



Published in final edited form as:

J Am Chem Soc. 2011 August 17; 133(32): 12350–12353. doi:10.1021/ja2033734.

Pyrrrolinone-pyrrolidine Oligomers As Universal Peptidomimetics

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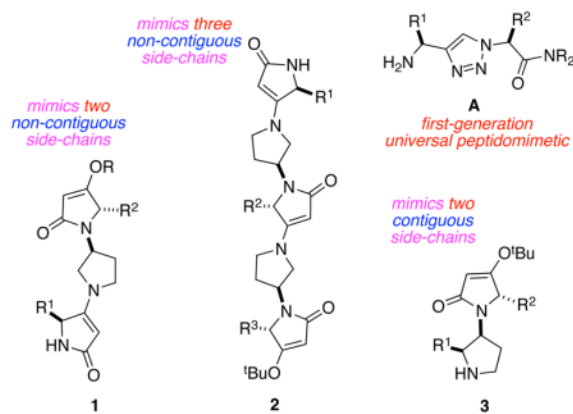
Abstract

Peptidomimetics **1** – **3** were prepared from amino acid-derived tetramic acids **7** as the key starting materials. Calculations show that preferred conformations of **1** can align their side-chain vectors with amino acids in common secondary structures more effectively than **3**. A good fit was found for a preferred conformation of **2** (an extended derivative of **1**) with a sheet-turn-sheet motif.

Minimalist peptidomimetics, that present amino acid side-chains without any structure to directly resemble peptide backbones, are the focal point of many recent studies inspired by Smith/Hirschmann^{1–5} and Hamilton.^{6–10} We recently designed sets of scaffolds, of which **A** is typical, that analog local pairs of amino acids (including non-contiguous ones) *in any secondary structure, ie universal peptidomimetics*.^{11–12} That work featured *five* peptidomimetic designs that together could be used to mimic almost all local pairs of amino acid side-chains in six of the most common secondary structure motifs. This paper describes an alternative scaffold design **1** that has preferred conformations which overlay well with pairs of amino acid residues in three different types of helix (3_{10} , α -, and π -), in β -strands, and in both parallel- and antiparallel- β -sheets. Further, an extended form of that same scaffold design **2** has a preferred conformation which overlays well with *three* amino acid side chains in a sheet/ β -turn/sheet-motif (an anti-parallel β -sheet). These two scaffolds are contrasted with **A** and a compound in series **3** where side chains are expressed on contiguous rings.

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Supporting Information Available: Preparations and spectra of compounds **1** – **12**, modeling procedures, overlays on secondary structures (preferred conformers of **1**, **1aa** and **2aaa** on secondary structure motifs in proteins {RMSE < 0.14 Å}; Hirschmann/Smith pyrrrolinones and Hamilton's terphenyl systems) and complete ref 2. CCDC 822217 – 822219 contains the supplementary crystallographic data, which can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.



For the preparation of scaffold **1**, an efficient procedure from Merck was used to decarboxylate *trans*-4-hydroxyproline to give more than 50 g of crystalline (*R*)-3-hydroxypyrrolidine.¹³ That pyrrolidine was *N*-protected to give the starting material indicated in Scheme 1. Nucleophilic displacement on a triflate-derivative of this (under conditions optimized to avoid elimination)¹⁴ gave the amino esters **4**. X-ray analysis of **4d·HCl** indicated its formation occurred via a single inversion. The crystalline hydrochloride salts of **4** were reacted with Bestmann's ylide^{15–17} to give the pyrrolinone **5**. Hydrogenolysis, then condensation of the free pyrrolidine-*NH* with 5-substituted 2,4-pyrrolidinediones (tetramic acids) **7** gave the featured trimers **1**. The tetramic acid derivatives **7** are useful starting materials because they can be prepared from *N*-Boc-protected amino acids via a one-pot procedure that affords tens of gram amounts without chromatography.^{18–21} NMR and X-ray analysis of compound **6d** indicated its formation was not complicated by epimerization. Condensation of the dimers **6** with *C*-deprotected derivatives of trimers **1** gave the pentamer **2**, hence the overall synthesis can be divergent-convergent as in Scheme 1.

NMR studies to detect preferred conformations in these types of molecules are inappropriate due to conformational averaging. Consequently, two complementary molecular modeling methods were used. Quenched molecular dynamics (QMD)^{22–25} probes *thermodynamic accessibilities* of conformational states, as described previously.¹¹ Briefly, this technique generates 600 minimized structures; ones that are energetically below a user-defined cut-off from the minimum energy conformer located (here 3.0 kcal/mol) are clustered into families based on RMS (root mean squared) deviations from user-defined atoms (0.5 Å). We have postulated¹¹ that matching *C α* - *C β* bond vectors forms a good basis for measuring fit to secondary structures, thus preferred conformations of scaffolds are defined by frameworks with only *Me*-side chains, *ie* Ala-analogs, like **1aa** and **2aaa**. For this reason, preferred conformers of **1aa** and **2aaa** were clustered based on *C α* - *C β* coordinates, and representative members of each cluster were tested for fit on *C α* - *C β* atom positions of ideal secondary structure motifs.

In our experience, a good fit of structures based on two amino acid side chains corresponds to an RMSD of 0.3 Å or less. Using this standard, Table 1 reveals preferred conformers of compound **1** fit *two* different residue pairs on the most elongated helical form (3_{10}), they fit *three* on an α -helix, and *three* on the most compressed helical form, the π -helix (with another at RMSD 0.31 Å). Other conformers overlay well on various side-chain pairs of β -strand and parallel- β -sheet residues, while the overall best match was for a parallel β -sheet. Interestingly, several of the favored overlays involved *non-contiguous* five-membered rings in structures **1** and **2** on *contiguous* amino acids in the secondary structures; this reflects the way the featured molecules can coil. All the preferred conformations highlighted in Table 1

were within 3.0 kcal/mol of the minimum conformation identified, hence these structures are *thermodynamically accessible*.

Table 1 also compares the overlay of scaffold **A** on the same elements of secondary structure. It shows scaffold **A** covers a more limited range of “secondary structure space”, as expected since the *Ca* atoms in this structure are held rigidly at one separation. A brief comparison was also made for the same elements of secondary structure with core building block of the Hirschmann/Smith pyrrolinones⁵ and Hamilton’s terphenyl systems (see supporting for structures). Hirschmann/Smith’s system overlaid well with parallel and antiparallel β -sheets, a β -strand, and a 3_{10} -helix (RMSD = 0.10 – 0.29 Å). A biphenyl representing part of Hamilton’s terphenyls overlaid better with 3_{10} - and π - than α -helices.²⁶ An outline of these studies is given in the supporting, and a more extensive comparison will be reported elsewhere.

It is harder to fit six coordinates than four, so matches of the mimics involving *three* side chains must have higher RMSD’s. In the event, the best match for pentamer **2** was with three residues of a sheet-turn-sheet motif (1.93 kcal/mol above the minimum energy one; RMSD: 0.46 Å; Figure 1). Moreover, we found one example of a protein-protein interaction (between monomers in the RAD52 undecamer) where **2aaa** matched three side-chains with an RMSD of only 0.14 Å (see supporting).

The next milestone in this study was to check that the different conformers are *kinetically accessible*. To do this, a density functional theory (DFT) method was used to investigate interconversion between the preferred states of **6a** (Figure 2a, and supporting). A maximum energy barrier of 5.10 kcal/mol was calculated using this method (Figure 2b). This indicates conformers of **6a** should rapidly interconvert on the ¹H NMR time scale, and experimentally this was shown to be the case.

Consideration of Newman projections through the bond indicated in Figure 2a indicates this molecule would tend to rest in predominantly three conformational states. This assertion is supported by the fact that three low energy conformational states emerged from both the DFT and QMD calculations (Figures 2b and c). The free energy differences from the DFT calculations predict that relative populations of the three conformers are on the ratio 1.00: 0.34: 0.12.

A synthesis of one compound in series **3** was developed to demonstrate that both heterocyclic rings in the “main-chain” of these peptidomimetics could be functionalized with amino acids. That route (Scheme 2) employs a known diastereoselective hydrogenation,²⁷ and is similar to Scheme 1 except that a thioamide was introduced (**9** to **10**) then reduced to the amine (**12** to **3**).

Extensive conformational analyses were performed for compound **3**. Full data is shown in the supporting material, but the key point emerges from Table 1. Specifically, preferred conformers of **3** do *not* fit pairs of amino acid side-chains in secondary structures as well as conformers of trimers **1** do. The side chains in **3** on contiguous residues are constrained in ways that preclude good overlap on common secondary structure motifs. This is supported by the modeling studies shown here and X-ray crystallographic analyses of compound **11** (supporting). Conversely, the *trimers 1* have at least one extra significant degree of freedom, and this allows them to flex into conformations that match secondary structures well.

The accessibility of tetramic acid derivatives **7** from different amino acids gives the syntheses here some considerable scope. We recently argued^{11–12} there are four *structural* criteria common to effective minimalist mimics: (i) facile syntheses with most amino acid side chains; (ii) kinetically and thermodynamically accessible conformations for induced fit;

(iii) only moderate loss of entropy on docking; and, (iv) appropriate $C\alpha - C\beta$ coordinates of an accessible conformation of the mimic matching those of the secondary structures. An advantage of defining a parameter such as (iv), *ie* matching side-chain $C\alpha - C\beta$ coordinates, is that the fit can be *quantitated* in terms of parameters like RMSD. This provides a firm basis for comparing abilities of peptidomimetics like **A** and **1 – 3** to mimic secondary structures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank The National Institutes of Health (GM087981) and The Robert A. Welch Foundation (A-1121) for financial support, Joseph H. Reibenspies for X-Ray crystallographic analysis, Dr. Marc Parisien for modification of the β -sheet builder program, and Dr. Dmytro Fedoseyenko for helpful discussions.

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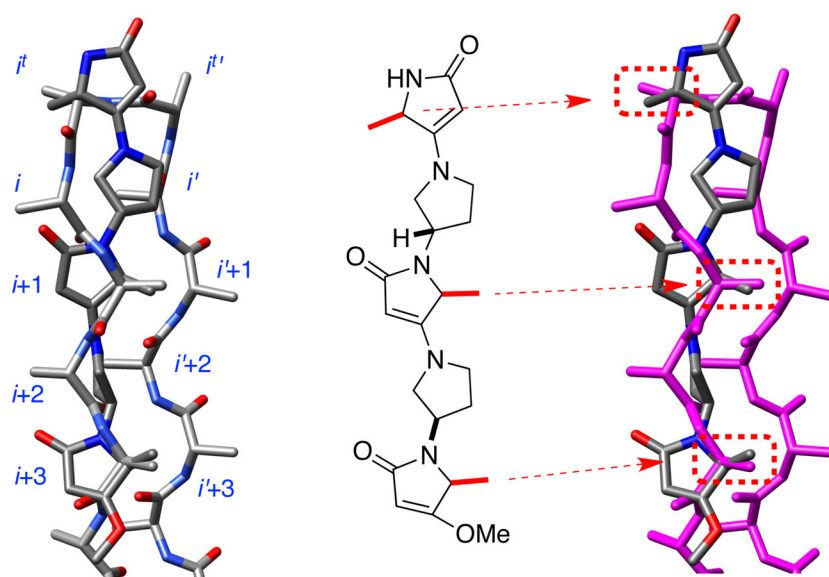


Figure 1.
Overlay of **2aaa** on sheet-turn-sheet motif.

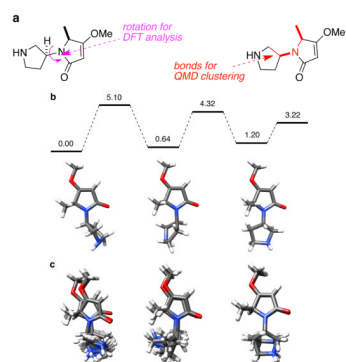
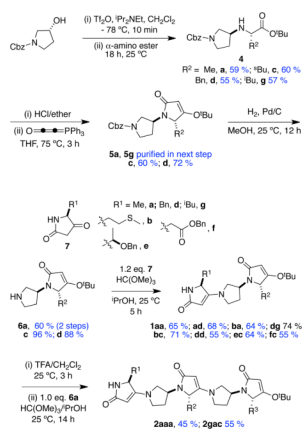
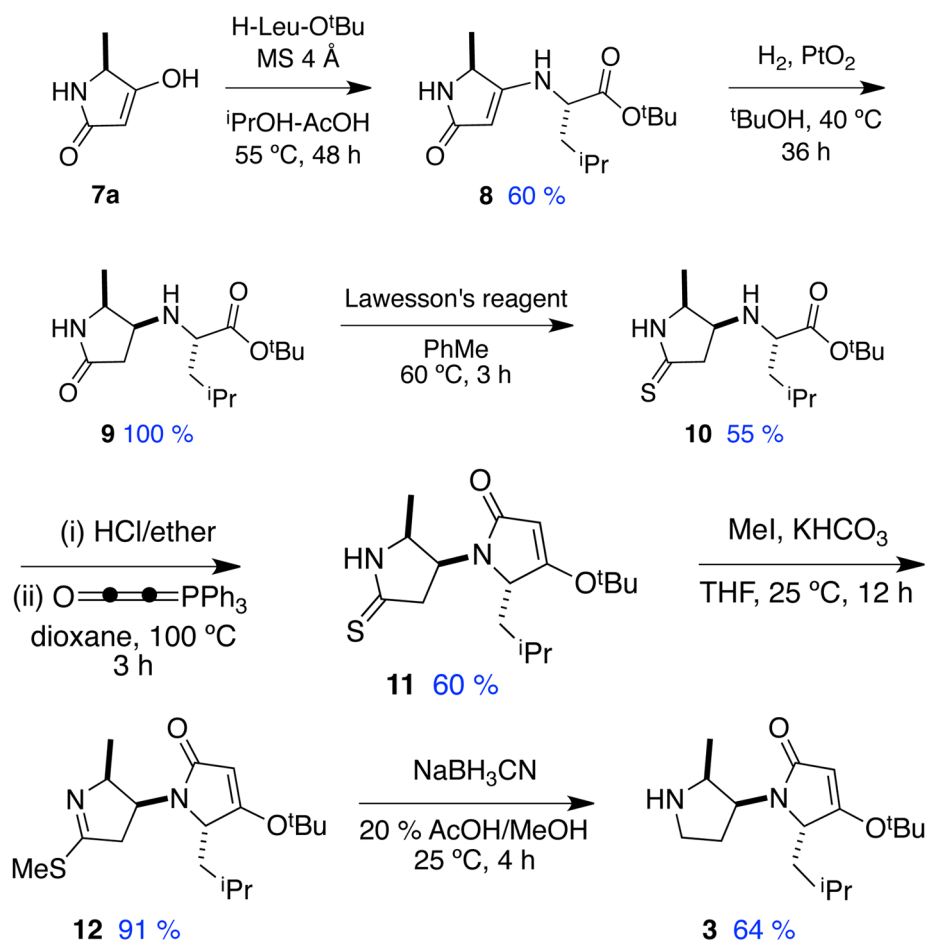


Figure 2. **a** Structures and parameters used for DFT and QMD analyses; **b** low energy conformers and energy barriers for interconversion from DFT calculations; **c** preferred conformers from QMD calculations. All energies shown are the free energies (ΔG°) in kcal/mol.



Scheme 1.
Syntheses of trimers **1** and pentamers **2**



Scheme 2.
Synthesis of **3** with side-chains on contiguous rings.

Table 1

Fit of most appropriate conformers of mimics **A**, **1**, and **3** on secondary structures (based on QMD analyses).^a

structure	seq.	A		1		3	
		ΔG	rmsd	ΔG	rmsd	ΔG	rmsd
3 ₁₀ -helix	<i>i-i+1</i>			2.55	0.22	2.09	0.55
	<i>i-i+2</i>	1.66	0.28	2.43	0.25		
	<i>i-i+3</i>			1.27	0.49		
α -helix	<i>i-i+1</i>			2.00	0.47	2.09	0.50
	<i>i-i+2</i>	1.62	0.25	2.55	0.30		
	<i>i-i+3</i>			0.73	0.14		
	<i>i-i+4</i>			1.27	0.18		
π -helix	<i>i-i+1</i>			2.55	0.14	2.09	0.54
	<i>i-i+2</i>	1.63	0.33	2.99	0.30		
	<i>i-i+3</i>			2.43	0.31		
	<i>i-i+4</i>			0.85	0.35		
	<i>i-i+5</i>			1.27	0.27		
β -strand	<i>i-i+1</i>					2.24	0.46
	<i>i-i+2</i>			1.19	0.19		
β -sheet (parallel)	<i>i-i+1</i>			1.01	0.10		
	<i>i-i+2</i>			2.55	0.20		
sheet/ β -turn/sheet	<i>i-i+1</i>	0.19	0.23			2.25	0.49
	<i>i-i+2</i>			1.19	0.12		
	<i>i-i'</i>			2.74	0.27		
	<i>i-i''+1</i>			0.59	0.08		
	<i>i-i'+2</i>			0.81	0.18		
	<i>i-i''</i>			1.38	0.09		
	<i>i-i'''+1</i>	0.14	0.06	0.75	0.25		
<i>i-i'''+1</i>			1.00	0.42			

^aAll RMSD values (Å) are for the conformers (within 3.0 kcal/mol of the minimum energy) that overlay on the secondary structures shown (ΔG values are in kcal/mol). See supporting for secondary structure templates.