JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Pyrrolinone–Pyrrolidine Oligomers as Universal Peptidomimetics

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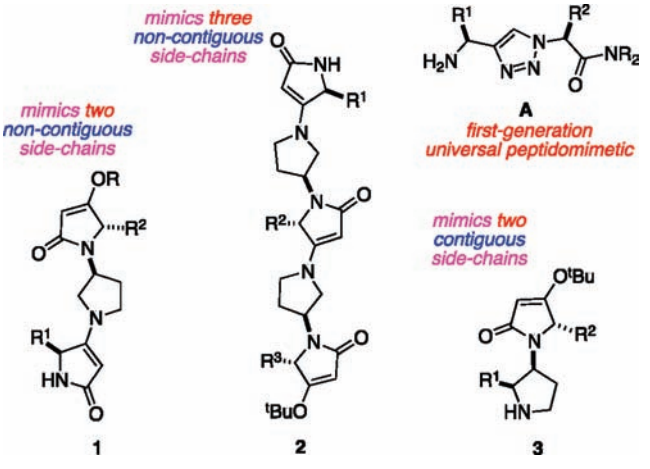
Supporting Information

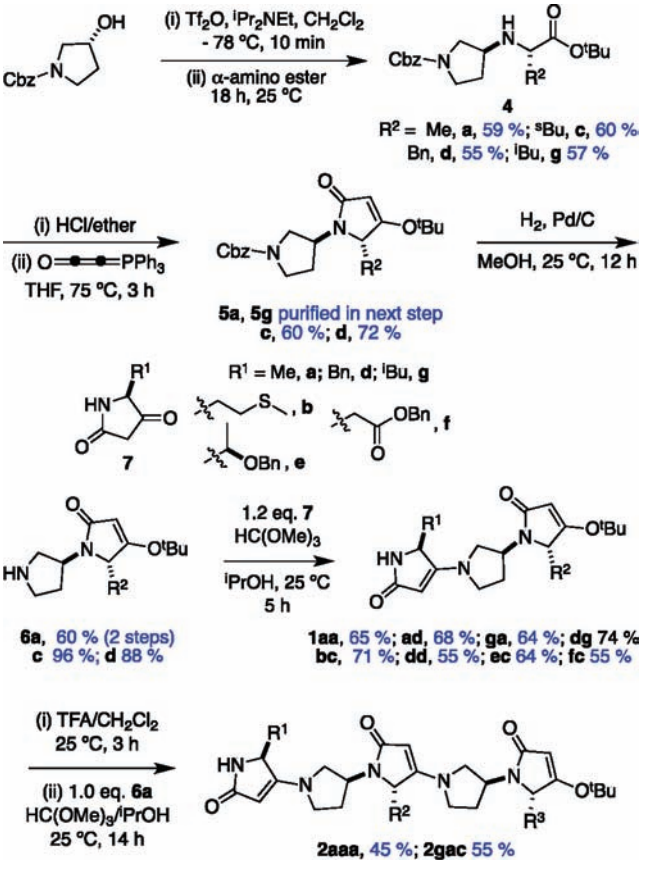
ABSTRACT: Peptidomimetics **1**–**3** were prepared from amino acid-derived tetramic acids **7** as the key starting materials. Calculations show that preferred conformations of **1** can align their side-chain vectors with amino acids in common secondary structures more effectively than conformations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

Minimalist peptidomimetics, which present amino acid side chains without any structure to directly resemble peptide backbones, are the focal point of many recent studies inspired by Smith and Hirschmann[1−5] and Hamilton.[6−10] We recently designed sets of scaffolds, of which **A** is typical, that are analogues of local pairs of amino acids (including noncontiguous ones) *in any secondary structure* (i.e., they are *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**, having preferred conformations that 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. Furthermore, an extended form of that same scaffold design, **2**, has a preferred conformation that overlays well with *three* amino acid side chains in a sheet/β-turn/sheet motif (an antiparallel β-sheet). These two scaffolds are contrasted with **A** and a compound in series **3** wherein side chains are expressed on contiguous rings.



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 amino esters **4**. X-ray analysis of **4d·HCl** indicated that its formation occurred via a single inversion. The crystalline hydrochloride salts of **4** were reacted with Bestmann's ylide[15−17] to give pyrrolinones **5**. Hydrogenolysis followed by condensation of the free pyrrolidine NH groups 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

Scheme 1. Syntheses of Trimers 1 and Pentamers 2



Received: April 25, 2011
Published: July 22, 2011

can be prepared from *N*-Boc-protected amino acids via a one-pot procedure that affords tens of grams without chromatography.[18−21] NMR and X-ray analysis of compound **6d** indicated that its formation was not complicated by epimerization. Condensation of dimers **6** with *C*-deprotected derivatives of trimers **1** gave pentamers **2**; hence, the overall synthesis can be divergent−convergent, as shown in Scheme 1.

NMR studies to detect preferred conformations in these types of molecules are inappropriate because of 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 cutoff from the minimum energy conformer (here 3.0 kcal/mol) are clustered into families based on root-mean-square deviations (RMSD's) from user-defined atoms (0.5 Å). We have postulated[11] that matching the Cα−Cβ bond vectors forms a good basis for measuring the fit to secondary structures, and thus, preferred conformations of the scaffolds are defined by frameworks with only Me side chains (i.e., Ala analogues), such as **1aa** and **2aaa**. For this reason, preferred conformers of **1aa** and **2aaa** were clustered based on Cα−Cβ coordinates, and representative members of each cluster were tested for their fit to Cα−Cβ 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. On the basis of this standard, Table 1 reveals that preferred conformers of compound **1** fit *two* different residue pairs on the most elongated

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

[a] All rmsd values (Å) are for the conformers (within 3.0 kcal/mol of the minimum-energy conformer) that overlay on the secondary structures shown (Δ*G* values are in kcal/mol). See the SI for secondary structure templates.

helical form (3_{10}), *three* on an α-helix, and *three* on the most compressed helical form, the π-helix (with another at an RMSD of 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 *noncontiguous* 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 of the preferred conformations highlighted in Table 1 were within 3.0 kcal/mol of the minimum conformation identified, so these structures are *thermodynamically accessible*.

Table 1 also compares the overlay of scaffold **A** on the same elements of secondary structure. It shows that scaffold **A** covers a more limited range of "secondary structure space", as expected since the Cα atoms in this structure are held rigidly at one separation. A brief comparison was also made for the same elements of secondary structure with the core building blocks of the Hirschmann/Smith pyrrolinones[5] and Hamilton's terphenyl systems [see the Supporting Information (SI) for structures]. The Hirschmann/Smith 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-helices than the α-helix.[26] An outline of these studies is given in the SI, 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 conformer; 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 the SI).

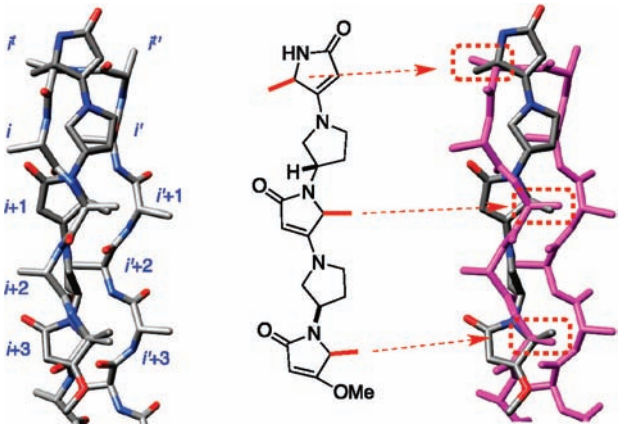


Figure 1. Overlay of **2aaa** on a sheet/β-turn/sheet motif.

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; also see the SI). A maximum energy barrier of 5.10 kcal/mol was calculated using this method (Figure 2b). This indicates that conformers of **6a** should rapidly interconvert on the ^{1}H NMR time scale, and experimentally this was shown to be the case.

Consideration of Newman projections through the bond labeled in Figure 2a indicates that this molecule would tend to rest predominantly in three conformational states. This assertion

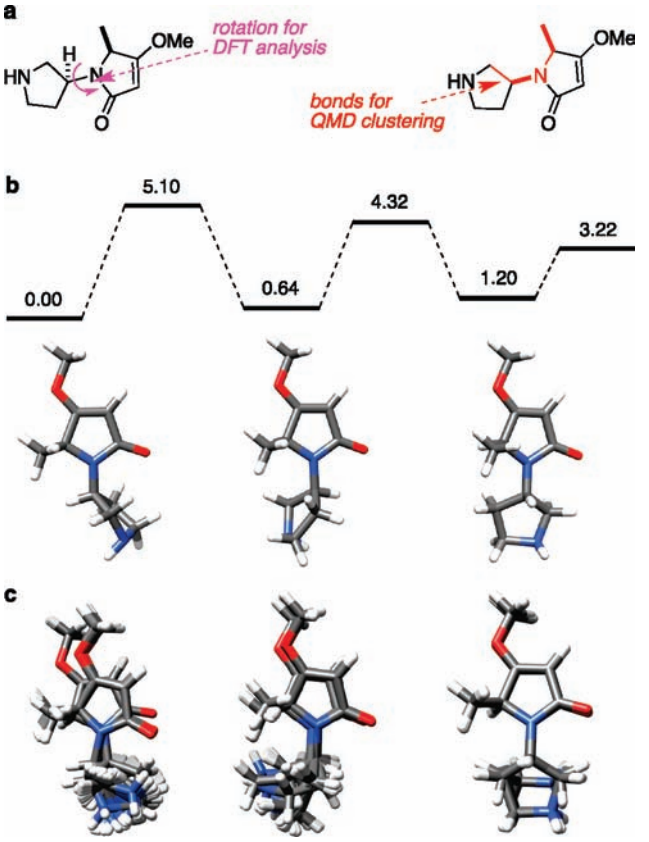


Figure 2. (a) Structures and parameters used for DFT and QMD analyses. (b) Low-energy conformers and energy barriers for interconversion from DFT calculations. (c) Preferred conformers from QMD calculations. All energies shown are free energies ($\Delta G°$) in kcal/mol.

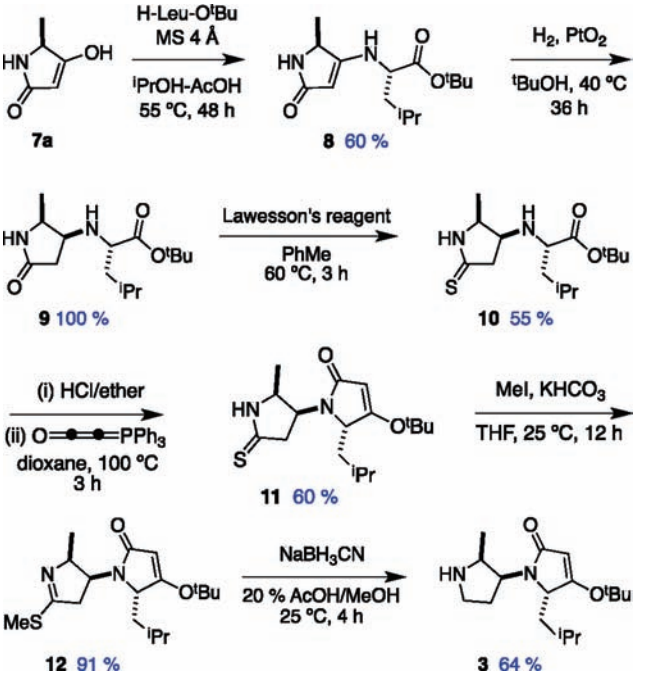
is supported by the fact that three low-energy conformational states emerged from both the DFT and QMD calculations (Figure 2b,c). The free energy differences from the DFT calculations predict that the relative populations of the three conformers should be in the ratio 1.00:0.34:0.12.

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**) and then reduced to the amine (**12** to **3**).

Extensive conformational analyses were performed for compound **3**. The full data set is shown in the SI, 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** (see the SI). Conversely, 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] that there are four *structural* criteria common to effective minimalist mimics: (i) facile syntheses with most amino

Scheme 2. Synthesis of 3 with Side Chains on Contiguous Rings



acid side chains; (ii) kinetically and thermodynamically accessible conformations for induced fit; (iii) only moderate loss of entropy on docking; and (iv) appropriate Cα−Cβ coordinates of an accessible conformation of the mimic matching those of the secondary structures. An advantage of defining a parameter such as (iv) (i.e., matching side-chain Cα−Cβ coordinates) is that the fit can be *quantitated* in terms of parameters such as RMSD's. This provides a firm basis for comparing the abilities of peptidomimetics such as **A** and **1**−**3** to mimic secondary structures.

■ ASSOCIATED CONTENT

Supporting Information. 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 {RMSD < 0.14 Å}; Hirschmann/Smith pyrrolinones and Hamilton's terphenyl systems) and complete ref 2 (as SI ref 10). This material is available free of charge via the Internet at http://pubs.acs.org. CCDC 822217−822219 contain 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.

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■ ACKNOWLEDGMENT

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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NOTE ADDED AFTER ASAP PUBLICATION

Scheme 1 and the corresponding information in the Supporting Information have been corrected and reposted August 10, 2011.