DOI: 10.1002/ange.200805901

Drug Design

Conformationally Homogeneous Heterocyclic Pseudotetrapeptides as Three-Dimensional Scaffolds for Rational Drug Design: Receptor-Selective Somatostatin Analogues**

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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. Although effective small-molecule drug scaffolds have been developed for the precise positioning of up to four functionalities primarily in two dimensions, an analogous rigid scaffold capable of the predictable juxtaposition of 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] however, one strategy that has not been broadly explored is the use of cyclic tetrapeptides.[2,3] Cyclic tetrapeptides offer an attractive platform for mimicking protein turn regions owing to their appropriate size, shape, and synthetic modularity. These structures remain largely unexplored as a result of poor synthetic efficiency in the construction of the strained 12-membered ring, an inability to control the *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]

Herein we report the synthesis and analysis of two classes of 13- and 14-membered-ring pseudotetrapeptides[5–7] that contain either one or two triazole moieties, respectively, and describe the design, synthesis, structural analysis, and binding affinity for the somatostatin (SST) receptor of a library of all 16 possible stereoisomeric compounds incorporating the somatostatin pharmacophore. These studies exploited the 1,4-disubstituted 1,2,3-triazole as a surrogate for a *trans* peptide bond.[7–10] Structural analysis of the library of diastereomers by NMR spectroscopy indicated that each of the peptide scaffolds adopts a distinct, rigid, conformationally homogeneous turnlike structure.[11] The three-dimensional pharmacophoric arrangement of the compounds can be altered systematically by varying the stereochemistry at different positions of the otherwise constitutionally identical scaffolds to yield both compounds with broad-spectrum activity against the five human SST-receptor subtypes and compounds with receptor selectivity. Our studies therefore provide a basis set of scaffolds with subtle but predictable differences in the spatial arrangement of amino acid side chains for the rational, structurally informed design of bioactive agents.

As part of a research program on the incorporation of triazoles into linear and cyclic peptide architectures,[9] we synthesized compounds **1**–**4** (Figure 1), which can be divided into two classes that differ by the presence of either one (class I) or two (class II) backbone triazole moieties (see the Supporting Information for the synthesis of compounds **1**–**4**). Encouraged by the relatively facile cyclization of these

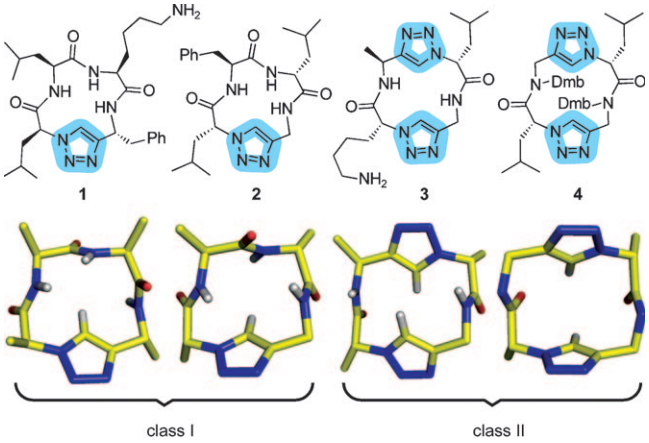

Figure 1. Chemical and molecular structures of representative members of two classes of cyclic-pseudotetrapeptide scaffolds. Structures were determined by multidimensional NMR spectroscopy (in the case of **1**–**3**) or X-ray crystallography (in the case of **4**). Some atoms have been omitted for clarity.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

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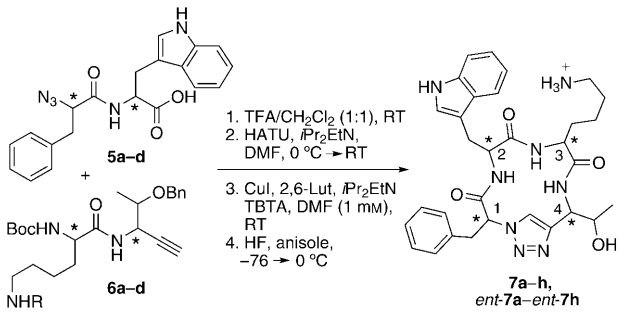
[**] We thank the NASA Earth and Space Science Fellowship Program (grant NNX07AR35H; J.M.B.), the Netherlands Organization for Scientific Research (NWO) for a travel grant (J.H.v.M.), and Dr. L. J. Leman for assistance with the manuscript. This research was supported in part by a grant from the National Institute of General Medical Sciences (GM52190) and by the Skaggs Institute for Chemical Biology.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200805901.

13- and 14-membered rings through triazole-ring formation, and motivated by their potential for a predictable side-chain arrangement, we undertook high-resolution structural analysis of these peptides. In all cases, the obtained structures revealed a rigid rectangular scaffold that could be superimposed well on idealized β-turn motifs (see Figure S2 in the Supporting Information). Importantly, the ^{1}H NMR spectra for all four compounds were sharp and consistent with a single conformation present in solution 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 with one another 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.

We wondered if the observed conformational homogeneity of **1**–**4** would hold for the entire series of 16 possible stereoisomeric pseudotetrapeptides 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 modeled easily) would provide a basis set of conformationally predictable, three-dimensional scaffolds suitable for drug design. Accordingly, we set out to synthesize the 16 possible stereoisomeric pseudotetrapeptides that incorporate 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 by using a β-turn motif.[12]

We prepared two sets of dipeptides, each comprising four diastereomers (compounds **5a**–**d** and **6a**–**d**), from which all 16 required diastereomeric tetramers could be synthesized (Scheme 1). The crude linear azidoalkynes underwent macrocyclization by copper(I)-mediated [3+2] Huisgen dipolar cycloaddition in 31–90% yield, as determined by HPLC. (The isolated products were obtained in 20–59% yield following deprotection; yields are based on the di-



Scheme 1. Synthetic strategy for the creation of the pseudotetrapeptide library. Compounds **5a**–**d**, compounds **6a**–**d**, and compounds **7a**–**h** differ only at the marked (asterisk) stereogenic centers. R = carbobenzyloxy (Cbz) or 2-Cl-Cbz; DMF = *N*,*N*-dimethylformamide, HATU = 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, TBTA = tris(benzyltriazolylmethyl)amine, TFA = trifluoroacetic acid.

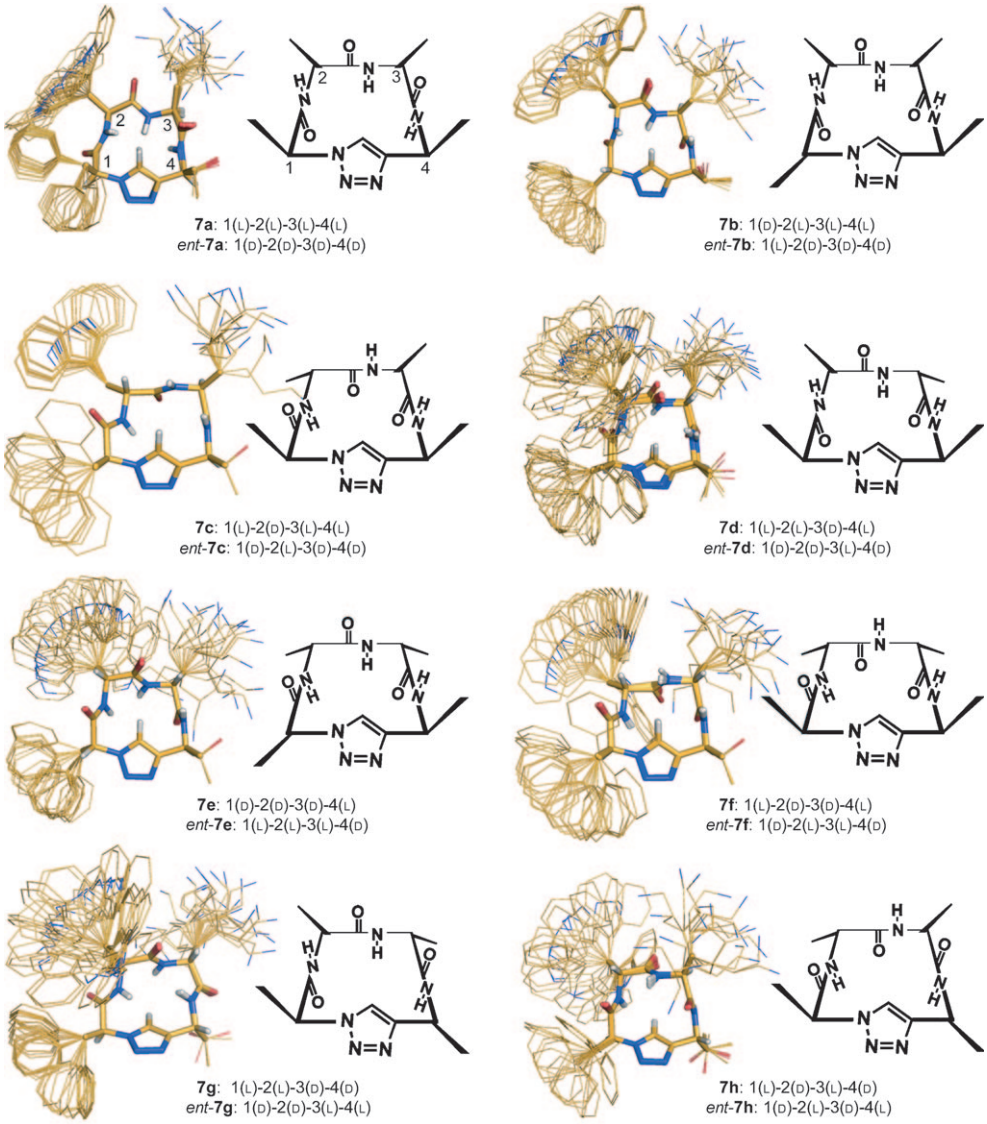


Figure 2. Structures determined by NMR spectroscopy in solution ([D$_6$]DMSO) and schematic diagrams for eight diastereomeric pseudotetrapeptide scaffolds. Stereocenter configurations are labeled according to the amino acid starting materials from which they are derived. For each structure shown, the corresponding enantiomer was also synthesized and assayed for hSSTR$_{1-5}$ binding.

peptide starting materials.) Alternative attempts to synthesize compounds of type **7** by macrolactamization resulted in racemization and poor cyclization yields, consistent with previous results.[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 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**).

Table 1: Binding affinities of $hSSTR_{1-5}$ for the heterocyclic pseudotetrapeptides.

Peptide	IC_{50} [nM][a]				
	$hSSTR_1$	$hSSTR_2$	$hSSTR_3$	$hSSTR_4$	$hSSTR_5$
SRIF-28[b]	1.8 ± 0.5	2.0 ± 0.3	2.4 ± 0.7	3.3 ± 0.3	2.4 ± 0.7
7a	> 10000	> 10000	1000 ± 100	2600 ± 200	2100 ± 400
7b	> 10000	> 10000	> 10000	1600 ± 200	> 10000
7c	5800 ± 500	> 10000	> 10000	> 10000	> 10000
7d	> 10000	> 10000	6700 ± 1500	5600 ± 500	> 10000
7e	2100 ± 200	> 10000	4200 ± 1600	570 ± 60	5000 ± 1500
7f	> 10000	> 10000	> 10000	2900 ± 300	> 10000
7g	> 10000	> 10000	> 10000	> 10000	> 10000
7h	110 ± 10	1200 ± 200	7700 ± 1100	2200 ± 300	3800 ± 800
ent-**7a**	> 10000	> 10000	2800 ± 600	980 ± 60	5100 ± 400
ent-**7b**	4100 ± 1200	> 10000	> 10000	5000 ± 700	6100 ± 600
ent-**7c**	> 10000	3300 ± 200	5400 ± 1300	4700 ± 1000	> 10000
ent-**7d**	180 ± 4	1300 ± 400	730 ± 200	790 ± 100	1600 ± 300
ent-**7e**	> 10000	> 10000	> 10000	> 10000	> 10000
ent-**7f**	> 10000	> 10000	> 10000	230 ± 50	> 10000
ent-**7g**	170 ± 20	160 ± 50	67 ± 30	140 ± 10	170 ± 50
ent-**7h**	> 10000	> 10000	3300 ± 400	2900 ± 700	> 10000

[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; nonselective [^{125}I]-(Leu^8, D-Trp^{22}, Tyr^{25}) SRIF-28 was used as the radioligand.[17] [b] For the chemical structure of SRIF-28, see Ref. [12d].

With the 16 stereoisomeric somatostatin analogues 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. We proceeded to determine the NMR solution structures of the eight compounds in one enantiomeric series by using the program CNS[15] (Figure 2). Each of the eight diastereomers exhibited a distinct and conformationally 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 configuration of the four stereogenic centers exerts the most dominant influence on the amide-backbone orientation and the distinct three-dimensional side-chain arrangement adopted by each scaffold.

The library of 16 stereoisomeric pseudotetrapeptides was screened for binding activity with 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 were observed, including a total lack of binding even at concentrations up to 10 μM (in the case of **7g**), selectivity for $hSSTR_1$ (in the case of **7h**) or $hSSTR_4$ (in the case of *ent*-**7f**), and broad-spectrum activity (in the case of *ent*-**7g**). The $hSSTR_2$ receptor appeared to have the most stringent binding requirements for these compounds, whereas $hSSTR_4$ bound nearly all 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 with 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 on *ent*-**7g** (the highest-affinity $hSSTR_4$ binder) by 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 configuration). After removing scaffolds with root mean square deviation (RMSD) values above a cutoff of 0.7 Å (see Table S1 in the Supporting Information for all RMSD values), we observed clustering into three conformational families (Figure 3a). Interestingly, the three qualitatively observed groupings correspond to the set of peptides that bind multiple hS 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 bind 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 the observed binding characteristics of the peptides.

To better understand the requirements for selective binding to $hSSTR_4$, the 16 compounds were overlayed differently onto *ent*-**7f** (the most selective $hSSTR_4$ binder): When the Thr C_α atom and additional backbone atoms (Lys carbonyl carbon atom and Trp amine) were used along with the fit atoms described above, and scaffolds with an RMSD value above a cutoff of 0.55 Å were discarded (see Table S1 in the Supporting Information for all RMSD values), the four most selective $hSSTR_4$ ligands were left (**7b**, **7e**, *ent*-**7a**, and *ent*-**7f**; Figure 3b). The most notable features that these compounds have in common are that all four contain a D-Phe

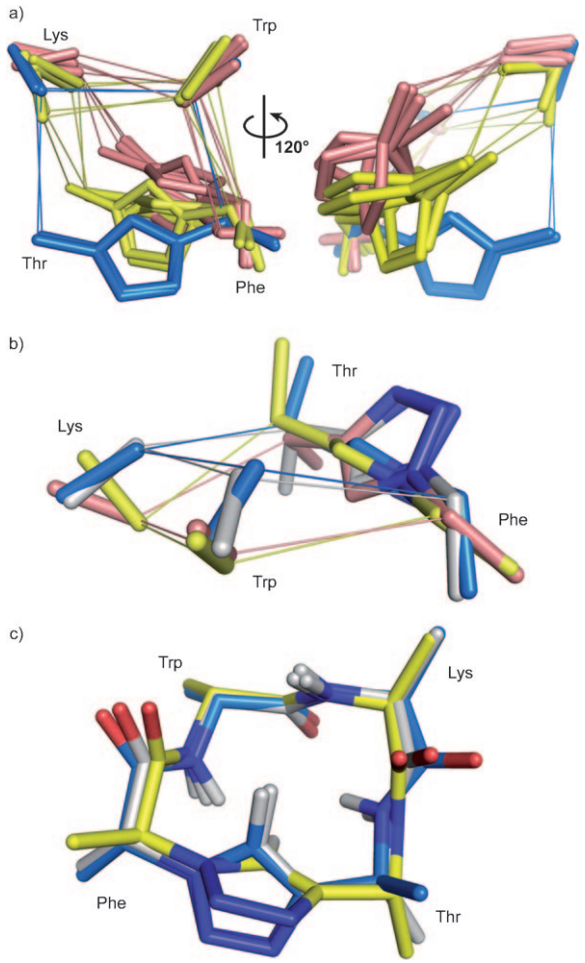


Figure 3. Pairwise fitting of pseudotetrapeptides to correlate structure with bioactivity. a) All 16 compounds were overlayed on *ent*-**7g** (highest-affinity $hSSTR_4$ binder) by using pair fits of the Trp, Lys, and Phe C_β atoms and the Phe C_α atom. Compounds with an RMSD value higher than 0.7 Å are not shown; compounds *ent*-**7g** and *ent*-**7d** (blue) bind multiple receptors, including $hSSTR_4$; compounds *ent*-**7f**, **7e**, *ent*-**7a**, and **7b** (yellow) are specific for $hSSTR_4$; compounds **7d**, **7g**, *ent*-**7c**, and *ent*-**7h** (pink) did not bind $hSSTR_4$ or bound $hSSTR_4$ 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 on *ent*-**7f** (most-selective $hSSTR_4$ binder; pink). An RMSD cutoff of 0.55 Å left compounds **7b** (yellow), **7e** (light blue), and *ent*-**7a** (white), which are the $hSSTR_4$-selective peptides. c) Overlay of the 16 compounds on **7h** (most-selective $hSSTR_1$ binder; yellow) by using pair fits of the C_α atoms of all four residues and the C_β atoms of Trp and Lys. An RMSD cutoff of 0.3 Å left only compounds *ent*-**7g** (light blue) and *ent*-**7d** (white), which are the only other high-affinity, albeit less selective, binders of $hSSTR_1$.

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 compounds with the lowest RMSD corresponded to the most selective $hSSTR_4$ ligands suggests that the avoidance of 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 on **7h** (the most selective $hSSTR_1$ binder) by using the C_α atoms of all four residues and the C_β atoms of Trp and Lys (Figure 3c). Molecules with an RMSD value greater than 0.3 Å were discarded (see Table S1 in the Supporting Information), and the remaining peptides (*ent*-**7g** and *ent*-**7d**) were the only other high-affinity, albeit less selective, binders of the $hSSTR_1$ receptor. When the bioactivities and the atoms required for this fit are considered, 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, whereas the Phe and Thr side chains influence receptor selectivity.

We hope that the well-defined diastereomeric structures described herein 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 with systematic and predictable differences in their spatial display of amino acid side chains could be useful in the delineation of the three-dimensional pharmacophoric requirements for receptor binding and selectivity, especially in cases in which high-resolution structural data are not readily available.

Coordinates for the NMR structures reported in this manuscript have been deposited at the BMRB databank (www.bmrb.wisc.edu, accession numbers 20036–20043).

Received: December 4, 2008
Published online: March 5, 2009

Keywords: conformation analysis · cyclic peptides · drug design · structure–activity relationships · triazoles

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