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Conformationally Homogeneous Heterocyclic Pseudo-Tetrapeptides as Three-Dimensional Scaffolds for Rational Drug Design: Mapping the Structural Basis Set and Application to the Design of Receptor-Selective Somatostatin Analogues

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Abstract

Many protein-protein interactions are mediated through the recognition of β-turn secondary structures. Consequently, small-molecule β-turn mimetics are invaluable as probes for assessing bioactive ligand conformations, establishing pharmacophoric requirements, and pursuing rational drug design. While effective drug scaffolds have been developed to precisely position up to four functionalities primarily in two-dimensions, an analogous rigid scaffold capable of predictably juxtaposing four amino acid side chains in three-dimensions has required the use of pentameric or larger cyclopeptides. Diverse approaches have been taken in efforts to constrain peptides into turn conformations,[1] but one strategy that has not been broadly explored is the use of cyclic tetrapeptides.[2,3] Cyclic tetrapeptides offer an attractive platform to mimic protein turn regions due to their appropriate size, shape, and synthetic modularity, but these structures remain largely unexplored due to poor synthetic efficiency in constructing the strained 12-membered ring, an

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inability to control *cis-trans* geometry of backbone amides, and the apparent requirement to sacrifice one of four amino acid residues to incorporate a proline or other turn-forming residue. [2,4,5]

Keywords

drug design; cyclic peptides; somatostatin; structure-activity relationship; triazole

Here we report the syntheses and analyses of two classes of 13-and 14-membered ring pseudo-tetrapeptides[5–7] containing either one or two triazole moieties, respectively, and describe the design, syntheses, structural analyses, and somatostatin (SST) receptor binding activities of a library of all 16 possible stereoisomeric compounds incorporating the somatostatin pharmacophore. These studies exploit the 1,4-disubstituted 1,2,3-triazole as a *trans* peptide bond surrogate.[7–10] Structural analysis of the diastereomeric library using NMR spectroscopy indicated that each of the peptide scaffolds adopts a distinct, rigid, conformationally homogeneous turn-like structure.[11] The three-dimensional pharmacophoric display of the compounds is systematically altered by varying the stereochemistry around the otherwise constitutionally identical scaffolds, yielding both compounds with broad-spectrum activity against the five human SST receptor subtypes (pan-somatostatins) and compounds with receptor selectivity. Our studies therefore provide a basis set of scaffolds having subtle but predictable differences in their spatial display of amino acid side chains that are useful for rational, structurally-informed design of bioactive agents.

As part of a research program investigating the incorporation of triazoles into linear and cyclic peptide architectures,[9] we synthesized compounds **1**–**4**, which can be divided into two classes that differ by the presence of either one (class I) or two (class II) backbone triazole moieties. Encouraged by the relatively facile cyclization of these 13- and 14-membered rings via triazole ring formation, and motivated by their potential for predictable side chain display, we undertook high resolution structural analyses of these peptides. In all cases, the obtained structures revealed a rigid rectangular scaffold that superimposed well on idealized β-turn motifs (Figure S2). Importantly, the ^{1}H NMR spectra for all four compounds were sharp and consistent with a single solution species present on the NMR time scale. Although the observed NOEs involving the triazole C-H atom indicate that the triazole populates multiple rotameric conformations in fast exchange on the NMR time scale, this variability has virtually no impact on the position of the rest of the atoms in the backbone or the C_{α}-C_{β} vectors (see Supporting Information for the synthesis of compounds **1**–**4**).

We wondered if the observed conformational homogeneity for **1**–**4** would hold for the entire series of 16 possible stereoisomeric pseudo-tetrapeptides of a given sequence. If so, structural determination of the eight diastereomers in a given enantiomeric series (from which the eight mirror image counterparts could be easily modeled) would provide a basis set of conformationally predictable, three-dimensional scaffolds amenable to drug design. Accordingly, we set out to synthesize the 16 possible stereoisomeric pseudo-tetrapeptides incorporating the pharmacophoric residues of somatostatin-14 (SRIF-14, Phe^{7}-Trp^{8}-Lys^{9}-Thr^{10}), a well-studied ligand known to bind its cognate receptors using a β-turnmotif.[12]

To synthesize the 16 stereoisomeric structures, we prepared two sets of dipeptides, each comprising four diastereomers (compounds **5a–5d** and **6a–6d**), from which all 16 required diastereomeric tetramers could be prepared (Scheme 1). Macrocyclizations of the crude linear azido alkynes via copper (I) mediated [3+2] Huisgen dipolar cycloaddition proceeded

in yields ranging from 31–90%, as determined by HPLC (isolated yields of 20–59% following deprotection, based on the dipeptide starting materials). Alternative attempts to synthesize compounds of type **7** via macrolactamization resulted in racemization and poor cyclization yields, consistent with previous reports.[7] Inclusion of the copper chelating ligand tris-(benzyltriazolylmethyl)amine (TBTA)[13] during the [3+2] macrocyclization step favored formation of the desired cyclic tetramer over formation of the cyclic octamer species that results from head-to-tail dimerization of two tetrapeptide substrates.[14] An attempt to facilitate macrocyclization by heating to 70 °C (for compound **7a**) resulted in complex ^{1}H NMR spectra that were ascribed to either multiple product conformations or racemization. On the other hand, cyclizations conducted at room temperature yielded conformationally homogeneous compounds that remained homogeneous after being heated to 70 °C (for compound **7d**).

With the 16 stereoiso meric somatostatin analogs in hand, we first assessed the extent of their conformational homogeneity. Encouragingly, all 16 compounds gave sharp ^{1}H NMR spectra consistent with a single conformation on the NMR time scale, so we proceeded to determine NMR solution structures using the program CNS[15] for the eight compounds in one enantiomeric series (Figure 2). Each of the eight diastereomers exhibited a distinct and conformationlly homogeneous structure, which is somewhat remarkable considering the conformational heterogeneity previously noted in cyclic tetrapeptides.[2,4,5] Not surprisingly, it appears that the relative chirality of the four stereogenic centers, exerts the most dominant influence on the amide backbone orientation and the distinct three-dimensional side chain display adopted by each scaffold.

The library of the 16 stereoisomeric pseudo-tetrapeptides were screened for binding activity to the five human somatostatin receptor subtypes ($hSSTR_{1–5}$) (Table 1). Despite the fact that all 16 compounds are constitutionally identical (having the same sequence of amino acids), a range of bioactivities was observed, including a total lack of binding even at concentrations up to 10 μM (**7g**), selectivity for $hSSTR_1$ (**7h**) or $hSSTR_4$ (***ent*-7f**), and broad-spectrum activity mimicking that of somatostatin (***ent*-7g**). The $hSSTR_2$ receptor appeared to have the most stringent binding requirements for these compounds, whereas $hSSTR_4$ bound nearly all of the compounds with some affinity.

To clarify the three-dimensional pharmacophoric determinants of affinity and specificity for these compounds, we next sought to identify structural similarities among the peptides having comparable activities. Because each scaffold is conformationally rigid, the backbone atoms and the C_α-C_β vectors can be used with relatively high confidence in structural analyses. To assess the $hSSTR_4$ binding profile, we overlayed all 16 compounds onto ***ent*-7g** (the highest affinity $hSSTR_4$ binder) using the C_β atoms of the Trp and Lys side chains (known to be the most important components of the pharmacophore) and the C_α and C_β atoms of the Phe side chain (because the highest affinity $hSSTR_4$ binders all contain a conserved D-Phe chirality). After removing scaffolds with RMSD values above a cutoff of 0.7 Å (see Table S1 for all RMSD values), we qualitatively observed clustering into three conformational families (Figure 3a). Interestingly, the three observed groupings correspond to the set of peptides that bind multiple hSSTR receptors (***ent*-7d** and ***ent*-7g**, blue atoms in Figure 3a), the peptides that are most selective for $hSSTR_4$ (**7b**, **7e**, ***ent*-7a**, and ***ent*-7f**, yellow atoms in Figure 3a), and peptides that bound with low affinity (**7d**, **7g**, ***ent*-7c**, and ***ent*-7h**, pink atoms in Figure 3a). In other words, when constrained by the overlay to place the pharmacophoric Phe, Lys, and Trp residues in a particular region of space (much as the receptor binding site would require a particular spatial arrangement of side chains), the rigidity of the scaffolds enforces different orientations that correlate with their observed binding characteristics.

To better understand the requirements for selective binding to $hSSTR_4$, a different overlay of the 16 compounds onto ***ent*-7f** (the most selective $hSSTR_4$ binder) was carried out. When the Thr C_α atom and additional backbone atoms (Lys carbonyl carbon and Trp amine) were used along with the fit atoms described above, discarding scaffolds above an RMSD cutoff of 0.55 Å (see Table S1 for all RMSD values) left the four most selective $hSSTR_4$ ligands (**7b**, **7e**, ***ent*-7a**, and ***ent*-7f**) (Figure 3b). The most notable similarities of these compounds are that all four contain a D-Phe residue and all four have Lys and Trp residues of the same chirality (both L- or both D-). The requirement that additional Thr and backbone atoms be included in the fit to ensure that the lowest RMSD compounds corresponded to the most selective $hSSTR_4$ ligands suggests that avoiding steric clashes near the Thr residue in the receptor binding pocket is an additional important factor in selective binding to $hSSTR_4$. The RMSD of these compounds from ***ent*-7f** follows the same trend as their affinity for the $hSSTR_4$ receptor (***ent*-7f** < **7e** < ***ent*-7a** < **7b**).

A similar structural analysis was conducted for $hSSTR_1$-binding ligands. The 16 diastereomers were overlayed onto **7h** (the most selective $hSSTR_1$ binder) using the C_α atoms of all four residues and the C_β atoms of Trp and Lys (Figure 3c). After discarding molecules with an RMSD cutoff of greater than 0.3 Å (see Table S1), the remaining peptides (***ent*-7g** and ***ent*-7d**) represent the only other high affinity, albeit less selective, binders of the $hSSTR_1$ receptor. Considering the bioactivities and the atoms required for this fit, it appears that the four C_α positions and the C_α-C_β vectors of Lys and Trp are the major determinants of $hSSTR_1$ affinity for our compounds, while the Phe and Thr side chains influence receptor selectivity.

We hope that the well-defined diastereomeric structures described here will serve as a basis set from which future structure-based drug design studies can be initiated. Furthermore, by determining the “negative structural image”[16] of receptor binding pockets, the use of small libraries of scaffolds having systematic and predictable differences in their spatial display of amino acid side chains could be useful in delineating the three-dimensional pharmacophoric requirements for receptor binding and selectivity, especially in cases where high-resolution structural data are not readily available.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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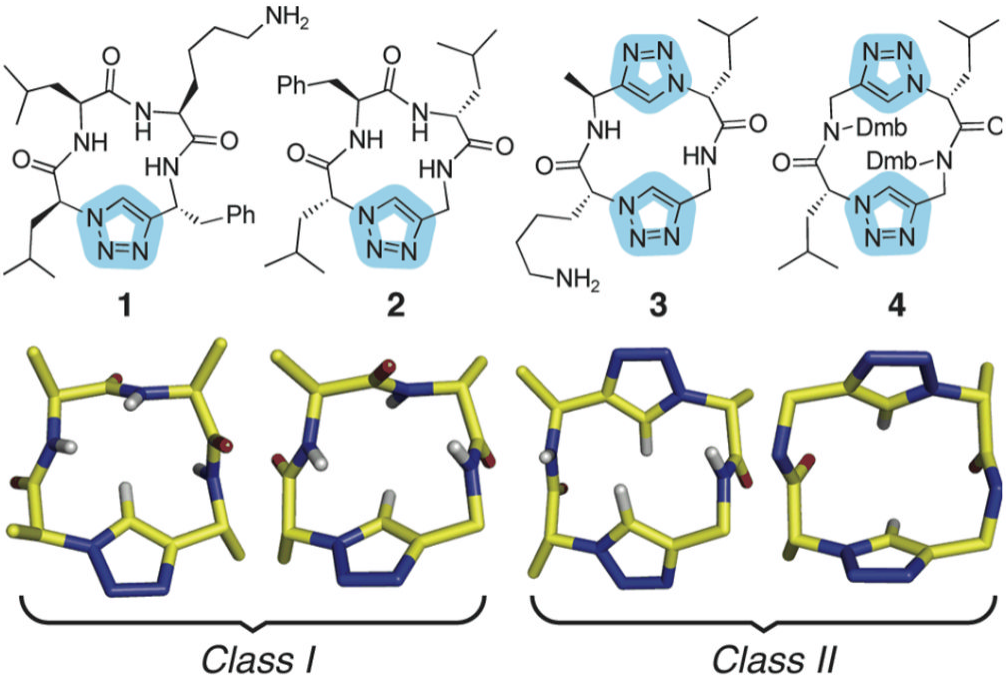


Figure 1.

Chemical and molecular structures of representative members of two classes of cyclic pseudo-tetrapeptide scaffolds. Structures were determined using multidimensional NMR (**1**–**3**) or X-ray crystallography (**4**). Some atoms have been omitted for clarity. Dmb = 2,4-dimethoxybenzyl.

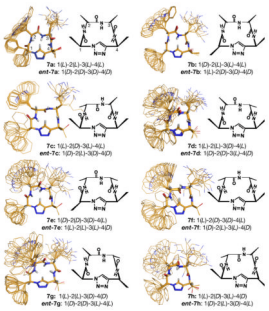


Figure 2.

NMR solution structures (d_6-DMSO) and schematic diagrams for eight diastereomeric pseudo-tetrapeptide scaffolds. Stereocenter configurations are labeled according to the amino acid starting materials from which they are derived. For each structure shown, the corresponding enantiomer was also synthesized and assayed for $hSSTR_{1-5}$ binding.

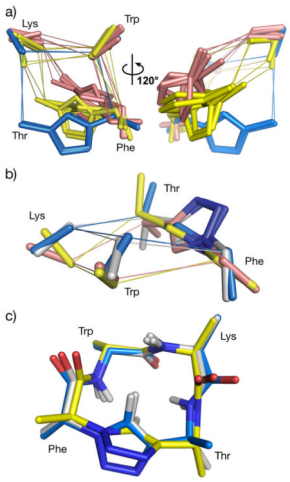
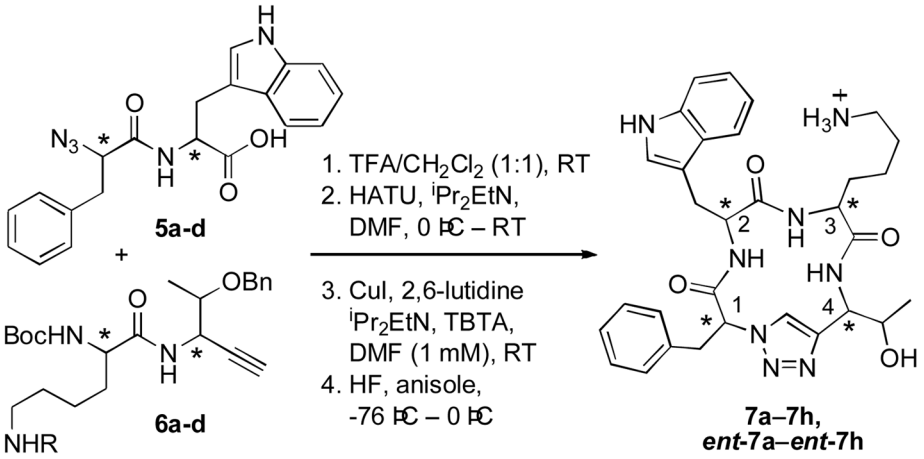


Figure 3.

Pairwise fittings of pseudo-tetrapeptides correlating structure with bioactivity. a) All 16 compounds were overlayed onto ***ent*-7g** (highest affinity $hSSTR_4$ binder) using pairfits of the Trp, Lys, and Phe C_β atoms and the Phe C_α atom. Compounds with an RMSD higher than 0.7 Å are not shown; compounds ***ent*-7g** and ***ent*-7d** (blue) bind multiple receptors including $hSSTR_4$; compounds ***ent*-7f**, **7e**, ***ent*-7a**, and **7b** (gold) are specific for $hSSTR_4$; compounds **7d**, **7g**, ***ent*-7c**, and ***ent*-7h** (pink) did not bind or bound with low affinity. Sticks are shown for the triazole atoms and the C_α and C_β atoms of Phe, Trp, and Lys. b) Overlay of the 16 compounds onto ***ent*-7f** (most selective $hSSTR_4$ binder) (pink). An RMSD cutoff of 0.55 Å left compounds **7b** (gold), **7e** (blue), and ***ent*-7a** (white), which represent the $hSSTR_4$-selective peptides. c) Overlay of the 16 compounds using pairfits of the C_α atoms from all four residues and Trp and Lys C_β atoms onto **7h** (most selective $hSSTR_1$ binder) (gold). An RMSD cutoff of 0.3 Å left only molecules ***ent*-7g** (blue) and ***ent*-7d** (white), which correspond to the only other high affinity, albeit less selective, binders of $hSSTR_1$.



Scheme 1.

Synthetic strategy for the pseudo-tetrapeptide library. Compounds **5a**–**d**, compounds **6a**–**d**, and compounds **7a**–**h** differ only at the marked (asterisk) chiral centers. R = Cbz or 2-Cl-Cbz. HATU = 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate, TBTA = tris-(benzyltriazolylmethyl)amine.

Table 1

$hSSTR_{1–5}$ binding affinities (IC_{50}, nM) for the heterocyclic pseudo-tetrapeptides.

	IC_{50} (nM)[a]				
peptide	**$hSSTR_1$**	**$hSSTR_2$**	**$hSSTR_3$**	**$hSSTR_4$**	**$hSSTR_5$**
SRIF-28[b]	1.8 ± 0.5	2.0 ± 0.3	2.4 ± 0.7	3.3 ± 0.3	2.4 ± 0.7
7a	>10,000	>10,000	1,000 ± 100	2,600 ± 200	2,100 ± 400
7b	>10,000	>10,000	>10,000	1,600 ± 200	>10,000
7c	5,800 ± 500	>10,000	>10,000	>10,000	>10,000
7d	>10,000	>10,000	6,700 ± 1,500	5,600 ± 500	>10,000
7e	2,100 ± 200	>10,000	4,200 ± 1,600	570 ± 60	5,000 ± 1,500
7f	>10,000	>10,000	>10,000	2,900 ± 300	>10,000
7g	>10,000	>10,000	>10,000	>10,000	>10,000
7h	110 ± 10	1,200 ± 200	7,700 ± 1,100	2,200 ± 300	3,800 ± 800
***ent*-7a**	>10,000	>10,000	2,800 ± 600	980 ± 60	5,100 ± 400
***ent*-7b**	4,100 ± 1,200	>10,000	>10,000	5,000 ± 700	6,100 ± 600
***ent*-7c**	>10,000	3,300 ± 200	5,400 ± 1,300	4,700 ± 1,000	>10,000
***ent*-7d**	180 ± 4	1,300 ± 400	730 ± 200	790 ± 100	1,600 ± 300
***ent*-7e**	>10,000	>10,000	>10,000	>10,000	>10,000
***ent*-7f**	>10,000	>10,000	>10,000	230 ± 50	>10,000
***ent*-7g**	170 ± 20	160 ± 50	67 ± 30	140 ± 10	170 ± 50
***ent*-7h**	>10,000	>10,000	3,300 ± 400	2,900 ± 700	>10,000

[a] Compounds were tested in triplicate. The IC_{50} values derived from competitive radioligand displacement assays reflect the affinities of the peptides for the cloned somatostatin receptors using the nonselective ^{125}I-[Leu^8, DTrp^{22}, Tyr^{25}] SRIF-28 as the radioligand.

[b] For the chemical structure of SRIF-28, see ref. 10d.