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Amphipathic α-Helix Mimetics Based on a 1,2-Diphenylacetylene Scaffold

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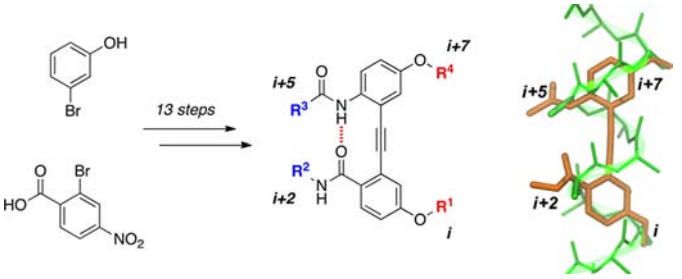
ABSTRACT



In order to mimic amphipathic α-helices, a novel scaffold based on a 1,2-diphenylacetylene was designed. NMR and computational modeling confirmed that an intramolecular hydrogen bond favors conformations of the 1,2-diphenylacetylene that allow for accurate mimicry of the *i*, *i* + 7 and *i* + 2, *i* + 5 side chains found on opposing faces of an α-helix.

α-Helices are the most common form of protein secondary structure,[1] and they play critical roles in mediating a diverse array of protein−protein interactions (PPIs) that include signal transduction, transcription, apoptosis and immune responses.[2] Molecules that can reproduce the orientations of key side chains of α-helices are of interest as novel biochemical tools and/or new leads for drug discovery.[3] Exhibiting random coil in solution and prone to metabolic degradation, short peptides themselves, however, do not represent attractive leads.[4] Instead, several novel strategies toward effective mimicry of the α-helix have been adopted, which include the introduction of side chain constraints into peptides, such as salt,[5] lactam[6] and disulfide[7] bridges, as well as "hydrocarbon-stapling".[8] β-Peptide foldamers that fold into helical structures have also been described.[9] Such preorganization not only increases binding affinity to target proteins but also improves metabolic stability.[8]

Complementary to these peptidomimetic approaches, Hamilton has pioneered a proteomimetic strategy, in which small-molecule, nonpeptidic scaffolds are suitably decorated to accomplish mimicry of the spatial and angular projections of key side chains of α-helices.[10] The original α-helix mimetic

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scaffold described by Hamilton is the terphenyl scaffold,[11] which has inspired a wide range of related frameworks.[12−16] Until recently, synthetic α-helix mimicry focused on replication of only the hydrophobic face of the α-helix, typically the *i*, *i* + 3/4 and *i* + 7 residues. Rebek and Hamilton later introduced α-helix mimetics in which heteroatoms were incorporated into the scaffold to improve aqueous solubility.[17] Around 50% of the α-helices that are found in proteins are amphipathic.[18] Synthetic α-helix mimetics that can mimic both faces of an α-helix might improve the synthetic ligand's binding affinity as well as its selectivity profile. Recently, elaboration of previously reported α-helix mimetic scaffolds has allowed for mimicry of both faces of the α-helix.[19] Novel and diverse amphipathic α-helix mimetics would be welcomed to enrich the pool of potential leads for drug discovery.

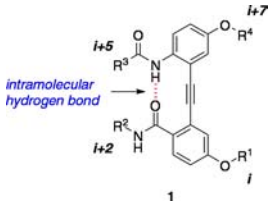

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Figure 1. A generic amphipathic α-helix mimetic based on a 1,2-diphenylacetylene scaffold.

Rotation about the central axis of 1,2-diphenylacetylenes can be biased through the formation of an intramolecular hydrogen bond.[20] Furthermore, 1,2-diphenylacetylenes constrained in this way have been proposed as potential β-strand mimetics.[21] Motivated by our interest in mimicking amphipathic BH3 α-helices of pro-apoptotic proteins, such as Bak and Bim, that engage the oncoproteins Bcl-x_L and Mcl-1,[22] we asked if the 1,2-diphenylacetylene framework incorporating a similar intramolecular hydrogen bond could function as a scaffold suitable to elicit mimicry of both faces of an α-helix. Toward this goal, we designed molecule **1** (Figure 1) in which the R groups are intended to mimic the *i* and *i* + 7 side chains on one face of an α-helix, and the *i* + 2 and *i* + 5 side chains on the other. It is hypothesized that the desired structural integrity of **1** will be maintained by an intramolecular hydrogen bond between the NH of the amide in the upper subunit and the CO of the amide in the lower subunit.[20] It should be noted that we do not seek a rigid hydrogen bond, since the *i*, *i* + 7 and the *i* + 2, *i* + 5 side chains of an α-helix are staggered, not eclipsed. The amides will restrict the flexibility of the R^2 and R^3 groups, further preorganizing the helix mimetic.

The synthesis of **2**, a derivative of **1** where $R^1 = R^2 = R^3 = R^4$ = Me, is illustrated in Scheme 1. Briefly, selective nitration of 3-bromophenol (**3**), followed by *O*-methylation and nitro group reduction afforded aniline **6**. A Sonogashira cross-coupling reaction of **6** with trimethylsilylacetylene delivered **7**, whose TMS group was subsequently removed with potassium carbonate in MeOH to yield **8**. Meanwhile, the carboxylic acid of 2-bromo-4-nitrobenzoic acid (**9**) was esterified, then the nitro group was chemoselectively reduced, diazotized and finally quenched with water to deliver methyl 2-bromo-4-hydroxybenzoate (**10**). After methylation of the hydroxyl group of **10**, a second Sonogashira reaction between **8** and **11** furnished 1,2-diphenylacetylene **12**. Acetylation, saponification, then coupling of the liberated carboxylic acid to methylamine gave the target molecule **2**.

To examine if the intramolecular hydrogen bond exists as proposed, we performed ^{1}H NMR (400 MHz) titrations of **2** in $CDCl_3$ with d_6-DMSO (Figure 2).[23] Hydrogen bonding results in deshielding, and an increasingly downfield-shifted proton resonance is indicative of stronger hydrogen bonding.[24] The acetamide NH (δ_H 9.14 ppm) is significantly downfield of both the benzamide NH in **2** (δ_H 6.10 ppm), and the acetamide NH in the control compound *N*-(4-methoxy-2-((trimethylsilyl)ethynyl)phenyl)acetamide (δ_H 7.78 ppm), neither of which can engage in intramolecular hydrogen bonding. Unlike the benzamide NH, the acetamide NH in **2** is protected from exchange with residual water in the sample (sharper resonance). Furthermore, with increasing concentrations of d_6-DMSO, the benzamide NH resonance shifts downfield as a consequence of stronger hydrogen-bonding interactions with DMSO. On the other hand, negligible changes in the acetamide NH chemical shift are observed, suggesting DMSO cannot contend with the postulated intramolecular hydrogen bond. As anticipated, other chemical shifts were largely unchanged. Collectively, these data confirm the existence of the designed intramolecular hydrogen bond.

In order to evaluate its most likely conformation under conditions that reflect those encountered in biological

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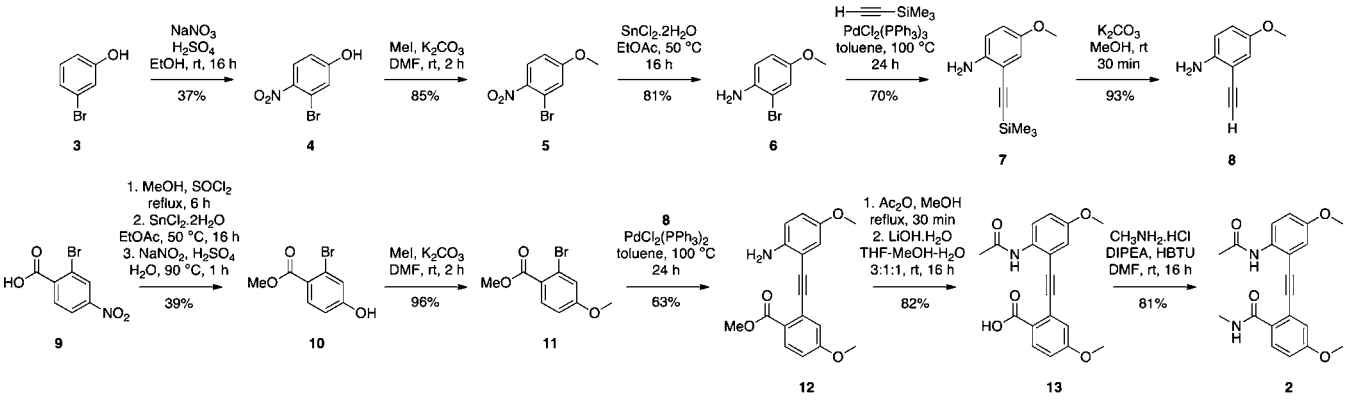
Scheme 1. Synthesis of a Generic Amphipathic α-Helix Mimetic Based on a 1,2-Diphenylacetylene Scaffold



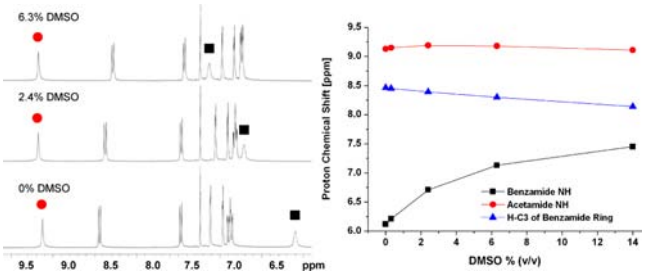


Figure 2. DMSO titration of **2** in $CDCl_3$ at 300 K. 1D ^1H NMR (400 MHz) spectra (left) and chemical shift vs [DMSO] (right). Black square = benzamide NH; red circle = acetamide NH.

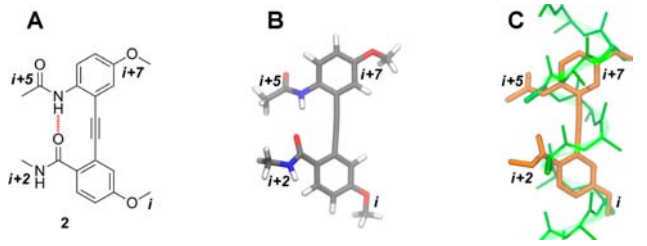


Figure 3. (A) Amphipathic α-helix mimetic **2**, dashed red line = hydrogen bond; (B) representative conformation of the peak population (RMSD = 0.52 Å) of **2** in explicit water; (C) superposition of **2** (orange) on a polyalanine α-helix (green), hydrogens omitted for clarity.

systems, we computationally analyzed **2** using 200 ns molecular dynamics (MD) simulations in explicit water with the program CHARMM.[25] Computational details can be found in the Supporting Information (SI). It was determined that the i and $i+7$ side chains in **2** project from the same face (and the $i+2$ and $i+5$ from the other) during 69% of the simulation time (Figures A and B in SI). Figure C in the SI implies that the strength of the NH···O=C hydrogen bond is ~0.5 kcal/mol in water. Together, these results demonstrate that the intramolecular hydrogen bond *biases* the conformation in aqueous medium, as desired, rather than locking it rigidly into place. A superposition of a representative snapshot (Figure 3B) of the peak population of **2** (Figures D and E, SI) on an idealized polyalanine α-helix is shown in Figure 3C (RMSD = 0.52 Å), which demonstrates that **2** replicates the spatial and angular projections of the i, $i+2$, $i+5$ and $i+7$ side chains of a native α-helix well. Interestingly, the RMSD is lowest for conformations with a $-37°$ twist between the phenyl rings (SI, Figure F), as demonstrated by a low-RMSD conformation viewed along the helical axis (SI, Figure G), and the relatively weak hydrogen bond of **2** in aqueous medium allows access to such desirable conformations of **2**.

One approach by which apoptosis is tightly regulated is through the antagonistic interaction of the pro-survival protein Mcl-1 on pro-death Bcl-2 family members, such as Bak and Bim.[22] More specifically, key residues of the Bim-BH3 α-helix that engage Mcl-1 are Leu62 (i), Ile65 ($i+3$) and Phe69 ($i+7$) on one face of the helix, and Arg63 ($i+1$) and Asp67 ($i+5$) on the opposing face.[26] We hypothesized that **1** could be elaborated to afford structural mimicry of an additional fifth residue. To this end, we designed amphipathic α-helix mimetic **14**. On the hydrophobic face, the isobutyl groups are intended as mimetics of the side chains of Leu62 and Ile65, while the benzyl group mimics Phe69. On the opposing face, a carboxylic acid reproduces the side chain of Asp67. It has been established that the *N*-methyl group of the benzamide moiety in **2** is a good mimetic of the $i+2$ side chain. We considered that, owing to the partial flexibility of the diphenylacetylene scaffold, this position might also reasonably mimic the $i+1$ side chain, particularly in the case of amino acids with flexible side chains. Therefore, mimicry of Arg63 was proposed by a basic ethylamine moiety (synthetically more accessible than ethylguanidine and more "drug-like" (lower polar surface area)) attached to the benzamide nitrogen.

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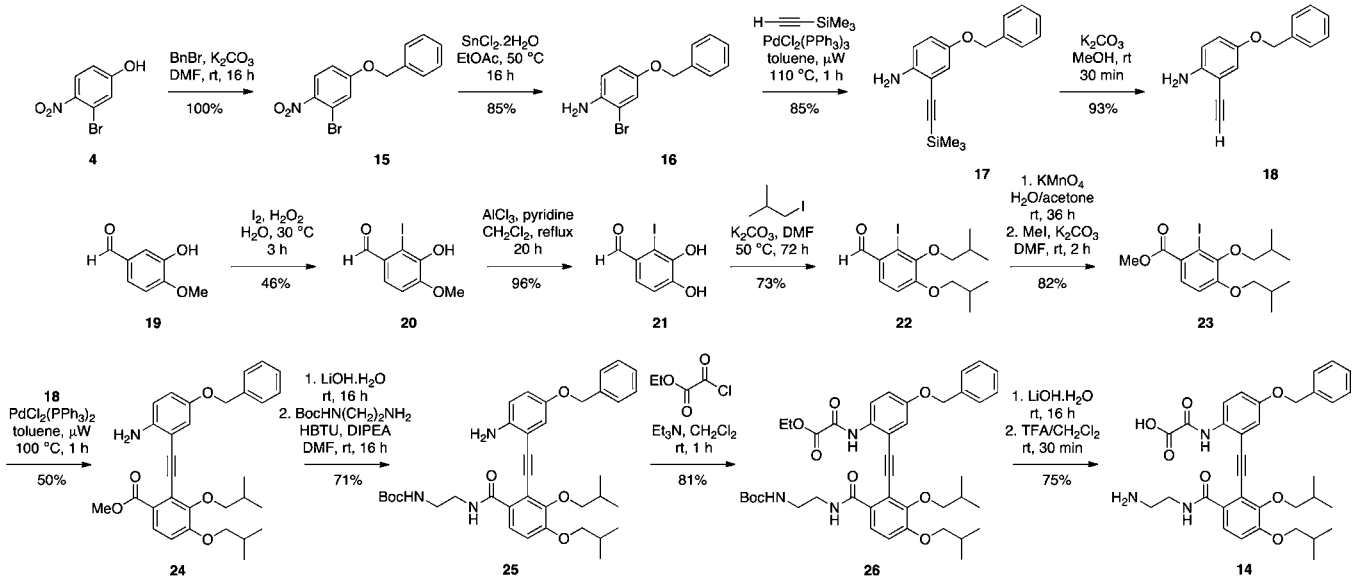
Scheme 2. Synthesis of Compound **14**, an Amphipathic α-Helix Mimetic of the Bim-BH3 Helix



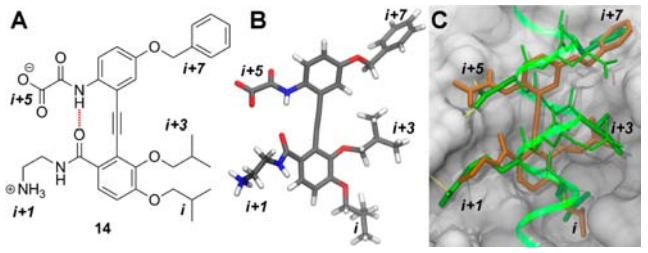


Figure 4. (A) Amphipathic α-helix mimetic **14**, dashed red line = hydrogen bond; (B) representative conformation of the peak population (RMSD = 1.91 Å) of **14** in explicit water; (C) superposition of **14** (orange) on the Bim BH3 α-helix (green).

Compound **14** was synthesized as depicted in Scheme 2. For the most part, the chemistry involved was similar to that for the synthesis of **2**, with the greatest difference the requirement to synthesize the more reactive iodoarene **24** to compensate for the increased steric hindrance in the Sonogashira cross-coupling reaction. As before, a 200 ns MD simulation in explicit water was used to determine the conformational preference of **14** under aqueous conditions. Here, the i and $i + 7$ side chains project from the same face 83% of the time. The greater preference for the desired syn conformation relative to **2** is apparently due to a transient salt bridge. Superposition of a representative snapshot (Figure 4B) of the peak population of **14** (Figures H–M, SI) on the corresponding side chains in an X-ray structure[27] of the Bim-BH3 helix in complex with Mcl-1 (Figure 4C, RMSD = 1.91 Å), reveals excellent mimicry of the five key residues of the Bim-BH3 helix. Preliminary studies indicate that **14** antagonizes the Mcl-1–Bak-BH3 complex with an IC_{50} of 3.24 ± 0.48 μM (SI).

In summary, we have designed a 1,2-diphenylacetylene scaffold that projects functional groups at the correct spatial and angular geometries to accurately mimic the i, $i + 7$ and $i + 2$, $i + 5$ side chains on opposite faces of an α-helix, and, therefore, allows for amphipathic α-helix mimicry. The synthesis is convergent, efficient and the introduction of side chain chemical diversity is, in most cases, reserved until later stages in the synthesis, which will facilitate the development of compound libraries. Finally, we achieved more superior α-helix mimicry by introducing additional functionality into the "lower" phenyl ring of the 1,2-diphenylacetylene scaffold, allowing for mimicry of the $i + 3$ side chain in addition to the i, $i + 1$, $i + 5$ and $i + 7$ side chains. To the best of our knowledge, this work represents the first-ever report of a novel, nonpeptidic, amphipathic α-helix mimetic that can emulate up to five amino acid side chains located across both faces and within two complete turns of the helix.

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Supporting Information Available. Computational details, complete syntheses of **2** and **14**, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.