

Universal Peptidomimetics

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Abstract: This paper concerns peptidomimetic scaffolds that can present side chains in conformations resembling those of amino acids in secondary structures without incurring excessive entropic or enthalpic penalties. Compounds of this type are referred to here as *minimalist mimics*. The core hypothesis of this paper is that small sets of such scaffolds can be designed to analogue local pairs of amino acids (including noncontiguous ones) *in any secondary structure*; i.e., they are *universal peptidomimetics*. To illustrate this concept, we designed a set of four peptidomimetic scaffolds. Libraries based on them were made bearing side chains corresponding to many of the protein-derived amino acids. Modeling experiments were performed to give an indication of *kinetic and thermodynamic accessibilities* of conformations that can mimic secondary structures. Together, peptidomimetics based on these four scaffolds can adopt conformations that resemble almost any combination of local amino acid side chains in any secondary structure. Universal peptidomimetics of this kind are likely to be most useful in the design of libraries for high-throughput screening against diverse targets. Consequently, data arising from submission of these molecules to the NIH Molecular Libraries Small Molecule Repository (MLSMR) are outlined.

Introduction

Peptides consist of polyamide main chains bearing substituents. The term “peptidomimetics”^{1,2} can encompass compounds designed to resemble peptide main chains, side chains, or both. Peptidomimetics that can present side chains on main-chain scaffolds containing amide bonds are ubiquitous because medicinal chemists frequently design analogues of bioactive peptides using this approach. For instance, peptidomimetics involving substitution of amide bonds with surrogates^{3–7} or transition-state analogues^{8–13} are common. Peptide modifications to produce molecules that resemble secondary structures

are also widespread. Many helical^{14–17} and turn^{18–23} mimics have been prepared like this, frequently by joining two amino acid substituents to constrain peptide fragments in relevant conformations.^{24–32}

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An important development in peptide mimicry has been the emergence of analogues of peptide secondary structures that mainly present selected side chains; i.e., the main-chain polyamide backbone is abbreviated or totally absent. This approach is appealing because small molecules without polyamide backbones are more likely to be orally bioavailable and proteolytically stable. Early examples of this type of mimic were from Hirschmann and Smith, who designed β -turn analogues based on sugar,^{33,34} steroid,³⁵ or even catechol³⁶ backbones. Similarly, Hamilton^{37–51} and others^{52–58} used biphenyl,⁵² terphenyl,^{38,39,41,47,48,59–61} and related^{37,39,40,44,47,62,63} scaffolds to mimic helices. In our laboratory, we refer to compounds that

present only selected side chains to resemble peptide secondary structures as *minimalist mimics*.^{64,65}

Literature on minimalist mimics may give the impression that these types of molecules have one preferred conformation in solution that corresponds to the target secondary structure, but that is *not* the case. Scaffolds like those in Figure 1a do *not* exist as a single conformation in solution. They equilibrate between forms representing local minima in Boltzman distributions of energy states, and their *global* minimum does not necessarily correspond to the target secondary structure. Instead, it is sufficient that the pertinent conformations for mimicry have energies similar to the global minima so that they are populated and that the transition-state energy barriers to arrive at them can be overcome at ambient temperatures—in other words, *that there are no insurmountable thermodynamic or kinetic obstacles to attaining the target conformations*. On the other hand, entropic considerations dictate that useful minimalist mimics cannot be *totally* flexible. Their scaffolds must have *limited degrees of freedom* to avoid significant entropic penalties on adopting the target secondary structure conformations.

One of the original helical mimics reported by Hamilton is discussed here to illustrate the validity of the assertions above. Figure 1b illustrates facets of the conformational equilibria for this helical mimic. It has only two significant degrees of freedom that affect the orientation of the side chains. Conformers (i)–(iv) approximate to minimum energy forms that alleviate steric interactions between the substituted phenyl groups.⁶⁶ There can be no conformer that is substantially lower in energy than these, so they are *thermodynamically accessible* with respect to the global minimum; i.e., they will all be significantly populated. In fact, they are approximately equal in energy, so the populations of each of these conformers are similar. Similarly, the transition-state energy barriers that need to be surmounted to equilibrate conformers of such substituted terphenyls can be overcome at room temperature (those energies for terphenyls^{67,68} depend on the *ortho* substituents involved),^{69,70} so states (i)–(iv) are also *kinetically accessible*.

Conformers (i) and (iv) in Figure 1b correspond to helical orientations of the side chains with opposite handedness, while (ii) and (iii) do *not* display side chains in helical conformations. *If this mimic binds to a substrate in the helical conformation (i), for instance, then it would do so by induced fit*. This is possible because the target conformation of the mimic is kinetically and thermodynamically accessible, and not because it is thermodynamically preferred.

Identification of conformations in equilibrating ensembles that are both kinetically and thermodynamically accessible can be done only by comparing with similar systems that have been

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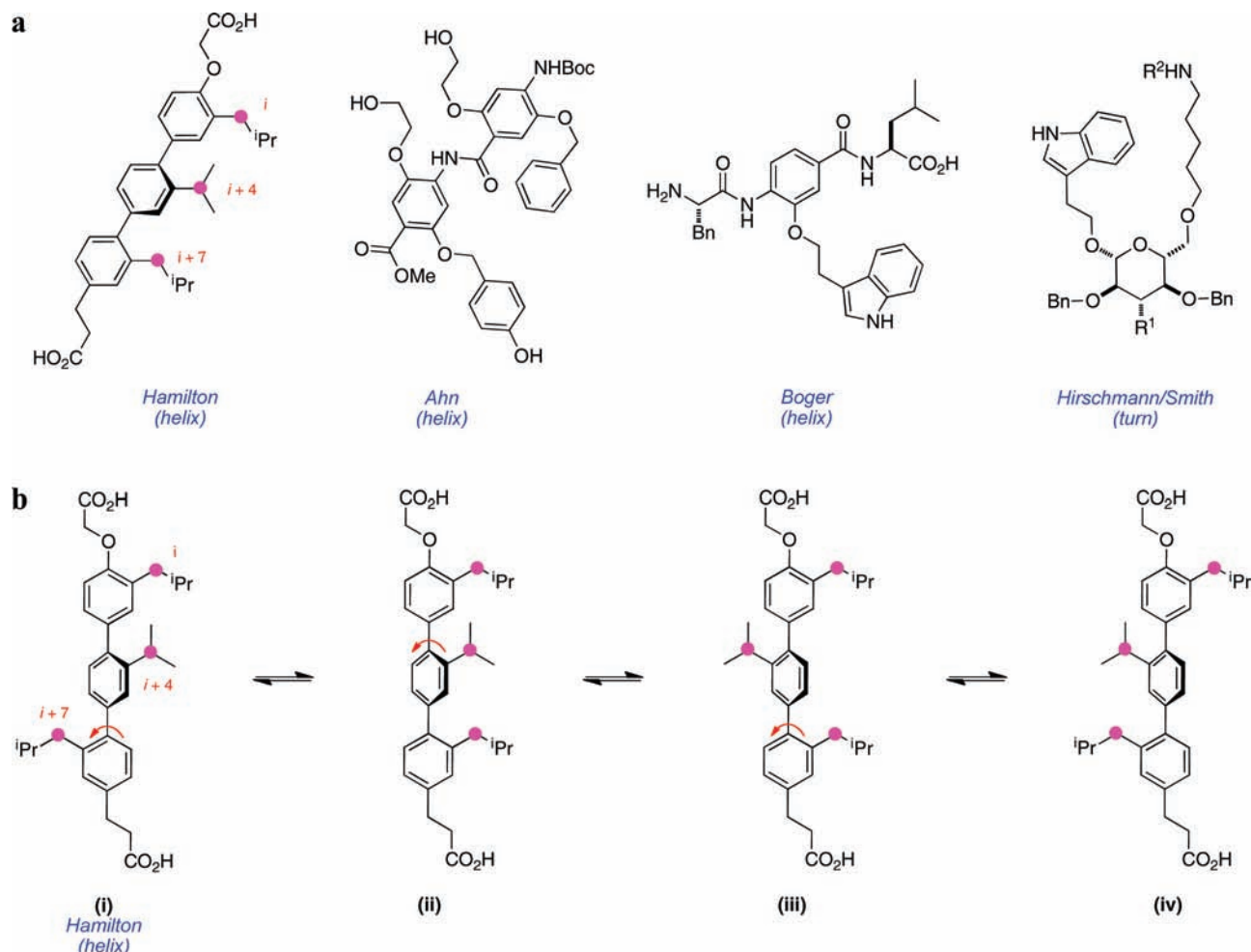


Figure 1. (a) Illustrative examples of minimalist mimics. (b) Favorable conformations of Hamilton's helical mimic shown in (a).

studied experimentally or via computational methods. Spectroscopic techniques like NMR cannot detect a preferred solution-state conformer of a mimic like that shown in Figure 1b at room temperature, because there is none. Coupling constants and NOE measurements for the scaffolds would reflect *conformational averaging* and give no useful data. Even if the mimic were crystallized, the conformational state(s) in the crystal would be determined by lattice packing forces. Consequently, computational methods are highly desirable to assess kinetically and thermodynamically accessible solution conformations of minimalist mimics.

This paper outlines structural design criteria for ideal minimalist mimics. In particular, it describes how $C^\beta-C^\beta$ distances can be used to estimate the correspondence of a minimalist mimic to a particular secondary structure, and how closer consideration of $C^\alpha-C^\beta$ vectors can be used as a more stringent test. It then describes how a small number of complementary scaffolds can be used to target *any* local pair of amino acids in *any* secondary structure, i.e., *universal mimics*.⁷¹ Several scaffold types were designed to give a set of putative universal

mimics, and libraries of each were generated to prove that a variety of amino acid side-chain substituents could be incorporated. Computational methods were identified to assess kinetic and thermodynamic accessibilities of relevant mimic conformations. Finally, an indication of the types of biophysical data that can be obtained with these compounds is presented.

Results

Criteria for the Design of Minimalist Mimics. To the best of our knowledge, criteria for design of minimalist mimics have never been specifically delineated before. Here we propose the following four *structural* design criteria:

- facile syntheses with most amino acid side chains (Arg, Trp, His, etc.);
- kinetically and thermodynamically accessible conformations for induced fit (i.e., not too rigid); but also
- only moderate loss of entropy on docking (i.e., only a few significant degrees of freedom that influence the side-chain orientations); and
- appropriate $C^\alpha-C^\beta$ coordinates of an accessible conformation of the mimic matching those of the secondary structure.

Other considerations can be important, too (e.g., water solubilities, toxicities, cell permeability, shelf lives). However, these four key parameters define the *structural* basis for

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designing minimalist mimics; if one of these is not satisfied, then concerns about the physical and pharmacological characteristics of the compounds may be inconsequential.

The first of the criteria listed above is self-evident from a practical perspective: a scaffold that cannot be easily made with most of the amino acid side chains found in proteins is generally not a good mimic. For instance, designs that can be conveniently prepared *only* with simple alkyl side chains would be of limited value if it was necessary to present Asn, Asp, Arg, Gln, His, Lys, Ser, Thr, Trp, and/or Tyr side chains.

The second and third criteria listed above, relating to the kinetic and thermodynamic accessibilities and to the significant degrees of freedom, have been addressed already. However, the fourth parameter, relating to correspondence of $C^\alpha-C^\beta$ vectors, is unique to this work and warrants further explanation.

Others before us have tried to reduce conformations of secondary structures to the simplest possible terms. For instance, β -turn mimics⁷² have been classified according to C^α -atom separations⁷³ or $C^\alpha-C^\beta$ vectors,⁷⁴ but, as far as we are aware, there have been no papers that suggest that *mimics* of *all* secondary structures could be broadly categorized using similar factors.

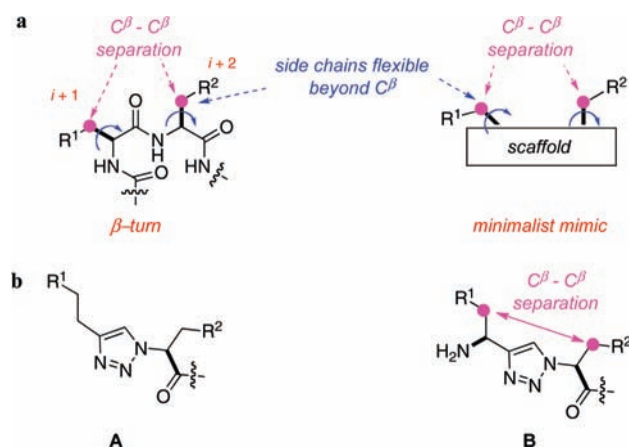


Figure 2. (a) A β -turn and a minimalist mimic consisting of a scaffold affording appropriate C^β atom separations. (b) Minimalist mimics **A** and **B** prepared by our group.

We recently proposed that C^β -atom separations are critical in the design of minimalist β -turn mimics.^{64,65} This is because the C^β positions represent the last atoms along the side chains that are held with some rigidity in these secondary structures; $C^\beta-C^\gamma$ side-chain vectors, and all those further removed from the backbone, are flexible (Figure 2a). Scaffolds **A** and **B** were prepared on the basis of this hypothesis (Figure 1b). Here we suggest that, in general, *accessibility of suitable distances between C^β atoms is a necessary condition for the design of minimalist peptidomimetics*. This concept is shown for a β -turn in Figure 2, and similar diagrams could be drawn for any secondary structure.

Intuitively, $C^\beta-C^\beta$ distances are useful as a “rough cut” to gauge the fit of a proposed minimalist mimic to a secondary structure. However, more accurate fitting requires a higher level

of sophistication. This comes by considering coordinates of the $C^\alpha-C^\beta$ bond vectors of side chains, because that parameter reveals how the side chain projects into space *as well as* $C^\beta-C^\beta$ distances. Matching $C^\alpha-C^\beta$ bond vectors to a particular secondary structure, therefore, gives a more realistic sense of the validity of the mimic. The only disadvantage is that considerations of $C^\alpha-C^\beta$ bond vectors require computation; hence, it is less convenient than the intuitive approach based on $C^\beta-C^\beta$ distances alone.

Analyzing the properties of minimalist mimics led us to conclude that there should be special subsets of these compounds that we now call *universal peptidomimetics*. The next section describes how $C^\beta-C^\beta$ distances (abbreviated to “ βs ”) can be used as an intuitive guide to the suitability of possible mimics as secondary structure mimics, and then how consideration of $C^\alpha-C^\beta$ bond vectors provides a more stringent test.

Candidate Universal Peptidomimetics Selected on the Basis of $C^\beta-C^\beta$ Distances. Minimalist peptidomimetics do not have fixed $C^\beta-C^\beta$ separations (βs values). For instance, peptidomimetics based on the triazoles **B** could have several different minimal energy conformations that correspond to preferred orientations of the two side chains. Indeed, energy barriers for interconversion between the conformers that place the two C^β atoms as close and as far apart as possible are likely to be relatively insignificant, so this particular type of peptidomimetic could access a range of βs values between these two extremes. In general, extremely contracted and expanded $C^\beta-C^\beta$ separations can be denoted as βs_c and βs_e , respectively.

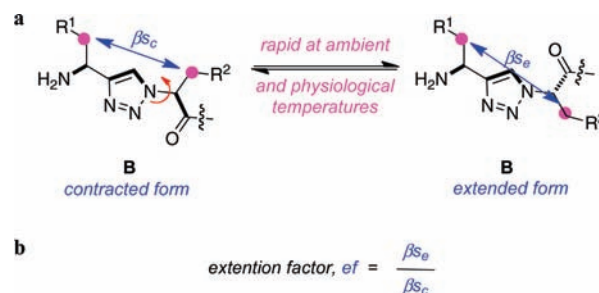


Figure 3. (a) Conformations of the triazole **B** can display side chains aligned with C^β atoms as close or as far apart as possible, and these conformations are easily interconverted at room temperature. (b) Definition of the term “extension factor”.

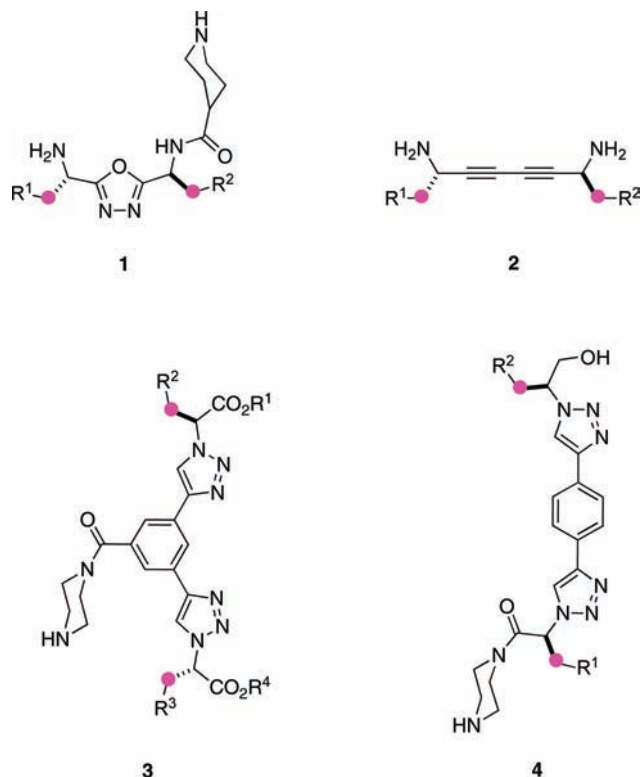
To calibrate the capacity of a minimalist peptidomimetic to span different $C^\beta-C^\beta$ distances, we have defined a parameter called the *extension factor*, $ef = \beta s_e/\beta s_c$, where βs_e and βs_c represent maximally extended and contracted $C^\beta-C^\beta$ separations among the conformers that are both kinetically and thermodynamically accessible (Figure 3). Some peptidomimetics could have kinetically or thermodynamically *inaccessible* $C^\beta-C^\beta$ separations between βs_e and βs_c , but for most, all conformations between βs_e and βs_c will be accessible. Peptidomimetics with small extension factors can mimic a limited range of $C^\beta-C^\beta$ separations, while those with large extension factors correspond to side-chain separations in more secondary structures. However, large extension factors are not *always* ideal in designs of universal peptidomimetics because they might also represent excessive flexibility in the scaffold, so ef values are predictors of *scope* rather than *quality*. On the other hand, scaffolds with large extension factors that can freeze in desired conformations with minimal loss of entropic stabilization are promising for the design of universal peptidomimetics.

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Four templates, **1–4** (listed in order of increasing average $C^\beta-C^\beta$ separations), were conceived using the structural criteria listed for minimalist mimics. Piperidine or piperazine residues in scaffolds **1**, **3**, and **4** were also included to facilitate assembly of these molecules into heterobivalent forms,^{64,65,75} but this is of no immediate consequence here.



The following procedure was used to calculate extension factors. First, the significant degrees of freedom in the minimized energy scaffold were identified. Typically these are bonds that can be rotated to adjust the $C^\beta-C^\beta$ separations. Rotations around these bonds were explored by setting this as the only variable and then monitoring energy as a function of rotation. This led to identification of the conformations that minimize and maximize the βs values; hence, the extension factors can be determined. Figure 4 shows these conformations for peptidomimetics **1**.

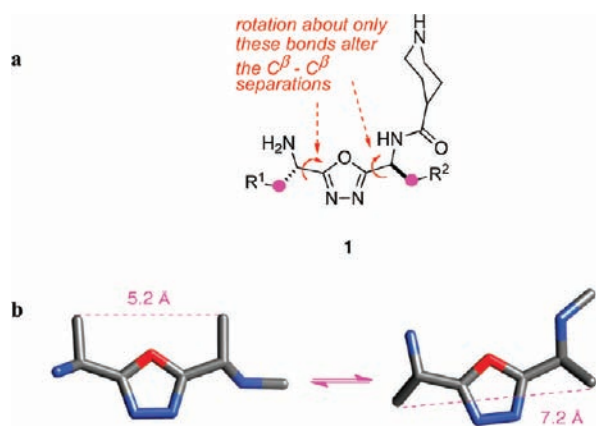


Figure 4. (a) “Significant degrees of freedom” for mimic **1** ($R^1 = R^2 = \text{Me}$); rotation about only these bonds in the scaffold alters the βs . (b) Conformations corresponding to the βs_c and βs_e in an abbreviated structure of compound **1** (piperidine ring omitted).

Table 1. Extension Factors Calculated for the Featured Peptidomimetics

monomer	βs_c (Å)	βs_e (Å)	ef
A	5.5	6.2	1.2
B	5.5	7.1	1.3
1	5.2	7.2	1.4
2	7.5	8.1	1.1
3	7.4	14.1	1.9
4	12.1	15.0	1.2

Table 1 shows the extension factors deduced for mimics **A**, **B**, and **1–4**. The data shown demonstrate that scaffold **1** allows the R^1 and R^2 side chains to approach more closely than any other in the series of compounds examined (i.e., βs_c is the smallest located). However, the value for βs_e is intermediate in the series; hence, the extension factor for this mimic is not the smallest of these compounds.

Table 2 shows data from modeling experiments we performed to assess $C^\beta-C^\beta$ separations (βs) for the common elements of secondary structure. It reveals considerable overlap between βs values for different residues in different secondary structures with the range of βs values accessible by each mimic. For instance, the i to $i + 3$ βs in a type I β -turn is about equal to that for the i to $i + 2$ in an inverse γ -turn (ca. 5.4 Å), so a scaffold that can access this βs can mimic those side chains in both secondary structures. Indeed, the extent of $C^\beta-C^\beta$ separation overlap in Table 2 indicates that almost all the side-chain $C^\beta-C^\beta$ separations could be mimicked with relatively few peptidomimetics. However, as noted above, analyses of $C^\beta-C^\beta$ separations provide rough approximations for the suitability of minimalist mimics. This parameter does not indicate the orientation of side chains; more stringent tests (based on $C^\alpha-C^\beta$ bond vectors) are required for this.

Each of the four mimic designs **1–4** are now considered separately. These scaffolds were very carefully designed to conform to the four parameters delineated above for minimalist peptidomimetics. Syntheses of small libraries based on these scaffolds are described to illustrate a range of amino acid side chains can be incorporated in each case. Density functional theory (DFT) calculations to explore the kinetic accessibility of the conformers are outlined. Thermodynamic accessibilities of the conformers pertinent to secondary structures are then described on the basis of a molecular dynamics and clustering procedure based on $C^\alpha-C^\beta$ bond vectors.

Universal Peptidomimetics 1: 1,3,4-Oxadiazole-Based. 1,3,4-Oxadiazoles^{80–82} have been used extensively in the design of pharmaceuticals^{83,84} and have been shown to have a range of bioactivities and applications, including bactericidal, fungicidal, analgesic, anti-inflammatory, antiproteolytic, anticonvulsant, nervous system depressant, sedative, and local anesthetic.^{85–89} A 1,3,4-oxadiazole-containing pharmaceutical of current importance is Merck’s anti-retroviral HIV drug, Raltegravir. 1,3,4-Oxadiazoles are also a compact five-membered heterocycle that might be ideal for the formation of universal peptidomimetics.

Scheme 1a describes the procedure used to form the oxadiazole^{80,83,84,90} mimics **1** via formation of amino acid

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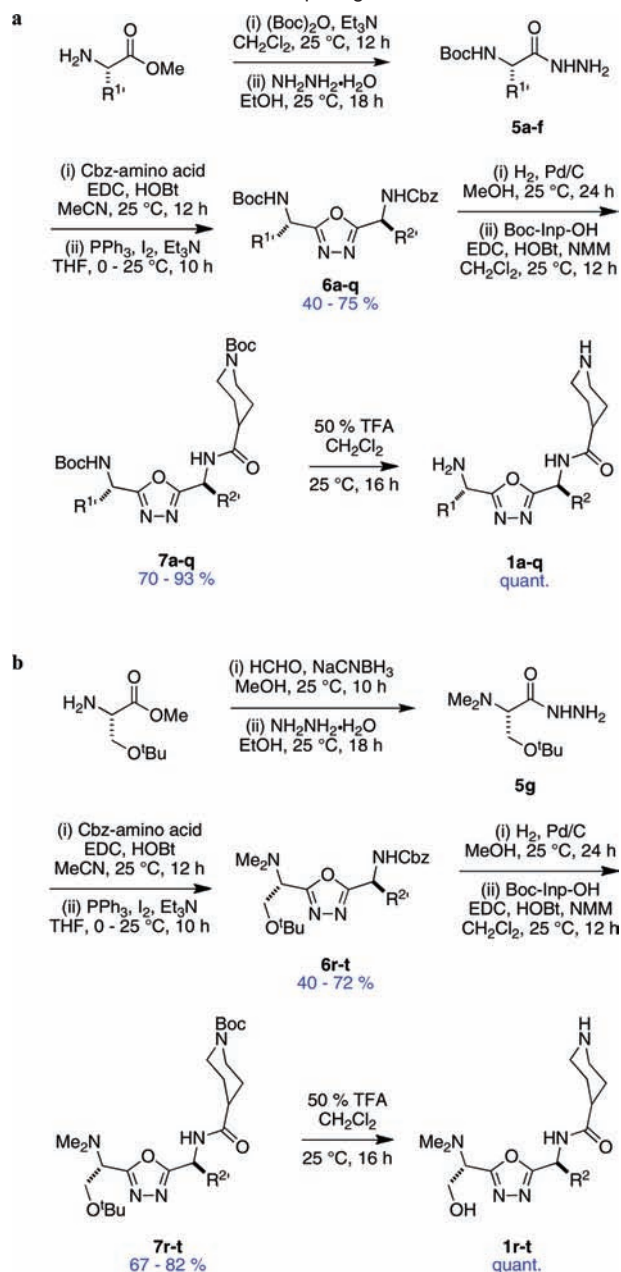
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Table 2. Correspondence of C^β–C^β Distances for Peptidomimetics A, B, and 1–4 with Common Secondary Structures^a

structure	sequence	βs(Å)	A	B	1	2	3	4
α-helix	<i>i-i+1</i>	5.2	■	■	■			
	<i>i-i+2</i>	7.1		■	■		■	
	<i>i-i+3</i>	5.6	■	■	■			
	<i>i-i+4</i>	6.5	■	■	■			
	<i>i-i+5</i>	9.8						
	<i>i-i+6</i>	10.8					■	
	<i>i-i+7</i>	10.7					■	
	<i>i-i+8</i>	12.9					■	■
β-turn (type-I)	<i>i-i+1</i>	5.7	■	■	■			
	<i>i-i+2</i>	5.1			■			
	<i>i-i+3</i>	5.4	■	■	■			
	<i>i+1-i+2</i>	5.2	■	■	■			
	<i>i+1-i+3</i>	7.5				■	■	
	<i>i+2-i+3</i>	5.6	■	■	■			
β-sheet (parallel)	<i>i-i+1</i>	5.8	■	■	■			
	<i>i-i+2</i>	7.1		■	■		■	
	<i>i-i+3</i>	11.1					■	
	<i>i-i+4</i>	13.2					■	■
	<i>i-i'</i>	5.5	■	■	■			
	<i>i-i'+1</i>	7.2		■	■	■		
	<i>i-i'+2</i>	9.0				■	■	
	<i>i-i'+3</i>	11.5					■	■
β-sheet (anti-parallel)	<i>i-i+1</i>	5.8	■	■	■			
	<i>i-i+2</i>	6.5	■	■	■			
	<i>i-i+3</i>	11.0					■	
	<i>i-i+4</i>	12.8					■	■
	<i>i-i'</i>	4.5	■	■	■			
	<i>i-i'+1</i>	7.6		■	■	■		
	<i>i-i'+2</i>	8.9				■	■	
	<i>i-i'+3</i>	12.7					■	■
γ-turn (classic)	<i>i-i+1</i>	4.7			■			
	<i>i-i+2</i>	7.2		■	■		■	
	<i>i+1-i+2</i>	5.1			■		■	
γ-turn (inverse)	<i>i-i+1</i>	5.7	■	■	■			
	<i>i-i+2</i>	5.4			■			
	<i>i+1-i+2</i>	6.2	■	■	■			

^a Templates for ideal type I β-turns^{54,76} and for γ-turns⁷⁷ were obtained from standard torsion angles. A standard template for overlays with an α-helix was obtained from Discovery Studio 2.5,⁷⁸ and a β-sheet template for overlays was obtained by β-sheet builder.⁷⁹

hydrazides **5**,⁹¹ coupling these to another amino acid,⁹² and then dehydrative cyclization to give **6**. Several sets of conditions were tested for the cyclization step, but the triphenylphosphine iodine system shown appeared to give the best results.^{93–95} Thereafter, a piperidine group was added (so that these monovalent compounds might later be assembled into bivalent ones),^{65,96} and the protecting groups were removed to give the target molecules **1**. This synthetic strategy involved six linear steps and only two chromatographic separations to the protected forms **7**. Those intermediates **7** probably have longer shelf-lives than the unprotected compounds **1** and are therefore the preferred

Scheme 1. Two Methods for Preparing Monovalent Mimics **1**

way to store these materials. Many of these steps can be performed in parallel to facilitate rapid formation of a chemical library.

A series of oxadiazole compounds that complement the ones shown in Scheme 1a was also made (Scheme 1b); these contain *N,N*-dimethyl serine fragments.⁹⁷ Three compounds containing this component were prepared via the slightly modified method shown in Scheme 1b. This is very similar to that in **1a** but involves an initial reductive amination step.

Table 3 lists the side chains incorporated into the oxadiazole-based mimics **1**. They are all derived from protein-based amino acids, including ones that have diverse and relatively reactive functionalities (e.g., Tyr, Lys, Glu, Ser, Asp, Ile, Thr). The patent literature contains a synthetic route similar to that shown in Scheme 1a⁹⁸ but does not encompass functionalized amino acids.

The following DFT method was used to investigate *kinetic accessibility* for compounds **1** and all the other scaffolds in the series. Briefly, Gaussian 03 was used at the B3LYP level of

Table 3. Oxadiazole-Based Peptidomimetics 1

compound 1	R ¹	R ²
a		
b		
c		
d		
e		
f	H	
g		
h		
i		
j		
k		
l		
m		
n		
o	H	
p		
q		
r		
s		
t		

theory with a 6-31+G(d') basis set and a polarized continuum solvation model with a dielectric of 80 (see Supporting Information). Figure 5 illustrates the data obtained for mimic

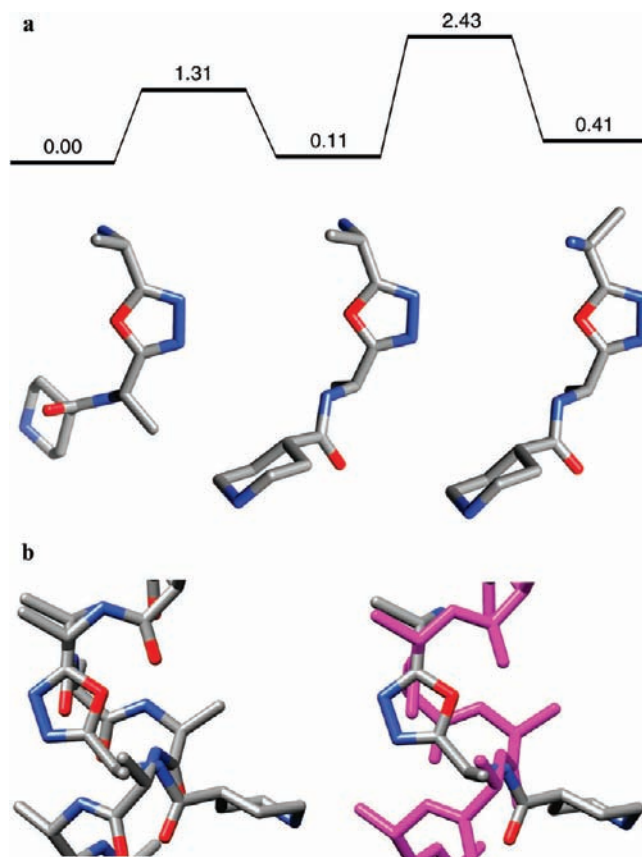


Figure 5. (a) Transposition of the global minima of **1** into a conformation that mimics the *i* and *i* + 3 residues in an α -helix by rotation around one of the significant bonds and then the other (ΔG° values shown in kcal/mol). (b) Overlay of the latter conformation on an ideal α -helix (shown color-coded on the left and in pink on the right).

1. The global minimum energy structure that was identified was set to 0 kcal/mol and is shown on the left side of Figure 5a.

Consideration of the likely energy barriers corresponding to rotations about the significant degrees of freedom for mimics **1** indicated that many conformations other than the global minimum would be accessible. Further, Table 2 indicates that scaffolds **1** can have $C^\beta-C^\beta$ separations corresponding to the *i* to *i* + 3 residues of an α -helix. The conformation of **1** that best overlays with the C^β atoms of the *i* and *i* + 3 residues in an α -helix is shown on the right-hand side of Figure 5a, and an overlay of this mimic with an α -helix is illustrated in Figure 5b. That conformation is then a target for further computational studies to monitor accessibility. Mapping the variation of energy with rotation about the two significant degrees of freedom reveals that it takes 1.31 kcal/mol to overcome one rotational energy barrier and 2.43 kcal/mol to surmount the other to arrive at the targeted α -helical conformations. Energy barriers of this magnitude are easily surmounted at room temperature, so induced fit is possible. Further, the targeted conformation is only 0.41 kcal/mol above the global minima, so these molecules will populate shapes required for docking the *i* and *i* + 3 residues in an α -helical form, even in the absence of other factors to induce that conformation.

The DFT method described above facilitates calculation of transition-state energies and relative energies of resting conformations, but it does not show the all the conformations that can be formed and rank their relative energies, i.e., *thermodynamic accessibility*. To do that, we used a complementary computational

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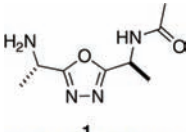
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Table 4. QMD Analysis of 1



1
abbreviated structure

	F1	F2	F3	F4	F5	F6	F7
population	66	21	72	11	7	1	2
ΔE (kcal/mol) from lowest energy conformer overall	0.00	0.12	0.15	0.26	0.28	0.34	0.40
min. $C^\beta - C^\beta$	6.32	6.07	5.91	5.93	5.65	6.50	6.92
max. $C^\beta - C^\beta$	6.93	6.32	6.48	6.53	5.92	6.50	6.92
corresponds to ^a			α - helix γ -turn		β - turn		

^a Representative structures in the families highlighted overlay with the secondary structures as represented in the figures accompanying this table.

technique: quenched molecular dynamics (QMD).^{99–102} In this technique, the molecule is minimized and then subjected to a molecular dynamics run at high temperature (1000 K) for a short time (600 ps); 600 conformational states are recorded during this run (i.e., every 1 ps) and minimized via molecular mechanics. The lowest energy structures below a user-defined cutoff are selected and then clustered into families on the basis of root-mean-square (rms) deviations from user-defined atoms (see Supporting Information).

The QMD technique applied to compound 1 ($R^1 = R^2 = \text{Me}$) illustrates the concept of universal peptidomimetics well.

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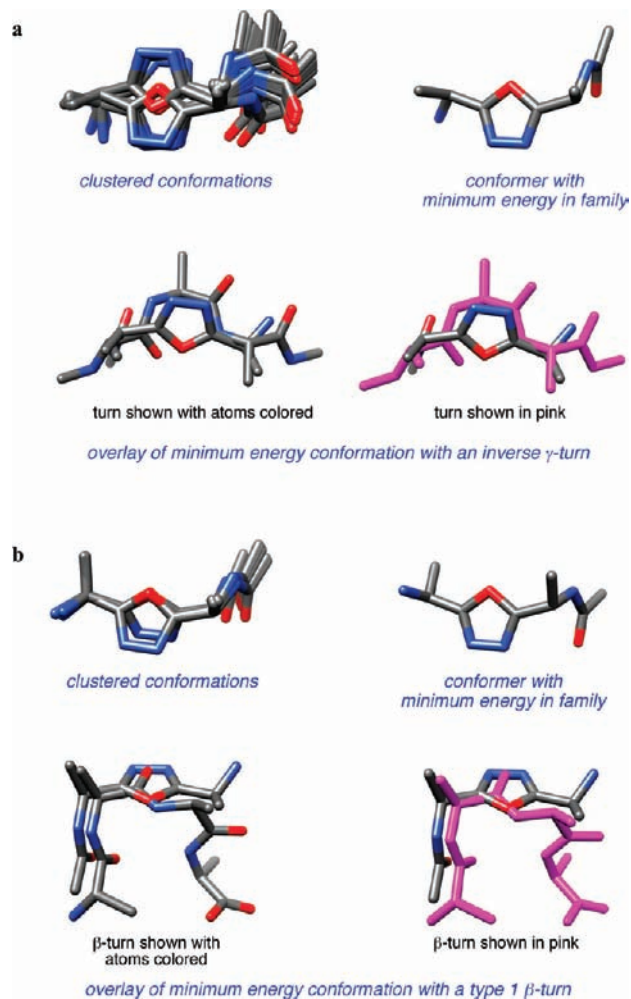


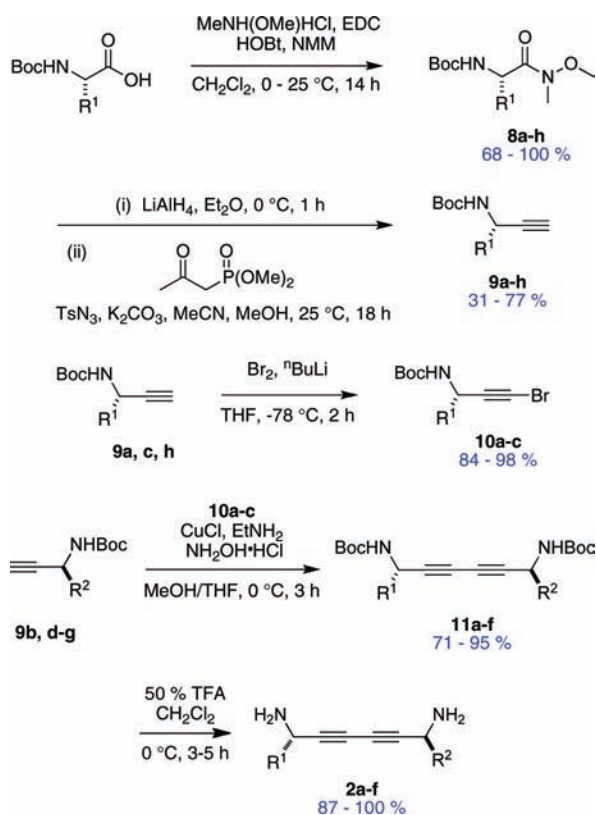
Figure 6. QMD data for compound 1 ($R^1 = R^2 = \text{Me}$). (a) Data from family 3, illustrating overlay with an inverse γ -turn (overlay with an α -helix is shown in Figure 5b). (b) Data from family 5, illustrating overlay with a type 1 β -turn.

Application of a 0.45 kcal/mol energy cutoff gave 180 (out of 600, see above) minimized conformations. These 180 structures were clustered by overlay of the C^α and C^β atoms (to within 0.3 Å rms deviation); this method was chosen because it matches conformations in terms of both $C^\beta - C^\beta$ distances and $C^\alpha - C^\beta$ bond vectors. Sorting in terms of $C^\alpha - C^\beta$ bond vectors as well as $C^\beta - C^\beta$ distances is a refinement that facilitates selection of the subsets of conformations with similar side-chain orientations from within the larger category of conformations with similar β_s values. This process gave seven families of conformations (Table 4). Family 3 has the most structures that overlaid (i.e., is the most “populated”), and the minimum energy structure in this family was only 0.15 kcal/mol above the overall minimum energy conformation of those sampled. Conformations within this family overlay well with the $i - i + 3$ side chains of an ideal α -helix conformation; this is the same conclusion that was reached using the DFT method (Figure 6a; see also Figure 5b above). However, structures in this family also overlay the $i - i + 2$ side chains of an ideal inverse γ -turn (Figure 6a). Furthermore, structures in family 5 overlay with an ideal type 1 β -turn at the $i + 1 - i + 2$ side chains (Figure 6b). Thus, the QMD technique illustrates how conformations of peptidomimetic 1, all of which are less than 0.45 kcal/mol from the overall minimum energy conformation identified, can mimic three

secondary structures. It obviates the requirement for exploring conformational space in the DFT method described above, i.e., that the user should have target conformations in mind. QMD facilitates sampling of conformation space, and the DFT approaches can be used to reveal if the desired conformations are kinetically accessible.

Table 1 was formulated by considering all the β s values that are open to a peptidomimetic and comparing them to common secondary structures. The QMD procedure described above is a more stringent test of whether a compound can be a minimalist mimic of a particular secondary structure. This is because C^α and C^β atoms are used to cluster conformations to within a specified rms deviation, and only low-energy conformations are considered (because of the application of low cutoff energies in the selection process). Thus, the QMD analyses find structures that resemble all the conformations suggested in Table 1, but suggest the most favored of those on the basis of C^β and C^α atom orientations. Several secondary structures were represented in the most favorable conformations found for compound 1; these include helical and turn conformers (both β and γ).

Scheme 2. Method for Preparing Diyne-Based Mimics 2



Universal Peptidomimetics 2: 1,3-Butadiyne-Based. 1,3-Butadiynes are atypical scaffolds for pharmaceuticals but are found in natural products.¹⁰³ They provide a scaffold suitable for syntheses of minimalist peptidomimetics. One reason for this is that both halves of the molecules 2 can be derived from amino acids, so almost any side chain can be incorporated.

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Table 5. 1,3-Butadiyne-Based Peptidomimetics 2

compound 2	R ¹	R ²
a		
b		
c		
d		
e		
f		

Scheme 2 illustrates how this concept was reduced to practice in a preparation of six peptidomimetics. Conversion of *N*-Boc amino acids into the corresponding alkynes 9 was achieved via routine chemistry with the Ohira–Bestmann modification^{104–106} of the Gilbert Seyferth reaction as the pivotal step; this proceeded without racemization. Coupling of two different alkynes to give unsymmetrical diynes rarely proceeds with good selectivity for the heterocoupling. After some experimentation, we found it best to convert the alkynes bearing the least functional side chain to the corresponding 1-bromo derivatives 10 and then couple these to the parent alkynes 9 via Cadiot–Chodkiewicz conditions.^{107,108} This procedure gave the unsymmetrical products 11 in good yields after chromatographic purification. Table 5 shows the side chains involved in the mimics 2 that were prepared.

There is only one significant degree of freedom involved in moving the two C^β atoms closer to or farther away from each other and simultaneously adjusting the orientation of the C^α – C^β vectors. Consequently, these peptidomimetics are

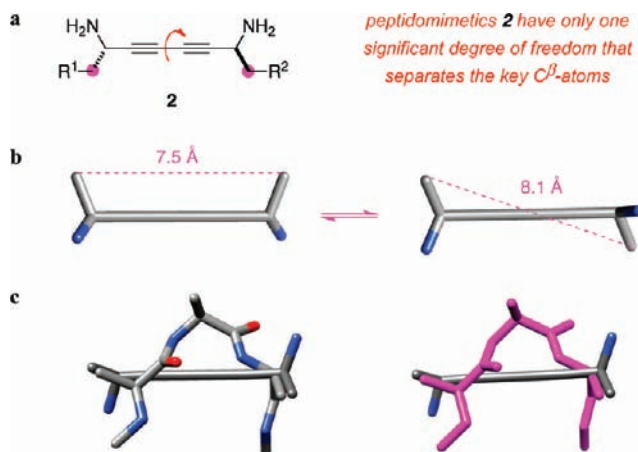
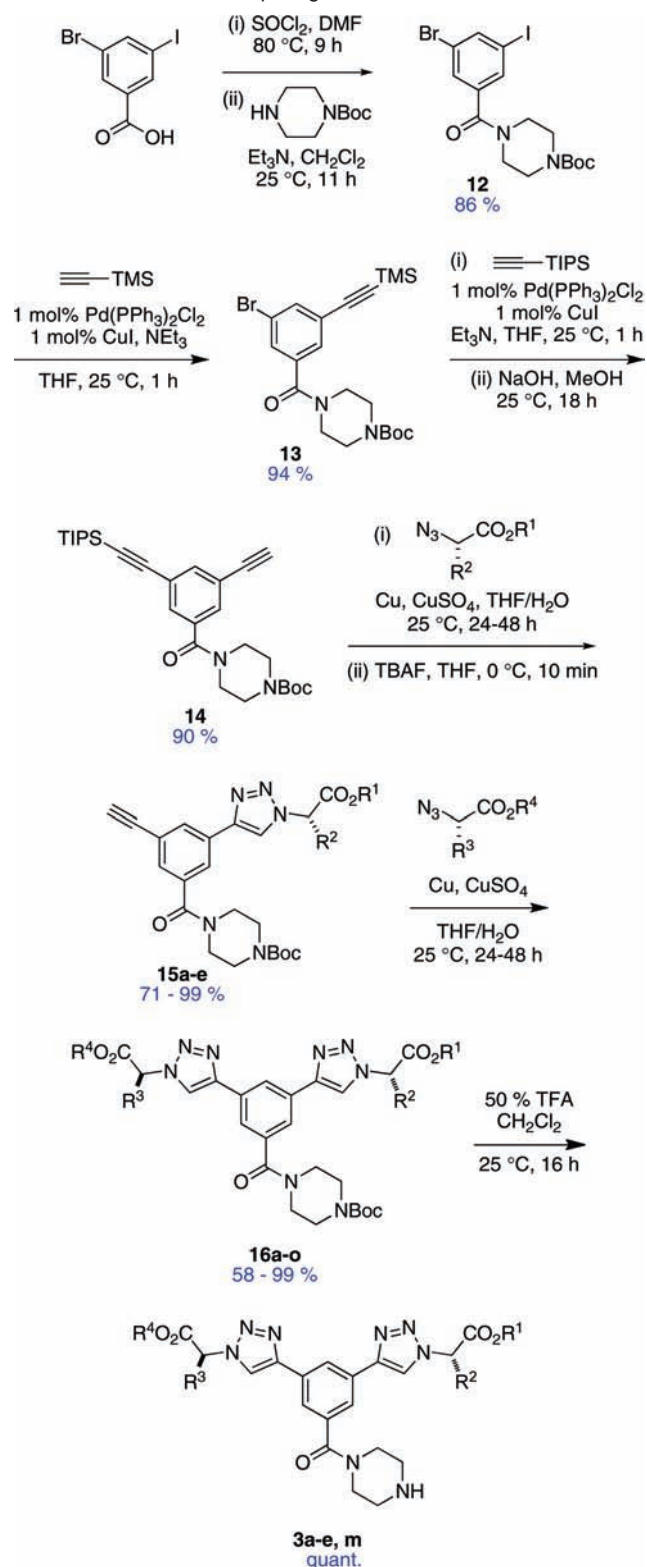


Figure 7. (a) Peptidomimetics 2 have only one significant degree of freedom ($R^1 = R^2 = \text{Me}$ shown). (b) Conformations corresponding to the β_s and $\beta_{s'}$. (c) Overlay of one conformation of 2 with the *i* and *i* + 2 side chains of a classical γ -turn.

Scheme 3. Method for Preparing Extended Mimics 3

highly constrained to give C^β separations between 7.5 and 8.1 Å (Figure 7), corresponding to an extension factor of 1.1 (Table 1).

C^α -alkyne and alkyne-alkyne bonds in compounds **2** are essentially free to rotate. Nevertheless, for completeness, DFT calculations were performed on this system. They revealed that the calculated energy maximum between the two conformations shown in Figure 7b was less than 0.01 kcal/mol, and the

Table 6. Kinked Bistriazole-Based Peptidomimetics 3

compound 3	R^1	R^2	R^3	R^4
a	Me			H
b	Me			Me
c	Me			H
d	Me			H
e	Me			Me
f	H			H
g	H			Me
h	H			H
i	H			Me
j	Me			H
k	Me			Me
l	Me			H
m	H			Me
n	H			H
o	H			Me

conformations were essentially equal in energy. Similarly, QMD calculations indicate that every possible conformation that holds the C^β atoms at distances between 7.5 and 8.1 Å has almost the same energy and is equally populated. These data show that this peptidomimetic would surrender very little entropy on induced fit docking that sets its C^β atoms between these ranges.

Being so constrained, it would be expected that compounds **2** have relatively low extension factors, and Table 1 shows that this parameter is lower than those for every other mimic in the series **A**, **B**, **1-4**. It follows that this compound should overlay

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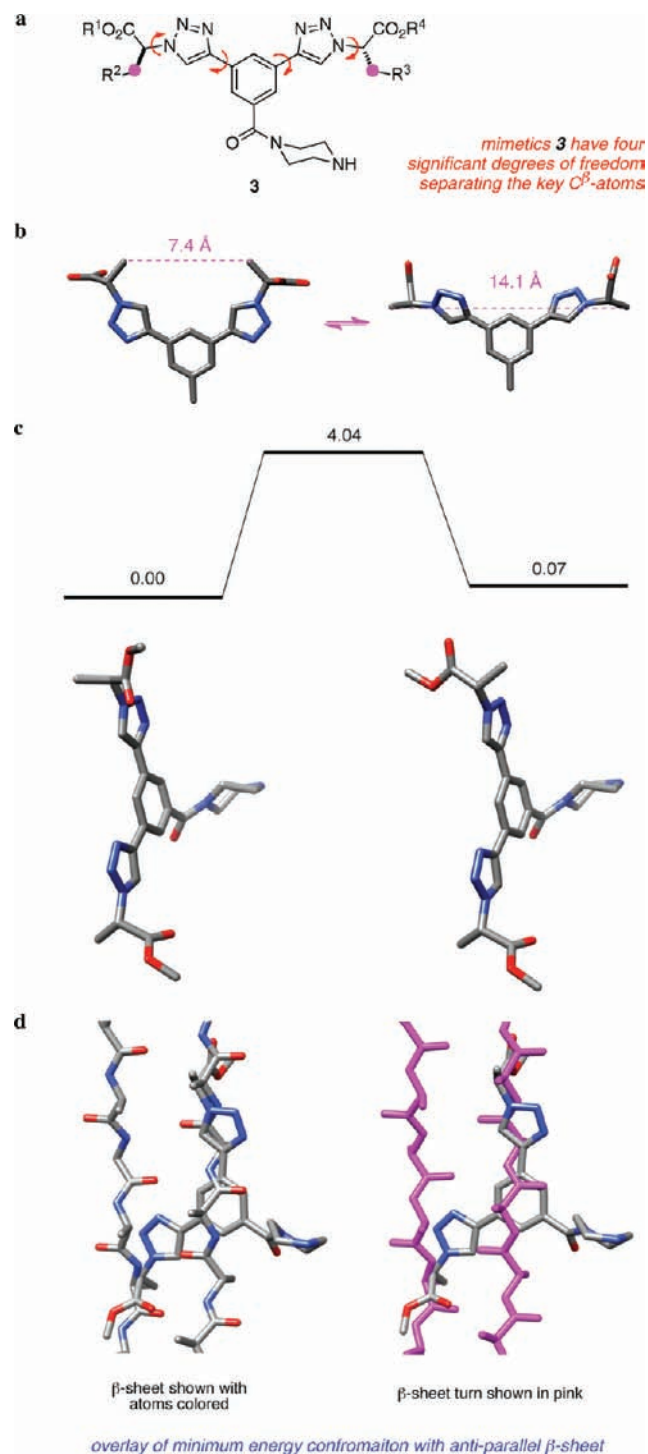


Figure 8. (a) Peptidomimetics **3** have four significant degrees of freedom ($R^1 = R^2 = \text{Me}$ shown). (b) Conformations corresponding to the β_{sc} and β_{sc} in an abbreviated model of structure **3** (piperazine ring omitted). (c) Transposition of the global minima of **3** into conformation that mimics the i and $i' + 3$ residues in an anti-parallel β -sheet (ΔG° values shown in kcal/mol). (d) Overlay of the latter conformation on an anti-parallel β -sheet (shown color coded on the left and in pink on the right).

side chains only with a limited number of secondary structures, and again, Table 1 indicates this is so. However, it still can overlay side chains with certain elements of β - and γ -turns and β -sheets; i.e., it is a useful member of the universal peptidomimetic set. For instance, Figure 7 shows the overlay of this with the i and $i + 2$ side chains of a classical γ -turn.

Table 7. QMD Analysis of **3**^a

abbreviated structure

	F1	F2	F3	F4	F5	F6	F7	F8
population	18	17	13	8	4	11	2	7
ΔE (kcal/mol) from lowest energy conformer overall	0.00	0.01	0.03	0.07	0.08	0.09	0.13	0.15
min. $C^\beta - C^\beta$	13.36	12.00	12.79	12.38	11.43	11.04	11.33	9.51
max. $C^\beta - C^\beta$	13.77	12.60	13.65	12.92	11.84	11.73	11.39	10.17
corresponds to ^b			α -helix	β -sheet ^c			β -sheet ^d	

^a A total of 15 families were identified, but F9–15 were omitted for clarity. F9 and F12–15 have only one structure, and F10 and F11 have four and five structures, respectively. All the conformers in F9–15 were more than 0.17 kcal/mol less stable than the global minimum.

^b Representative structures in the families highlighted overlay with the secondary structures as represented in the figures accompanying this table. ^c Antiparallel form. ^d Parallel form.

Universal Peptidomimetics 3: “Kinked” Bistriazole-Based. Compounds that present side chains at relatively large separations are necessary in a set of universal peptidomimetics if together they are to be comprehensive mimics of secondary structures. Minimalist mimetics **3** and **4** are included in this set to provide such extended C^β separations.

A monoprotected diyne scaffold similar to compound **14** has been prepared before,¹⁰⁹ so preparation of that particular intermediate was relatively straightforward (Scheme 3). Sequential click–deprotect–click reactions using amino acid-derived azides afforded the mimics **3** with a diverse set of side-chain functionalities corresponding to Glu, Ile, Leu, Lys, Ser, Trp, and Tyr in the examples we elected to make (Table 6).

Figure 8a shows the *four* significant degrees of freedom for the scaffold **3**. An extension factor of 1.9, the maximum in the series, was calculated for this framework, indicative of a concertina-like ability to expand and contract the distance between the C^β atoms (Figure 8b). Table 2 indicates that this design is capable of presenting C^β atoms at separations that correspond to distal side chains in a variety of secondary structures.

Calculations for the viability of interconversion of favored conformers (*density functional theory* method) show such processes are relatively facile for compounds **3**. For instance, changing the global minimum conformation into one that overlays with “cross strand” $i-i' + 3$ residues of an anti-parallel β -sheet involves surmounting an energy barrier of only 4.04 kcal/mol and costs only 0.07 kcal/mol on arrival at that conformation (Figure 8c and 8d). Thus, the conformers are comparable in energy and will interconvert rapidly at room temperature.

Table 7 collects the QMD data for an abbreviated structure of peptidomimetic **3** to illustrate the range of conformers that

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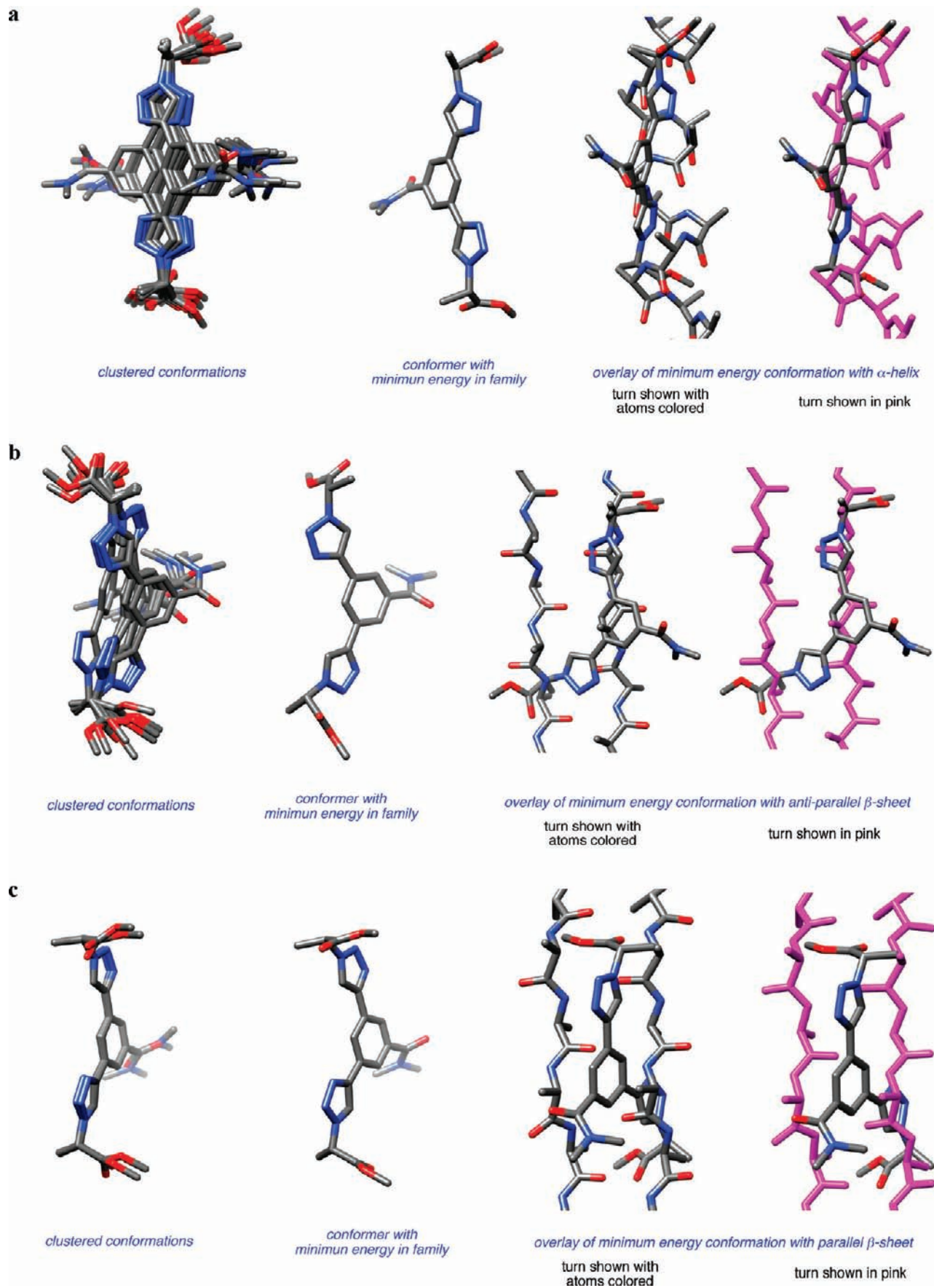
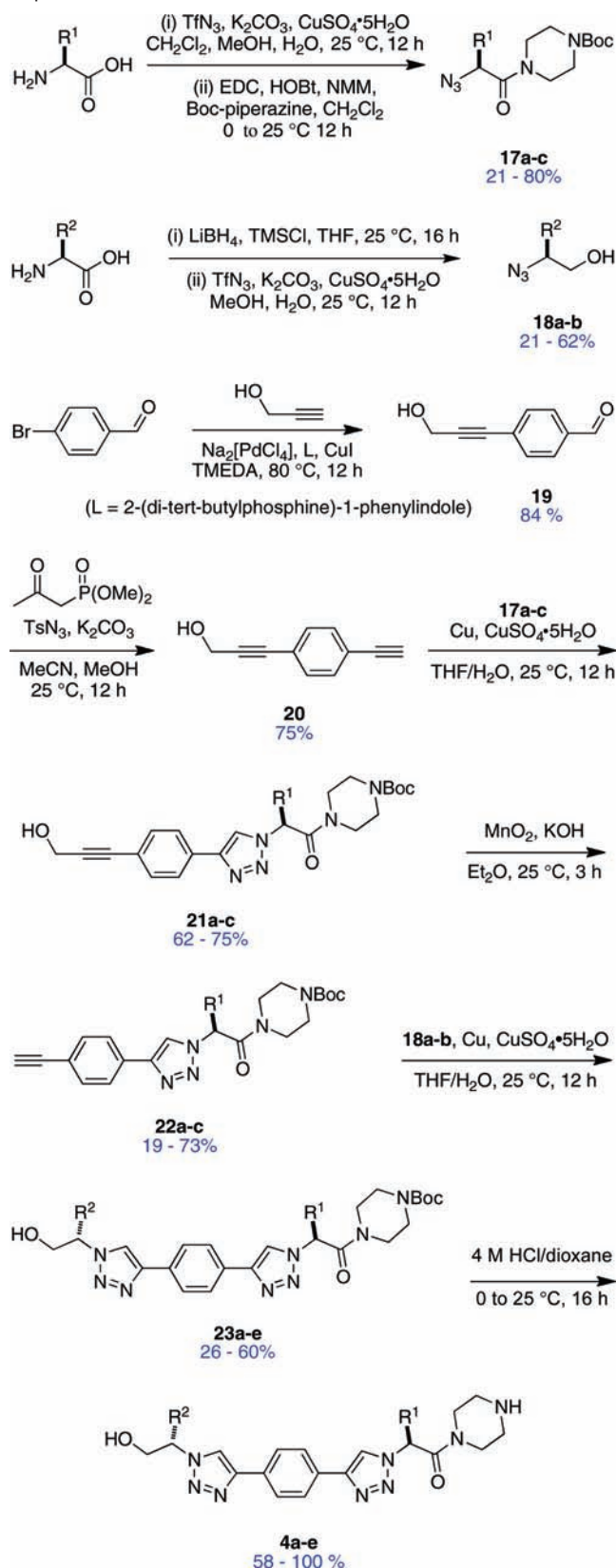


Figure 9. Conformers from QMD studies on compounds **3** (see Table 7). (a) Family 3 overlaid with an α -helix; (b) family 4 overlaid with an antiparallel β -sheet; and (c) family 7 overlaid onto a parallel β -sheet.

Scheme 4. Synthesis of the “Linear” Bistriazole-Based Peptidomimetics **4**

are accessible. Application of an energy cutoff value of 0.3 kcal/mol and clustering to within an rms deviation of 0.3 Å gave eight major families. Conformers in family 3 overlaid with α -helix $i-i+8$ residues, while members of family 4 contained conformers that overlaid well with the $i-i'+3$ residues of an

Table 8. Synthesis of the “Linear” Bistriazole-Based Peptidomimetics **4**

compound 4	R^1	R^2
a		
b		
c		
d		
e		

antiparallel β -sheet (Figure 9), just as shown in the *density functional theory* calculations for this molecule. The lowest energy conformers in families 3 and 4 were only 0.03 and 0.07 kcal/mol less stable than the observed global minimum, respectively. Members of family 7 overlaid well with the $i-i+4$ residues of a parallel β -sheet (lowest energy conformer 0.13 kcal/mol above observed global minima).

Universal Peptidomimetics 4: “Linear” Bistriazole-Based. Peptidomimetics **3** feature a 1,3-disubstituted benzene as the central ring. An analogous 1,4-disubstituted benzene system gives a greater possible span between the two pertinent side chains; the resulting design is peptidomimetics **4**. These compounds can have $\text{C}^\beta-\text{C}^\beta$ separations of up to 15.0 Å (Figure 10), but their extension factors are toward the smaller end of the range given in Table 1. Scheme 4 shows the strategy that was performed to obtain these materials; it is similar to that used in Scheme 3, except that the dialkyne framework was built during the synthesis rather than at the beginning. Table 8 summarizes the side chains involved and the compounds formed.

Table 9. QMD Analysis of **4**^a

4
abbreviated structure

	F1	F2	F3	F4	F5	F6	F7
population	25	19	16	13	17	5	6
ΔE (kcal/mol) from lowest energy conformer overall	0.00	0.03	0.16	0.24	0.33	0.50	0.85
min. $\text{C}^\beta-\text{C}^\beta$	13.92	13.18	14.10	13.63	12.62	13.35	13.78
max. $\text{C}^\beta-\text{C}^\beta$	14.48	13.56	14.32	14.24	13.13	13.44	14.11
corresponds to ^b		β -sheet ^c			α -helix		

^a A total 10 families were identified, but F8–10 were omitted. F8 and F10 have only one structure, and F9 has three. All the conformers in F8–10 were at least 0.92 kcal/mol less stable than the global minimum. ^b Representative structures in the families highlighted overlay with the secondary structures as represented in the figures accompanying this table. ^c Parallel form.

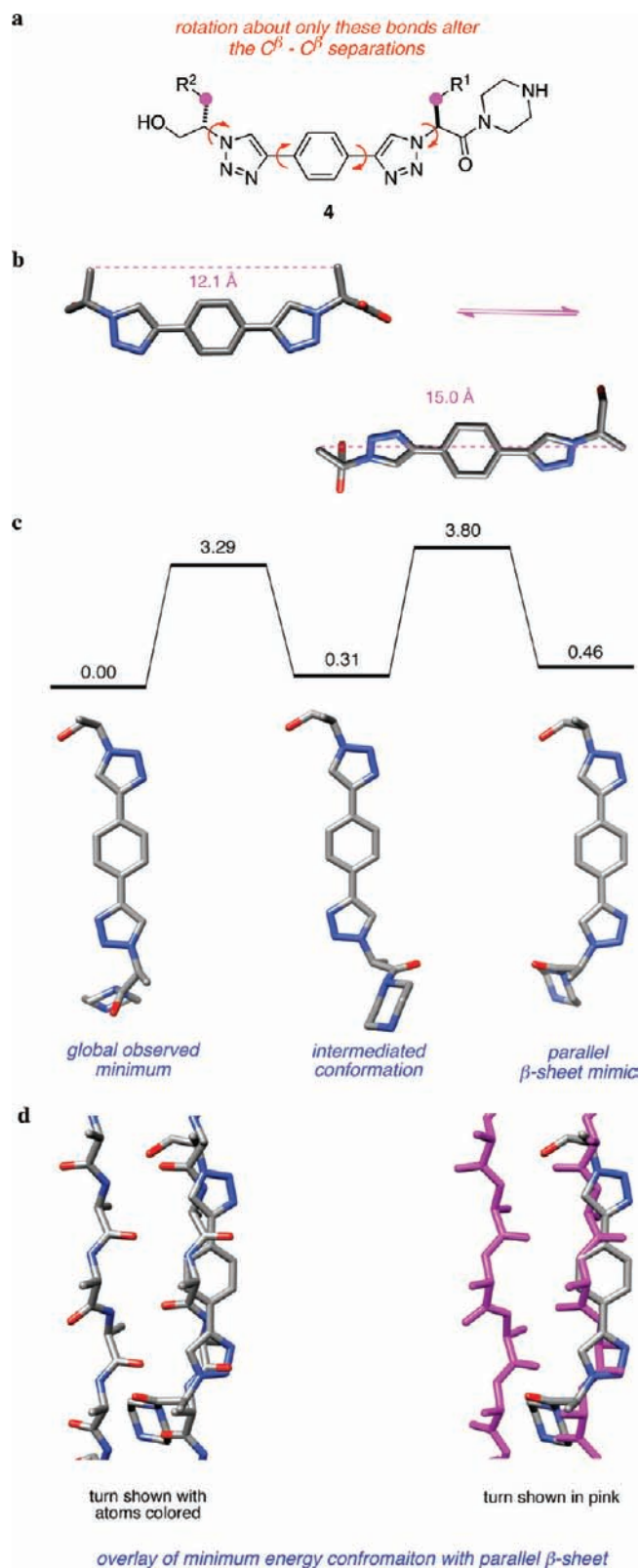


Figure 10. (a) Peptidomimetics **4** have four significant degrees of freedom ($R^1 = R^2 = \text{Me}$ shown). (b) Conformations corresponding to the β_{sc} and β_{sc} in an abbreviated model of structure **4** (piperazine ring omitted). (c) Global minimum conformation of **4** from the *density functional theory* method must surmount energy barriers of just over 3 kcal/mol to reach conformations that mimic the i and $i + 4$ residues in a parallel β -sheet (ΔG° values shown in kcal/mol). (d) Overlay of the latter conformation on a parallel β -sheet (shown color coded on the left and in pink on the right).

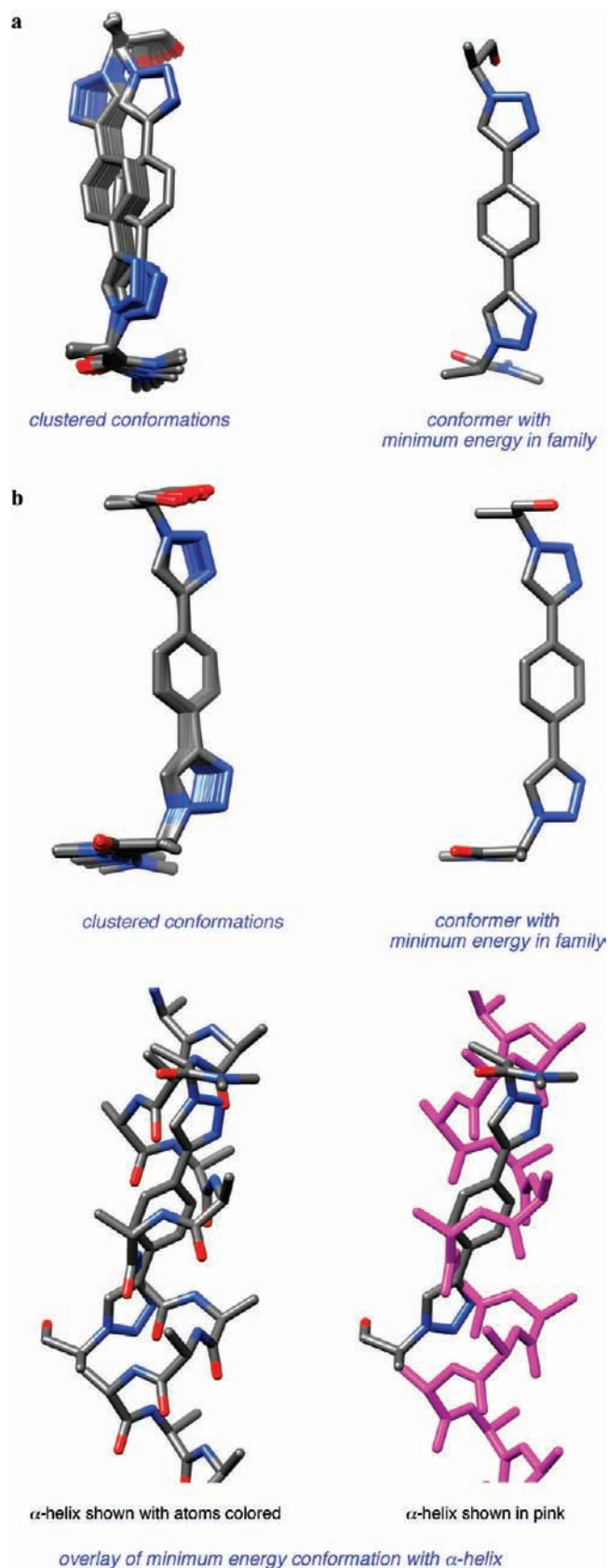


Figure 11. Conformers generated in QMD (see Table 7): (a) family 2 overlays with a parallel β -sheet, as in Figure 10, and (b) family 5 overlays with an α -helix.

Table 2 indicates that peptidomimetics **4** conformers might overlay with distal side chains of several secondary structures,

including the $i-i+4$ C^β atoms from the same strand of a parallel β -sheet. Figure 10 shows that relatively small energy barriers must be surmounted to reach this conformation from the observed global minimum, and only 0.46 kcal/mol must be expended to do so.

QMD analyses of peptidomimetics **4** gave seven major families; of these, family 2 conformers overlaid well with a parallel β -sheet, placing the i and $i+4$ C^β atoms at appropriate distances (Table 9). Similarly, family 5 overlaid with an α -helix. Clustered conformations in these two families, and the minimum energy conformation in each, are shown in Figure 11.

Biophysical Data. Scaffolds **A** and **B** were originally designed to be mimics of β -turns in neurotrophins. There is considerable evidence that the turn regions in the neurotrophins constitute hot-spots for their interactions with the Trk receptors.^{110,111} This assertion is supported by two studies from these laboratories that are now published. In the first, fluorescently labeled derivatives of compounds in series **A** were found to bind cells stably transfected with the TrkA receptor.⁶⁵ In the second study, compounds in series **B** were subjected to direct binding, survival and neuritogenic, and biochemical signal transduction assays; these revealed eight agonistic ligands binding to the ectodomain of TrkC. These peptidomimetics afford discrete signals leading to either cell survival or neuritogenic differentiation, effects that are both attributed to the parent neurotrophin, NT-3.⁶⁴

Universal peptidomimetics are likely to be most useful in the design of libraries for high-throughput screening against diverse targets. This is because they can mimic a range of secondary structures and present any protein amino acid side chain; this impacts the diversity space that corresponds to hot-spots¹¹² in protein–protein interactions. Universal peptidomimetics are also likely to be useful for known targets where exact binding conformations are *unknown*. This represents a large number of situations, including all those for which only one protein in a protein–protein interaction has been structurally characterized and those for which solid-state structure analyses show a possible mode of association that is actually different in solution or in a cellular environment. Conversely, rigid peptidomimetics that are not universal tend to be more useful when the binding conformation is known with a high degree of confidence and can be accurately matched by the mimic.

Motivated by the reasoning presented above, we submitted compounds **A**, **B**, **1**, and **3** (and protected precursors to these) to the NIH Molecular Libraries Small Molecule Repository (MLSMR) for screening. This is ideal for testing these compounds, many of which were not designed for any particular target but simply as secondary structure mimics in general. Screening centers test compounds in the MLSMR and publish the data on PubChem; thus, samples submitted from this work can be exposed to a wide variety of screens—many more than it would be practical to establish in one laboratory. These screening centers have already identified cases where our molecules promote or inhibit protein–protein interactions of particular interest. A summary

is given in the Supporting Information, and an abbreviated outline is given here.

An assay for the protein–protein interactions involving the Bcl-2 family proteins Mcl-1 and Bid (PubChem Assay ID (AID) 1021) showed some activities for compounds **1f** (PubChem Structure ID (SID) 24708217) and **3e** (SID 24707927). Another active compound, the peptidomimetic with scaffold **A**, where $R^1 = n$ -butyl, $R^2 = \text{Lys}$ (SID 24708065), was detected in a related study, also dealing with interactions between Bcl-2 proteins, but this time Bcl-XL with Bim (AID 2129). This is interesting because scaffolds **A**, **1**, and **3** are all pertinent to helical conformations from the studies presented above, and interactions with helical protein segments in a binding cleft is characteristic of protein–protein interactions involving the Bcl-2 family proteins.^{47,113}

Two assays identified small molecules that inhibited the PB1-domain interaction of MEK5 with either native or a Lys-Ala mutant of MEKK2. Peptidomimetics **3a** (SID 24708115) and **3b** (SID 24708166) and two mimics derived from scaffold **B** ($R^1 = \text{Leu}$, $R^2 = \text{Trp}$, SID 24707989, and also $R^1 = \text{Ile}$, $R^2 = \text{Trp}$, SID 24708162) were active in the assay with the native MEKK2 protein (AID 1531). Compound **3b** was also active in the assay using a Lys-Ala mutant of MEKK2 (AID 1530). In confirmation assays, compound **3b** showed inhibition of MEK5 with MEK Kinase 2 (WT, AID 1897), with an EC_{50} value of 5.51 μM . For the inhibition of MEK5 with MEK Kinase 2 (Lys-Ala mutant, AID 1895), the compound was active, with an EC_{50} value of 4.44 μM .

Conclusion

1,3,4-Oxazoline-based mimics **1** were predicted to be useful mimics of many pairs in amino acids in conformations that resemble all common secondary structures (Table 2). Modeling data for this scaffold indicated that conformations with a diverse set of β s values would be accessible; a typical energy maximum for interconversion of the conformers was only 2.43 kcal/mol (Figure 5). This assertion was born out by the range of conformations that emerged from the QMD studies, which also highlighted low-energy conformers that resemble elements of α -helix, γ -turn, and β -turn structures.

There are insignificant barriers to interconversion of the 1,3-butadiene peptidomimetics **2** between conformational states that resemble amino acid pairs in γ -turns, β -turns, and β -sheets. These are relatively tight minimalist mimics, having only one significant degree of freedom for separation of the C^β atoms, hence their small extension factors, but it takes very little energy to interconvert between them. Conversely, the kinked bistriazole scaffolds **3** allow high β s variability. Favored conformations of this scaffold can mimic, for instance, relatively close residues of an antiparallel β -sheet or closer side chains in α -helices. All possible C^β atom separations are calculated to be accessible, as they are for all the mimics **1–4**. Mimics **4** are similar to **3** except that they orient the triazole units *para* rather than *meta*. This scaffold is a tool for mimicking relatively distal side chains, even in relatively extended structures like the i and $i+4$ C^β atoms from one strand of a parallel β -sheet.

Over the past decade, there has been considerable interest in generic libraries prepared to supply high-throughput

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screening projects, particularly in the pharmaceutical industry, with “drug-like” compounds to test. The small heterocyclic structures **1–4** and **B** can be decorated with the most pertinent pharmacophores for peptide and protein targets: amino acid side chains. These specific non-peptidic structures may have some commercial potential, and a patent application on them has been published.¹¹⁴ However, it should not be difficult to conceive other scaffolds for universal peptidomimetics, and it is our hope that the concepts described here will facilitate this process.

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LBMS-Applications Laboratory, headed by Dr. Shane Tichy and Dr. Yohannes Rezenom, provided mass spectrometric support, and Dr. Gillian Lynch at University of Houston supported our QMD work. We thank Shuhei Shimizu for some additional experiments.

Supporting Information Available: Procedures for modeling experiments, synthesis, and characterization data for all peptidomimetics, and complete ref 34. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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