Toward Proteomimetics: Terphenyl Derivatives as Structural and Functional Mimics of Extended Regions of an α-Helix

Brendan P. Orner, Justin T. Ernst, and Andrew D. Hamilton*

Department of Chemistry, Yale University
P.O. Box 208107, New Haven, Connecticut 06510-8107

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The design of synthetic structures that mimic large and noncontiguous regions of a protein surface remains an elusive goal.[1] There has been considerable success in the field of small *peptidomimetics* that reproduce features of short peptides in extended[2] or β-turn conformations.[3] However, much less progress has been made in the search for *proteomimetics* or nonpeptide structures that mimic larger areas of the protein surface[4] such as an α-helix.[5] This is remarkable given the ubiquitous role of α-helical regions in mediating protein−protein interactions.[6] The difficulty clearly lies in the large and elongated surface area that is presented by 2−4 turns of an α-helix. One strategy involves the covalent or noncovalent stabilization of a 16−20-mer peptide in a helical conformation either through side chain contacts,[7] end capping templation,[8] specific folding[9] or use of β-peptides.[10] As part of our interest in helix surface recognition,[11,12] we sought an entirely nonpeptidic scaffold that could be synthesized in a modular fashion and project side chain functionality with similar distance and angular relationships to those found in α-helices. We herein report a new family of *proteomimetics*, based on a functionalized terphenyl scaffold, that are structural mimics of two turns of the myosin light chain kinase α-helix and show functional analogy in binding with high affinity to calmodulin.

In α-helix-protein complexes critical interactions are often found along one face of the helix, involving side chains from the *i*, *i* + 3, and *i* + 7 residues.[6] The relative positions of these groups in an all-Ala α-helix are shown in Figure 1 A−D and compared to the projection of substituents in a tris-functionalized 3,2′,2″-terphenyl derivative.[13] This is an attractive template for proteomimetic design due to the simplicity of the structure and the potential for an iterative synthesis. The alternating arrangement of *i*, *i* + 3, and *i* + 7 groups through two turns in the helix compares well with the 3,2′,2″-substituents when the terphenyl is in a staggered conformation with dihedral angles of 68° and 36° between the phenyl rings.[14] In this easily accessible conformation, the three subsituents project from the terphenyl core with similar angular relationships and 4−25% shorter distances than between the *i*, *i* + 3, and *i* + 7 β-carbons in an α-helix (Figure 1C and D).[15]

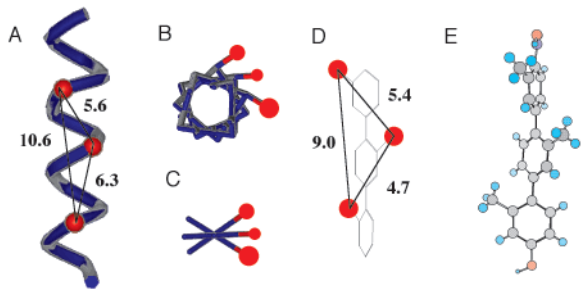

Figure 1. (A) Schematic representation of an α-helical 12-mer peptide with *i*, *i* + 3, and *i* + 7 substituents, side view; (B) top view; (C) 3,2′,2"-trisubstituted terphenyl, top view; (D) side view; (E) X-ray crystal structure of **1**.

A modular synthesis of the 3,2′,2″-tris-substituted-terphenyl derivatives was developed on the basis of sequential Negishi coupling reactions. The terminal 3-substituted phenyl triflate was linked to the central 4-iodo-3-substituted phenyl silyl ether by zinc transmetalation of the iodide and palladium-mediated coupling. Deprotection and triflation of the resulting biphenyl was followed by reaction with a second 4-iodo-3-substituted phenyl silyl ether to give the terphenyl silyl ether. The final steps involved deprotection and alkylation of the hydroxyl group with solubility modulating groups such as acetate.

An X-ray crystal structure of 3,2′,2″-trimethyl-4-nitro-4″-hydroxy-terphenyl derivative **1** (Figure 1E) showed the molecule in a staggered conformation (dihedral angles, 59.1° and 120.7°) with rings A and C projecting their Me substituents on the same face of ring B.[16] The distances between the Me groups are 5.10 (3,2′), 6.28 (2′,2″), and 8.83 Å (3,2″), in reasonable correspondence to the *i*, *i* + 3, and *i* + 7 β-carbons in an α-helical peptide. Other low-energy conformers will be present in solution; however, the solid-state structure and the low rotational barrier of related biphenyls[17] point to the desired terphenyl conformation (Figure 1C and D) being accessible, particularly in the presence of a complementary recognition site.

To test the idea of α-helix mimicry by terphenyl derivatives, we focused on the interaction between calmodulin (CaM) and an α-helical domain of smooth muscle myosin light-chain kinase (smMLCK).[18] CaM also represents an interesting target in our continuing search[19] for molecules that influence cell cycle events.[20]

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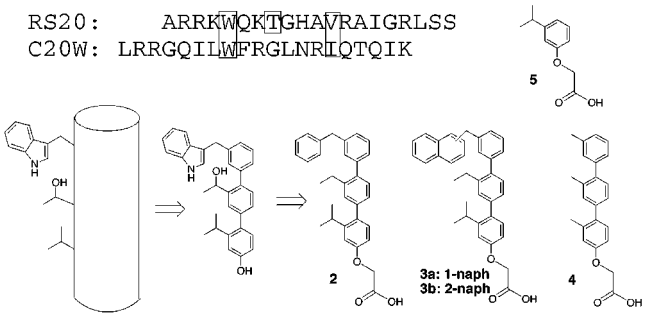

Figure 2. Design of the smMLCK mimetic.

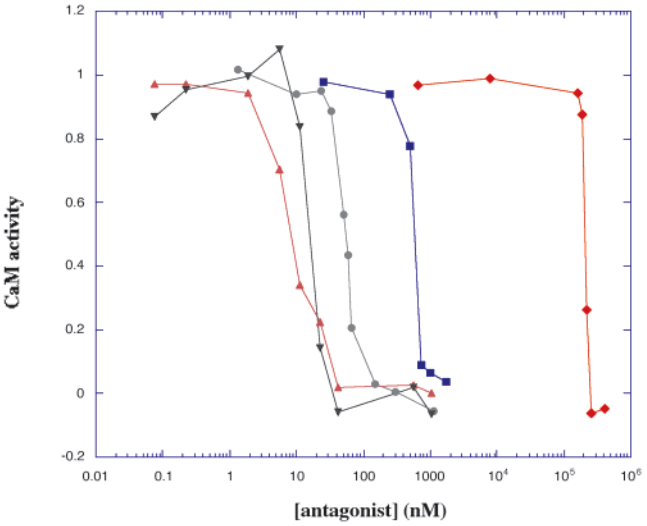

Figure 3. Antagonism of CaM by monitoring PDE hydrolysis of mant-cGMP. ◆ = **5**, ■ = **2**, ● = **RS-20**, ▼ = **3b** ▲ = **3a**. 13.9 nM CaM, 50 mu/mL PDE, 8 μM mant-cGMP, 10 mM Mops, 0.5 mM $MgCl_2$, 90 mM KCl, 0.73 mM $CaCl_2$, 0.2% DMSO, pH 7.0.

Moreover, earlier work from DeGrado has shown that CaM provides an effective recognition surface for a variety of peptides in an α-helical conformation.[21] The sequence of a 20-mer fragment (RS20) in smMLCK is shown in Figure 2, and mutational studies have established a key role for three *i*, *i* + 3, and *i* + 7 residues (Trp800, Thr803, and Val807) in binding to the C-terminal domain of CaM in a complex that also involves the collapsed N-terminal region.[22] The hydrophobic side chains of this key trio of residues can be mimicked by the corresponding 3,2′,2″-terphenyl **2** which, in a staggered conformation, should project them with a similar rise and angle to that in smMLCK. For synthetic simplicity we changed the indole of Trp800 to phenyl and removed the hydroxyl of Thr803. Using Negishi couplings of differently substituted phenyltriflates, we prepared terphenyl **2** as a mimic of the calmodulin binding face of smMLCK. The free hydroxyl at the end of the iterative terphenyl synthesis was alkylated with benzyl bromoacetate, the ester was hydrolyzed, and the resulting carboxylic acid was converted to the ammonium salt of **2**, which proved to be surprisingly soluble in buffer with <1% DMSO.

Passage of **2** through an avidin-based affinity resin derivatized with biotinylated CaM led to retention of the terphenyl on the column. Discrete complex formation to CaM was indicated by the release of **2** from the column, as monitored by HPLC, on elution with 5 M biotin. The control experiment with an underivatized avidin resin resulted in no observable retention of **2**. In addition, polyacrylamide gel permeation chromatography (6 kD cutoff) showed that **2** alone (70 μM in buffer) was retained on the column but passed through when mixed with 1 equiv of CaM, due presumably to the formation of a CaM:**2** complex. To amplify the binding event between CaM and **2**, an enzymatic assay was also employed. CaM activates the enzyme 3′-5′-cyclic nucleotide phosphodiesterase (PDE) through an interaction that is thought to involve the same hydrophobic region that binds to smMLCK.[23] Addition of **2** to a solution of CaM and PDE caused a dose-dependent reduction in the ability of CaM to activate the enzyme for the hydrolysis of substrate (Figure 3).[24] The inhibitory potency of **2** (IC_{50} = 800nM) in this PDE assay is only 10-fold less than the 20-mer α-helical peptide RS20 (IC_{50} = 80nM) and much stronger than the trimethylterphenyl **4** (IC_{50} > 20μM)[25] that lacks the trio of binding substituents or monophenyl **5** (IC_{50} = 150μM). This result suggests that **2** acts as a functional mimic of a natural CaM substrate by antagonizing the binding interaction between CaM and PDE.

Evidence that **2** also acts as a structural mimic of smMLCK, and binds to the same region in the C-terminal domain of CaM, came from competition experiments using the helical peptide C20W (Figure 2). This is a helical region of the plasma membrane calcium pump protein that binds exclusively to the C-terminal domain of CaM, in the same area as smMLCK.[26] Addition of dansylated C20W to CaM results in an increase in the fluorescence emission intensity due to complex formation. This effect is reversed on titration of an excess of **2** into a solution of the complex CaM:C20W-dansyl, clearly suggesting that **2** and C20W are competing for the same binding site on CaM.[27] If this model is correct, we should be able to enhance the binding of **2** by optimizing the fit between the terphenyl substituents and the binding pockets on CaM. To test this we prepared, using modifications of the synthetic route above, 1- and 2-naphthyl derivatives **3a** and **3b** as improved analogues of the Trp800 indole side chain in smMLCK. Figure 3 shows that both **3a** and **3b** are very potent inhibitors of CaM activation of PDE enzyme activity with IC_{50} values of 9 nM and 20 nM, respectively. For **3a** this potency corresponds to an 8-fold improvement over the helical peptide RS20 from smMLCK and renders it among the most active CaM antagonists known. However, the full extent of helix mimicry by **2**, **3a**, and **3b** in terms of their precise conformations and modes of binding to CaM awaits high-resolution structures of these complexes.

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Supporting Information Available: Details of the characterization of the binding interaction of calmodulin with the terphenyl derivatives, the phosphodiesterase activity assay, the X-ray crystallographic analysis of **1**, and the syntheses of **2**, **3a** and **3b** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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