ORGANIC LETTERS

2009
Vol. 11, No. 19
4418−4421

Novel Amphiphilic α-Helix Mimetics Based on a Bis-benzamide Scaffold

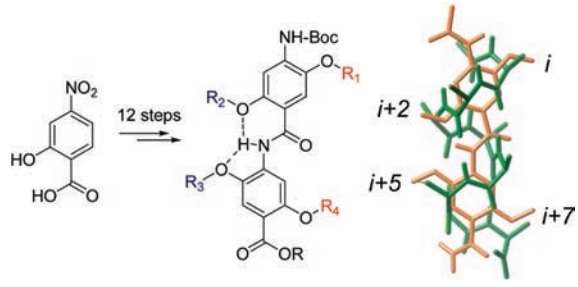
Srinivasa Marimganti, Murthy N. Cheemala, and Jung-Mo Ahn*

Department of Chemistry, University of Texas at Dallas, 800 West Campbell Road, Richardson, Texas 75080

jungmo@utdallas.edu

Received August 3, 2009

ABSTRACT



To mimic amphiphilic α-helices, a new scaffold was designed based on a bis-benzamide that places four side-chain functional groups found at the *i*, *i*+2, *i*+5, and *i*+7 positions of a helix. Its two hydrogen bonds fix the conformation and provide accurate bifacial arrangement of the four substituents, simultaneously representing two opposing helical sides. An efficient synthetic route was achieved for the construction of bis-benzamides, and their superior α-helix mimicry was confirmed by X-ray crystallography.

As one of the most abundant protein secondary structures,[1] α-helices are often found to play an important role in protein complex formations through which diverse regulatory processes like gene expression, enzyme activity, signal transduction, immune response, and apoptosis are modulated.[2] This makes α-helical peptides a versatile tool to investigate protein−protein and peptide hormone−receptor interactions as well as to ultimately develop potent therapeutic candidates. However, short peptide fragments are prone to have a random coil or less characterized structure in solution when taken out of proteins.[3] In addition, short peptides generally suffer from serious limitations, such as high susceptibility to enzymatic degradation, poor bioavailability, and difficulty in penetrating membranes, which must be improved for effective use in biomedical applications.

To preserve α-helicity in short peptides, several strategies have been developed including the formation of a lactam bridge,[4] a salt bridge,[5] and a disulfide bridge[6] between amino acid residues. Hydrocarbon stapling by olefin metathesis was also found to effectively stabilize α-helices.[7] In addition, a variety of foldamers like β-peptides were developed to mimic helical structures.[8] These conformational restrictions not only increase binding affinity to target proteins by stabilizing the required helical structure but also improve enzymatic stability and bioavailability.

Another approach is de novo design of nonpeptide α-helix mimetics. Using rigid and preorganized scaffolds, α-helix mimetics place side-chain functional groups in a proper

(1) Barlow, D. J.; Thornton, J. M. *J. Mol. Biol.* **1988**, *201*, 601.
(2) (a) Cochran, A. G. *Chem. Biol.* **2000**, *7*, R85–R94. (b) Peczuh, M, W.; Hamilton, A. D. *Chem. Rev* **2000**, *100*, 2479.
(3) (a) Scholtz, J. M.; Baldwin, R. L. *Annu. Rev. Biophys. Biomol. Struct.* **1992**, *21*, 95. (b) Schneider, J. P.; Kelly, J. W. *Chem. Rev.* **1995**, *95*, 2169.
(4) (a) Ospay, G.; Taylor, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 6966. (b) Phelan, J. C.; Skelton, N. J.; Braisted, A. C.; McDowell, R. S. *J. Am. Chem. Soc.* **1997**, *119*, 455.
(5) Marqusee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898.
(6) Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9391.
(7) (a) Blackwell, H. E.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **1998**, *37*, 3281. (b) Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 5891. (c) Patgiri, A.; Jochim, A. L.; Arora, A. S. *Acc. Chem. Res.* **2008**, *41*, 1289.
(8) (a) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399. (b) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366.

10.1021/ol901785v CCC: $40.75
Published on Web 08/31/2009

orientation as found in α-helices to interact with target proteins. The absence of peptide structure also results in high enzymatic stability and enhances bioavailability. In the past decade, several α-helix mimetics[9] were developed by using scaffolds like indans,[10] terphenyls,[11] tris-pyridylamides,[12] ladder-like polycyclic ethers,[13] tris-benzamides,[14] and pyridazines.[15] High structural rigidity in these scaffolds easily places three functional groups corresponding to the *i*, *i*+3 (or *i*+4), and *i*+7 positions in an α-helix that organizes one helical face.

However, most of the α-helices found in proteins and biologically active peptides are amphiphilic, possessing a hydrophilic surface on the opposite side of a hydrophobic one. In particular, α-helical segments in peptide hormones highly rely on amphiphilicty for maximal interaction with target receptor proteins. This clearly suggests that presenting only three functional groups found on a single side of a helix, as observed in the existing α-helix mimetics, does not model essential helical amphiphilicity. The lack of functional groups found on the opposite side of a helix in the structure of "one-sided" α-helix mimetics may result in not only suboptimal affinity to target proteins but also lowered selectivity that would significantly limit their utility by potential promiscuity.

In an effort to achieve superior α-helix mimicry with higher affinity and selectivity to taget proteins, we report herein novel amphiphilic α-helix mimetics based on a bis-benzamide scaffold (Figure 1). A suitable scaffold for amphiphilic α-helix mimetics should facilitate the installation of functional groups on both sides of the scaffold and also ensure rigid conformation by its preorganized framework. Satisfying these criteria, the bis-benzamide scaffold easily places four functional groups corresponding to the *i*, *i*+2, *i*+5, and *i*+7 positions in a helix by employing its four hydroxyl groups.

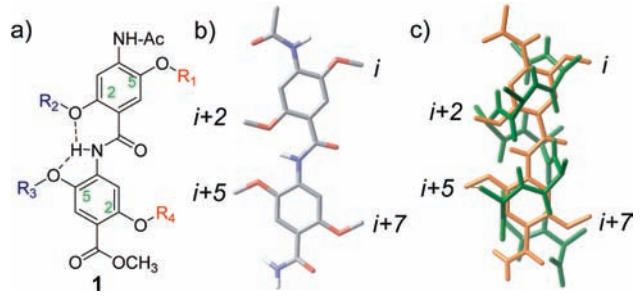
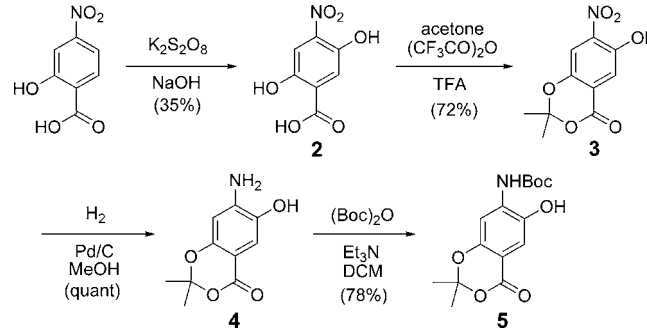


Figure 1. (a) Amphiphilic α-helix mimetic **1** based on a bis-benzamide scaffold; (b) its lowest-energy conformation; (c) superimposition of **1** (orange) on an α-helix (green).

A Monte Carlo conformational search and subsequent energy minimization of a bis-benzamide by using Macro-Model (version 9, Schrödinger) demonstrated high structural rigidity resulting from two hydrogen bonds between a benzamide proton and two adjacent alkoxy substituents (R_2 and R_3). These hydrogen bonds lock the conformation of the bis-benzamide and arrange the two functional groups (R_2 and R_3) on the same side, with the other two (R_1 and R_4) positioned on the opposite side. Superimposition of its lowest energy conformation on an α-helix shows that the four functional groups of a bis-benzamide are well overlaid on the corresponding side chains of a helix, properly mimicking helical amphiphilicity.

Scheme 1. Synthesis of an Amphiphilic Subunit **5**



Since a bis-benzamide comprises of two identical subunits, a protected form of a 4-amino-2,5-dihydroxybenzoic acid **5** was first synthesized (Scheme 1). Starting with 2-hydroxy-4-nitrobenzoic acid, an Elbs persulfate oxidation reaction[16] was carried out to introduce a 5-hydroxy group. The regioselectivity of the oxidation at the 5-position was confirmed by ^{1}H NMR with two singlets in aromatic region. Then, the two hydroxyl groups at the 2- and 5-positions of the hydroxyquinone **2** were easily differentiated by the formation of a ketal between 1-carboxylate and 2-hydroxy groups. Reduction of a nitro group in the ketal **3** and subsequent protection of the resulting amine with a Boc group produced the amphiphilic builidng block **5** for the construction of bis-benzamides.

Having the protected benzoate **5** in hand, we sought to mimic α-helical regions of peptide hormones to demonstrate proof-of-concept. Since numerous α-helical peptide hormones utilize both hydrophobic and hydrophilic surfaces for maximal interaction with their receptors, amphiphilic α-helix mimetics based on the bis-benzamide scaffold would be effective in simultaneously emulating both helical faces and result in high affinity and selectivity. This approach would

(9) For reviews on α-helix mimetics, see: (a) Davis, J. M.; Tsou, L. K; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326. (b) Garner, J.; Harding, M. M. *Org. Biomol. Chem.* **2007**, *5*, 3577. (c) Che, Y.; Brooks, B. R.; Marshall, G. R. *J. Comput.-Aided Mol. Des.* **2006**, *20*, 109.

(10) Horwell, D. C.; Howson, W.; Ratcliffe, G. S.; Willems, H. M. G. *Bioorg. Med. Chem.* **1996**, *4*, 33.

(11) (a) Yin, H.; Lee, G.-I.; Park, H. S.; Payne, G. A.; Rodriguez, J. M.; Sebti, S. M.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 2704. (b) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382.

(12) Ernst, J. T.; Becerril, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 535.

(13) Oguri, H.; Oomura, A.; Tanabe, S.; Hirama, M. *Tetrahedron Lett.* **2005**, *46*, 2179.

(14) Ahn, J.-M.; Han, S.-Y. *Tetrahedron Lett.* **2007**, *48*, 3543.

(15) Volonterio, A.; Moisan, L.; Rebek, J. *Org. Lett.* **2007**, *9*, 3733.

(16) Bhattachryya, S. C.; Seymour, D. E. *J. Chem. Soc.* **1950**, 1139.

also faciliate a rational design of peptidomimetics for biologically important helical peptides. Compared to a random screening campaign, a rational design approach has many advantages including facile optimization of initial leads by taking hints from structure−activity studies of target peptides.

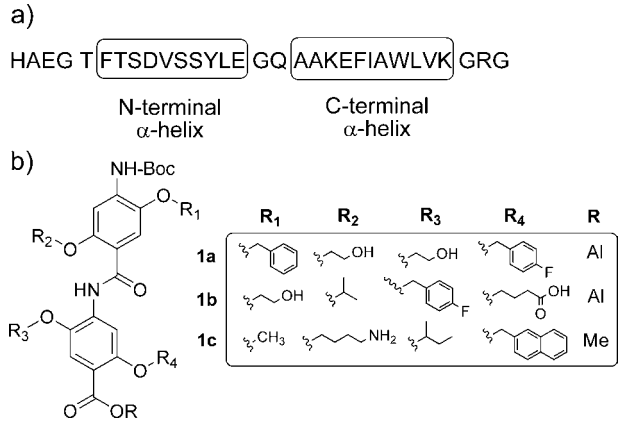
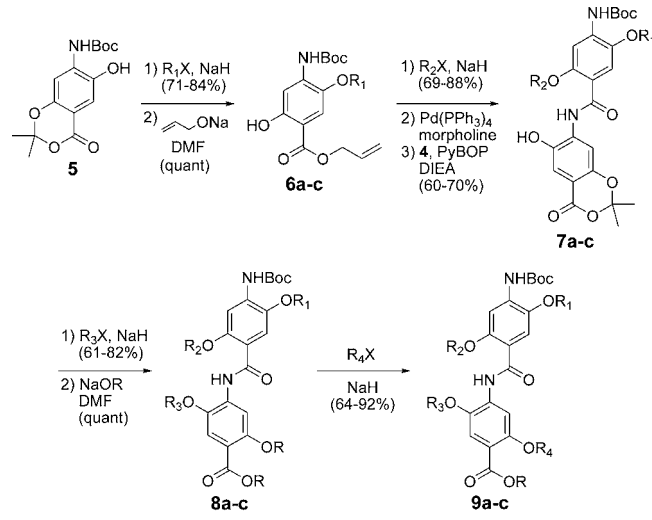


Figure 2. (a) Sequence of GLP-1; (b) amphiphilic α-helix mimetics **1a**−**c** designed for *N*- and *C*-terminal helices in GLP-1.

To evaluate the bis-benzamide scaffold, we have focused on helical segements in glucagon-like peptide-1 (GLP-1). GLP-1 is a 30 amino acid-containing peptide hormone and plays a critical role in glucose homeostasis by stimulating insulin secretion and restoring pancreatic β-cell mass, which are highly beneficial to treat type 2 diabetes.[17] Structural analysis by 2D-NMR,[18] CD spectroscopy,[19] and X-ray crystallography[20] showed that GLP-1 has two α-helical regions between residues 13−20 and 24−34, and their importance in receptor interaction was confirmed by our recent cyclization scanning study.[21] Since amino acids on both sides of helices, such as Phe[12], Ser[14], Tyr[19], Ile[29], and Trp[31], are found to be important for receptor binding and activation,[22] the two α-helical segments of GLP-1 appear to be suitable targets for amphiphilic α-helix mimetics.

Three amphiphilic α-helix mimetics **1a**−**c** were designed on the basis of the bis-benzamide scaffold to represent the two helical segments in GLP-1 (Figure 2). One of the *N*-terminal α-helix mimetics **1a** presents side-chain functional groups of Phe[12], Ser[14,17], and Tyr[19] and organizes a hydrophobic face by using Phe[12] and Tyr[19] and a hydrophilic one with Ser[14,17]. On the other hand, the other *N*-terminal α-helix mimetic **1b** includes side chains of Ser[14], Val[16], Tyr[19], and Glu[21],whereas the *C*-terminal α-helix mimetic **1c** contains Ala[24], Lys[26], Ile[29], and Trp[31]. Due to synthetic difficulties previously observed,[13] 4-fluorobenzyl and 2-naphthylmethyl groups were used as surrogates for the side chain functional groups of Tyr and Trp, respectively.

Scheme 2. Synthesis of Amphiphilic α-Helix Mimetics (**9a**−**c**)



As described in Scheme 2, the first *O*-alkylation was carried out by treating the protected amphiphilic subunit **5** with an alkyl halide and NaH to install a side-chain functional group (R_1) at the *i* position. After quantitative removal of the ketal and the formation of an allyl ester **6a**−**c**, the released 2-hydroxy group was alkylated to introduce a functional group (R_2) at the *i*+2 position. Then removal of the allyl ester with Pd^0 and a coupling reaction with the amphiphilic subunit **4** produced a bis-benzamide **7a**−**c**. Two functional groups (R_3 and R_4) for the *i*+5 and *i*+7 positions were added by repeating *O*-alkylation and ketal removal steps to complete the synthesis of the amphiphilic α-helix mimetics **9a**−**c**. The side chain protecting groups in **9a**−**c** (TBS, TMS, MOM, Aloc) were removed with tetrabutylammonium fluoride, TFA, or Pd^0, respectively, and the resulting final compounds **1a**−**c** will be examined for biological activity.

To evaluate α-helix mimicry, we have crystallized the bis-benzamide **1a**. As shown in Figure 3, the crystal structure of **1a** confirms two hydrogen bonds between the benzamide proton and two adjacent 2-hydroxyethoxy groups, which arranges two substituents (R_2 and R_3) on the same side. The hydrogen bonds also drive the other two functional groups (R_1 and R_4) to the opposite side as designed. Well overlaid on the corresponding α-helical peptide segment in GLP-1,

(17) (a) Baggio, L. L.; Drucker, D. J. *Gastroenterology* **2007**, *132*, 2131. (b) Drucker, D. J.; Nauck, M. A. *Lancet* **2006**, *368*, 1696. (c) Aaboe, K.; Krarup, T.; Madsbad, S.; Holst, J. J. *Diabetes Obes. Metab.* **2008**, 1.

(18) (a) Thornton, K.; Gorenstein, D. G. *Biochemistry* **1994**, *33*, 3532. (b) Neidigh, J. W.; Fesinmeyer, R. M.; Prickett, K. S.; Andersen, N. H. *Biochemistry* **2001**, *40*, 13188.

(19) Runge, S.; Schimmer, S.; Oschmann, J.; Schiødt, C. B.; Knudsen, S. M.; Jeppesen, C. B.; Madsen, K.; Lau, J.; Thøgersen, H.; Rudolph, R. *Biochemistry* **2007**, *46*, 5830.

(20) Runge, S.; Thøgersen, H.; Madsen, K.; Lau, J.; Rudolph, R. *J. Biol. Chem.* **2008**, *283*, 11340.

(21) Murage, E. N.; Schroeder, J. C.; Beinborn, M.; Ahn, J.-M. *Bioorg. Med. Chem.* **2008**, *16*, 10106.

(22) Adelhorst, K.; Hedegaard, B. B.; Knudsen, L. B.; Kirks, O. *J. Biol. Chem.* **1994**, *269*, 6275.

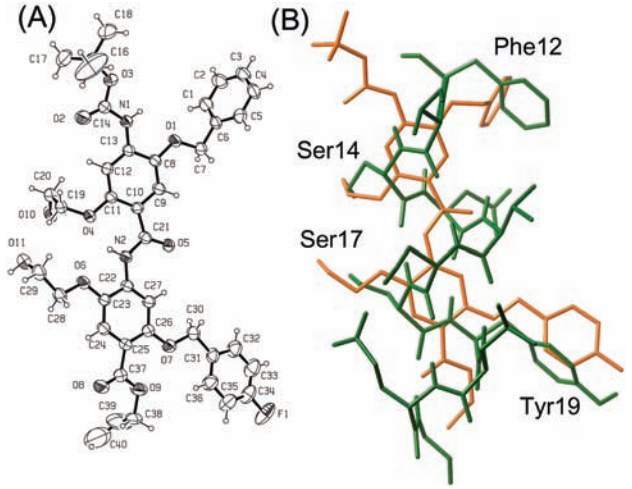


Figure 3. (A) X-ray crystal structure of an amphiphilic α-helix mimetic **1a** (ellipsoids depited at the 30% probablility level.[23]) and (B) superimposition of **1a** (orange) over the corresponding helical segment in GLP-1 (green).

the crystal structure of **1a** clearly shows superior α-helix mimicry accounting for helical amphiphilicity (Figure 3B).

(23) CCDC 732238 contains the supplementary crystallographic data that can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

In summary, we have designed a bis-benzamide scaffold to construct amphiphilic α-helix mimetics, and four functional groups installed on bis-benzamides represent amino acid side chains at the *i*, *i*+2, *i*+5, and *i*+7 positions accounting for helical amphiphilicity. Comprising two indentical amphiphilic subunits, an efficient synthetic route was achieved by repetitive *O*-alkylations and amide bond formation reactions. To evaluate α-helix mimicry, three amphiphilic α-helix mimetics were designed and synthesized on the basis of the sequences of an α-helical peptide hormone GLP-1. An X-ray crystal structure confirms that the bis-benzamide scaffold shows superior α-helix mimicry by simultaneously representing both helical faces with the preorganized framework, which will potentially provide high affinity and selectivity to target proteins.

Acknowledgment. This work was supported by the American Diabetes Association (7-07-JF-02), the Welch Foundation (AT-1595), and the Texas Advanced Research Program (009741-0031-2006).

Supporting Information Available: Experimetal procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901785V