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Enaminone-Based Mimics of Extended and Hydrophilic α-Helices

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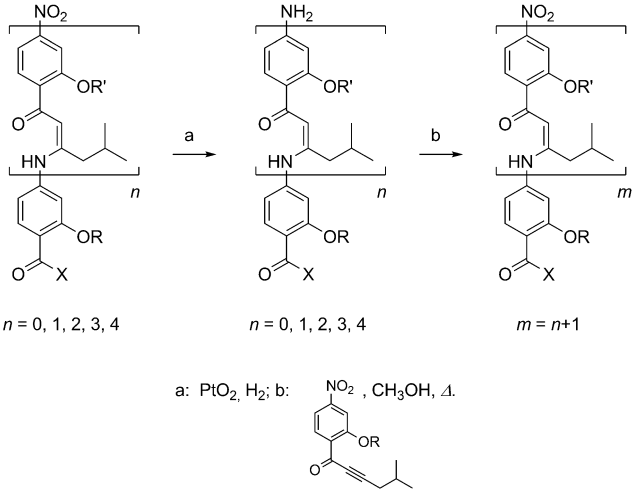
The pursuit of structural and functional mimics of α-helices has been a fruitful area of research over the past decade. The evolution of scaffold design has led to the progression from molecules that are difficult to synthesize, solubilize, and derivatize to structural motifs that can be easily assembled, elongated, and diversified. These advancements have allowed α-helix mimics to be generated more quickly and thus to be more easily evaluated.[1]

To this point, much α-helix mimetic research has focused on molecules that mimic up to two turns of an α-helix containing principally hydrophobic residues.[2] Although many interesting and biologically active compounds have been made and investigated, there has been limited synthetic work done on either extended scaffolds[3] or those that can be assembled in the presence of hydrophilic amino acid-like functional groups without the use of extensive protecting group strategies.[4]

Extended and hydrophilic helices are of considerable biological significance. For example, the seven-helix transmembrane bundles of G-protein-coupled receptors not only span the width of the bilayer membrane (≈8 turns), but also are often stabilized by interhelix interactions of hydrophilic side chains.[5] Other important elongated helices include leucine zippers, structural motifs in the DNA-binding region of transcription factor proteins.

A major goal in the field of helix mimicry is the development of scaffolds that can be easily elongated and functionalized for use in probing the nature of these important biological molecules. Herein, an oligoenaminone-based scaffold that can readily mimic elongated α-helices is described. The principal advantages of the enaminone design are its ease of synthesis and the presence of intramolecular hydrogen bonds, which stabilize the conformation.

We have previously reported the synthesis of α-helicomimetic enaminones, through hydrogenation of a nitroarene to an aniline followed by conjugate addition to the corresponding ynone-functionalized monomeric unit.[6] This paper describes how this strategy can be applied to a range of side-chain substituents beyond the simply hydrophobic as well as to the iterative construction of a range of extended helix mimetics, with full structural characterization of the sequential oligomers (Scheme 1).



Scheme 1. General synthesis of enaminones.

We first tested the ability of this synthetic route to support more polar substituents. Enaminones with free carboxylate groups in the lower monomer were assembled through the reaction of aniline **1** with ynone **2** to give enaminone **3** (Scheme 2). The nitro substituent in **3** could be readily reduced to amino acid **4**, which not only improved water solubility, but also readies it for potential further elongation. A major advantage of this route is that it is executed without protecting groups.

A second test of the approach came for helix mimetics with polar side chains, such as hydroxyl group containing **6**. This derivative was assembled by an analogous route (Figure 1 a) and, again, in the absence of protecting groups. A crystal structure of **6** showed that the intramolecular hydrogen bond was maintained in the solid state despite the presence of a nearby hydroxyl group (Figure 1 b).

The enaminone synthetic route is also amenable to ready elongation. The first series of extended mimics was based on an amide-terminated scaffold. The synthetic approach fol-

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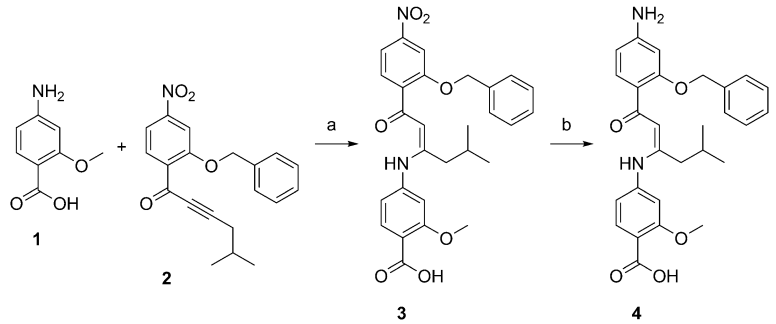


Scheme 2. Synthesis of carboxy-terminated enaminones.

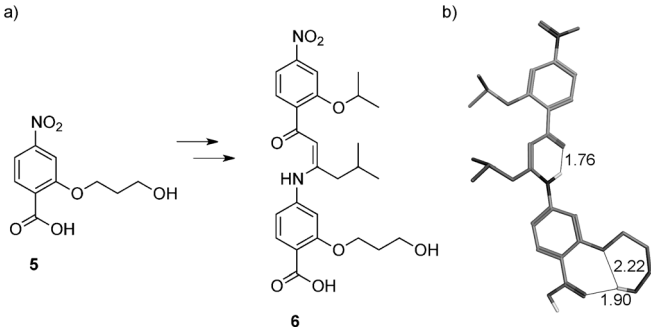


Figure 1. a) Synthesis and b) crystal structure of hydroxyl-bearing enaminone **6**.

lowed our earlier strategy of converting the 2-alkoxy-4-nitroarylcarboxylic acid monomer into its ynone derivative, followed by nucleophilic attack by the 2-alkoxy-4-amino-arylcarboxamide lower component.[6] Reduction of the free nitro group followed by conjugate addition to a second ynone unit gave the double-enaminone series **7**–**11** with a variety of hydrophobic substituents (Scheme 3).

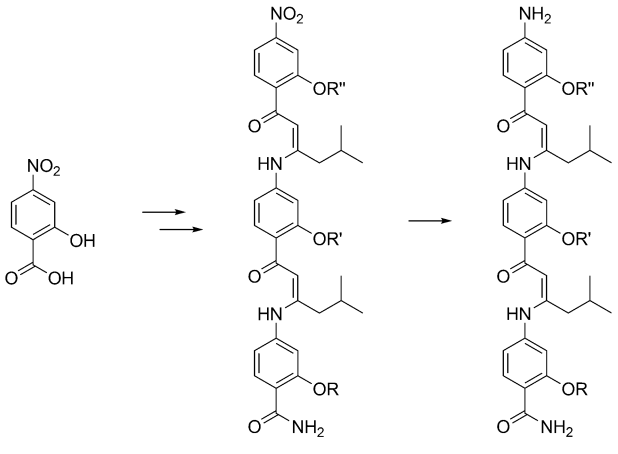
Spectroscopic investigation of compounds **7**–**14** showed that, in all cases, the intramolecular hydrogen bonds are intact in solution, as was evidenced by the pronounced ^{1}H NMR analysis downfield shifts of all enaminone NH protons ($\approx$13 ppm). A low-energy structure (as determined by molecular modeling by using the MOE program) of nitroenaminone **9** displays excellent overlap between its side chains and the relevant residues on a key helix in the thymidylate synthase–dihydrofolate reductase (TS–DHFR) enzyme complex of *Cryptosporidum hominis*;[8] studies on the functional mimicry of this helix are in progress (Figure 2).

We were also interested in the use of extended enaminones as structural mimics of leucine zipper protein domains. These motifs offer elegant examples of residue-specific hydrophobic interactions as stabilizing forces in protein–protein complexes. To this end, we undertook the iterative synthesis of ester-terminated elongated enaminones containing hydrophobic substituents to imitate the leucines projecting from a single face of a helix in the coiled coil of the zipper.

The synthesis of these elongated mimics was accomplished by using the iterative process previously outlined (Scheme 4). Crystal structures were obtained for two- and four-turn mimics of the leucine zipper helix (**17** and **20**, respectively). As in the shorter structures, the intramolecular hydrogen bonds are intact in these derivatives the solid state and serve to stabilize the helicomimetic core. Compound **23**, which structurally mimics ten turns of a leucine zipper monomer, marks the longest synthetic helix mimetic of any type characterized to date.

Complementary packing of the isopropyl side groups is evident in the crystal structure of compound **17** (Figures 3), which contains two enaminone molecules in each asymmetric unit. The interlocking character of these interactions is closely analogous to the packing observed in solid-state structures of leucine zipper dimers.

In addition to two intramolecular hydrogen bonds and many intermolecular hydrophobic contacts, the structure of the pentamer **20** shows a distinctive conformation. The scaffold structure lies in two distinct planes defined by the lower three rings at an angle to the upper two (Figure 4). The side chains project at different angles from the scaffold and, reminiscent of the side chains of an α-helix, cover a considerable amount of three-dimensional space. Access to conformations of this type in the solid state bodes well for the structural mimicry of extended single faces of α-helices in solution.



7: R = *i*Pr, R' = *i*Pr, R" = Bn
8: R = Bn, R' = *i*Pr, R" = *i*Pr
9: R = *i*Pr, R' = *i*Pr, R" = *i*Pr
10: R = Bn, R' = *i*Pr, R" = Bn
11: R = Bn, R' = Bn, R" = Me

12: R = *i*Pr, R' = *i*Pr, R" = Bn
13: R = Bn, R' = *i*Pr, R" = *i*Pr
14: R = *i*Pr, R' = *i*Pr, R" = *i*Pr

Scheme 3. Synthesis of elongated amidoenaminones.

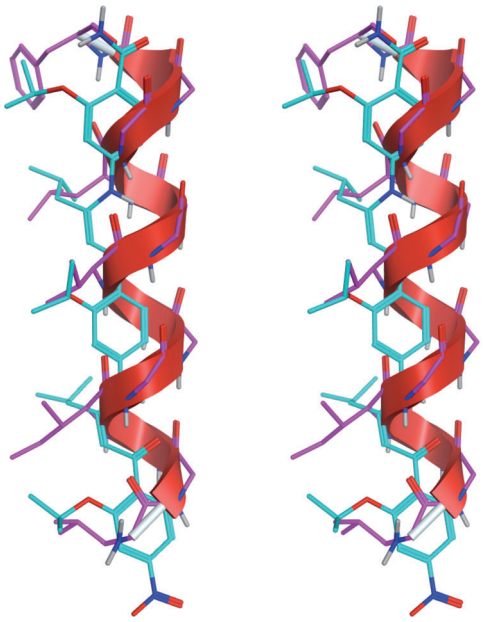
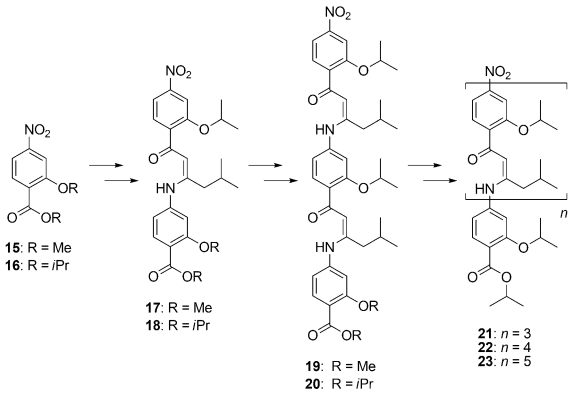


Figure 2. Stereoview of the overlap of a low-energy structure of **9** with *C. hominis* TS–DHFR crossover helix.



Scheme 4. Synthesis of elongated helix mimics.

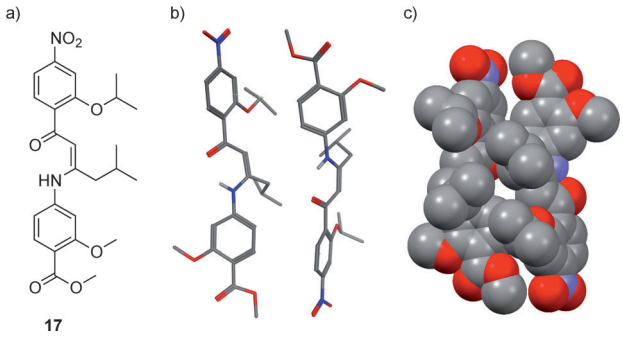


Figure 3. a) X-ray crystal structure of dimeric asymmetric unit of **17** in b) stick and c) spacefill views.

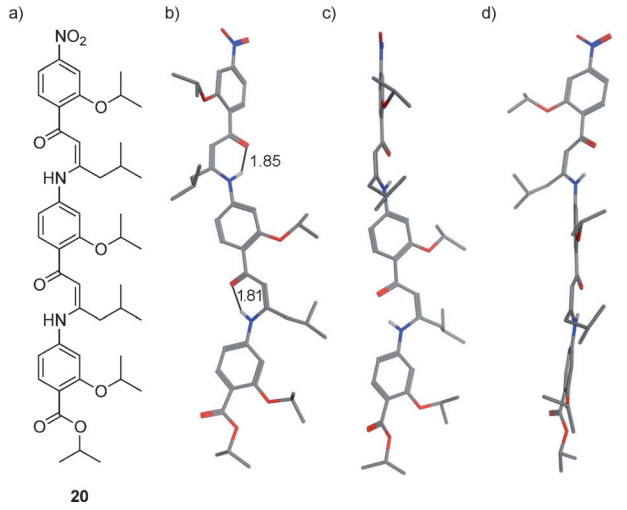


Figure 4. a) X-ray crystal structure of **20** from three different angles, highlighting the b) intramolecular hydrogen bonds and c),d) planarity of the structure.

This publication details the synthesis of a variety of enaminones, demonstrating molecules of this type to be versatile scaffolds for the mimicry of an array of α-helices. In addition to incorporating hydrophobic and hydrophilic side chains, we have shown that molecules capable of mimicking up to ten turns of an α-helix can be assembled and characterized. This work serves to expand the toolkit for small-molecule peptide mimicry.

Experimental Section

Synthesis of all enaminones and precursors was performed according to previously published synthetic routes.[6] Full characterization for all compounds can be found in the Supporting Information. CCDC-871189 (**6**), CCDC-871188 (**17**), and CCDC-871187 (**20**) contain the supplementary crystallographic data for this paper. These data 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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Keywords: α-helix · enzymes · hydrogen bonds · peptidomimetics · synthesis design

[1] a) M. J. Adler, A. G. Jamieson, A. D. Hamilton, *Curr. Top. Microbiol. Immunol.* **2010**, *348*, 1–23; b) C. G. Cummings, A. D. Hamilton, *Curr. Opin. Chem. Biol.* **2010**, *14*, 341–346; c) J. M. Davis, L. K. Tsou, A. D. Hamilton, *Chem. Soc. Rev.* **2007**, *36*, 326–334; d) N. T. Ross, W. P. Katt, A. D. Hamilton, *Philos. Trans. R. Soc. London Ser. A* **2010**, *368*, 989–1008.

[2] a) W. P. Nolan, G. S. Ratcliffe, D. C. Rees, *Tetrahedron Lett.* **1992**, *33*, 6879–6882; b) I. C. Kim, A. D. Hamilton, *Org. Lett.* **2006**, *8*, 1751–1754; c) O. Kutzki, H. S. Park, J. T. Ernst, B. P. Orner, H. Yin, A. D. Hamilton, *J. Am. Chem. Soc.* **2002**, *124*, 11838–11839; d) B. P. Orner, J. T. Ernst, A. D. Hamilton, *J. Am. Chem. Soc.* **2001**, *123*, 5382–5383; e) J. Edgar, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 891–893.
[3] a) J. M. Rodriguez, A. D. Hamilton, *Tetrahedron Lett.* **2006**, *47*, 7443–7446; b) J. M. Rodriguez, A. D. Hamilton, *Angew. Chem.* **2007**, *119*, 8768–8771; *Angew. Chem. Int. Ed.* **2007**, *46*, 8614–8617; c) J. M. Rodriguez, N. T. Ross, W. P. Katt, D. Dhar, G.-i. Lee, A. D. Hamilton, *ChemMedChem* **2009**, *4*, 649–656; d) J. P. Plante, T. Burnley, B. Malkova, M. E. Webb, S. L. Warriner, T. A. Edwards, A. J. Wilson, *Chem. Commun.* **2009**, 5091–5093; e) J. Plante, F. Campbell, B. Malkova, C. Kilner, S. L. Warriner, A. J. Wilson, *Org. Biomol. Chem.* **2008**, *6*, 138–146; f) F. Campbell, J. P. Plante, T. A. Edwards, S. L. Warriner, A. J. Wilson, *Org. Biomol. Chem.* **2010**, *8*, 2344–2351.
[4] a) J.-M. Ahn, S.-Y. Han, *Tetrahedron Lett.* **2007**, *48*, 3543–3547; b) S. Marimganti, M. N. Cheemala, J.-M. Ahn, *Org. Lett.* **2009**, *11*, 4418–4421; c) I. Saraogi, C. D. Incarvito, A. D. Hamilton, *Angew. Chem.* **2008**, *120*, 9837–9840; *Angew. Chem. Int. Ed.* **2008**, *47*, 9691–9694; d) J. A. Hebda, I. Saraogi, M. Magzoub, A. D. Hamilton, A. D. Miranker, *Chem. Biol.* **2009**, *16*, 943–950; e) I. Saraogi, J. A. Hebda, J. Becerril, L. A. Estroff, A. D. Miranker, A. D. Hamilton, *Angew. Chem.* **2010**, *122*, 748–751; f) S. Thompson, R. Vallinayagam, M. J. Adler, R. T. W. Scott, A. D. Hamilton, *Tetrahedron* **2012**, *68*, 4501–4505.
[5] N. J. Gibson, J. Y. Cassim, *Biophys. J.* **1989**, *56*, 769–780.
[6] M. J. Adler, A. D. Hamilton, *J. Org. Chem.* **2011**, *76*, 7040–7047.
[7] Energy minimizations were performed by using Molecular Operating Environment (MOE), MMFF94, 10000 iterations, default settings.
[8] M. A. Vargo, W. E. Martucci, K. S. Anderson, *Biochem. J.* **2009**, *417*, 757–764.

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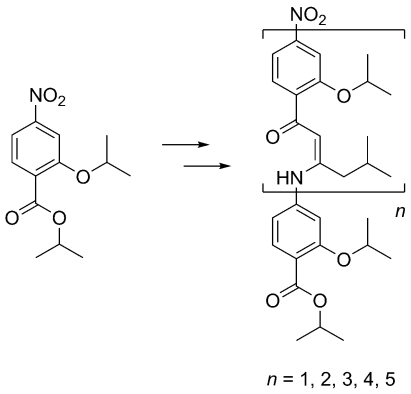
Synthetic molecules capable of the mimicry of α-helices that are elongated and/or contain hydrophilic side chains have been largely elusive. However, the oligophenylenaminone structure can surmount both of these challenges (see scheme).



Peptidomimetics

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