptides through three simultaneous hydrogen bonds on one face of a peptide strand (Figure 17).

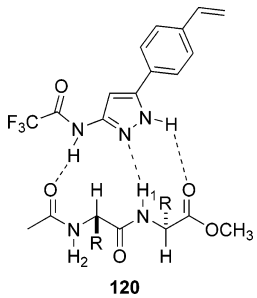
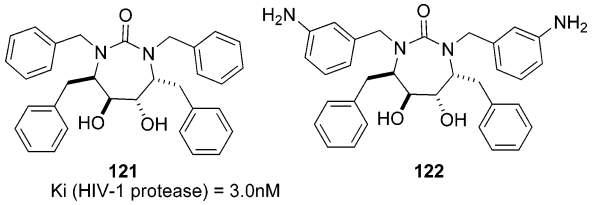
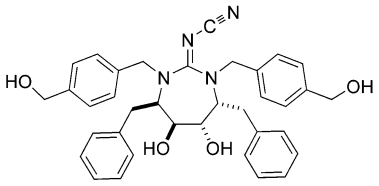


Figure 17. Schematic representation of hydrogen bonding between **120** and a peptide chain. Hydrogen bonds are indicated by dashed lines. Adapted from ref 294.

Expansion of the ring to include seven atoms leads to the use of urea scaffolds. Similar to the concept discussed in section 6, a series of *cyclic urea* inhibitors of HIV-1 protease have used five- to seven-membered cyclic ureas, such as **121**,[295] as scaffolds to project benzylic and related hydrophobic substituents into appropriate P2, P1, P1′, and P2′ positions





for interaction with the enzyme.[295−297] The carbonyl group of the urea ring serves to replace the water molecule within the enzyme (water 301 molecule for HIV-1 protease). The central location of the cyclic urea scaffold enables projection of substituents into the enzyme pockets as illustrated in Figure 18 by the

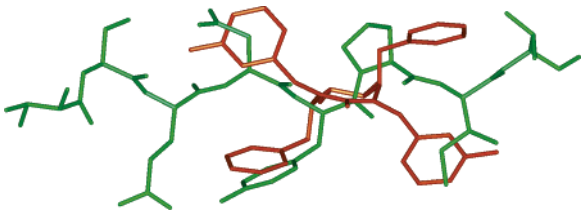


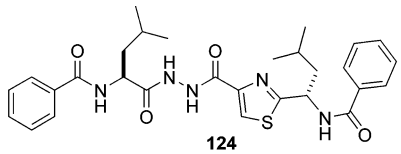
Figure 18. Comparison HIV-1 protease binding conformations for cyclic urea inhibitor **122** (PDB entry: 1dmp) (orange) and linear peptidic substrate (PDB entry: 1mt7) (green). Images were generated using Insight II.

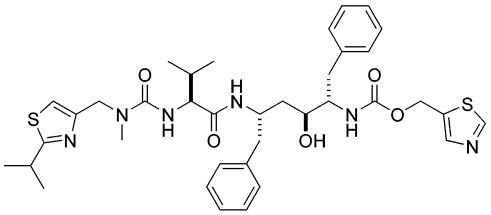
complex of **122** with HIV-1 protease[298] (PDB entry: 1dmp). In extensions of this work, guanidines also were developed as cyclic inhibitors capable of displacing the structural water molecule. As found in the crystal structure of **123** bound to HIV-1 protease[299] (PDB entry: 1hvh), the P1 and P1′ groups retained a symmetrical conformation. The P2 and P2′ groups of the guanidine **123** were in an unsymmetrical conformation due mainly to the presence of the cyano group.

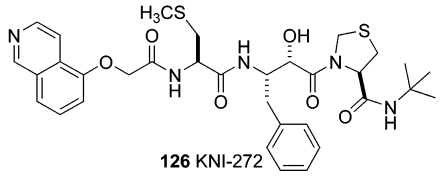
8. Ligands Containing One Ring with Two Heteroatoms (N, S) or Three Heteroatoms (N, N, S or N, N, N)

Alternative five-membered heterocyclic rings used as proline replacements in medicinal chemistry include thiazoles, thiazolines, thiazolidines, their corresponding oxazoles and oxazolines, and their condensation products with other amino acids to form dipeptide surrogates. The *thiazole* ring is planar and rigid providing a different directional scaffold for side chains to those previously discussed. As seen from the crystal structure of **124** bound to cathepsin K[300] (PDB entry: 1ayv), the catalytic cysteine thiol binds to the central carbonyl of **124**. The thiazole acts as a scaffold directing the 2,5-substituents in an extended conformation across both S and S′ sides of the active site, with the thiazole ring on the S′ side of the active site. Alternatively, the thiazole ring is at the end of the peptide chain, as in the potent inhibitor of HIV-1 protease ritonavir **125**. Although possessing rigidifying thiazoles at either end, a urea and carbamate, ritonavir **125** still has conformational flexibility and thus pays an entropic penalty for reorganizing to the extended β-strand that binds to the protease.[301]

Unsaturation of the thiazole ring gives a heterocyclic ring with a degree of conformational flexibility, a *thiazolidine*. The potent inhibitor of HIV-1 protease **126**,[302,303] known as KNI-272, possesses an allophenylnorstatine transition state isostere, a hydrophobic quinoline at P3, and a bulkier thiazolidine instead of proline at P1′. The thiazolidine has an important β-strand inducing role to play in this molecule, which has a solution structure that is almost identical to its conformation in its crystal structure with HIV-1 protease.[304] This indicates that KNI-272 and analogues are pre-organized in β-strands for receptor binding.[302,303]



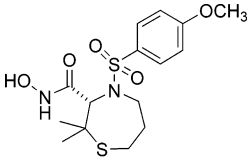




The heterocycle has been expanded to the six- or seven-membered ring, *thiazine* or *thiazepine*, in a series of matrix metalloproteinase inhibitors. From a crystal structure of the complex of **127** with MMP-3[305] (PDB entry: 1d5j), the thiazepine ring was orientated in a pseudo-chair conformation and one of the methyl groups directed toward Val163 of the enzyme. The heterocyclic scaffold imparts structural rigidity and an extended conformation to the molecule, and the methoxyphenyl sulfonyl amide is directed toward the S1′ site and is stabilized by additional hydrogen bonds between the sulfonyl group and Leu164, and the amide NH and Ala165 of the enzyme.

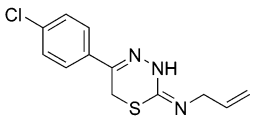
In considering a six-membered ring with three heteroatoms, 1,3,4-thiadiazine scaffolds have been identified as potent inhibitors of matrix metalloproteinases. The crystal structure of the inhibitor **128** with MMP-8[306] (PDB entry: 1jh1) provided insights to the unique binding of the 6H-1,3,4-thiadiazine based MMP-inhibitors. In particular, both of the ring nitrogens of **128** are involved in specific hydrogen

bonds with the backbone of MMP-8 through the NH group of Alal163 and the COOH group of Glu198 and provides an extended structure in inhibitor **128**, however leaving the S1′ pocket of the enzyme unfilled.



127

IC_{50} (MMP-3) = 0.7 nM

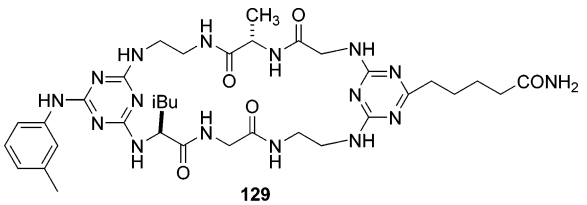


128

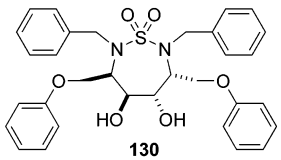
Ki (MMP-8) = 41 μM

In another variation, the *1,3,5-triazine* moiety has been used as a core for unnatural amino acids and can be used to incorporate a wide variety of functional groups into an oligomer. The triazine participates in hydrogen-bonding patterns analogous, if not identical, to the conventional hydrogen-bonding interactions involving peptide backbones. The short β-strand mimetic **129** was prepared to demonstrate the capacity of the triazine scaffold as a useful model of β-strand conformation.[307]

Introduction of a third heteroatom into a seven-membered ring can be illustrated in the C_2-symmetrical HIV-1 protease inhibitor, the *cyclic sulfonamide* **130**. Structurally, **130** is similar to the cyclic ureas and has similar binding features to those discussed in section 7. However, a solid-state structure shows **130** bound to HIV-1 protease[308] (PDB entry: 1ajv) with an unanticipated ligand conformation in which the P1′ and P2′ side chains were transposed, in comparison in the P1′and P2′ to the cyclic ureas.

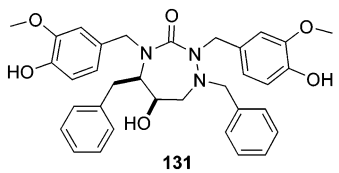


129



130

Ki (HIV-1 protease) = 19.1 nM



131

Ki (HIV-1 protease) = 0.005nM

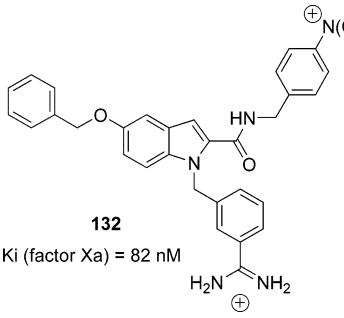
Analogous eight-membered *cyclic sulfamides* have also been reported.[309] A series of unsymmetrical inhibitors have been reported, where inclusion of a third nitrogen atom in the ring allowed for the incorporation of different ligands for the S1′, S2, and S2′ substrate binding sites of HIV-1 protease. In one example, the crystal structure of the complex of azacyclic urea **131** with HIV-1 protease[310] (PDB entry: 1pro) indicated that the phenolic oxygens in the S2 and S2′ subsites had strong hydrogen-bonding interactions with the enzyme backbone through the N−H bonds of Asp30 and Asp130.

As seen above, the use of monocyclic heterocyclic rings as scaffolds for either participating in β-strand hydrogen bonding or projecting substituents into an extended conformation are numerous and varied. A logical extension is to consider heterocyclic systems containing two or more rings. Such systems offer increased opportunity for control of hydrogen bonding and substituent projection, thus promoting β-strand conformations for mimetic design.

9. Ligands Containing Two Rings with One Heteroatom (N or O)

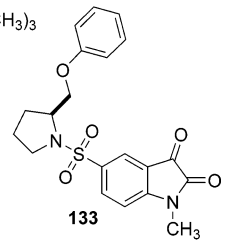
The use of conformationally constrained scaffolds to induce a β-strand has been explored predominantly with fused bicycles containing more than one heteroatom. Fused bicycles have been studied extensively as dipeptide mimetics to induce turn conformations. With the realization that the extended conformation is a recognition element for biological function,[2,3,7,311] a number of bicyclic scaffolds have now been developed to mimic the β-strand.

Indole represents a parent heteroaromatic scaffold that is rigid and provides possibilities for directing substituents toward the pockets of an enzyme. From a series of 1H-indole-2-carboxamides, the crystal structure of the complex of inhibitor **132** with the blood coagulation factor Xa was obtained[312] (PDB entry: 1lpg). The scaffold indole in **132** provides an extended conformation as verified by the placement of the benzamide group in the S1 pocket, and the amidino benzyl group into the S4 pocket of factor Xa. When oxidation sites are introduced to the indole ring, as in isatin **133**, the heterocyclic ring can engage in hydrogen bonding. The crystal structure of the inhibitor **133** with caspase 3[313] (PDB entry: 1gfw) displayed β-strand-type hydrogen bonding of the carbonyl oxygen atoms of the isatin core, with direction of the pyrrolidine ring into the S2 subsite.



132

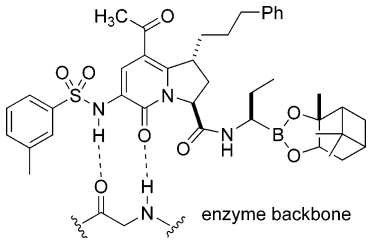
Ki (factor Xa) = 82 nM



133

Ki (caspase-3) = 0.015 μM

In a variation to indole, functionalization of the heterocyclic ring is seen in the *6-amino-5-oxotetrahydroindolizine* **134**,[314] a potent nonpeptidic inhibitor of HCV NS3 protease. In the scaffold of **134**, the heterocyclic ring C=O group and NH of the ring form a pair of β-sheet hydrogen bonds with the enzyme as confirmed by related crystal structures[250,315] (Figure 19), and thus effectively mimics the extended conformation of peptides as a β-strand mimetic.

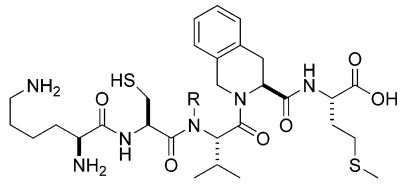


134

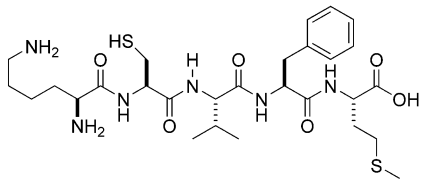
IC_{50} (HCV-NS3 protease) = 0.12 μM

Figure 19. Schematic representation of potential hydrogen bonding between **134** and HCV-NS3 protease. Hydrogen bonds are indicated by dashed lines.

The heterocyclic scaffold can be used to position peptide side chains. In comparison with CA1A2X peptide inhibitors of farnesyl transferase (FTase) *in vitro*[5,161] (section 2), the introduction of conformationally constrained amino acids (A1 and A2) as is **135** and **136**,[162] allowed the extended character of the pentapeptides to be reinforced or diminished compared to the linear parent pentapeptide **137**. The (*L*)-*1,2,3,4-tetrahydro-3-isoquinoline* residue demonstrated a strong influence on the extended conformations as well as a potent inhibitor against FTase.

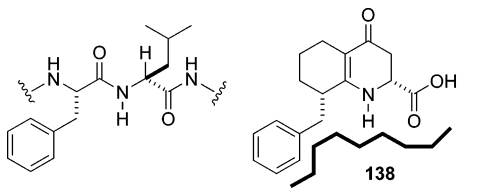


135 R = H 80% extended structure IC_{50} (Ftase) = 20 nM
136 R = CH_3 95% extended structure IC_{50} (Ftase) = 5 nM



137 67% extended structure IC_{50} (Ftase) = 1 μM

Another approach has been to incorporate a bicyclic scaffold that mimics the conformation of a dipeptide constrained within a β-strand. This work was stimulated by structural studies that identified the exposed elements of the β-strand of the 43−46 segment of Phe−Leu−Thr−Lys of CD4, the cellular receptor protein for gp120.[316−318] A cyclic vinylamide scaffold



segment Phe43-Leu44 of CD4

138

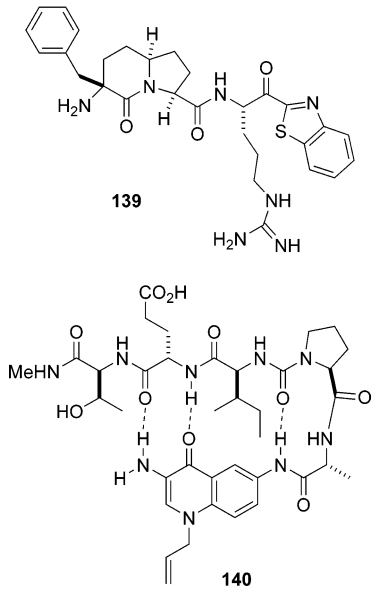
a cyclic compound designed to mimic segment Phe43-Leu44 of CD4

Figure 20. Mimicry of the Phe43−Leu44 segment of CD4 by compound **138**. The bold line indicates the region of mimicry where constraints are made from a single side. Adapted from refs 319 and 320.

138 has been designed to mimic the segment Phe43−Leu44 with constraints made from a single side (Figure 20).[319,320]

Using a similar rationale, β-strand bicyclic scaffolds have been developed as thrombin inhibitors with P1′ groups that adopt an extended β-strand motif. Bicyclic compound **139** is an example of a P3 to P1′ β-strand mimetic. In the crystal structure of **139** bound to thrombin[321] (PDB entry: 1a5g), an antiparallel β-strand hydrogen-bonded interaction was observed between the carbonyl oxygen and amino nitrogen atom of the bicyclic scaffold **139** and the Ser214−Gly216 main chain segment of thrombin.

Bicyclic heterocycles have also been used as β-strand inducers. In NMR studies of tripeptide derivatives of the bicyclic *3,6-diaminoquinolone* **140**,[27] the hydrogen-bonding scaffold, induced a rigid β-strand conformation in attached amino acids. A hydrogen atom was required on the amino group at position 3 of the quinone scaffold for formation of one of the necessary hydrogen bonds of the β-strand conformation (Figure 21).

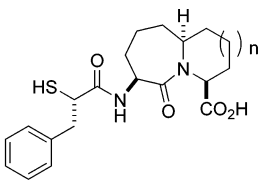


139

140

Figure 21. Schematic representation of hydrogen bonding in **140**. Hydrogen bonds are indicated by dashed lines. Adapted from ref 27.

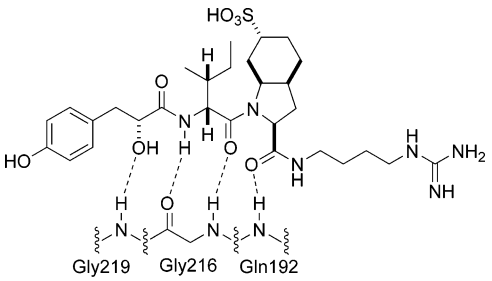
Less rigid scaffolds have been explored. In extension of work discussed in section 4, incorporation of a seven-membered lactam in the 7−5 fused **141** and the 7−6-fused bicyclic azepinone **142** led to dual acting ACE/NEP inhibitors.[248] The extra ring presumably restricts the conformational mobility of the lactam and promotes the required configuration of the amide bond for an extended conformation.



141 (n=0) IC_{50} (NEP) = 18 nM
IC_{50} (ACE) = 5 nM

142(n=1) IC_{50} (NEP) = 25 nM
IC_{50} (ACE) = 11 nM

Alternative placement of the side chains on the saturated bicyclic heterocycle is seen in the *octahydroindole* aeuroginosin 98-B (**143**), an inhibitor of trypsin. In the crystal structure of **143** with trypsin[322] (PDB entry: 1aq7), the scaffold tethered to the enzyme by the sulfate group orientates the side chains to achieve an extended hydrogen-bonding pattern that is illustrative of antiparallel β-strand binding (Figure 22).

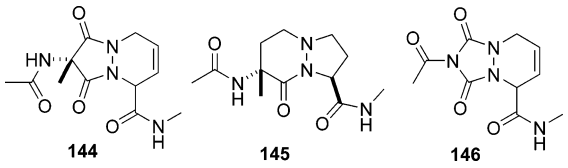


143

Figure 22. Schematic representation of antiparallel β-strand hydrogen bonding between **143** and trypsin. Hydrogen bonds are indicated by dashed lines.

10. Ligands Containing Two Rings with Two or Three Heteroatoms (N, N or N, S or N, N, N)

Introduction of a further heteroatom into scaffolds with two rings has been considered. Molecular modeling and conformational searches indicated that the 5,6-fused bicyclic scaffolds **144**, **145**, and **146** would meet the spatial requirements of a dipeptide β-strand mimetic, while facilitating versatile incorporation of substitutents.[323]



Extremely potent enzyme inhibitors have been realized with these scaffolds, which act as a mimetic of the D-Phe-Pro orientation of PPACK (D-Phe-Pro-ArgCH_2Cl). This thrombin inhibitor reached phase II clinical trials,[324] and reproduces the three-dimensional orientation of the substrate and its hydrogen-bonding patterns. Numerous inhibitors based on the sequence of PPACK have been reported,[325] and include compounds such as **147**, **148**, and **149**.[326] A crystal structure of MOL-126 **147** bound to thrombin[321] (PDB entry: 1a5g) (Figure 23) revealed anti-

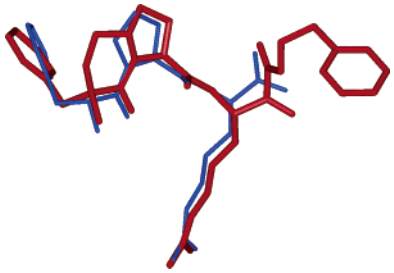


Figure 23. Comparison of the active site thrombin-binding conformations of bicyclic β-strand mimetic **147** (PDB entry: 1a5g) (red) and the peptidic inhibitor PPACK (PDB entry: 1abj) (blue). Images were generated using Insight II.

parallel β-strand hydrogen-bonded interactions between the carbonyl oxygen and the amino nitrogen of the diazabicyclo[4.3.0]nonane scaffold, and the Ser214 to Gly216 main chain segment of thrombin[326−328] (Figure 24).

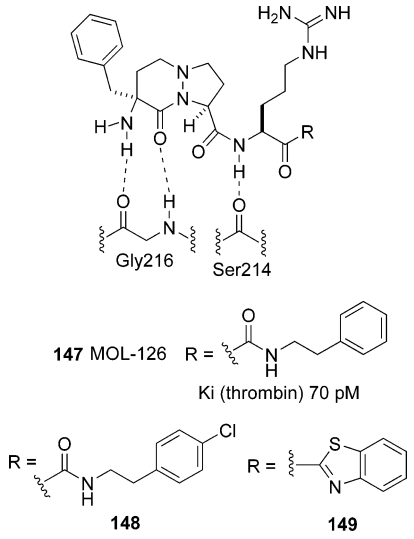
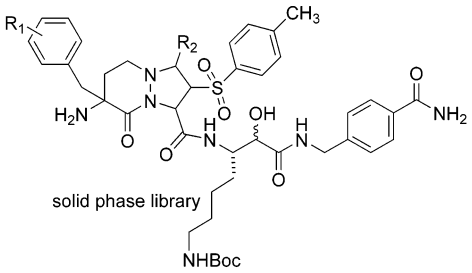
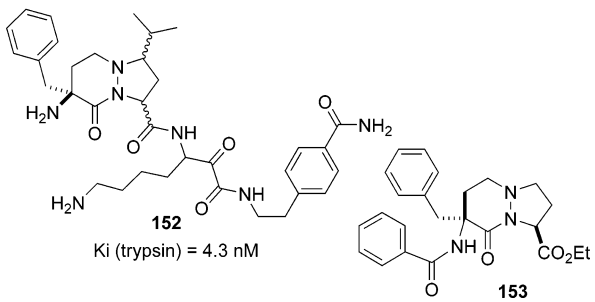


Figure 24. Schematic representation of the hydrogen bonding pattern between MOL-126 **147**, **148**, or **149** and thrombin. Hydrogen bonds are represented by dashed lines.

A practical synthetic route to the constrained β-strand mimetic scaffold in **145** has been achieved by regioselective 1,3-dipolar addition of azomethine imines with vinyl sulfones in solution[327] or the solid phase.[329] This led to development of inhibitors **150**,[329] **151**,[329] and **152**.[327] Since this work, the first enantioselective synthesis of this class of β-strand mimetic was reported for **153**.[330]



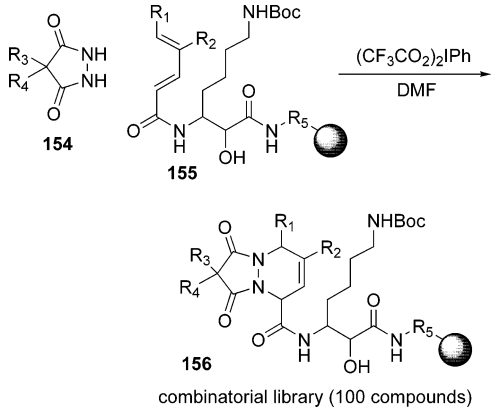
150 R_1= 4-t-Bu, R_2= Ph 65% inhibition (trypsin) at 100 nM
151 R_1= 3-Fluoro, R_2 = furyl 23% inhibition (factor viia) at 100 nM



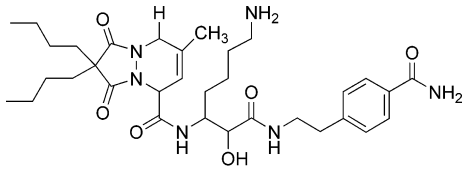
Use of the 5,6-fused bicyclic scaffold in **144** has been reported in the solid-phase library synthesis of a hundred β-strand mimetics **156**.[323] A cycloaddition reaction between a resin-bound substituted pentadienoic acid amide **155**, and a 3,5-pyrazolinedione

generated in situ from the 3,5-pyrazolidinedione **154**, gave the Diels−Alder adducts **156** (Scheme 3). Cleavage from resin gave a range of analogues and inhibitor **157** was identified as a potent inhibitor of thrombin.[323]

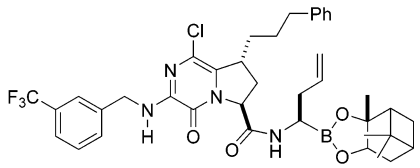
Scheme 3



A bicyclic scaffold with two N atoms, the *pyrazinone* ring, has been used as a β-strand mimetic. Analogous to the hydrogen bonding observed for **147**, inhibitor **158** makes three hydrogen-bond interactions with the hepatitis C virus-NS3 protease (HCV−NS3) enzyme backbone, two using the pyrazinone C=O and NH, the third from the P1 amide NH.[331]



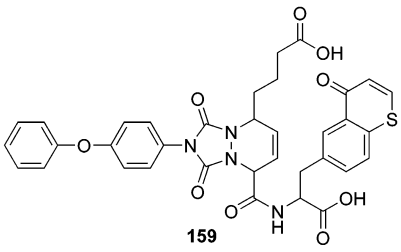
Ki (thrombin) = 0.2 nM



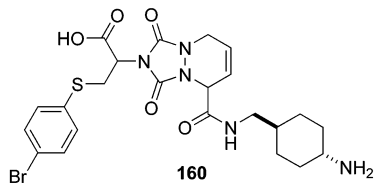
IC_{50} (HCV-NS3 protease) = 0.06 μM

Incorporation of another N heteroatom into the bicyclic core has led to the synthesis of compound libraries containing the triazolopyridazine β-strand scaffold.[323,332,333] The putative binding mode of the peptidomimetic triazolopyridazine scaffold places the substituents on the ring into the S1, S2, and S3 pockets of thrombin. From such studies, potent inhibition has been reported for protein tyrosine phosphatase (PTPase) by **159**,[332] thrombin by **160**,[333] and trypsin by compound **161**.[323] Many diverse analogues based on the parent 5−6 fused bicyclic scaffold in **161** have been reported in the patent literature.[334]

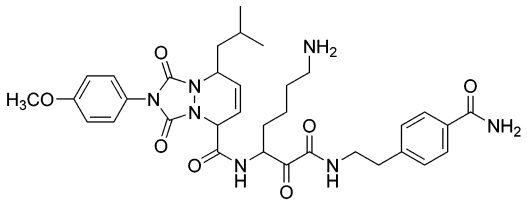
In a modification of the β-strand triazolopyridazine scaffold, an asymmetric synthesis has been reported for the enantiomers of the saturated analogue of MOL-376, a potent and selective inhibitor of thrombin (K_i (thrombin) = 1.2 nM). Conformational analysis indicated that the S-enantiomer **162** is a better fit for an idealized antiparallel β-strand conformation.[335] Further inclusion of an oxidation site on the bicyclic core led to development of the modified β-strand scaffold **163**.[336] Using a combinatorial library approach, **163** was shown to selectively inhibit the activator protein-1 transcription without affecting nuclear factor κB transcription or thioredoxin.



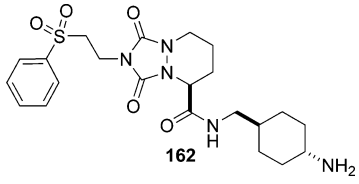
IC_{75} (PTP1B) = 10 μM

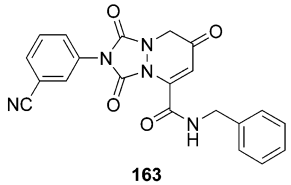


Ki (thrombin) = 0.023 nM



Ki (trypsin) = 0.28 nM



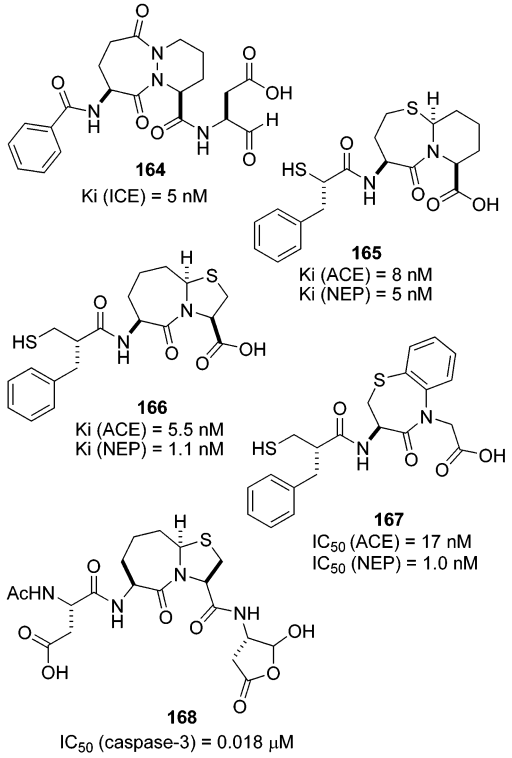


IC_{50} (activator protein-1) = 20 μM

The scaffolds that have been discussed in this section have a number of similarities to the monocyclic lactam inhibitors developed for ICE, ACE, and NEP (section 4). With respect to ICE, it was found that high potency required a constrained peptide mimetic which retained the P3 NH, P3 CO, and P1 NH hydrogen-bonding functionalities in the correct position for β-sheet hydrogen bonding with enzyme, and led to development of the scaffold found in compound **164**.[337]

Similar bicyclic scaffolds **165**,[244] **166**,[242] and **167**[243,338] were used as conformationally restricted surrogates

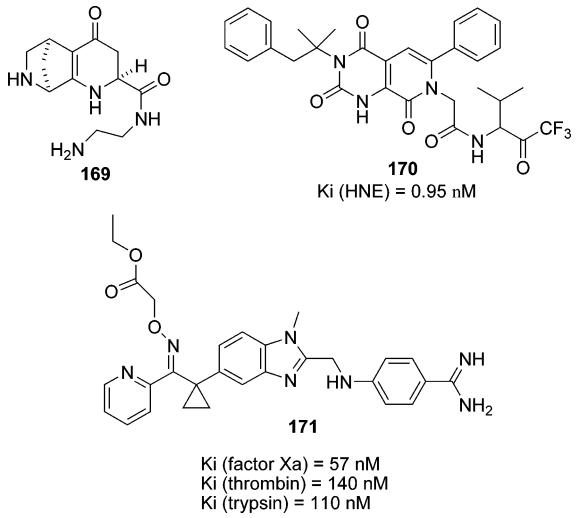
of Ala-Pro and have been developed as dual metalloprotease inhibitors of ACE and NEP.[244] A structure activity study of analogues of **167** demonstrated that replacement of the seven-membered thiazepine by six-membered thiazine changed the extended conformation to reduce activity versus ACE (2-fold) but enhanced activity versus NEP (5-fold).[243] However, incorporating a P2−P3 conformationally constrained dipeptide mimetic led to **168** as a highly selective inhibitor of caspase-3.[339]



Fused bicyclic dipeptide β-strand mimetics have been developed to inhibit the interaction of CD4, the cellular receptor for HIV, with the viral protein gp120, after a crystal structure indicated that interactions occurred through β-stranded regions. In a variation of compound **138** (Figure 20), **169** was designed to mimic the Thr45−Lys46 module of the four-residue segment of CD4.[319,320] Although **138** and **169** have been coupled to form a four-residue analogue, the efficacy of these cycles is yet to be determined.

A few other bicyclic heterocyclic ring systems have been used as scaffolds to append substituents. Pyridopyrimidine trifluoromethyl ketones have been designed to extend the concept of the related pyridine trifluoromethyl ketones (section 4). A rigidly positioned carbonyl group was incorporated to participate in a hydrogen-bonding interaction with the backbone NH groups of Gly218 and Gly219 and the C=O and NH portion of Val216 of the HNE enzyme. The pyrimidine ring serves as a scaffold to orient the substitutents toward the S5 and S4 subsites of the enzyme's extended binding pocket. Among a series of pyridopyrimidines, analogue **170** displayed potent inhibition of elastase.[252]

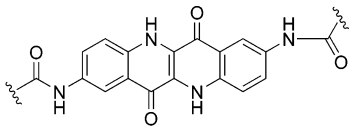
Another rigid scaffold is the benzimidazole of the dual specific inhibitor of thrombin and factor Xa, **171**. Crystal structures have been reported for **171** with thrombin[340] (PDB entry: 1g30), factor Xa[340] (PDB entry: 1g2l) and trypsin[340] (PDB entry: 1oyq supersedes 1g34). Compound **171** lies either flat (factor Xa) or perpendicular (thrombin) to the protein surface, in an extended conformation within the enzyme active site cleft, and efficiently occupies the S2 and S4 subsites by placing the methyl substituent into the S2 subsite and the pyridinyl moiety into the S4 subsite.



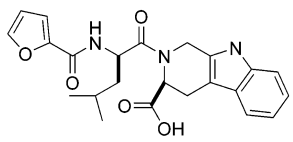
The final examples of scaffolds are those of polycyclic heterocyclic rings. In the early literature, the epindolidione **172** has been used as a scaffold for nucleating β-sheet structure in an attached polypeptide chain.[13,341,342] Appending short polypeptides at either end resulted in a parallel β-sheet structure, in which the polycyclic core acted as a β-strand scaffold through hydrogen bonding of the carbonyl and NH groups of the epindolidione **172**.

More recently, the structure of adamalysin II, a metalloendopeptidase (MEP) isolated from the venom of the Eastern diamond-back rattle snake, has been reported in a complex with the peptidomimetic inhibitor **173**[343] (PDB entry: 3aig). The polycyclic ring derives from the amino acid Trp cyclizing with an adjacent Leu residue. The inhibitor **173** binds to the S′-side of the protease, inserting between two protein segments, and establishing a mixed parallel−antiparallel three-stranded β-sheet, thereby acting as a β-strand template.

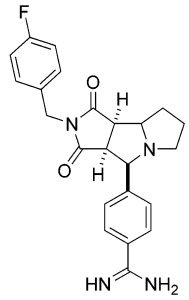
In other tricyclic core examples, the rigid inhibitor **174** showed extended geometry in the binding site, complemented by hydrogen bonding, in its crystal structure with trypsin[344] (PDB entry: 1oyt). The hydroxyproline derived diketopiperazine template of general structure **175** has been used as a rigid, structure-directing template for peptidic receptor arms. These receptor arms can form noncovalent interactions with free side chains and protected side chains that are presumably in extended conformations.[345,346] The screening of several dye-marked diketopiperazine receptors against an encoded side chain protected tripeptide library indicated high binding specificities and a relationship between structural changes and binding preferences.[346]



172

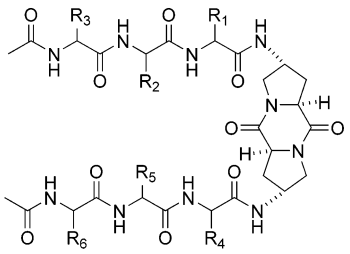


173



174

Ki (trypsin) = 0.057 μM



R_1, R_2, R_3, R_4, R_5, R_6 = amino acid side chain

175

11. Concluding Remarks

It has been only recently that the extended peptide β-strand has been recognized as a universal motif for certain classes of biomolecular receptors. Relatively few methods have been reported for fixing β-strand conformations to produce potent enzyme inhibitors. However, compounds that have been molded into β-strand-mimicking structures have been shown to have high affinity for biomolecular receptors. It still does not seem to be well appreciated that fixing molecules in a β-strand conformation can substantially increase affinity for receptors that recognize the β-strand.[2]

Efforts should be made in future to discover whether designed molecules actually do structurally mimic the β-strand well, as relatively few cases exist in which rigid constraints have been specifically demonstrated to be β-strand mimetics. The issues of the extent of conformational flexibility in solution versus the organized interactions within a crystal structure of an inhibitor−enzyme complex needs to be approached carefully when designing β-strand mimics. In particular, the $^3J_{NHC\alpha}$ coupling constant in proton NMR spectra can be particularly diagnostic (9−10 Hz) for β-strands of peptidomimetics in solution.[96]

Many potential β-strand mimetics are examples of so-called "privileged" structures,[347−351] composed of scaffolds that could be used in many different types of β-strand mimetics with multiple potential uses. Examples have been given above of one- and two-ring scaffolds that have been adapted to inhibit several different proteases. Such multipurpose privileged scaffolds can be valuable for creating initial drug leads, especially if they are "druggable" entities that confer appropriate bioavailability characteristics. However, this commonality of scaffold can also create problems. If the scaffold is too simple and minimally decorated with substituents, they may bind promiscuously to multiple receptors leading to toxicities.[352] This is a major problem in the area of protease inhibitors.

The trend in drug discovery today is to synthetically elaborate such scaffolds with sufficient foliage to engender high selectivity for the target receptor. Getting the balance right between the common use of β-strand-mimicking scaffolds and appending sufficient substituents to create selectivity in receptor binding is clearly a big challenge in this field. Likewise, there is a fine balance needed between using structural constraints (e.g., a bicyclic heterocycle ring), steric effects, and hydrogen bonds to organize the geometry and conformation of a peptide into a β-strand, and considerably more research needs to be done to elucidate the relative importance of these influences in dictating strand structure.

β-Strand mimetics are also expected to find emerging uses in coming years in the design of compounds that can interfere with peptide/protein β-strand aggregation. There are already over 30 known "amyloid diseases" in which proteins are thought to misfold into β-strands that aggregate into β-sheet structures,[28−35] the toxic agents being thought to be small aggregates. β-Strand mimetics may offer opportunities to build druggable agents that prevent or reverse such β-sheet formation and aggregation. In another context, β-sheets represent some 30% of protein structure, so β-strand mimetics could therefore conceivably have many promising uses in destabilizing protein structures and mimicking or antagonizing protein−protein or protein−DNA interactions that are mediated by β-sheets.

The availability of new drug-like scaffolds that can be used to preorganize molecules in β-strand shapes, either alone or in combination, looks to be a promising strategy for developing enzyme inhibitors, receptor antagonists, and perhaps strand-containing vaccines. Since we now know of receptor classes that specifically recognize the peptide β-strand, this strategy may be successful for receptors of such classes even when the specific receptor structure is unknown. Strand mimetics also raise the possibility, because of their rigidity, of potential uses in protein surface mimetics as part of long scaffolds designed to separate protein/peptide motifs by long distances. β-Strand mimetics might also be incorporated into polypeptides to investigate effects of templating in protein folding. Directed strategies toward the development of nonpeptidic analogues of the peptide β-strand, in combination with serendipitous discovery of β-strand

mimetics, should expand significantly in the coming years. We encourage further studies to create new designed β-strand mimetics and to identify the importance of the β-strand structure in other examples of protein recognition.

12. Abbreviations

Ac	acetyl
ACE	angiotensin converting enzyme
Boc	*tert*-butoxycarbonyl
Bu	butyl
DMF	dimethylformamide
FTase	farnesyl transferase
HFC	human fibroblast collengenase
HCV	hepatitis C virus
HIV-1	human immunodeficiency virus type-1
HLE	human leukocyte elastase
HNE	human neutrophil elastase
HRV	human rinovirus
ICE	interleukin-1β converting enzyme
MHC	major histocompatibility complex
MMP-1	matrix metalloproteinase-1
MMP-2	gelatinase
MMP-3	stromelysin
MMP-7	matrilysin
MMP-8	human neutrophil collengenase
MMP-13	metallomatrix protein human collengenase-3
MMP-15	membrane type-2 matrix metalloprotease
NEP	metalloprotease neutral endopeptidase
NMR	nuclear magnetic resonance spectroscopy
PDB	Protein Data Bank
PPE	porcine pancreatic elastase
PTP	protein tyrosine phosphatase

13. Acknowledgments

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