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Protein–Protein Interface Mimicry by an Oxazoline Piperidine-2,4-dione

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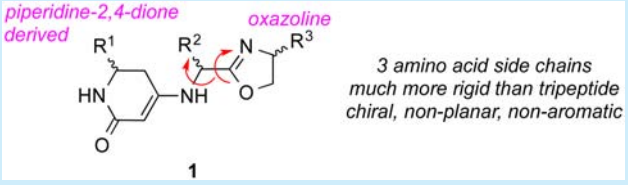
Supporting Information

ABSTRACT: Representative minimalist mimics **1** were prepared from amino acids. Scaffold **1** was not designed to mimic any particular secondary structure, but simulated accessible conformations of this material were compared with common ideal secondary structures and with >125000 different protein–protein interaction (PPI) interfaces. This data mining exercise indicates that scaffolds **1** can mimic features of sheet-turn-sheets, somewhat fewer helical motifs, and numerous PPI interface regions that do not resemble any particular secondary structure.



Early minimalist mimics of secondary structures inspired by Hamilton's terphenyls[1] featured planar aromatic units that display side chains in appropriate orientations. More recently, this field has turned toward chiral and heterocyclic designs that exist in coiled conformations and/or tend to have superior water solubilities.[2] It is convenient if mimics of this kind can be produced from amino acid starting materials; otherwise, it is difficult to incorporate all the different side chains. The recently reported oligooxopiperazines, for example, are derived from amino acids.[3]

Even though effective minimalist mimics are more rigid than peptides,[4] most populate multiple solution conformers that display side chains in different orientations.[5] We developed two strategies, EKO[6] and EKOS (exploring key orientations on secondary structures),[7] to ascertain how conformations of minimalist mimics resemble protein–protein interface regions and ideal secondary structures, respectively. Application of EKO exposes the enormous diversity of PPI interfaces: even a small fraction of these could not be accurately represented by all the secondary structure mimics reported in the literaure to date. Consequently, there is a need to develop and understand new chemotypes for the key issue of *interface mimicry*.

This paper introduces chiral, nonaromatic, interface mimics **1** composed of piperidine-2,4-dione and oxazoline fragments linked by –NHCHR– units (Figure 1). The objectives of this study were to develop a synthesis of molecules **1** that could incorporate many genetically encoded amino acid side chains, to elucidate the bias of this scaffold toward all the common ideal secondary structures and to show illustrative cases where EKO predicts an excellent match of accessible conformers of **1** on PPI interface regions.

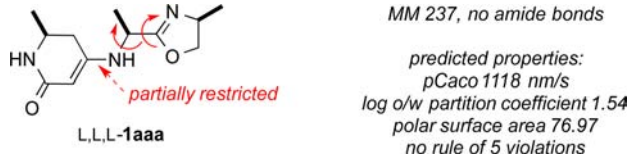


Figure 1. Scaffold **1** is a minimalist mimic of secondary structures with favorable predicted properties for cell and oral bioavailability.

Scheme 1 shows how the oxazoline fragments were prepared from Fmoc-protected or Cbz-protected amino acids and amino alcohols. Throughout this paper, compounds are numbered according to the scaffold (or scaffold intermediate), and lower case one-letter codes are used to relate the side chains R^1–R^3 to the closest amino acids [e.g., **d′** for the $-CH_2CO_2{}^tBu$ of Asp (**d**) and **k′** for the $-(CH_2)_4NHCbz$ of Lys (**k**)]. After a routine coupling to obtain molecules **2**, the primary alcohol was mesylated and then treated with base to initiate oxazoline formation. Some Fmoc-protected compounds related to **3** have been reported prior to this work,[8] but most of the systems with the side chains indicated in Scheme 1 have not been prepared before. The cyclization conditions in Scheme 1 were arrived at after some optimization; they are a modification of those used in Sigman's aminooxazoline syntheses.[8a] Many other conditions that did not use DMAP or relied upon activation via PPh_3/CCl_4 gave poor product yields. Removal of the FMOC protecting group from the protected amines **3** gave the aminooxazolines **4**. A similar procedure was used, but with *N*-Cbz protected Phe, to access the **ff** chiron.

Having obtained a set of aminooxazolines, we developed two methods (**A** and **B**[9] in Scheme 2) to add these to the piperidine-2,4-dione derivatives **5**;[10,13,14] product was obtained using either approach, but the yields differed on a case-by-case basis. Overall, the synthetic route is divergent–convergent because any ketone **5** can be condensed with any amine **4**.

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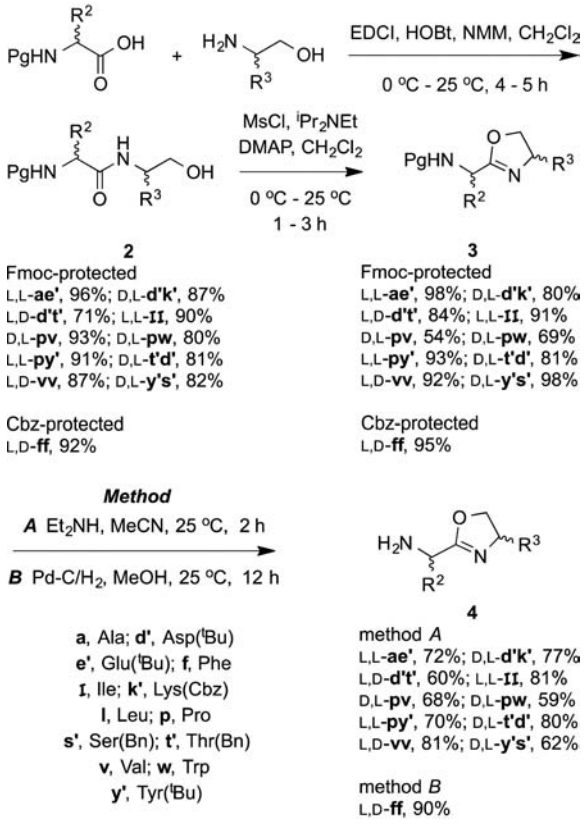
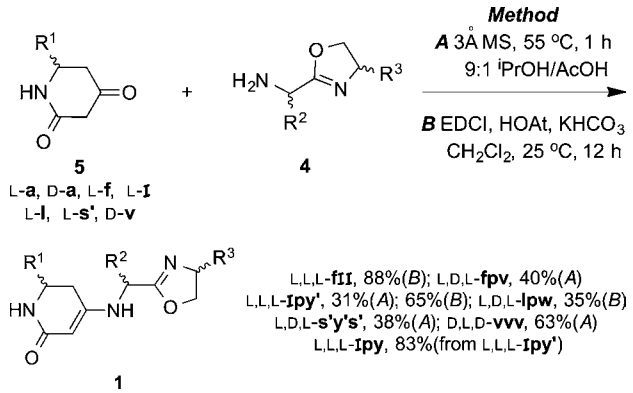
Scheme 1. Fmoc Approach Was Used To Obtain Most Chirons 4, but Cbz Was Used To Access the ff Chiron



Scheme 2. Syntheses of the Target Compounds 1



EKOS was used to relate the ensemble of simulated accessible conformers of **1aaa** to ideal secondary structures. This process was carried out for all stereoisomers of **1aaa**; full data are shown in the Supporting Information, and two select examples are given here. First, conformers of LLL-**1aaa** matched better on an ideal α-helix than on any of the other secondary structures (Figure 2a; for a full explanation of these plots see ref 10). In our experience, it is much harder to design good helical mimics than ones that overlay other motifs. Our application of EKOS on oligooxopiperazines[3] (all stereoisomers, unpublished data) indicate they have accessible conformations that overlay well on an ideal α-helix with a RMSD of 0.44 Å (based on the 6 *Cα* and *Cβ* coordinates), and that is better than nearly all of the other mimics of ideal α-helical conformations in the literature.[7] However, LLL-**1aaa** appears to be a superior α-helical mimic

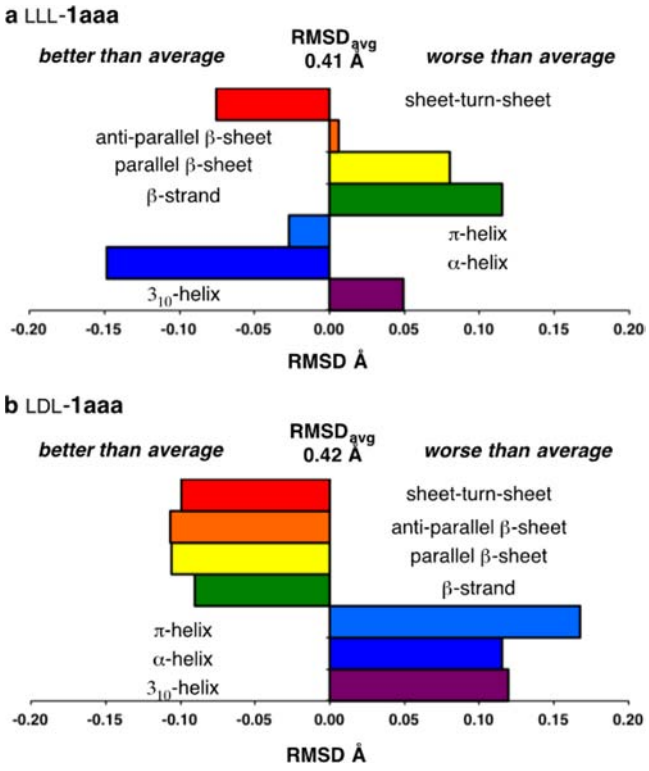


Figure 2. RMSD (Å) for the simulated conformers in the ensemble that best overlay the indicated ideal secondary structures, relative to the average values for the best conformers overlaid on each of the seven motifs, are shown.

since it can adopt a conformation that matches an ideal α-helix with a RMSD of only 0.26 Å.[7]

Achiral minimalist mimics like terphenyls have only one isomer to compare with ideal secondary structures. Conformations of *chiral* minimalist mimics, however, are stereochemically dependent, and we offer two observations related to this. First, stereochemical changes can *significantly* alter the conformational bias of many minimalist mimics such that one isomer can match extended conformations whereas another is more closely overlaid on helical motifs; comparison of LLL-**1aaa** with LDL-**1aaa** illustrates this. Thus, even though the LLL-isomer is disposed to α-helical conformations (blue bar) and can match sheet-turn-sheet motifs almost as well (red bar in Figure 2a), the LDL-form is biased toward extended motifs and *not* helical ones (Figure 2b). The second observation is that correlations of mimic stereochemistries and conformational biases are beyond what the human mind can perceive; systematic data mining (the EKOS strategy) is essential for this.

Most of the compounds **1** prepared here were not solids, though in one case, LLL-**1fii**, we were able to collect crystals and obtain an X-ray structure. That molecule crystallized in *two* similar conformations (differing by RMSD 0.28 Å, based on the 6 *Cα* and *Cβ* coordinates, see the Supporting Information), but which nevertheless project the side chains in slightly different orientations. We recently outlined another technique based on exploring key orientations that can be used to relate X-ray structures to simulated solution conformations: EKOX.[10] Application of EKOX to LLL-**1fii** in the crystal reveals that one conformer fits well on one strand of a sheet−turn−sheet motif (Figure 3a), consistent with the predictions in Figure 2a (red bars, respectively); this is interesting because minimalist

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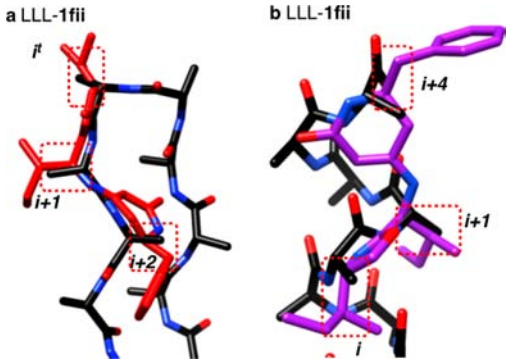

Figure 3. One conformer in the crystal structure of LLL-**1fii** optimally overlays with a sheet-turn-sheet motif (a), while neither overlaid particularly well on an ideal α-helix (b shows the best match).

strand mimics are rare. However, neither of the conformers in the crystal structure overlaid particularly well on an α-helix; the best one is shown in Figure 3b.

When accessible conformers of LLL-**1aaa** were simulated and data mined on over 125,000 PPIs in the PDB, 257 "hits" were found. The applied definition of a hit was that the three methyl side-chain coordinates of the mimic overlaid with three interface side chains with an RMSD of 0.3 Å or better. The best overlay occurred on an interface sheet−turn−sheet motif as shown in Figure 4 (RMSD 0.12 Å). The other 256 hits are tabulated in the Supporting Information.

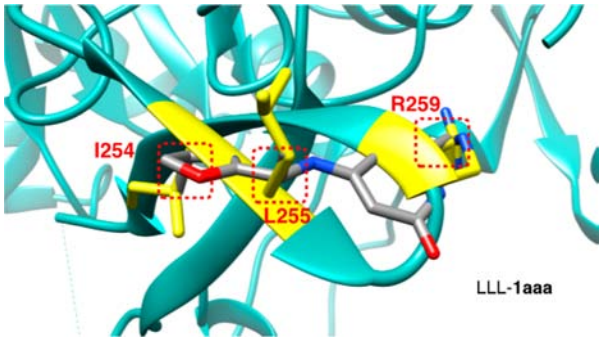

Figure 4. Accessible simulated conformer of LLL-**1aaa** overlays with excellent correspondence on the interface region of inosine 5′-monophosphate dehydrogenase (pdbid = 4ff0, RMSD = 0.12 Å).

Despite Figure 2a, it would be incorrect to assume that simulated accessible conformers of LLL-**1aaa** overlay well on only sheet−turn−sheet and helical motifs at interfaces. The statistical distribution of overlays in each of the featured ideal secondary structures is depicted in Figure 5. Consistent with the findings from EKOS based on *ideal* secondary structures (Figure 2a), α-helical and sheet-turn-sheet motifs are the most common matches for LLL-**1aaa** at interfaces. However, Figure 5 shows over 50% of the overlays occur on nearly consecutive amino acids ("single segments" in Figure 5) that are not part of any ideal secondary structure. Moreover, the next most common type of overlay was on amino acid sequences from different parts of the chain, and which also do not resemble any ideal secondary structure (*ie* "multiple segments"). Consequently, *most of the potential for* LLL-***1aaa*** *in interface mimicry appears not to be correlated with any particular secondary structure.*

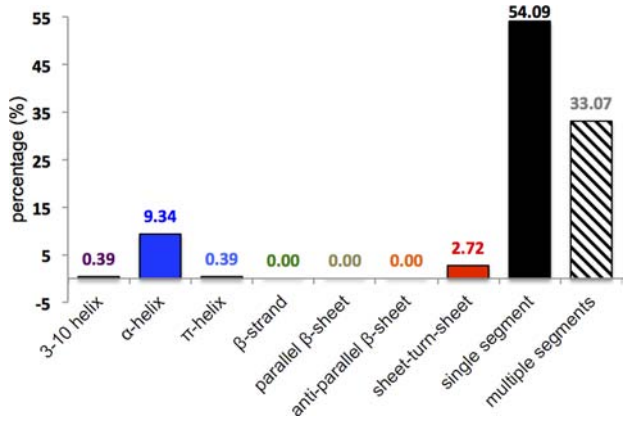

Figure 5. Statistical distribution of the best 257 overlays of preferred conformers of LLL-**1aaa** (generated using EKO; all RMSD < 0.31 Å).

Some parameters of scaffolds **1** relevant to their use as cellular probes were also considered. Molecule **1aaa** has a low molecular mass and no amide bonds; these characteristics are favorable for cellular- and oral-permeability. QikProp[11] was used to predict some other key parameters of **1aaa** (Figure 1). Simulated permeability of **1aaa** through Caco cells is excellent (i.e., >500 nm/s), and the estimated log octanol/water partition coefficient, 1.54, is near the midpoint of the optimal range (−2.0 to +6.5). Moreover, there are no rule of five[12] violations for this structure. Obviously, these properties will be modulated when side chains other than methyl are involved, but the scaffold provides a good framework for probe development.

Overall, we conclude that compounds based on LLL-**1aaa** can be excellent helical mimics, but they may adopt a range of conformations that overlay well on other secondary structures, notably sheet−turn−sheet motifs. Like many other minimalist mimics, however, molecules **1** can overlay on diverse interface regions, most of which are not directly related to secondary structures.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data for the new compounds, procedure for mathching on ideal secondary structures, X-ray data, and best hits from EKO mining for the featured chemotypes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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