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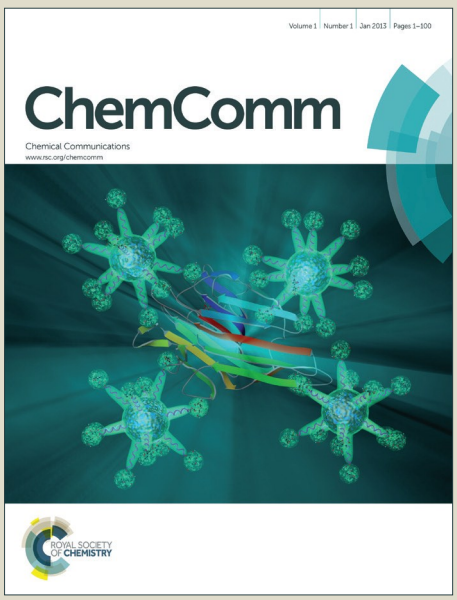

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β-Strand mimics based on tetrahydropyridazinedione (tpd) peptide stitching

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Short peptides featuring a tetrahydropyridazinedione (tpd) backbone tether exhibit reduced conformational flexibility external to the heterocyclic constraint. Analysis by NMR, molecular modeling and X-ray crystallography suggests both covalent and non-covalent stabilization of extended peptide conformations. An efficient solid-phase protocol was developed for the synthesis of a new class of β-strand mimics based on oligomeric tpd subunits.

Recognition events between protein secondary structures mediate myriad biological processes and have emerged as targets for both small molecule and peptide-based therapeutics. The β-sheet is the second most common protein structural motif and its associations make up a sizable fraction of the protein interactome.[1, 2] Modulating β-sheet/strand interactions with designed mimics has thus garnered considerable interest.[3-5] The large surface area-to-pharmacophore ratio associated with β-sheet domains poses a significant peptidomimetic design challenge due to complex molecular topology, increased conformational flexibility, and potentially poor bioavailability. Since the folding and stability of ordered peptides are compromised outside the context of surrounding tertiary structure, covalent tethering remains a promising approach for stabilizing peptide folds. In β-strands, these approaches typically involve macrocyclization via side chains[3, 4, 6-8] or the use of turn-templated β-hairpin motifs.[9-16] There remains a need for additional minimalist constraints capable of stabilizing short β-strand peptides.

We recently reported the synthesis of a novel tetrahydropyridazinedione (tpd) tether designed to restrict accessible conformations within a dipeptide subunit.[17] The tpd heterocycles are derived from chiral α-hydazino acid building blocks that are chemoselectively incorporated into a growing peptide chain on solid support. Given the appropriate side chain stereochemistry, we envisioned that the tpd subunit could constrain short sections of the backbone into extended conformations. Moreover, multiple tpd tethers within a peptide strand would afford novel oligomeric β-sheet mimics that potentially take advantage of both covalent and internal H-bond stabilization (Figure 1). Since conformational flexibility and polar surface area are well-recognized impediments to peptide-based drug development, these features may further serve to enhance the drug-likeness of tpd-'stitched' sequences.[18] Here, we report the structural analysis of tpd-constrained model peptides as well as the synthesis and conformation of a new class of β-sheet mimics based on oligomeric tpd subunits.

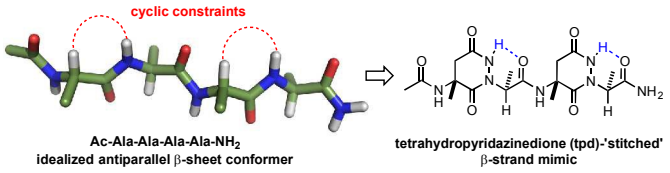


Figure 1. Design of tpd-based β-strand mimics.

Covalent tethering of the D-Asp side chain to the preceding backbone nitrogen locks the amide bond in the trans configuration and severely restricts the ϕ_i and ψ_i torsions. Less obvious was the potential impact of tpd cyclization on backbone torsions external to the heterocycle (*i*+1 residue). We first employed quantum mechanics calculations (M06-2X-D3/6-31G* level[19]) with implicit solvent on the model dipeptides Ac-Gly-Ala-NH_2 and Ac-tpd-Ala-NH_2 and carried out relaxed dihedral energy coordinate scans on the Ala ψ and ϕ angles (the Gly-Ala amide bond, ω, in the control dipeptide was locked in a trans conformation for these simulations). Relaxed coordination scans perform a geometry optimization for each constrained conformation. The respective Ramachandran-like energy plots show markedly fewer accessible Ala conformations in the tpd variant relative to the Ac-Gly-Ala-NH_2 parent compound (Figure 2). The tpd dipeptide Ala residue is also more restricted to the β-strand region of the plot with a highly restricted ϕ angle and extended ψ torsion to create an optimal energy well centered at a ψ angle of 150° and ϕ angle of -75°. The angles in this energy well allow for the possibility of a N-H···O=C hydrogen bond between the tpd nitrogen and Ala carbonyl oxygen. However, residues in idealized β-sheets exhibit characteristic ϕ angles in the -119° to -139°range.

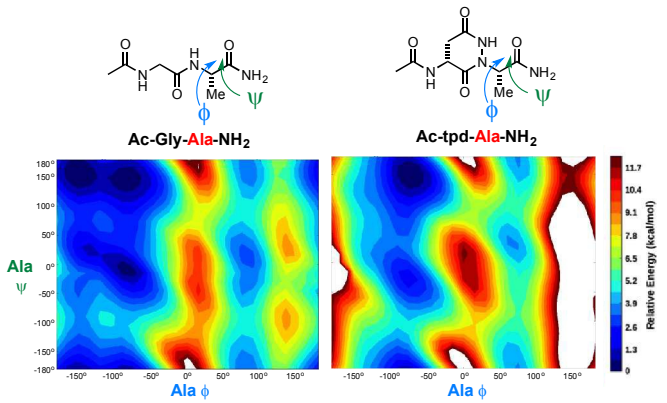


Figure 2. Ramachandran plots from M06-2X-D3/6-31G* level QM calculations with implicit solvent for Ala residue ψ and ϕ torsions in control and tpd dipeptides.

The increase in exocyclic torsional rigidity was next investigated experimentally by ^{1}H NMR. We previously reported the synthesis of tpd-constrained tetrapeptide **1**, which gave rise to a very well resolved NMR spectrum absent of rotational isomers.[17] Although the N'H proton did not give rise to a discernable ^{1}H NMR signal (precluding deuterium exchange experiments), the Gly α protons in **1** exhibited an unusually large diastereotopic separation (0.72 ppm) suggesting conformational restriction of the Ala ψ and ϕ torsions. We then synthesized acyclic control peptide **2** resulting from formal scission of the N-N bond. In contrast to **1**, the Gly α protons in **2** appear within 0.17 ppm of each other, presumably due to an increase in flexibility and conformational averaging. As expected, diastereotopic separation of the D-Asp β protons was also enhanced after tpd cyclization, although not as dramatically as the *exocyclic* Gly α signals (see Supporting Information).

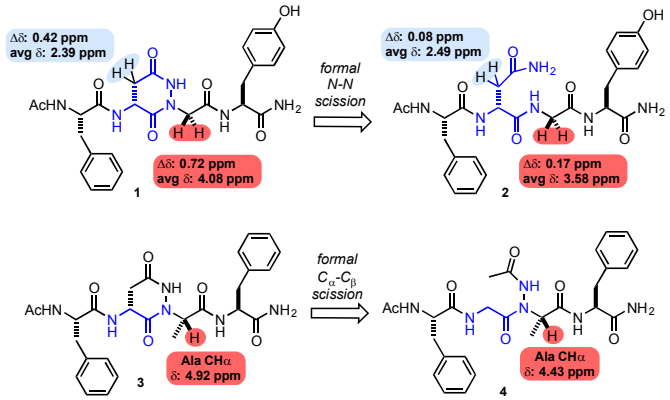


Figure 3. Conformational impact of tpd constraint in ^{1}H NMR.

The α□protons of residues in β-strand/sheet peptides are known to appear downfield relative to their random coil counterparts by ^{1}H NMR.[20] This downfield shift was also observed in the case of the Gly α protons in **1**. However, reference peptide **2** was deemed an unsuitable control due to the electronically distinct environment introduced by the *N*-(*N*'-acyl)amino backbone substituent. We then compared tpd-constrained peptide **3** to *N*-acetamido control compound **4** (resulting from formal scission of the tpd Cα-Cβ bond). The Ala residues in this matched pair of peptides thus experience the same electronic inductive effects from the backbone substituent group. As with the Gly residue in **1**, The Ala α proton **3** exhibited a 0.5 ppm downfield shift by ^{1}H NMR. This effect can be attributed entirely to conformational restriction imparted by tpd cyclization.

To further characterize the preferred conformation of small tpd peptides, we set out to prepare hydrophobic derivatives for crystallographic analysis. Constrained dipeptides **10-12** were synthesized by aminolysis of the (*N*'-Boc)-hydrazino esters, coupling to a preactivated D-Asp residue, and acid-catalyzed Boc cleavage/cyclization. Both **10** and **11** readily crystallized out of EtOH and single crystals were subjected to X-ray diffraction. The backbone ϕ and ψ torsions are in close agreement with the values expected of antiparallel β-sheet peptides. Moreover, the solid-state structure of each dipeptide showed the presence of an intramolecular H-bond between the tpd N'H and the Ala/Phe carbonyl oxygen. The observed N-H···O=C distances were in the 2.3-2.4 Å range and the H-bond angles were 119.5 and 121.1° for **10** and **11**, respectively. Though the contribution of this interaction to conformational stability in solution is not clear, the N···O donor-acceptor distances of ~2.9 Å in the solid state are well within the cutoff for medium strength H-bonds.[21] The observed H-bonds are more easily accommodated by the larger ϕ_{i+1} dihedral angle in the X-ray crystal structures of **10** and **11** relative to the -75° torsion calculated for the model dipeptide in Figure 2. Given indirect NMR evidence of the presence of an intramolecular H-bond in solution, it is possible that QM calculations underestimate the importance of this interaction within tpd-constrained structures.

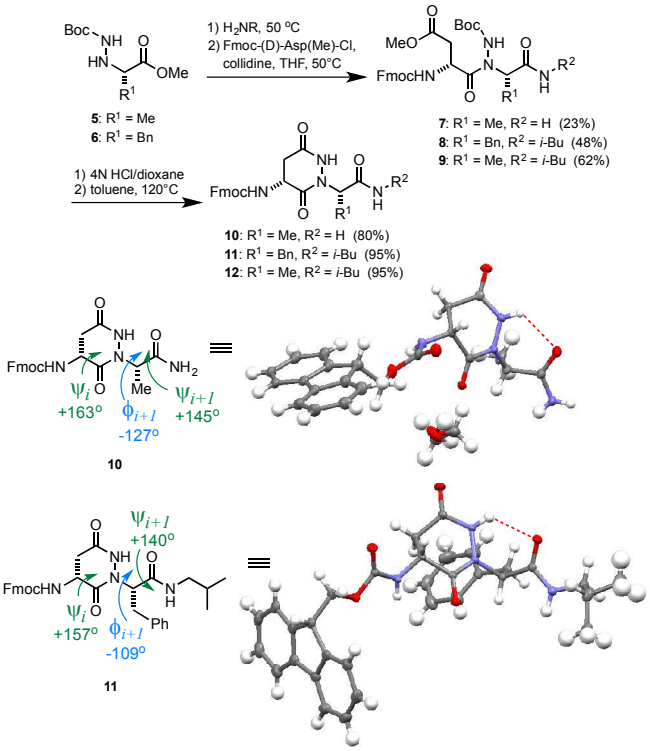
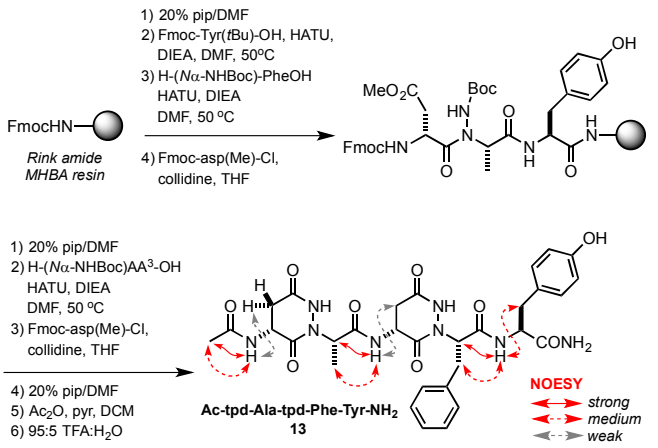


Figure 4. X-Ray crystal structures of tpd dipeptides.

The absolute values of the relevant ϕ and ψ backbone torsions in **11** are slightly lower than those in **10**, which may be due in part to further conformational restriction via a CH-π interaction between the Phe aromatic ring and one of the tpd β protons. The pseudoaxial tpd

β proton is positioned almost directly above the Phe benzene ring in the X-ray structure of **11**, resulting in an Hβ-π centroid distance of 3.55 Å. This conformation appears to also exist in solution, as we observed a large diastereotopic separation (Δδ = 1.49 ppm) between the tpd β protons resulting from anisotropic shielding by NMR. A ROESY correlation between only one of the two tpd β protons and the Phe aromatic hydrogens in **11** was also observed. Although Ala-containing variant **12** did not yield diffraction quality crystals, its NMR spectrum revealed a far less pronounced diastereotopic separation (tpd Hβ Δδ = 0.42 ppm).

The ability to mimic longer sections of a β-strand prompted us to explore the synthesis of oligomeric 'stitched' tpd-containing peptides. By alternating chiral α-hyrazino acid residues with D-Asp derivatives, we successfully prepared a series of polycyclic peptidomimetics on solid support, as shown in Figure 5. Incorporation of (*N*'-Boc) hydrazino acids was achieved chemoselectively using HATU/DIEA. Intermediates were then reacted directly with Fmoc-protected D-aspartic acid chloride and bis-ring closure effected by treatment with TFA. These peptidomimetics retain native hydrogen bonding capacity and side chain functionality on one face of the putative β-strand across 5 or more residues. In each case the major byproducts were those arising from incomplete tpd cyclization and/or trifluoroacylation of the *N*-amino substitutents during cleavage from the resin. Despite this, the desired di-tpd products **13-18** were obtained in 11-21% overall yield following RP-HPLC purification.



compound	sequence	$[M+H]_{obs}$	$[M+H]_{cal}$	isolated yield (%)
13	Ac-tpd-Ala-tpd-Phe-Tyr-NH_2	665.2661	665.2678	14
14	Ac-tpd-Ala-tpd-Ala-Tyr-NH_2	589.2356	589.2365	21
15	H-Leu-tpd-Phe-tpd-Leu-Asn-NH_2	729.3680	729.3679	16
16	H-Leu-tpd-Ile-tpd-Leu-Asp-NH_2	696.3680	696.3675	15
17	H-Leu-tpd-Leu-tpd-Leu-Glu-NH_2	710.3831	710.3831	13
18	H-Leu-tpd-Val-tpd-Val-Gln-NH_2	681.3670	681.3679	11

Figure 5. Synthesis of di-tpd β-strand mimics.

The representative pentapeptide mimic **13** was further analyzed by NMR. As with other tpd-constrained peptides, the ^{1}H NMR spectrum of **13** was remarkably well resolved, showing no evidence of amide rotamers. Unequivocal resonance assignments were made on the basis of TOCSY and NOE spectra. The α protons of the hydrazino acid residues (Ala and Phe) again appeared far downfield (> 0.5 ppm) of their natural amino acid counterparts. Anisotropic shielding of *one* of the four tpd β protons by the Phe aromatic ring was observed within this pentapeptide, consistent with the solution and solid state conformation of dipeptide **11**. Peptides in β-strand/sheet conformations exhibit characteristic NOE correlations between the $H\alpha_i$ and NH_{i+1} protons. Both the Ala and Phe α protons showed strong sequential correlations to the tpd NH protons. NOE correlations between the tpd amide NH and the Ala/Phe β protons were present, confirming the all-trans amide configuration across the peptapeptide. Weak intraresidue correlations were also observed between the tpd NH protons and *only* the pseudoaxial tpd β protons.

Using our solid-phase protocol, we synthesized a highly constrained heptapeptide mimic featuring three sequential tpd subunits (**19**). Although a significant amount of bicyclic byproduct was obtained in this case, the desired tricyclic compound could be isolated in 10% overall yield after RP-HPLC purification. The ROESY spectrum of **19** again exhibited strong and medium $H\alpha_i$-NH_{i+1} and $H\beta_i$-NH_{i+1} correlations, respectively, confirming the presence of trans amide bonds throughout the molecule.

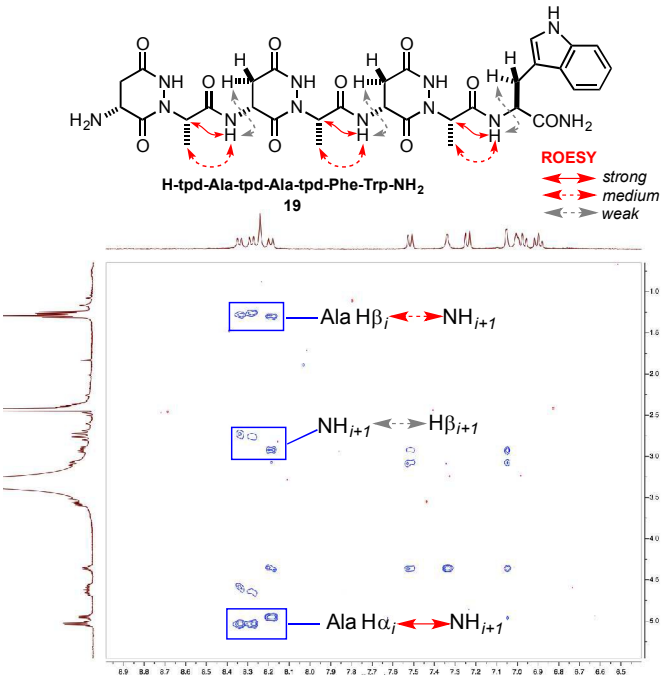


Figure 6. Non-sequential NH ROESY correlations within a tri-tpd β-strand mimic (DMSO-d_6).

In summary, we have described a novel class of β-strand mimics based on tetrahydropyridazinedione (tpd) peptide stitching. We have carried out conformational analysis on tpd-constrained peptides and described the first solid-phase synthesis of derivatives featuring oligomeric tpd-Xaa subunits. A combination of molecular modeling, X-ray, and NMR data demonstrate that the tpd constraint imposes significant conformational restriction external to the newly formed heterocycle. Evidence for the predicted $N'H_{tpd}\cdots O{=}C_{Xaa}$ H-bond, as well as an unusual CH-π interaction, highlights the structural impact of the cyclic azodicarbonyl motif. The combination of covalent tethering and non-covalent interactions within these structures cooperate to promote extended peptide conformations. We anticipate that tpd peptides will thus find broad utility as non-proteinogenic β-strand/sheet mimics that are readily accessible via solid-phase peptide synthesis.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures and spectroscopic data. See DOI: 10.1039/c000000x/

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