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Design, Synthesis, and Binding Affinities of Pyrrolinone-Based Somatostatin Mimetics

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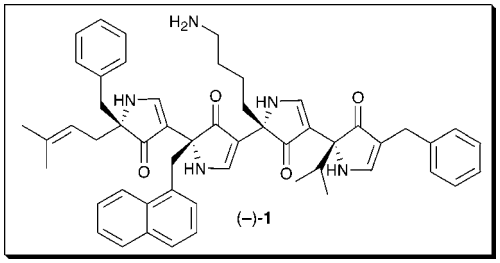
ABSTRACT



Tetrapyrrolinone somatostatin (SRIF) mimetics (cf. 1), based on a heterochiral (D,L-mixed) pyrrolinone scaffold, were designed, synthesized, and evaluated for biological activity. The iterative synthetic sequence, incorporating the requisite functionalized coded and noncoded amino acid side chains, comprised a longest linear synthetic sequence of 23 steps. Binding affinities at two somatostatin receptor subtypes (hsst 4 and 5) reveal micromolar activity, demonstrating that the D,L-mixed pyrrolinone scaffold can be employed to generate functional mimetics of peptide β-turns.

The design of novel β-turn mimetics holds great promise as a tactic for drug discovery to overcome the pharmacokinetic problems commonly associated with peptides. Research from this laboratory has established the 3,5-linked (nitrogen displaced) homochiral pyrrolinone scaffold (Figure 1) as a competent β-strand/β-sheet peptidomimetic both in solution and in the solid state.[1] The biological relevance of the pyrrolinone β-strand was subsequently demonstrated by the design and synthesis of potent pyrrolinone-based inhibitors of proteolytic enzymes, including HIV-1 protease,[2] renin,[3] and matrix metalloproteases.[4] In addition, in collaboration with Olson,[5] we devised a high affinity peptide-pyrrolinone hybrid ligand for the class II major histocompatability

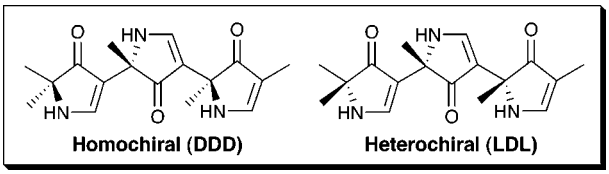


Figure 1. Backbone stereogenicity of a homochiral (DDD) and heterochiral (LDL) polypyrrolinone chain.

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complex (MHC) protein HLA-DR1, which Wiley and coworkers[6] subsequently demonstrated, via X-ray analysis, to bind with remarkable similarity to the native peptide. More recently, we disclosed that heterochiral (D,L-mixed)[7] tetrapyrrolinones adopt β-turn-like conformations in solution.[8] This result suggests that the pyrrolinone scaffold is capable of mimicking both the β-strand and β-turn conformations of peptides simply by modification of the backbone stereogenicity.

A stringent test for a peptidomimetic would be to devise an active ligand for a biologically important receptor. It is, however, important to recognize that observation of affinity with a receptor known to recognize a particular conformation (i.e., β-turn) does not necessarily establish the active conformation but, when taken together with physical data, provides circumstantial evidence about the bioactive conformation of the ligand. With this caveat in mind, we chose to test the biological relevance of the pyrrolinone β-turn via the design and synthesis of pyrrolinone-based somatostatin (SRIF-14) mimetics, both because of our long standing interest in nonpeptide somatostatin mimetics[9] and because the β-turn of SRIF-14 has been shown to be necessary and sufficient for both receptor binding and signal transduction.[10]

Somatostatin (Somatotropin Release Inhibiting Factor, SRIF-14) is an endogenous, cyclic tetradecapeptide hormone with numerous biological activities, including the regulation of both endocrine secretion (i.e., growth hormone, insulin, glucagon, and secretin) and exocrine secretion (i.e., gastric acid).[11] In addition, SRIF acts both as a neurotransmitter in cell signaling and as an inhibitor of cell proliferation. To date five human somatostatin receptor subtypes (hsst 1−5) belonging to the G-protein coupled receptor (GPCR) family have been identified.[12] The short biological half-life (<3 min)[13] of SRIF-14 has led to intense efforts to design stable, orally bioavailable peptide and nonpeptide mimetics of somatostatin.[14] The former, but not the latter, has been achieved. Numerous studies, principally by the Merck group,[10] revealed that the biologically active pharmacophore of SRIF comprises a type I β-turn projecting the essential Phe7, Trp8, and Lys9 side chains with appropriate trajectories to interact with the receptor. Peptidal peptidomimetics were subsequently pioneered by Spatola.[15] Research initiated in 1987 at the University of Pennsylvania has gone a long way to put *designed* nonpeptidal peptidomimetics of neuropeptide hormones solidly on the map.[9] From the beginning, we appreciated that there is an important difference in the molecular nature of the interaction of peptides with enzymes versus those with receptors such as the G-protein coupled receptors (GPCRs). The former involve the interaction of the side chains and the peptide backbone of *both* the enzymes and their peptidal substrates. For binding and signal transduction of G-protein coupled receptors with their peptidal ligands, only side-chain interactions are thought to be required. Having initially envisioned the polypyrrolinones for use as protease inhibitors, the scaffold was designed to interact with the backbone of the proteins, in addition to providing side-chain interactions.

Nonpeptide peptidomimetics incorporating tryptophan and lysine mimicking side chains attached to a variety of turn scaffolds have produced a wide spectrum of biologically active SRIF mimetics (including scaffolds based on β-D-glucose,[9] benzodiazepines,[16] spirolactams,[17] tripeptide heterocycles,[18] and β-peptides[19]).

We envisioned that a D,L-mixed *tetra*pyrrolinone would provide the requisite turn scaffold[8] upon which to design SRIF mimetics as ligands for their G-protein coupled receptors. Incorporation of side chains onto the β-turn mimic defined by the Phe7, Trp8, Lys9, Thr10 sequence suggested a pool of potential SRIF mimetics (Figure 2). Monte Carlo conformational searches[20] predicted that the desired turn structure would be the low energy conformation of heterochiral oligopyrrolinones. For example, Figure 3 displays a

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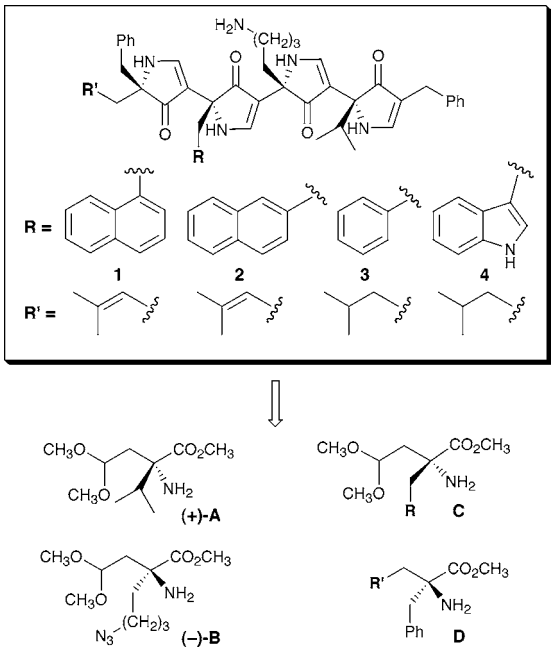

Figure 2. Pool of prospective pyrrolinone-based SRIF mimetics and the requisite building blocks.

stereoview of the low energy conformation of prospective pyrrolinone SRIF mimetic **2**, overlayed with the solution structure of cyclic hexapeptide L-363,301, a potent SRIF mimetic.[21] Particularly pleasing is the excellent overlap of the tryptophan and lysine mimicking side chains that occupy the critical $i + 1$ and $i + 2$ positions of SRIF-14.

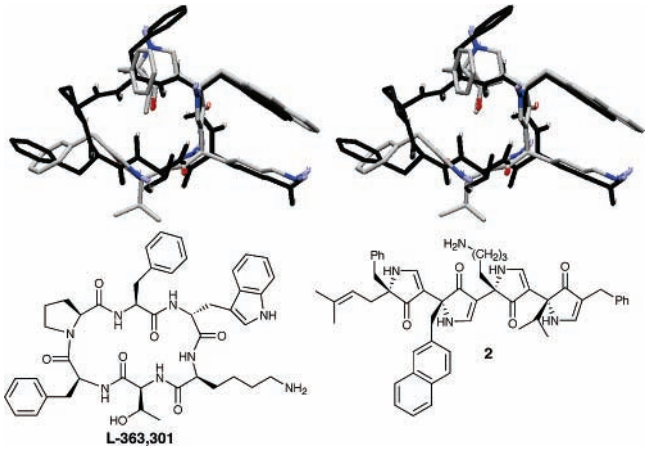

Figure 3. Stereoview: overlay of L-363,301 (black) with **2** (gray).

The initial target selected for synthesis was tetrapyrrolinone **4**, incorporating an indole side chain. Experience with polypyrrolinone synthesis suggested the use of the dimethyl acetal based pyrrolinone synthetic protocol, developed in our laboratory.[8] The requisite building blocks are illustrated in Figure 2. Not unexpectedly, however, and despite our best efforts, complications with introduction of the indole side chain led us to focus on several aromatic surrogates for the indole ring. Naphthyl- and phenyl-based side chains have been employed successfully as SRIF indole replacements without significantly decreasing biological activity[9,22] and, as such, held greater promise for ready incorporation into the polypyrrolinone-based scaffold (vide infra).

Toward this end, tetrapyrrolinones (**1**−**3**) were envisioned to arise from four α,α-disubstituted amino ester precursors (**A**−**D**), exploiting an iterative C to N extension of the pyrrolinone chain. The requisite building blocks were readily prepared via the enantioretentive alkylation tactic developed by Karady[23a] and Seebach.[23b] Toward this end, amino esters derived from phenylalanine and valine were prepared via procedures developed early in our pyrrolinone synthetic program;[8] the amino esters possessing noncoded amino acid side chains (α- and β-naphthyl), (−)-**C**$_\alpha$ and (−)-**C**$_\beta$, and the lysine mimic (−)-**B** were prepared exploiting our "universal oxazolidinones synthetic protocol."[24] Experimental details for construction of **A**−**D** are available in the Supporting Information.

With the necessary amino esters in hand, we began iterative construction of the tetrapyrrolinones (Scheme 1). Condensation of (+)-**A** with hydrocinnamaldehyde employing azeotropic removal of water afforded the corresponding imine, which upon treatment with KHMDS undergoes metallo-enamine promoted cyclization to generate monopyrrolinone (−)-**5**, effectively capping the C-terminal pyrrolinone with a benzyl group. Hydrolysis of the dimethyl acetal then affords the corresponding monopyrrolinone aldehyde, which is condensed with amino ester (−)-**B** and in turn subjected to the same KHMDS protocol to provide bispyrrolinone (+)-**6**, a common intermediate for prospective β-turn mimetics **1**−**3**.

The third iterative pyrrolinone ring construction, the point of synthetic diversion, permits incorporation of three aromatic tryptophan-mimicking side chains into the growing polypyrrolinone rings. Yields here were modest. Not withstanding the modest yields, the fourth pyrrolinone synthetic iteration introduced a benzyl side chain in each case, employing amino ester (−)-**D** for trispyrrolinones (−)-**7** and (−)-**8** and the reduced congener (+)-**10** for trispyrrolinone (−)-**9**. Staudinger reduction or hydrogenation of the azide to generate the lysine side chain mimic provided tetrapyrrolinone SRIF mimetics **1**−**3**. The syntheses of **1**−**3** were achieved via a longest

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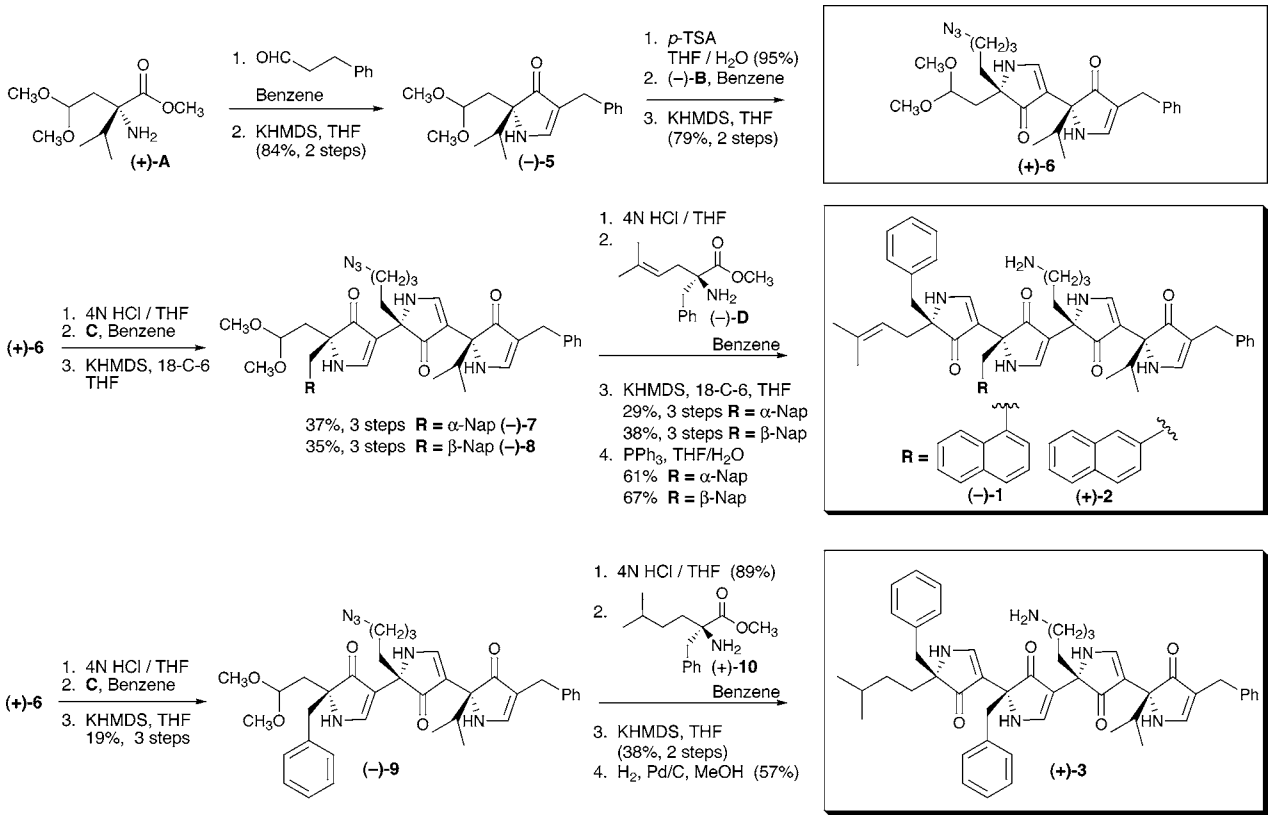
Scheme 1. Preparation of SRIF Mimetics **1**−**3**



linear sequence of 23 steps [12 steps from (+)-**A**] in an overall yield of ca. 1.0%.

Binding affinities of the three tetrapyrrolinone SRIF mimetics were determined via a radioligand binding assay with the [^{125}I]-SRIF$_{14}$ ligand at two somatostatin receptors[25] (hsst 4[26] and 5[27]) (Table 1). All three designed ligands possessed low micromolar affinity for hsst 4, with (−)-**1** and (+)-**3** exhibiting an IC$_{50}$ of approximately 2 μM and (+)-**2** possessing an IC$_{50}$ of 4 μM. At the hsst 5 receptor, only the naphthyl-containing compounds displaced binding of [^{125}I]-SRIF$_{14}$. Despite the modest binding affinities of these compounds relative to SRIF, our experience with the glucose-based SRIF mimetics suggests that micromolar activity provides sufficient and relevant validation for the potential of nonpeptidal scaffolds. In addition, as observed with our β-D-glucose turn mimics, optimization of mimetics with micromolar activity can lead to nanomolar ligands.[9] Therefore, the observation that SRIF mimetics **1**−**3** displace [^{125}I]-SRIF$_{14}$ binding to somatostatin receptors in vitro at low micromolar concentrations supports the potential utility of the pyrrolinone scaffold as a β-turn peptidomimetic. Equally important, these results establish polypyrrolinones as a privileged scaffold capable of generating functional mimetics of multiple peptidal secondary structures. Efforts to develop more potent β-turn peptidomimetics based on the 3,5-linked pyrrolinone scaffold continue in our laboratory.

Table 1. Binding Affinities of Pyrrolinone-Based SRIF Mimetics

ligand	IC$_{50}$ hsst 4	IC$_{50}$ hsst 5
(−)-**1**	2.14 μM	2.44 μM
(+)-**2**	4.04 μM	1.27 μM
(+)-**3**	2.05 μM	38% at 10 μM
SRIF-14	0.111 nM	0.362 nM

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Supporting Information Available: Schemes for the preparation of the amino ester building blocks and experimental details for all synthetic transformations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. IC$_{50}$ values were determined by a nonlinear, least squares regression analysis using Data Analysis Toolbox (MDL Information Systems, San Leandro, CA).

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