

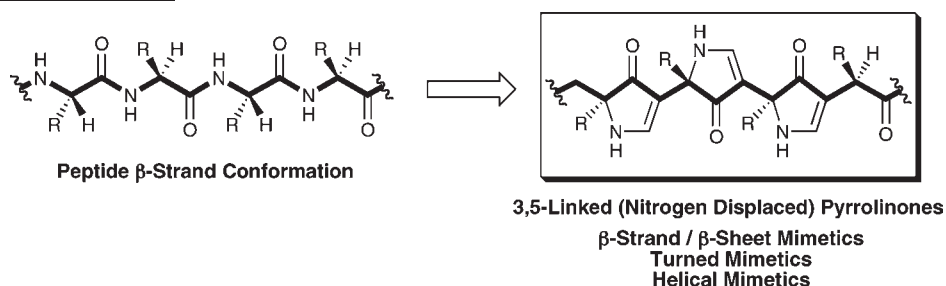
Pyrrolinone-Based Peptidomimetics. “Let the Enzyme or Receptor be the Judge”

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CONSPECTUS



Peptides and proteins, evolved by nature to perform vital biological functions, would constitute ideal candidates for therapeutic intervention were it not for their generally poor pharmacokinetic profiles. Nonpeptide peptidomimetics have thus been pursued because they might overcome these limitations while maintaining both the potency and selectivity of the parent peptide or protein. Since the late 1980s, we have sought to design, synthesize, and evaluate a novel, proteolytically stable nonpeptide peptidomimetic scaffold consisting of a repeating structural unit amenable to iterative construction; a primary concern is maintaining both the appropriate peptide-like side-chains and requisite hydrogen bonding. In this Account, we detail how efforts in the Smith–Hirschmann laboratories culminated in the identification of the 3,5-linked polypyrrolinone scaffold.

We developed effective synthetic protocols, both in solution and on solid supports, for iterative construction of diverse polypyrrolinones that present functionalized peptide-like side-chains. As a result of the rigid nature of the pyrrolinone scaffold, control over the backbone conformation could be exerted by modulation of the stereogenicity of the constituent monomers and the network of intramolecular hydrogen bonding. The extended conformation of the homochiral 3,5-linked polypyrrolinone scaffold proved to be an excellent mimic for β -strands and β -sheets. Application to enzyme inhibitor design and synthesis led not only to modest inhibitors of the aspartic acid protease renin and the matrix metalloprotease class of enzymes, but importantly to bioavailable HIV-1 protease inhibitors with subnanomolar binding constants.

The design and synthesis of a competent peptide–pyrrolinone hybrid ligand for the class II major histocompatibility complex (MHC) antigen protein HLA-DR1 further demonstrated the utility of the 3,5-polypyrrolinone motif as a mimic for the extended polyproline type II peptide backbone. Equally important, we sought to define, by synthesis, the additional conformational space accessible to the polypyrrolinone structural motif, with the ultimate goal of accessing pyrrolinone-based turn and helix mimetics. Toward this end, a mono-*N*-methylated bispyrrolinone was found to adopt an extended helical array in the solid state. Subsequent synthesis of *D,L*-alternating (heterochiral) tetrapyrrolinones both validated the expected turn conformations in solution and led to a functionally active mimetic of a peptidal β -turn (similar to somatostatin). Finally, the design, synthesis, and structural evaluation of both acyclic and cyclic heterochiral (that is, *D,L*-alternating) hexapyrrolinones yielded nanotube-like assemblies in the solid state. Taken together, these results illustrate the remarkable potential of the 3,5-linked polypyrrolinone scaffold as β -strand, β -sheet, β -turn, and potentially helical peptidomimetics.

Introduction

In the late 1980s Hirschmann, Nicolaou, and Smith initiated collaborative research programs at the University of Pennsylvania to develop novel nonpeptide peptidomimetic

scaffolds with improved pharmacokinetic properties.¹ This collaboration was based on the Hirschmann hypothesis that secondary amide bonds of peptides and proteins were primarily responsible for their poor pharmacokinetic

properties.² Two conceptually different programs were initiated: one directed at the design of nonpeptide peptidomimetic receptor antagonists/agonists,³ and the second focused on the development of protease enzyme inhibitors.⁴ For the receptor antagonists/agonists program, the concept, innovative at the time, entailed peptide backbone replacement with a scaffold that would display the requisite peptide-like side-chains with trajectories similar to those found in turned peptide ligands, without regard for ligand backbone or receptor hydrogen bonding. For the protease inhibitor program, scaffolds were sought to mimic the well-known extended β -strand conformation of native peptide substrates, including both side-chain trajectories and importantly substrate–enzyme hydrogen bonding. This Account will focus on the evolution of pyrrolinone-based nonpeptide peptidomimetic program, highlighting the design, synthesis, and biological validation of pyrrolinone based scaffolds.

Initial Design

Proteolytic enzymes (cf. aspartic acid and serine proteases) bind substrates in an extended β -strand conformation via an extensive array of backbone-to-backbone hydrogen bonds, in conjunction with side-chain interactions (Figure 1A).^{5,6} Thus, in contrast to the design of β -turn mimics that bind receptors, β -strand peptidomimetics for use as protease inhibitors require not only appropriate side-chain trajectories but also optimal hydrogen-bond registration with the enzyme backbone.

Early on, a decision was made to devise a β -strand mimetic that would incorporate a repeating structural unit, thus facilitating general application to a spectrum of problems by structural unit modification. The requirements of a repeating unit, that would appropriately project peptide-like side-chains, maintain the requisite hydrogen bonds, and prove proteolytically stable, led to the design of a vinylogous amide⁷ based repeating core, attractive for the following reasons: (1) the amide and vinylogous amide NH possess similar pK_a values;⁸ (2) the nitrogen and carbonyl of amides and vinylogous amides display similar hydrogen bonding potential; (3) vinylogous amides are proteolytically stable; and (4) the vinylogous amide moiety provides backbone rigidity with an element of preorganization. To optimize side-chain and hydrogen bond registration, vis-à-vis a native peptide sequence, the vinylogous amide was incorporated into a five-membered *pyrrolinone* ring (Figure 1B). For translation of a peptide chain into a “nitrogen-displaced” polypyrrolinone mimic, see Figure 1C. A conceptually similar exercise, involving displacement of the carbonyl groups, provides an

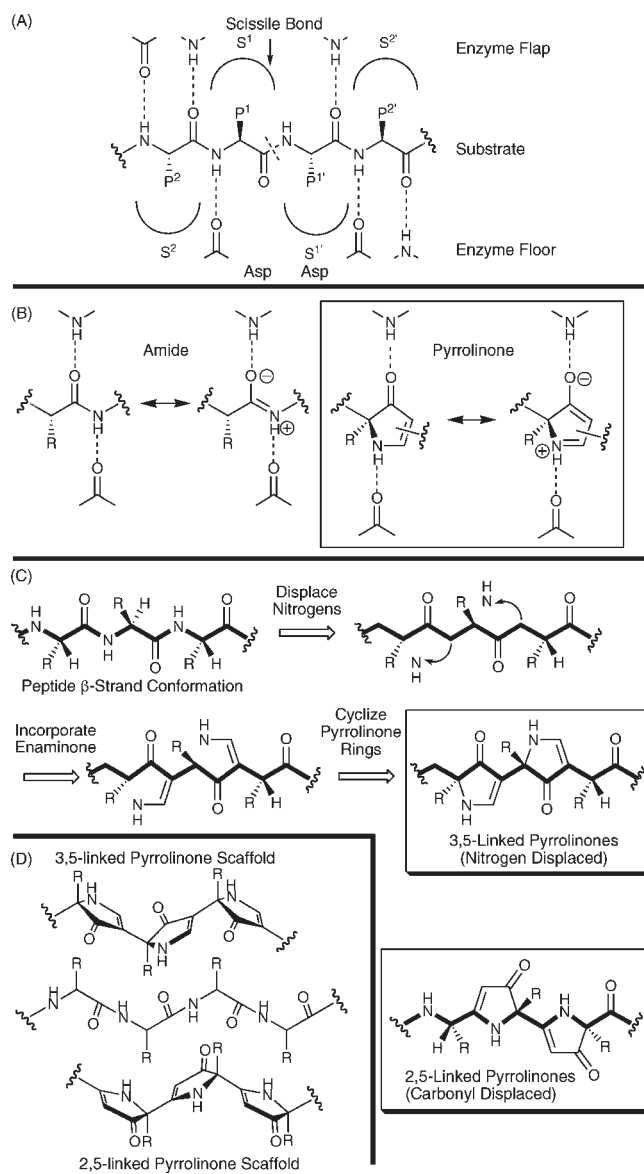


FIGURE 1. (A) Typical aspartic acid protease active site, (B) Comparison of amide and pyrrolinone units, (C) Design of 3,5- and 2,5-pyrrolinones, and (D) Registrations of 3,5- and 2,5-linked pyrrolinone scaffolds.

alternate peptidomimetic backbone, termed 2,5-linked “carbonyl displaced” polypyrrolinones. Compared to a peptide β -strand, the pyrrolinone rings occupy somewhat different registrations relative to the pleats of β -strands (Figure 1D). Thus, unique chemical, structural, and biological characteristics for each scaffold could be envisioned (vide infra).

Monte Carlo conformational searches for model nitrogen and carbonyl displaced tetrapyrrolinones (**1** and **2**) were employed to determine the most favorable conformation. For the 3,5-linked nitrogen displaced tetrapyrrolinone (**1**), three low energy conformational classes were observed (Figure 2A). In contrast, only a single low energy class, possessing an

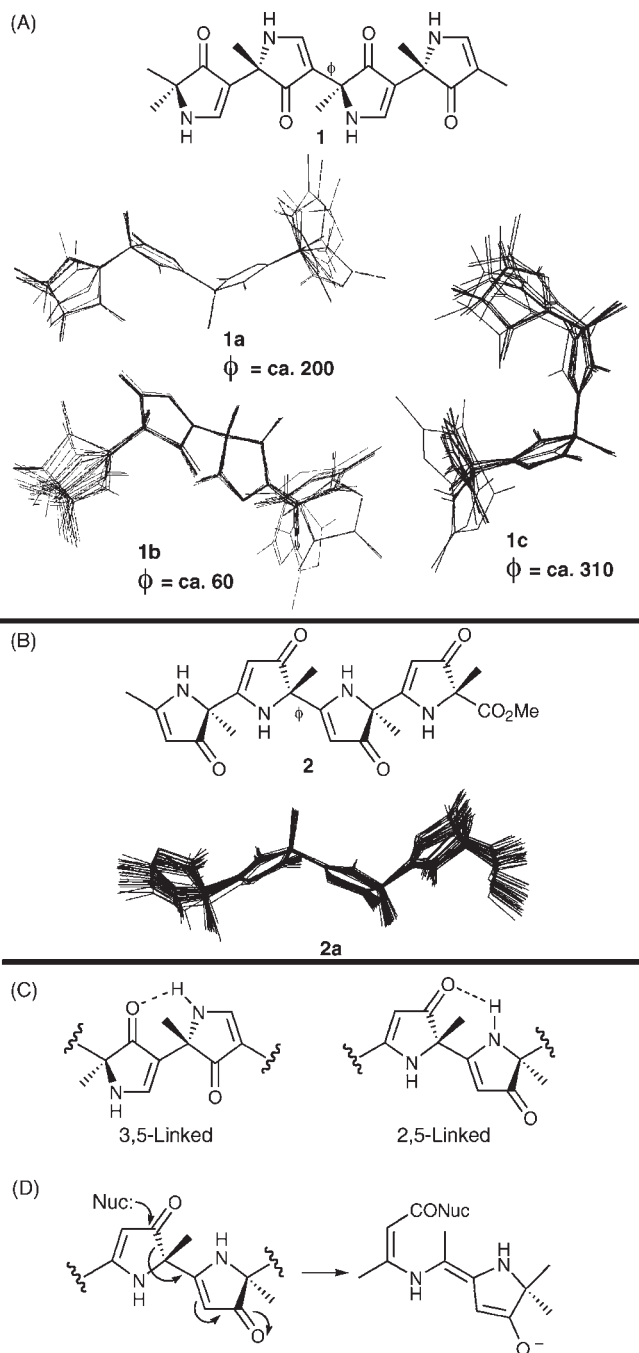


FIGURE 2. Monte Carlo generated backbone conformations for model tetrapyrrolinones **1** (A) and **2** (B); (C) Predicted intramolecular hydrogen bond between adjacent pyrrolinone rings; (D) Potential susceptibility of the 2,5-linked pyrrolinone scaffold to nucleophilic decomposition.

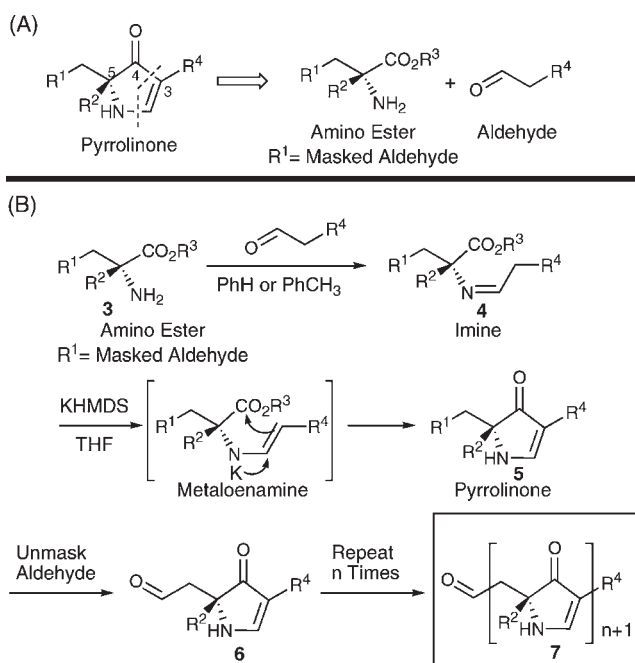
extended backbone conformation (**2a**), was observed for the 2,5-linked carbonyl displaced tetrapyrrolinone **2** (Figure 2B). Importantly, both extended low energy conformations (**1a** and **2a**) incorporate repeating intramolecular hydrogen bonds between the carbonyl of the pyrrolinone ring and the adjacent pyrrolinone NH hydrogen (Figure 2C), anticipated to stabilize the extended conformation of polypyrrolinone scaffolds.

Somewhat worrisome, however, was the potential for decomposition of 2,5-linked polypyrrolinone scaffold upon attack of a nucleophile (Figure 2D). We thus decided to focus initially (and principally) on the 3,5-linked scaffold.⁹

Construction of 3,5-Linked Polypyrrolinones

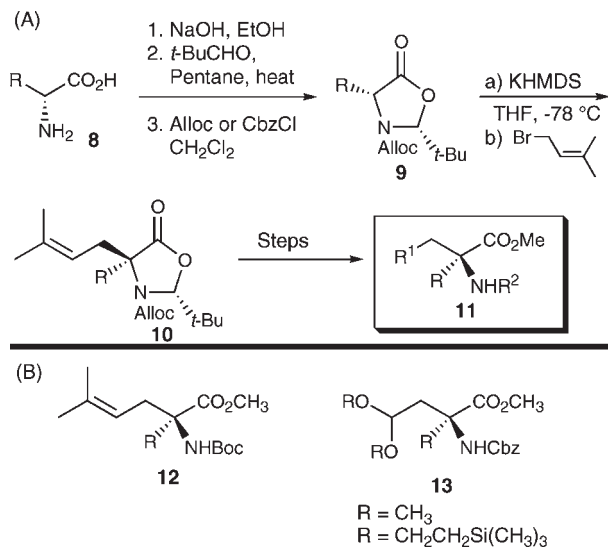
The Hiroi retron¹⁰ (Scheme 1A), involving condensation of aminoesters with *preestablished* α -stereogenicity and aldehyde building blocks, was selected as the foundation for our pyrrolinone synthetic program. Application and extension of this sequence to iterative construction of polypyrrolinones was substantially validated in our laboratory (Scheme 1B).¹¹

SCHEME 1. (A) Retrosynthetic Analysis of the 3,5-Pyrrolinone Unit; (B) Iterative 3,5-Pyrrolinone Synthesis via Metalloenamine Mediated Cyclization



To construct the requisite amino acid ester building blocks, we adopted a modification of the Seebach¹²/Karady¹³ chemistry for the self-regeneration of stereogenic centers (Scheme 2A), initially exploiting a *tert*-butyl carbamate (Boc) protecting group for the amines and an olefin (i.e., prenyl group) for the masked aldehydes (cf. **12**). To expedite iterative polypyrrolinone construction, a second protecting group strategy was introduced that employed a Cbz-carbamate and an acetal (cf. **13**).¹⁴ Use of acetals both eliminated the need for oxidative cleavage of the olefin for subsequent iterations, a transformation that proved incompatible with some amino-acid-like side chains, and provided flexibility vis-à-vis deprotection.¹⁵

Validation of the polypyrrolinone scaffold as a β -strand mimetic (*vide infra*) prompted extension of the second-generation

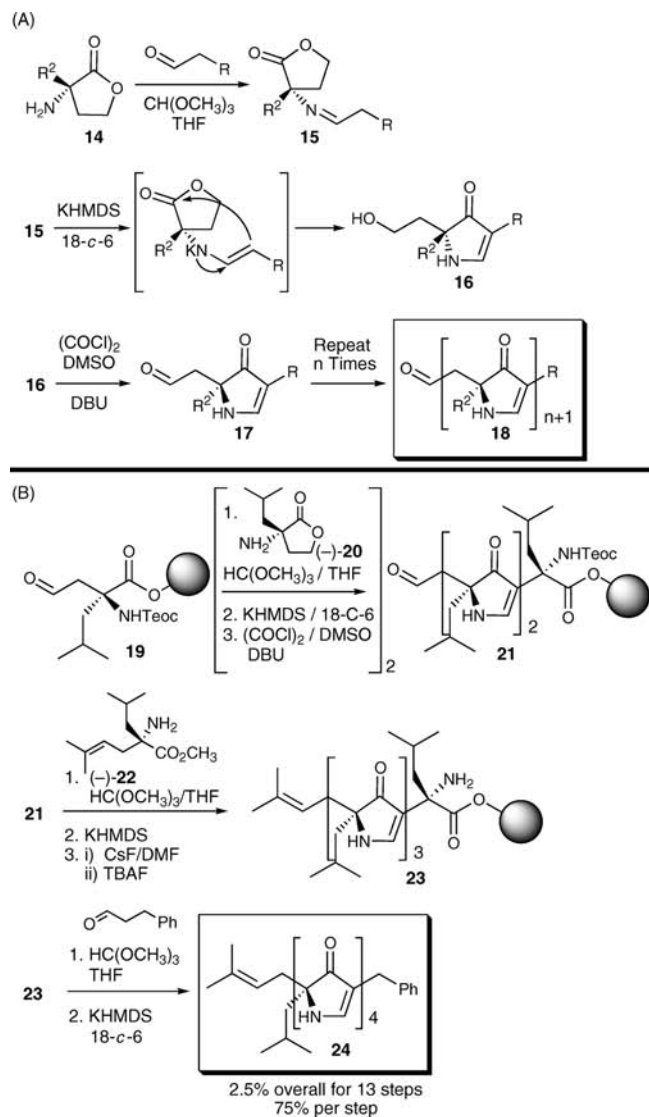
SCHEME 2. (A) Synthesis of Amino Ester and Aldehyde Building Blocks; (B) Amino Ester Building Blocks for Approaches A and B


pyrrolinone synthetic protocol to solid support to permit construction of potential polypyrrolinone libraries. Here a third-generation strategy was required,¹⁶ employing amino lactones (cf. **14**). The sequence retained the earlier two-step imine/metalloenamine cyclization (Scheme 1B), which when applied to the lactone releases an alcohol requiring only mild oxidation to generate the aldehyde for iterative chain extension (Scheme 3A). Importantly, the requisite α -aminolactone building blocks proved readily available,¹⁷ thus facilitating the synthesis of polypyrrolinones on solid-support (Scheme 3B). Equally important for library construction, we developed a cross-coupling protocol to access diverse C-terminal peptidomimetics from a common precursor (Scheme 3C).¹⁸

Validation of the Extended 3,5-Polypyrrolinone Conformation as a β -Strand Mimic

Prior to the development of prospective pyrrolinone-based enzyme inhibitors, experimental support for the proposed extended β -strand/ β -sheet-like conformation was sought. Trispyrrolinone (–)**27**, a potential mimic of the Precigoux equinine tetrapeptide methyl ester, H-Leu-Leu-Val-Tyr-OMe,¹⁹ was designed and synthesized. Single crystal X-ray analysis revealed the anticipated extended β -strand-like conformation in the solid state (Figure 3A), with both the side-chain trajectories and carbonyl orientations overlaying remarkably well with the corresponding tetrapeptide (Figure 3B).⁴ Analysis of the unit cell further revealed interstrand hydrogen bonding with head-to-tail molecule stacking, similar to that found in antiparallel β -sheets (Figure 3C).

Single crystal X-ray analysis of the des-Boc trispyrrolinone [cf. amine (–)**28**] also revealed an extended β -strand-like

SCHEME 3. (A) Third-Generation Pyrrolinone Synthesis; (B) Synthesis of Polypyrrolinones on Solid Support; (C) Palladium-Catalyzed Functionalization of C-terminal Pyrrolinones


conformation; however, now stacking in the solid state in a *parallel* β -sheet like arrangement, as observed for the equinine tetrapeptide (Figure 4).⁴ That the nitrogen displaced pyrrolinone scaffold forms interstrand hydrogen bonds, stabilizing, respectively, antiparallel and parallel sheet formation, was also evident in the crystallographic packing of **27** and **28**.

In similar fashion, solution Fourier transform infrared (FT-IR) studies demonstrated that the NH and the carbonyl of adjacent pyrrolinone rings, as predicted, participate in a six-membered

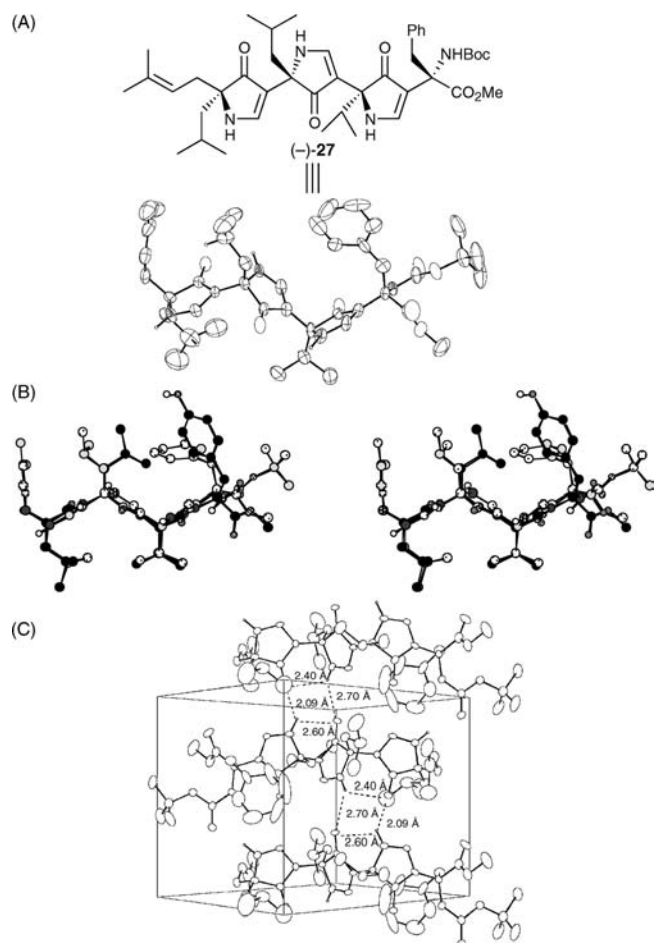


FIGURE 3. (A) ORTEP plot of trispyrrolinone (–)-**27**; (B) Overlay with the crystal structure of the corresponding tetrapeptide (stereoview); (C) Unit cell illustration highlighting the antiparallel packing of (–)-**27**. Adapted with permission from ref 20. Copyright 1994 American Chemical Society.

ring *intramolecular* hydrogen bond (Figure 5).²¹ Variable temperature ¹H NMR studies were also informative. The N-terminal pyrrolinone NH proton in (–)-**29**, which cannot form an intramolecular H-bond, exhibits a large temperature chemical shift dependence, whereas the C-terminal pyrrolinone NH proton displays only a small chemical shift temperature dependence due to intramolecular H-bonding. From the outset, however, we recognized that a more definitive test demonstrating the extended conformation would be the successful application of the polypyrrolinone structural motif to a relevant biological problem, or as Professor Hirschmann often stated: “*Let the enzyme or receptor be the judge!*”.

Enzyme Inhibition Employing the 3,5-Linked Pyrrolinone Scaffold

As a first test of the 3,5-linked polypyrrolinone scaffold, we sought to design inhibitors of renin, a critical target in the late

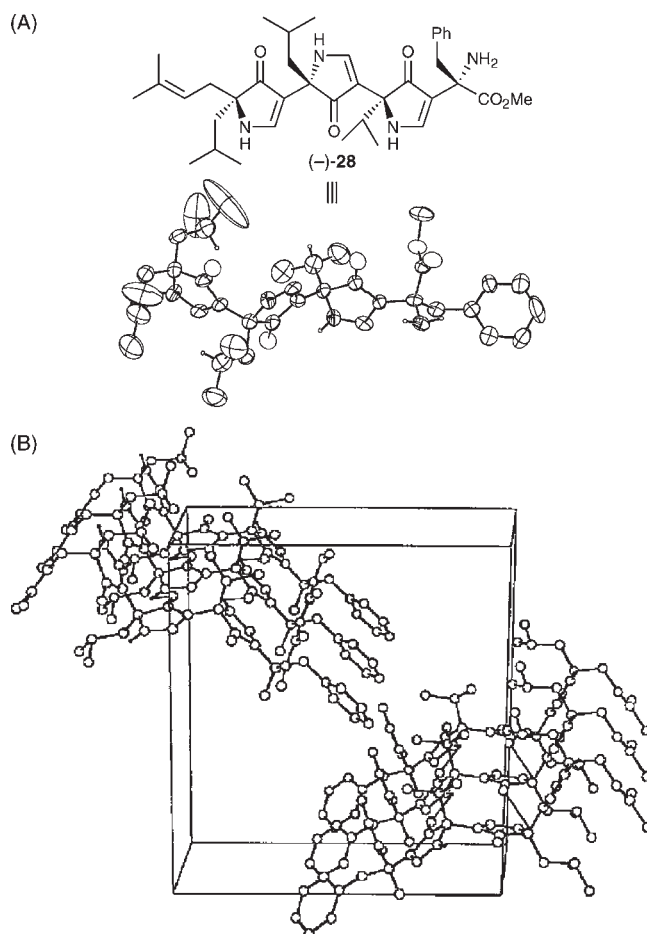


FIGURE 4. ORTEP plot (A) and unit cell (B) for trispyrrolinone amine (–)-**28**. Adapted with permission from ref 20. Copyright 1994 American Chemical Society.

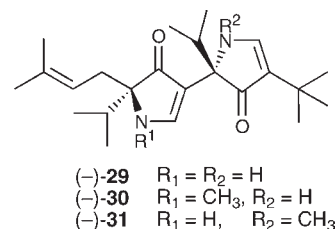


FIGURE 5. Pyrrolinones used in solution phase structural studies.

1980s for intervention in the renin-angiotensinogen cascade regulating blood pressure.²² Elements of several known renin inhibitors²³ were employed in our initial design. Ultimately, monopyrrolinone **32** and bispyrrolinone **33** were selected based on modeling studies and constructed via our first generation synthetic protocol (Figure 6). Pleasingly, *in vitro* assays of (+)-**32** and (–)-**33** revealed IC₅₀ values of 18 and 0.6 μM, respectively.²⁴ Observation of activity, albeit modest, was taken as the first evidence that the 3,5-pyrrolinone scaffold in fact held promise as a β-strand mimic.

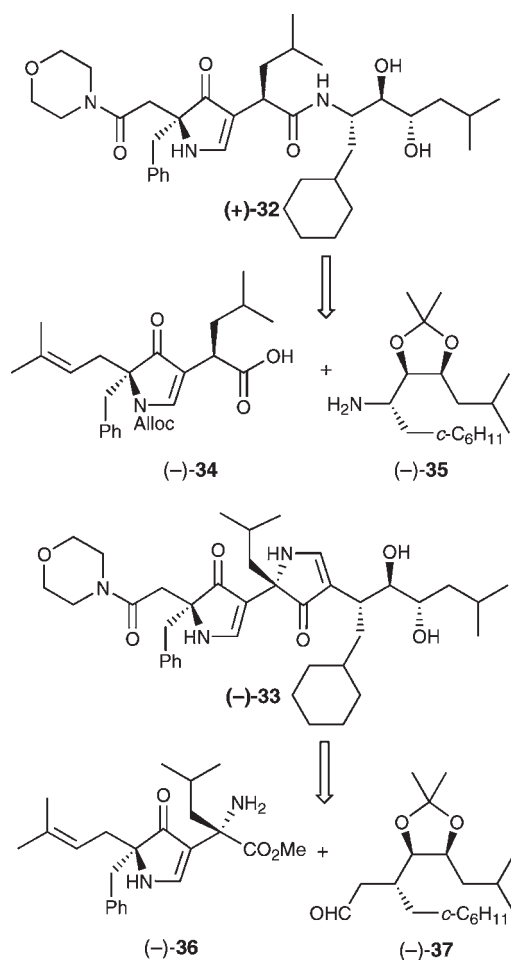


FIGURE 6. Design and synthesis of pyrrolinone-based renin inhibitors.

Design, Synthesis, and Biological Evaluation of HIV-1 Protease Inhibitors

In 1988, the identification that the HIV-1 protease was an aspartic acid protease proved seminal in the search for effective interventions in the HIV pandemic.²⁵ Not surprisingly, we turned to the design and synthesis of pyrrolinone-based HIV-1 protease inhibitors. Initially, we employed the early Merck peptidyl protease inhibitor L-682,679 (Figure 7)²⁶ as a design template. Replacement of the P1'-P2' dipeptide with a bispyrrolinone, possessing both the appropriate side-chains and the P2-P1 and P3' units, led to a series of prospective bispyrrolinone HIV-1 protease inhibitors (cf. **38–40**).^{24,27}

When tested for activity both in enzyme inhibitory (IC_{50}) and cellular (CIC_{95}) assays, furanyl carbamates (–)-**39** and (–)-**40** proved potent at the low nanomolar range (Table 1). That in purified enzyme assays (IC_{50}), (–)-**39** and (–)-**40** proved less active than L-682,679, while in cell-based assays (CIC_{95}) more active [i.e., lower CIC_{95} to IC_{50} (C/I) ratios], suggested that the inhibitors were more cell-permeable than

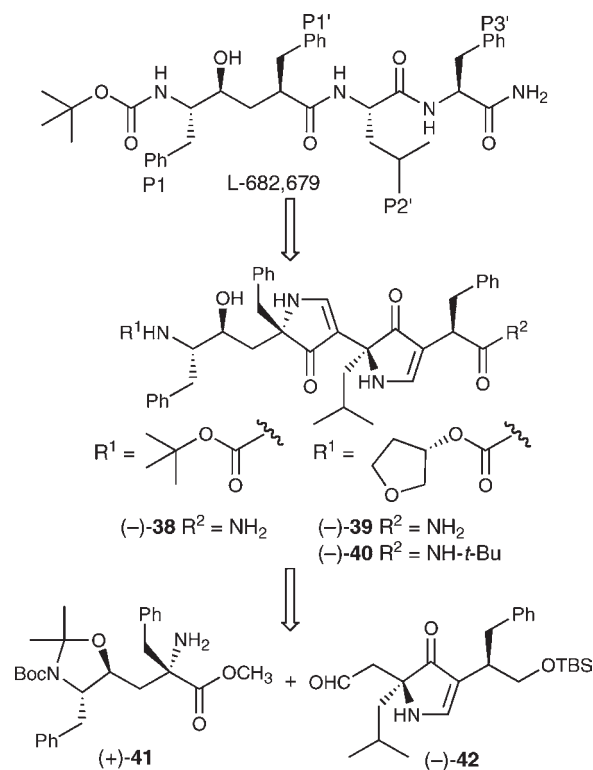


FIGURE 7. Design and synthesis of prospective bispyrrolinone-based HIV-1 protease inhibitors.

the analogous peptides.²⁸ The improved transport properties were attributed to the presence of the *intramolecular* hydrogen bonds between the adjacent pyrrolinone rings, that decreased the desolvation energy required for passage from the extracellular aqueous phase into and through the cellular membrane.

TABLE 1. Biological Activity of Bispyrrolinone HIV-1 Protease Inhibitors^a

| inhibitor | N-terminus | C-terminus | IC_{50} (nM) | CIC_{95} (nM) | C/I |
|----------------|----------------------|-----------------|----------------|-----------------|--------|
| L-682,679 | Boc | NH ₂ | 0.6 | 6000 | 10 000 |
| (–)- 38 | Boc | NH ₂ | 10 | 1500 | 150 |
| (–)- 39 | furanyl carbamate | NH ₂ | 1.3 | 800 | 615 |
| (–)- 40 | furanyl carbamate | NH-t-Bu | 3.3 | 800 | 242 |

^a50% inhibitory concentration, IC_{50} ; cellular 95% inhibitory concentration, CIC_{95} .

Monopyrrolinone HIV-1 Protease Inhibitors

While translation of a peptidyl protease inhibitor into bispyrrolinone congeners proved successful, the bispyrrolinone inhibitors were not orally bioavailable in dogs, presumably due, at least in part, to their high molecular weight (ca. 730). A series of lower molecular weight *monopyrrolinone* inhibitors typified by (–)-**43** (MW = 583), based on L-685,807

and Indinavir,²⁹ were therefore designed and synthesized (Figure 8).³⁰

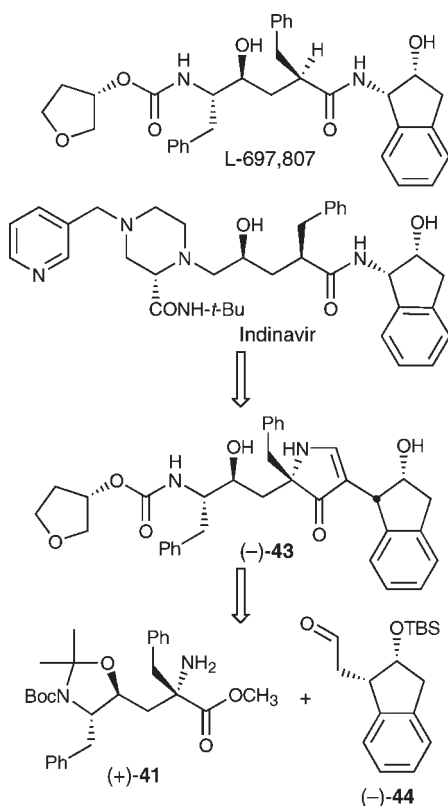


FIGURE 8. Design of monopyrrolinone HIV-1 protease inhibitors.

Biological evaluation of (-)-43 and related (P2/P1') congeners revealed that the inhibitors exploiting the monopyrrolinone scaffold were indeed quite potent against the wild-type HIV-1 protease (Table 2), displaying lower C/I ratios than either the peptide or bispyrrolinones based inhibitors, and thus were anticipated to have improved cell membrane transport properties. Administration of (-)-43 in two dogs revealed oral bioavailability of ca. 13%.

TABLE 2. HIV-1 Protease Bioassay Data for Monopyrrolinone and Related Amide-Based Inhibitors^a

| inhibitor | IC ₅₀ (nM) | CIC ₉₅ (nM) | C/I |
|-----------|-----------------------|------------------------|--------|
| Indinavir | 0.36 | 25–100 | 69–277 |
| L-682,679 | 0.6 | 6000 | 10 000 |
| (-)-39 | 1.3 | 800 | 615 |
| L-697,807 | 0.03 | 3 | 100 |
| (-)-43 | 2.0 | 100–250 | 50–125 |

^a50% inhibitory concentration, IC₅₀; cellular 95% inhibitory concentration, CIC₉₅.

Equally important, X-ray analysis of (-)-43 co-crystallized with the HIV-1 protease^{30a} provided the foundation for an extended Penn/Merck program to design additional modified monopyrrolinone-based inhibitors with improved

binding affinity. From 1994 to 2005, repeated rounds of design and synthesis, employing molecular modeling and X-ray analysis of co-crystal structures, culminated in the discovery of (-)-48,³¹ the most potent (in vitro) monopyrrolinone-based HIV-1 protease inhibitor prepared to date in our laboratory (Figure 9). Thus, our early hypothesis that the pyrrolinone scaffold holds considerable potential as a β -strand mimetic had been validated.

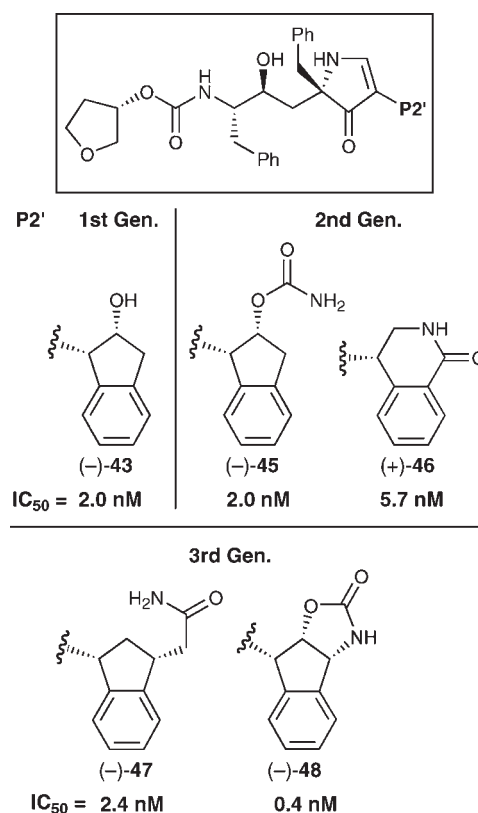


FIGURE 9. Development of monopyrrolinone-based HIV-1 protease inhibitors.

Major Histocompatibility Complex Hybrid Ligands and Matrix Metalloproteases Inhibitors

Building on the success of the aspartic acid protease program, we turned to other proteins and enzymes of biomedical significance, known to prefer binding extended β -strand conformations. The class II major histocompatibility complex (MHC) comprises a series of extracellular membrane-bound proteins found on specialized antigen-presenting T-cells, with the MHC protein HLA-DR1 specifically linked to increased susceptibility toward rheumatoid arthritis.³² In 1994, the late Don Wiley and colleagues established that class II MHC molecules bind antigenic peptides in an extended, polyproline type II conformation.³³ In collaboration with Olsen at Hoffmann–La Roche, we initiated a program

with Wiley to design a pyrrolinone-peptide hybrid ligand for HLA-DR1.³⁴ The design was based on an analogue of the potent peptide HA 306-318 (Figure 10).³⁵ We sought to mimic the peptide with pyrrolinone-peptide hybrid **50** (Figure 10). The bispyrrolinone segment was constructed via our second-generation protocol to provide Fmoc-protected bispyrrolinone amino acid (–)**51**, that was incorporated into peptide **50** via Fmoc-based solid-phase synthesis.

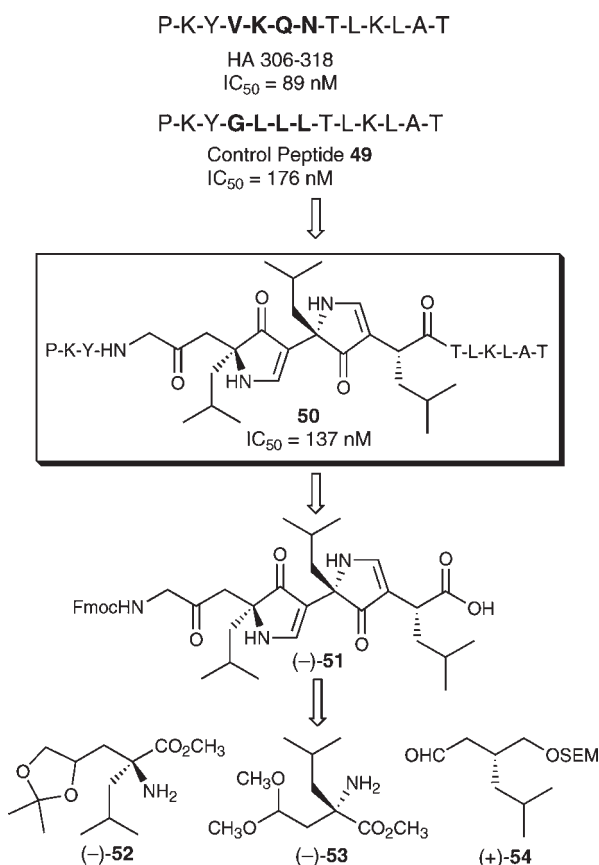


FIGURE 10. Pyrrolinone–peptide hybrid ligand for the class II MHC HLA-DR1.

Affinity-binding experiments revealed that the pyrrolinone-peptide hybrid (**50**) was a competent ligand for HLA-DR1 ($IC_{50} = 137 \text{ nM}$), compared to both HA 306-318 (89 nM) and the control peptide **49** (176 nM).³⁴ Equally exciting, the X-ray structure of co-crystallized **50** and HLA-DR1 obtained by the Wiley group^{34b} mimicked closely the polyproline type II conformation of peptide HA-306-318, a result that demonstrates that the pyrrolinone scaffold can serve as a direct replacement of an amino acid sequence in a bioactive peptide, involving an extended conformation.

We also explored the design of inhibitors for matrix metalloproteases (MMPs), a family of zinc-containing enzymes known to bind substrates in extended conformations,

which have been implicated in a variety of disease states.³⁶ Employing peptidyl inhibitor Ro-31-4724 ($IC_{50} = 9 \text{ nM}$ for MMP-1)³⁷ as the design template, bispyrrolinones (–)**55** and (–)**56** were synthesized (Figure 11).³⁸ Although the bispyrrolinone MMP inhibitors displayed only modest activity (ca. low μM), conformation of the pyrrolinone scaffold as β -strand mimics had again been achieved.

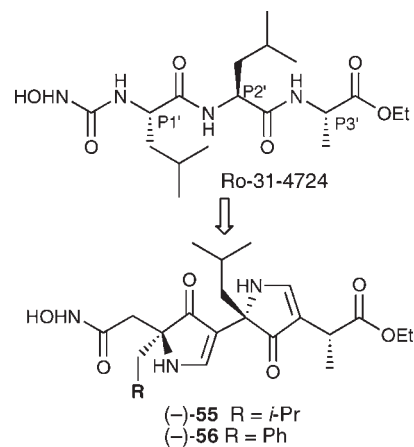


FIGURE 11. Pyrrolinone-based MMP inhibitors.

Alternative Conformations for the 3,5-Linked Polypyrrolinone Scaffold

An evolving interest of the Smith–Hirschmann collaboration was the accessibility of alternative conformational space for 3,5-linked polypyrrolinones. Toward this end, early molecular modeling calculations (Figure 2A) had suggested that the 3,5-linked backbone could not only adopt the extended β -strand conformation, stabilized by an intramolecular hydrogen bond, but also turned and twisted conformations similar to the other secondary conformations of peptides and proteins (Figure 2A).⁴ Tactics to access the broader range of polypyrrolinone conformational space were envisioned to include modulation of the α -stereogenicity, the side-chain structure, and/or the presence of intramolecular hydrogen bonding.

N-Methylated 3,5-Linked Pyrrolinones: Disruption of Intramolecular Hydrogen Bonding

To explore the hypothesis that disrupting the intramolecular hydrogen bond between the pyrrolinone units would lead to additional backbone conformations, structural analysis of a series of model N-methylated bispyrrolinones such as (–)**31** (Figure 5) was undertaken.³⁹ Crystallographic analysis revealed that (–)**31** had a ϕ angle of 177° and that the individual molecules assembled to form a beautiful helical

array in the solid-state (Figure 12).³⁹ Although attempts to enforce a helical array by covalent linking the bispyrrolinone units proved unrewarding,^{39b} observation of a helical array in the solid state of (–)-**31** provided the first evidence of the wider range of conformational diversity available to the 3,5-linked polypyrrolinone structural motif.

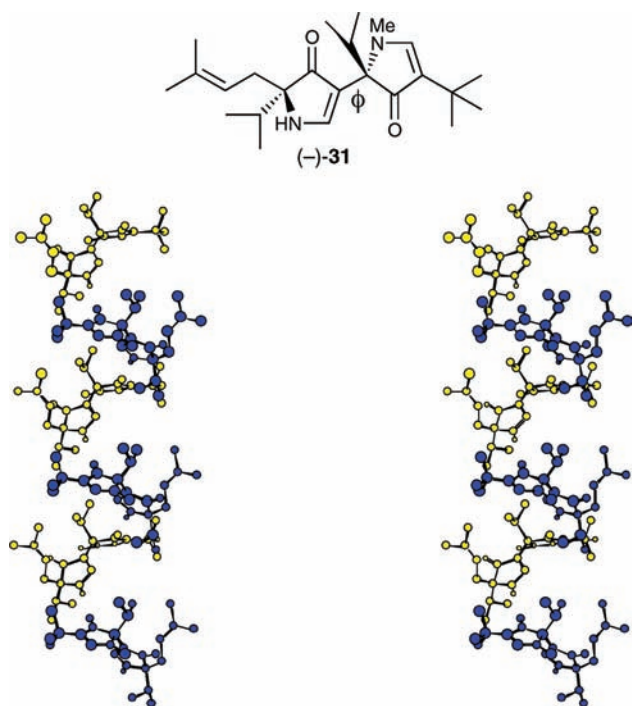


FIGURE 12. Stereoview of the X-ray structure of (–)-**31**. Reprinted with permission from ref 40. Copyright 1999 Elsevier.

D,L-Alternating Polypyrrolinones: Computational Analysis, Synthesis, and Structural Evaluation

In addition to disrupting intramolecular hydrogen bonding, we targeted modulation of the stereogenicity α to the pyrrolinone carbonyl to expand the 3,5-polypyrrolinone conformational space. Based on the established ability of D-amino acids to stabilize β -turns,⁴¹ as well as the demonstrated turn conformations observed with peptides containing D- and L-amino acids,⁴² we reasoned that a sequence of alternating D,L-linked pyrrolinones might preferentially adopt a turn structure.

Computational analysis of *heterochiral* D,L-alternating 3,5-linked pyrrolinones revealed that the low energy conformations not only adopt turn conformations (Figure 13),¹⁴ but importantly predicted that the family of turn conformations would again accommodate intramolecular hydrogen bonding between the adjacent pyrrolinone rings. Moreover, the intramolecular hydrogen bonding would enforce the

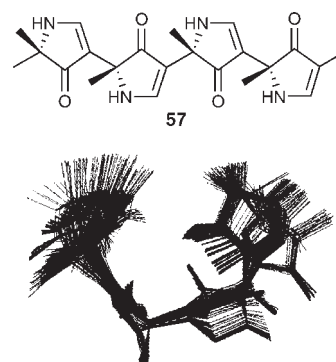


FIGURE 13. D,L,D,L-Tetrapyrrolinone **57** and the low energy structures from a Monte Carlo conformational search.

β -turn-like conformation. With this as background, the synthesis of an initial D,L-alternating tetrapyrrolinone (–)-**58** was achieved exploiting the second-generation protocol. A series of variable concentration NMR, 2D-NMR, and FT-IR experiments revealed that intramolecular hydrogen bonding within tetrapyrrolinone (–)-**58** did in fact lead to a turned conformation in solution (Figure 14).¹⁴

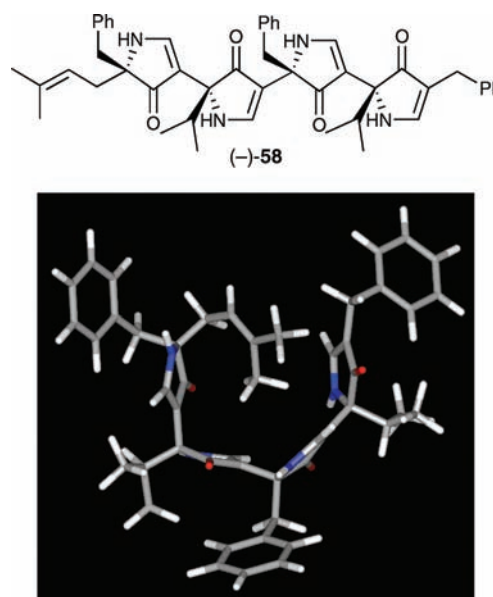


FIGURE 14. Solution structure of (–)-**58**.

Having demonstrated by rational design that a *tetrapyrrolinone* scaffold can adopted a β -turn-like conformation, we next constructed a D,L-alternating *hexapyrrolinone* (**59**, Figure 15A).⁴³ A series of 2D-NMR experiments again revealed a flat, G-shaped turn conformation of (–)-**59** in CDCl₃ (Figure 15B). Pleasingly, X-ray analysis of crystalline (–)-**59** confirmed the flat G-shaped structure (Figure 15C), similar to the low energy conformation observed in solution. Of equal interest, the unit cell revealed that (–)-**59** self-assembles into

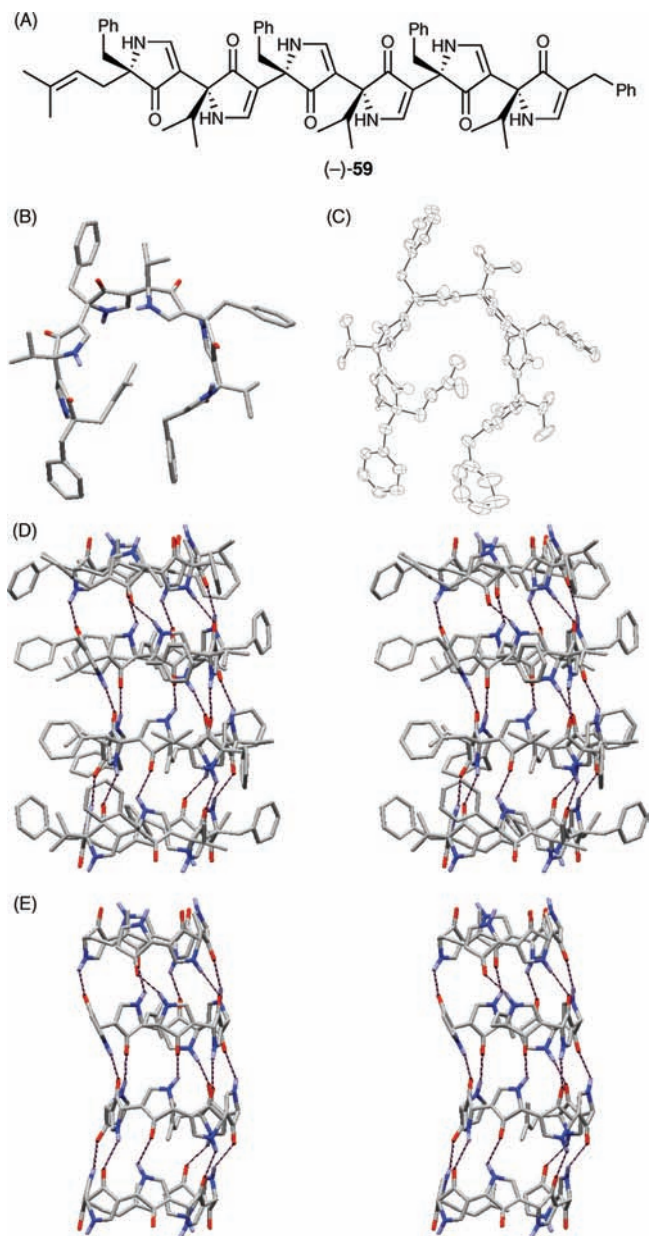


FIGURE 15. (A) *D,L*-Alternating hexapyrrolinone (–)-**59**; (B) Predicted solution structure of (–)-**59**; (C) ORTEP diagram of (–)-**59**; Stereoviews illustrating a nanotube-like assembly of (–)-**59** in the solid state; (D) Side view with intermolecular hydrogen bonds illustrated and (E) with side-chains removed.

a nanotube-like quaternary structure (Figure 15D and E), with the monomers arrayed in an antiparallel fashion.

A Biologically Relevant β -Turn Peptidomimetic Based on the Polypyrrolinone Structural Motif

To validate a heterochiral (*D,L*-alternating) polypyrrolinone turn mimic in a biologically relevant system, recall the “Hirschmann motto”, we turned to somatostatin (somatotropin

release inhibiting factor, SRIF-14), the endogenous, cyclic tetradecapeptide hormone that regulates endocrine and exocrine secretion. Somatostatin was of course well-known to Hirschmann and colleagues, having demonstrated at Merck that a β -turn is both necessary and sufficient for somatostatin receptor binding and signal transduction.⁴⁴ A series of *D,L*-mixed tetrapyrrolinones, incorporating the turn side-chain sequence of L-363,301 (cf. Phe7, Trp8, Lys9, Thr10)⁴⁴ were envisioned as prospective pyrrolinone-based SRIF mimetics (Figure 16).

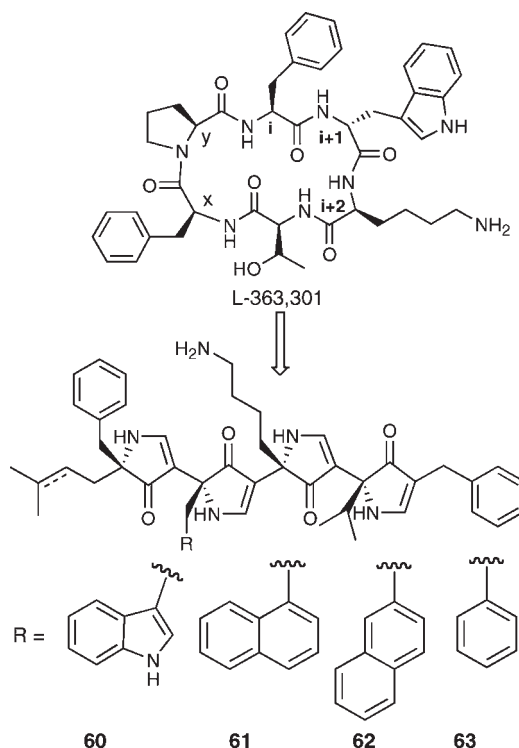


FIGURE 16. Prospective pyrrolinone-based SRIF mimetics.

Although the synthesis of tetrapyrrolinone **60** possessing an $i + 1$ indole side-chain mimic proved elusive, three *D,L*-alternating tetrapyrrolinone SRIF mimetics (–)-**61**, (+)-**62**, and (+)-**63** displaying aromatic indole surrogates were constructed.⁴⁵ Binding affinities were determined at two somatostatin receptors (hsst 4 and 5, Table 3). Despite the modest affinities relative to SRIF, the potential utility of the pyrrolinone scaffold as a β -turn peptidomimetic had been validated.

TABLE 3. Binding Affinities of Pyrrolinone-Based SRIF Mimetics

| ligand | IC ₅₀ hsst 4 | IC ₅₀ hsst 5 |
|----------------|-------------------------|-------------------------|
| (–)- 61 | 2.14 μ M | 2.44 μ M |
| (+)- 62 | 4.04 μ M | 1.27 μ M |
| (+)- 63 | 2.05 μ M | 38% at 10 μ M |
| SRIF-14 | 0.111 nM | 0.362 nM |

Macrocyclic D,L-Alternating Hexapyrrolinones: Design, Synthesis, and Structure Evaluation

The nanotube-like architecture of (–)-**59** in the solid state suggested ring closure to achieve macrocyclic hexapyrrolinones **64** and **65** (Figure 17). Importantly, Monte Carlo

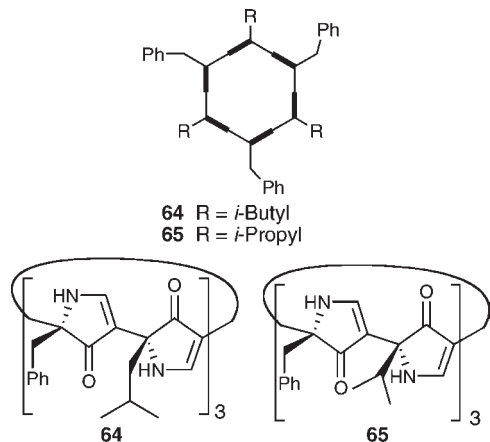
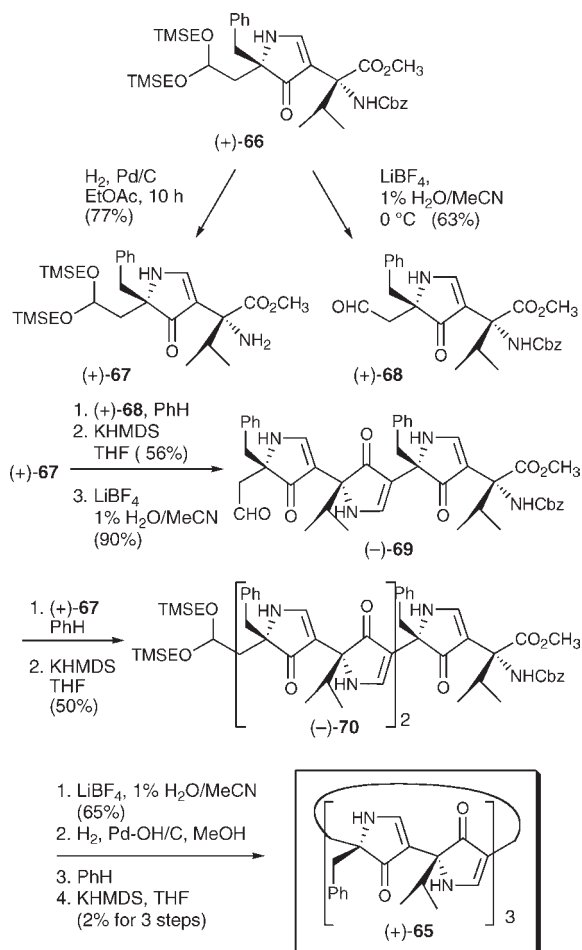


FIGURE 17. Designed macrocyclic hexapyrrolinones.

SCHEME 4. Convergent Synthesis of Macrocyclic Hexapyrrolinone (+)-**65**



conformational searches for **64** predicted a flat conformation for the monomers with the potential for an antiparallel stacking arrangement, in agreement with the observed stacking in the crystalline 17open-chain hexapyrrolinone (–)-**59**.⁴³

Macrocyclic hexapyrrolinone **64** was subsequently prepared,⁴⁶ although the yield for the macrocyclization step proved quite modest (ca. 12%). Importantly, the propensity of macrocycle (+)-**64** to self-assemble in solution was demonstrated via a series of ¹H NMR studies. Unfortunately, crystals of (+)-**64** suitable for X-ray analysis were not forthcoming. Lacking a crystal structure of (+)-**64**, an alternate hexapyrrolinone **65** was designed and synthesized (Scheme 4), with the expectation that the reduced flexibility of the isopropyl side-chains would facilitate crystal growth.

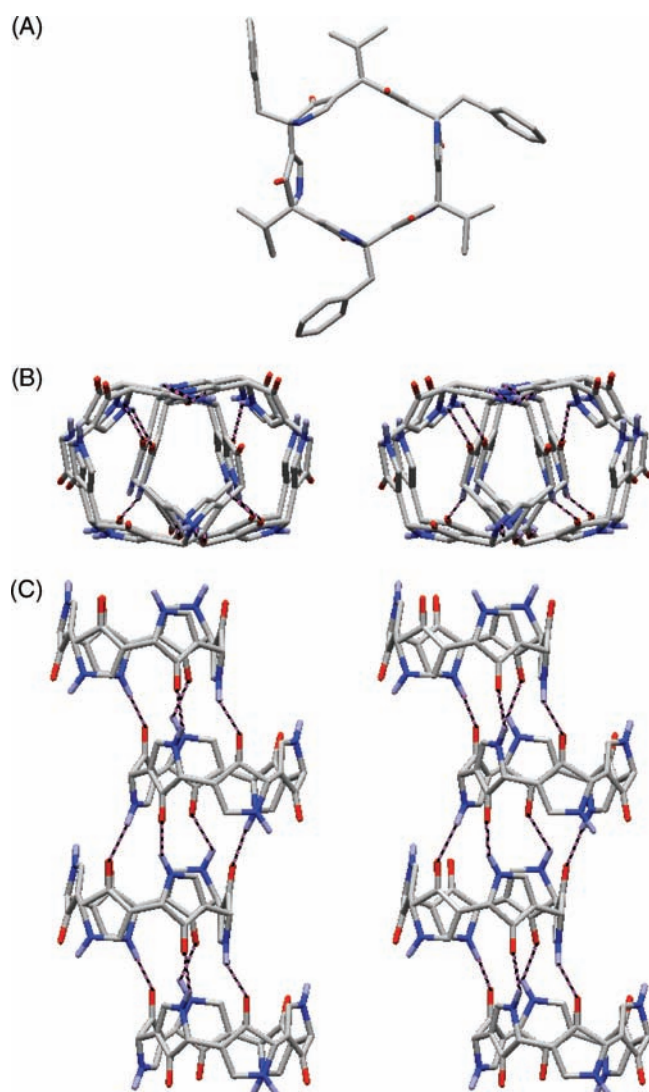


FIGURE 18. (A) X-ray structure of (+)-**65**; nanotube-like assembly of (+)-**65** in the solid state (side-chains omitted); stereoview from the top (B) and side view (C).

Pleasingly, crystals suitable for X-ray analysis of (+)-**65** were obtained; however, unlike the open chain hexapyrrolinones (–)-**59**, (+)-**65** was found to assemble into an infinite, staggered, nanotube-like array, with four pyrrolinone rings participating in intermolecular hydrogen bonding (Figure 18).⁴⁶ The first steps toward the design, synthesis, and structural characterization of novel pyrrolinone-based nanostructures had thus been achieved with the synthesis and structural characterization of (+)-**64** and (+)-**65**.

Summary

The polypyrrolinone scaffold, designed as a nonpeptide peptidomimetic incorporating both hydrogen bond capacity and side-chain diversity, led to the development of a robust metalloenamine-based synthesis, followed by expansion to include diverse amino-acid-like side-chains, flexible protecting-group strategies, and importantly translation to solid-support for the eventual construction of polypyrrolinone libraries. Homochiral 3,5-linked polypyrrolinones adopt extended β -strand/sheet-like conformations, while heterochiral (D,L-alternating) polypyrrolinones lead to turned structures. Importantly homochiral mono- and bispolypyrrolinones were validated as inhibitors of renin, matrix metalloproteases, and potent bioavailable HIV-1 protease inhibitors, as well as a competent peptide–pyrrolinone hybrid ligand for the class II MHC HLA-DR1. Finally, the turned conformations of heterochiral polypyrrolinones were shown to be both biologically relevant as β -turn mimics, capable of producing novel nanotube-like structures. Taken together, the results of the Hirschmann–Smith pyrrolinone program illustrate the remarkable potential of the pyrrolinone structural motif as a privileged scaffold for molecular mimicry, capable of generating β -strand/ β -sheet, β -turn, and potential helical peptidomimetics.

A.B.S. dedicates this Account to the memory of Ralph Hirschmann, friend, colleague, and mentor. We thank our many co-workers and collaborators who have contributed greatly to this research program, and whose names appear in the references cited. Financial support was provided by the NIH, through Grant GM-41821, and by the Merck Research Laboratories and Hoffmann–La Roche.

BIOGRAPHICAL INFORMATION

Amos B. Smith, III was born in Lewisburg, PA, in 1944, and in 1966 completed Bucknell University's inaugural B.S.-M.S. degree in chemistry with Professor H. W. Heine. After a year of medical school at the University of Pennsylvania, he entered The

Rockefeller University, completing his Ph.D. degree in 1972 and spending a year as a Research Associate, both with Professor W. C. Agosta. He then joined the University of Pennsylvania, where he is currently the Rhodes-Thompson Professor of Chemistry and a Full Member of the Monell Chemical Senses Center. From 1988 to 1996, he served as Chair of the Department of Chemistry. In addition, he holds Honorary Memberships in the Kitasato Institute and the Pharmaceutical Society of Japan and serves as the inaugural Editor-in-Chief of *Organic Letters*. In 2009, he was awarded a D.Sc. (honoris causa) from Queens University Belfast (Northern Ireland) for his contributions to Organic Chemistry.

Adam K. Charnley was born in 1978 and spent his early years in Lapeer Michigan. He obtained a B.S. degree from the University of Notre Dame in 2000 as a combined chemistry-business major. In 2005, he completed his Ph.D. in Chemistry at the University of Pennsylvania under the guidance of Professor Amos B. Smith, III. During that time, he was awarded a Bristol-Myers Squibb Graduate Fellowship in Synthetic Organic Chemistry. From 2006 to 2008, he worked in the laboratory of Professor Erik J. Sorensen at Princeton University as an NIH NRSA Postdoctoral Fellow. In 2008, he joined GlaxoSmithKline, where he is currently a Principal Scientist in the Pattern Recognition Receptor DPU of the Immuno-Inflammation Center of Excellence for Drug Discovery.

Ralph F. Hirschmann (1922–2009) was born in Bavaria, Germany, and came to the U.S. in his teens. He graduated from Oberlin College and then served in the U.S. Army in the Pacific Theater during World War II. He resumed his education at the University of Wisconsin, Madison, where he was the Sterling Winthrop Fellow. In 1950, he completed his Ph.D. studies under the guidance of W. S. Johnson and joined Merck & Co., Inc. In 1987, at age 65, he retired from Merck, where he was Senior Vice President for Basic Research, and joined the faculty at the University of Pennsylvania as the Makineni Professor of Bioorganic Chemistry. At Merck, his team discovered Mevacor, Vasotec, Prinivil, Primaxin, Proscar, and Ivermectin. In 1969, Robert G. Denkwalter, Hirschmann, and their collaborators reported simultaneously with the Merrifield group (Rockefeller University) the first total synthesis of an enzyme in solution.

FOOTNOTES

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§Ralph Hirschmann, 1922–2009.

REFERENCES

- (a) Hirschmann, R. F.; Nicolaou, K. C.; Angeles, A. R.; Chen, J. S.; Smith, A. B., III The β -D-Glucose Scaffold as a β -Turn mimetic. *Acc. Chem. Res.* **2009**, *42*, 1511–1520. Also see *Acc. Chem. Res.* **2008**, *41*, issue 10.
- Smith, A. B., III; Keenan, T. P.; Holcomb, R. C.; Sprengeler, P. A.; Guzman, M. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. Design, Synthesis, and Crystal Structure of a Pyrrolinone-Based Peptidomimetic Possessing the Conformation of a β -Strand: Potential Application to the Design of Novel Inhibitors of Proteolytic Enzymes. *J. Am. Chem. Soc.* **1992**, *114*, 10672–10674.
- (a) Nicolaou, K. C.; Salvino, J. M.; Raynor, K.; Pietranico, S.; Reisine, T.; Freidinger, R. M.; Hirschmann, R. In *Design and Synthesis of a Peptidomimetic Employing β -D-Glucose for Scaffolding*; Rivier, J. E., Marshall, G. R., Eds.; ESCOM: Leiden, 1990; pp 881–884. (b) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B., III.; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. Nonpeptidic Peptidomimetics with β -D-Glucose Scaffolding. A Partial Somatostatin Agonist Bearing a Close Structural Relationship to a Potent, Selective Substance P Antagonist. *J. Am. Chem. Soc.* **1992**, *114*, 9217–9218.

- 4 Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P.; Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. De Novo Design, Synthesis, and X-Ray Crystal Structures of Pyrrolinone-Based β -Strand Peptidomimetics. *J. Am. Chem. Soc.* **1994**, *116*, 9947–9962.
- 5 For a review, see: Tyndall, J. D. A.; Nall, T.; Fairlie, D. P. Proteases Universally Recognize Beta Strands In Their Active Sites. *Chem. Rev.* **2005**, *105*, 973–999.
- 6 Rich, D. H. In *Comprehensive Medicinal Chemistry*, Sammes, P. G., Taylor, J. B., Eds.; Pergamon: New York, 1990; Vol. 4, pp 391–441.
- 7 Greenhill, J. V. Enaminones. *Chem. Soc. Rev.* **1977**, *6*, 277–294.
- 8 (a) Huisgen, R.; Brade, H.; Huisgen, R. Die Basizitätskonstanten Offenkettiger Carbonsäure-Amide. *Chem. Ber.* **1957**, *90*, 1432–1436. (b) Greenhill, J. V. Enaminones. *Chem. Soc. Rev.* **1977**, *6*, 277–294.
- 9 For the synthesis and structural analysis of a 2,5-trispyrrolinone, see: Smith, A. B., III; Knight, S. D.; Sprengeler, P. A.; Hirschmann, R. The Design and Synthesis of 2,5-Linked Pyrrolinones. A Potential Non-Peptide Peptidomimetic Scaffold. *Bioorg. Med. Chem.* **1996**, *4*, 1021–1034.
- 10 Hiroi, K.; Achiwa, K.; Yamada, S.-I. Stereochemical Studies. IX. Asymmetric Synthesis of 2-Alkylcyclo-hexanones with Enamine Alkylation. *Chem. Pharm. Bull.* **1972**, *20*, 246–257.
- 11 Smith, A. B., III; Holcomb, R. C.; Guzman, M. C.; Keenan, T. P.; Sprengeler, P. A.; Hirschmann, R. An Effective Synthesis of Scalemic 3,5,5-Trisubstituted Pyrrolin-4-Ones. *Tetrahedron Lett.* **1993**, *34*, 63–66.
- 12 (a) Seebach, D.; Naef, R. Enantioselective Generation and Diastereoselective Reactions of Chiral Enolates Derived from α -Heterosubstituted Carboxylic Acids. Preliminary Communication. *Helv. Chim. Acta* **1981**, *64*, 2704–2708. (b) Seebach, D.; Fadel, A. N. O-Acetals from Pivalaldehyde and Amino Acids for the α -Alkylation with Self-Reproduction of the Center of Chirality. Enolates of 3-Benzoyl-2-(tert-butyl)-1,3-oxazolidin-5-ones. *Helv. Chim. Acta* **1985**, *68*, 1243–1250. For a review, see: (c) Seebach, D.; Sting, A. R.; Hoffmann, M. Self-Regeneration of Stereocenters (SRS) - Applications, Limitations, and Abandonment of a Synthetic Principle. *Angew. Chem., Int. Ed.* **1996**, *35*, 2708–2748.
- 13 (a) Karady, S.; Amato, J. S.; Weinstock, L. M. Enantioselective Alkylation of Acyclic Amino Acids. *Tetrahedron Lett.* **1984**, *25*, 4337–4340. (b) Amato, J. S.; Weinstock, L. M. Karady, S. U.S. Patent, 4,508,921, 1985.
- 14 Smith, A. B., III; Wang, W.; Sprengeler, P. A.; Hirschmann, R. Design, Synthesis, and Solution Structure of a Pyrrolinone-Based β -Turn Peptidomimetic. *J. Am. Chem. Soc.* **2000**, *122*, 11037–11038.
- 15 Smith, A. B., III; Benowitz, A. B.; Favor, D. A.; Sprengeler, P. A.; Hirschmann, R. A Second-Generation Synthesis of Scalemic 3,5,5-Trisubstituted Pyrrolin-4-Ones: Incorporation of Functionalized Amino Acid Side-Chains. *Tetrahedron Lett.* **1997**, *38*, 3809–3812.
- 16 (a) Smith, A. B., III; Liu, H.; Hirschmann, R. A Second-Generation Synthesis of Polypyrrolinone Nonpeptidomimetics: Prelude to the Synthesis of Polypyrrolinones on Solid Support. *Org. Lett.* **2000**, *2*, 2037–2040.
- 17 Smith, A. B., III; Liu, H.; Okumura, H.; Favor, D. A.; Hirschmann, R. Synthesis of Polypyrrolinones on Solid Support. *Org. Lett.* **2000**, *2*, 2041–2044.
- 18 Chamley, A. K. Ph.D. Thesis, University of Pennsylvania, 2005.
- 19 Precigoux, G.; Courseille, C.; Geoffre, S.; Leroy, F. Crystal Structure of the 10–13 Sequence of Angiotensinogen L-leucyl-L-leucyl-L-valyl-L-tyrosyl Methyl Ester. *J. Am. Chem. Soc.* **1987**, *109*, 7463–7465.
- 20 Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P.; Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. De Novo Design, Synthesis, and X-Ray Crystal Structures of Pyrrolinone-Based β -Strand Peptidomimetics. *J. Am. Chem. Soc.* **1994**, *116*, 9947–9962.
- 21 Guzman, M. C. Ph.D. Thesis, University of Pennsylvania, 1994.
- 22 Brewster, U. C.; Setaro, J. F.; Perazella, M. A. The Renin-Angiotensin-Aldosterone System: Cardioresenal Effects and Implications for Renal and Cardiovascular Disease States. *Am. J. Med. Sci.* **2003**, *326*, 15–24 and references cited therein.
- 23 (a) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Dihydroxyethylene Transition-State Mimic at the Scissile Bond To Impart Greater Inhibitory Potency. *J. Med. Chem.* **1988**, *31*, 2264–2276. (b) Izuka, K.; Kamijo, T.; Kubota, T.; Akahane, K.; Umeyama, H.; Kiso, Y. New Human Renin Inhibitors Containing an Unnatural Amino Acid, Norstatine. *J. Med. Chem.* **1988**, *31*, 701–704.
- 24 (a) Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Akaishi, R.; Guzman, M. C.; Jones, D. R.; Keenan, T. P.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Holloway, M. K.; Schleif, W. A. Design and Synthesis of Peptidomimetic Inhibitors of HIV-1 Protease and Renin. Evidence for Improved Transport. *J. Med. Chem.* **1994**, *37*, 215–218.
- 25 Brik, A.; Wong, C.-H. HIV-1 Protease: Mechanism and Drug Discovery. *Org. Biomol. Chem.* **2003**, *1*, 5–14.
- 26 Vacca, J. P.; Guare, J. P.; deSolms, S. J.; Sanders, W. M.; Giuliani, E. A.; Young, S. D.; Darke, P. L.; Zugay, J.; Sigal, I. S.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Anderson, P. S.; Huff, J. R. L-687,908, A Potent Hydroxyethylene Containing HIV Protease Inhibitor. *J. Med. Chem.* **1991**, *34*, 1225–1228.
- 27 Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Guzman, M. C.; Yokoyama, A.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. Pyrrolinone-Based HIV Protease Inhibitors. Design, Synthesis, and Antiviral Activity: Evidence for Improved Transport. *J. Am. Chem. Soc.* **1995**, *117*, 11113–11123.
- 28 Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Darke, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. Synthesis and Antiviral Activity of a Series of HIV-1 Protease Inhibitors with Functionality Tethered to the P1 or P1' Phenyl Design. *J. Med. Chem.* **1992**, *35*, 1685–1701.
- 29 (a) Lyle, T. A.; Wiscourt, C. M.; Guare, J. P.; Thompson, W. J.; Anderson, P. S.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Dixon, R. A. F.; Sigal, I. S.; Huff, J. R. Benzocycloalkyl Amines as Novel C-termini for HIV Protease Inhibitors. *J. Med. Chem.* **1991**, *34*, 1228–1230. (b) Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.-W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. L-735,524: An Orally Bioavailable Human Immunodeficiency Virus Type 1 Protease Inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096–4100.
- 30 (a) Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Yao, W.; Sprengeler, P. A.; Holloway, M. K.; Kuo, L. C.; Chen, Z.; Darke, P. L.; Schleif, W. A. An Orally Bioavailable Pyrrolinone Inhibitor of HIV-1 Protease: Computational Analysis and X-ray Crystal Structure of the Enzyme Complex. *J. Med. Chem.* **1997**, *40*, 2440–2444. (b) Smith, A. B., III; Cantin, L.-D.; Pasternak, A.; Guise-Zawacki, L.; Yao, W.; Chamley, A. K.; Barbosa, J.; Sprengeler, P. A.; Hirschmann, R.; Munshi, S.; Olen, D. B.; Schleif, W. A.; Kuo, L. C. Design, Synthesis, and Biological Evaluation of Monopyrrolinone-Based HIV-1 Protease Inhibitors. *J. Med. Chem.* **2003**, *46*, 1831–1844.
- 31 Smith, A. B., III; Chamley, A. K.; Harada, H.; Beiger, J. J.; Cantin, L.-D.; Kenesky, C. S.; Hirschmann, R.; Munshi, S.; Olsen, D. B.; Stahlhut, M. W.; Schleif, W. A.; Kuo, L. C. Design, Synthesis and Biological Evaluation of Monopyrrolinone-Based HIV-1 Protease Inhibitors Possessing Augmented P2 Side-Chains. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 859–863.
- 32 Todd, J. A.; Acha-Orbea, H.; Bell, J. I.; Chao, N.; Fronek, Z.; Jacob, C. O.; McDermott, M.; Sinha, A. A.; Timmerman, L.; Steinman, L.; McDevitt, H. O. A Molecular Basis for MHC Class II-associated Autoimmunity. *Science* **1988**, *240*, 1003–1009.
- 33 Stern, L. J.; Brown, J. H.; Jardetzky, T. S.; Gorga, J. C.; Urban, R. G.; Strominger, J. L.; Wiley, D. C. Crystal Structure of the Human Class II MHC Protein HLA-DR1 Complexed with an Influenza Virus Peptide. *Nature* **1994**, *368*, 215–221.
- 34 (a) Smith, A. B., III; Benowitz, A. B.; Sprengeler, P.; Barbosa, J.; Guzman, M. C.; Hirschmann, R.; Schweiger, E. J.; Bolin, D. R.; Nagy, Z.; Campbell, R. M.; Cox, D. C.; Olsen, G. L. Design and Synthesis of a Competent Pyrrolinone-Peptide Hybrid Ligand for the Class II Major Histocompatibility Complex Protein HLA-DR1. *J. Am. Chem. Soc.* **1999**, *121*, 9286–9298. (b) Lee, K. H.; Olsen, G. L.; Bolin, D. R.; Benowitz, A. B.; Sprengeler, P. A.; Smith, A. B., III; Hirschmann, R.; Wiley, D. C. The Crystal Structure of a Pyrrolinone-Peptide Hybrid Ligand Bound to the Human Class II MHC Protein HLA-DR1. *J. Am. Chem. Soc.* **2000**, *122*, 8370–8375.
- 35 (a) Hammer, J.; Belunis, C.; Bolin, D.; Papadopoulos, J.; Walsky, R.; Higelin, J.; Danho, W.; Sinigaglia, F.; Nagy, Z. A. High-Affinity Binding of Short Peptides to Major Histocompatibility Complex Class II Molecules by Anchor Combinations. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4456–4460. (b) Bolin, D.; Campbell, R. Hoffmann-La Roche Inc., unpublished results.
- 36 For recent reviews of matrix metalloproteases, see: Matrix Metalloproteinases. *Biochim. Biophys. Acta*, 2010, *1803*, 1–150.
- 37 Johnson, W. H.; Roberts, N. A.; Borkakoti, N. Collagenase Inhibitors: Their Design and Potential Therapeutic Use. *J. Enzyme Inhib.* **1987**, *2*, 1–22.
- 38 Smith, A. B.; Nittoli, T.; Sprengeler, P. A.; Duan, J. J.-W.; Liu, R.-Q.; Hirschmann, R. F. Design, Synthesis, and Evaluation of a Pyrrolinone-Based Matrix Metalloprotease Inhibitor. *Org. Lett.* **2000**, *2*, 3809–3812.
- 39 (a) Smith, A. B., III; Favor, D. A.; Sprengeler, P. A.; Guzman, M. C.; Carroll, P. J.; Furst, G. T.; Hirschmann, R. Molecular Modeling, Synthesis, and Structures of N-Methylated 3,5-Linked Pyrrolin-4-Ones Toward the Creation of a Privileged Nonpeptide Scaffold. *Bioorg. Med. Chem.* **1999**, *7*, 9–22. (b) Favor, D. A. Ph.D. Thesis, University of Pennsylvania, 1998.
- 40 Smith, A. B., III; Favor, D. A.; Sprengeler, P. A.; Guzman, M. C.; Carroll, P. J.; Furst, G. T.; Hirschmann, R. Molecular Modeling, Synthesis, and Structures of N-Methylated 3,5-Linked Pyrrolin-4-Ones Toward the Creation of a Privileged Nonpeptide Scaffold. *Bioorg. Med. Chem.* **1999**, *7*, 9–22.
- 41 Nutt, R. F.; Veber, D. F.; Saperstein, R.; Hirschmann, R. Somatostatin Analogs Which Define the Role of the Lysine-9 Amino Group. *Int. J. Peptide Protein Res.* **1983**, *21*, 66–73.
- 42 (a) Karle, I. L.; Handa, B. K.; Hassall, C. H. The Conformation of the Cyclic Tetrapeptide L-Ser(O-t-Bu)- β -Ala-Gly-L- β -Asp(OMe) Containing a 14-Membered Ring. *Acta Crystallogr.* **1975**, *B31*, 555–560. (b) De Santis, P.; Morosetti, S.; Rizzo, R. Conformational Analysis of Regular Enantiomeric Sequences. *Macromolecules* **1974**, *7*, 52–58. (c) Tomasic, L.; Lorenzi, G. P. Some Cyclic Oligopeptide with S_{2n} Symmetry. *Helv. Chim. Acta* **1987**, *70*, 1012–1016. (d) Clark, T. D.; Buriak, J. M.; Kobayashi, K.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. Cylindrical β -Sheet Peptide Assemblies. *J. Am. Chem. Soc.* **1998**, *120*, 8949–8962 and references cited therein. (e) Hanessian, S.; Vinci, V.; Fettes, K.; Maris, T.; Viet, M. T. P. Self-Assembly of Noncyclic Bis-D- and L-tripeptides into Higher Order Tubular

- Constructs: Design, Synthesis, and X-ray Crystal Superstructure. *J. Org. Chem.* **2008**, *73*, 1181–1191.
- 43 Smith, A. B., III; Wang, W.; Chamley, A. K.; Carroll, P. J.; Kenesky, C. S.; Hirschmann, R. Design, Synthesis and Structural Analysis of *D,L*-Mixed Polypyrrolinones 1: From Nonpeptide Peptidomimetics to Nanotubes. *Org. Lett.* **2010**, *12*, 2990–2993.
- 44 Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. Conformationally Restricted Bicyclic Analogs of Somatostatin. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, 2636–2640.
- 45 Smith, A. B., III; Chamley, A. K.; Mesaros, E. F.; Kikuchi, O.; Wang, W.; Benowitz, A.; Chu, C.-L.; Feng, J.-J.; Chen, K.-H.; Lin, A.; Cheng, F.-C.; Taylor, L.; Hirschmann, R. Design, Synthesis, and Binding Affinities of Pyrrolinone-Based Somatostatin Mimetics. *Org. Lett.* **2005**, *7*, 399–402.
- 46 Smith, A. B., III; Xiong, H.; Chamley, A. K.; Brenner, M.; Mesaros, E. F.; Kenesky, C. S.; Di Costanzo, L.; Christianson, D. W.; Hirschmann, R. Design, Synthesis, and Structural Analysis of *D,L*-Mixed Polypyrrolinones. 2. Macrocyclic Hexapyrrolinones. *Org. Lett.* **2010**, *12*, 2994–2997.