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Synthesis of Pyridazine-Based Scaffolds as α-Helix Mimetics

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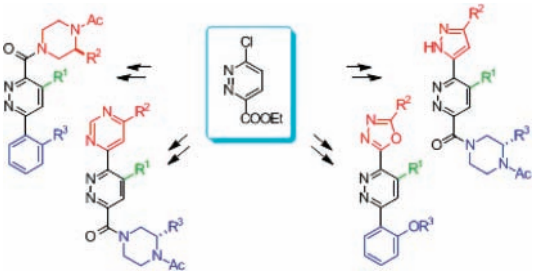
ABSTRACT



The synthesis of several amphiphilic, nonpeptidic scaffolds that mimic the presentation of *i*, *i* + 3 or *i* + 4, and *i* + 7 residues of a peptide α-helix is described. The approach uses a pyridazine core, and the synthesis involves only a few steps and minimizes the number of C–C bond-forming reactions. The versatility of the synthesis makes it suitable for the preparation of small libraries of low molecular weight α-helix mimetics that could be targeted to certain protein/protein interactions.

α-Helices are the most common protein secondary structures and play a pivotal role in many protein−protein interactions. Frequently the critical interactions are found along a "face" of the helix involving side chains from the *i*, *i* + 3 or *i* + 4, and *i* + 7 residues. These project from the α-helix with well-known distances and angular relationships.[1] Molecules that can predictably and selectively reproduce these projections could be valuable as tools in molecular biology and, potentially, as leads in drug discovery.[2] The syntheses of peptidomimetics having a stabilized α-helical conformation have been achieved by introducing synthetic templates into the peptidic chain[3] by using *β*-hairpin mimetics,[4] *β*-peptide sequences,[5] and unnatural oligomers with discrete folding propensities (foldamers).[6] Small synthetic molecules able to mimic the surfaces of constrained peptides offer the advantage of improved stability, lower molecular weight, and in some cases better bioavailability. Although synthetic small molecules that adopt various well-defined secondary structures are well-documented,[7] the first useful mimetics for an α-helix were reported only recently by Hamilton and coworkers: the terphenyl scaffold,[8] and its pyridine[9] and terephthalic acid[10] analogues.

Here we describe the synthesis of small libraries of new classes of low molecular weight α-helix mimetics having a pyridazine ring in the central position and hydrophobic amino acid side chains of the key *i*, *i* + 3 or *i* + 4, *i* + 7

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positions. These include the pyrazole−pyridazine−piperazine scaffold **1** and the oxadiazole−pyridazine−phenyl scaffold **2** (Figure 1).

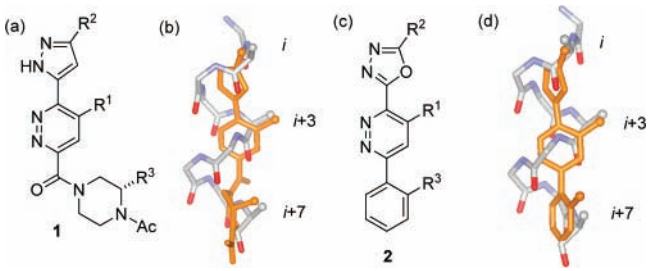
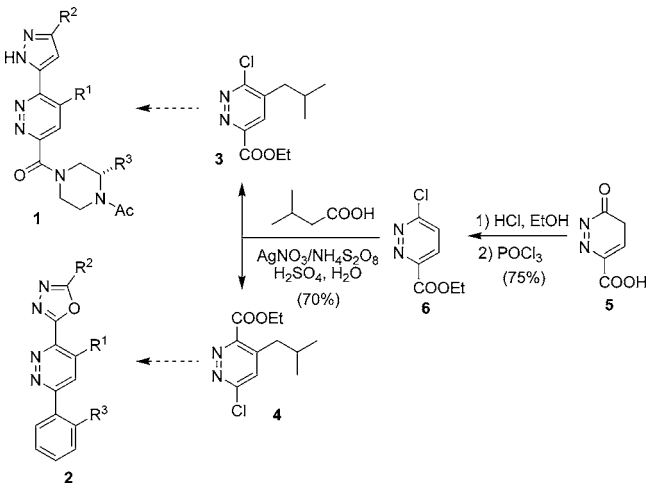


Figure 1. (a) Pyrazole−pyridazine−piperazine scaffold. (b) Superimposition of **1** (orange) on the *i*, *i* + 3, *i* + 7 positions of an α-helix. (c) Oxadiazole−pyridazine−phenyl scaffold. (d) Superimposition of **2** (orange) on the *i*, *i* + 3, *i* + 7 positions of an α-helix.

Inspired by the Hamilton terphenyl, we sought improved synthetic accessibity, and an amphiphilic structure with hydrophobic surface for recognition and a "wet edge" for enhanced solubility.

As depicted in Scheme 1, compounds **1** and **2** could be obtained in a few steps involving a minimum number of C−C bond-forming reactions, starting from two regioisomeric 4- and 5-alkyl-3-chloro-6-carboxypyridazine ethyl esters **3** and **4**, respectively. The latter could be obtained by nucleophilic alkylation of 3-chloro-6-carboxypyridazine ethyl ester **6**[11] by alkyl free radicals that are known to react with electron-poor protonated heteroaromatics such as 3,6-dichloropyridazine.[12] Accordingly, esterification of commercially available 6-oxo-1,6-dihydropyridazine-3-carboxylic acid **5** followed by treatment with $POCl_3$ gave **6**.[13] This underwent homolytic alkylation by free isobutyl radical, generated by silver-catalyzed oxidative decarboxylation of isovaleric acid, and led to a mixture (ca 2:1 regioisomeric ratio) of regioisomers **3** and **4** that were easily separated by flash chromatography.

Scheme 1. General Routes for the Synthesis of α-Helix Mimetics Based on Pyridazine **6**



The structures of regioisomers **3** and **4** were assigned on the basis of the chemical shifts of the aromatic protons (see the Supporting Information). Moreover, it has been reported that similar pyridazines having a carbonitrile group instead of the ethyl ester function reacted with pivalic acid under the same conditions to yield a 7:3 mixture of two regioisomers, the major one having the same regiochemistry (confirmed by X-ray analysis) of **3**.[14]

The major regioisomer **3** underwent Sonogashira coupling[15] with benzyl and isobutyl alkynyl alcohols **7a**,**b**[16] and eventually led to pyridazines **8a**,**b**, respectively, in good yields (Scheme 2). Oxidation of **8a**,**b** to the corresponding ketones **9a**,**b** was achieved in high yields with the Dess−Martin periodinane reagent, while oxidation with MnO_2 gave good results only with compound **8a** (R^2 = isobutyl). Acetylenic ketones such as **9** are known to undergo heteroannulation reactions with bis-nucleophiles like ureas, guanidines, hydrazines, and others.[17] Accordingly, compounds **9a**,**b** were reacted with hydrazine in MeOH at 0 °C affording after 1 h the pyrazole derivatives **10a**,**b** in high yields. After hydrolysis of the ethyl ester function with LiOH

(7) (a) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568−6570. (b) Gennari, G.; Salom, B.; Potenza, D.; Williams, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2067−2069. (c) Gude, M.; Piarulli, U.; Potenza, D.; Salom, B.; Gennari, C. *Tetrahedron Lett.* **1996**, *37*, 8589−8592. (d) Cho, C. Y.; Moran, E. J.; Cherry, S. R.; Stephans, J. C.; Fodor, S. P. A.; Adams, C. L.; Sundaram, A.; Jacobs, J. W.; Schultz, P. G. *Science* **1993**, *261*, 1303−1305. (e) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1996**, *118*, 7529−7541. (f) Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 1066−1072. (g) Lokey, R. S.; Iverson, B. L. *Nature*, **1995**, *375*, 303−305. (h) Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010−4011. (i) Antuch, W.; Menon, S.; Chen, Q.-Z.; Lu, Y.; Sakamuri, S.; Beck, B.; Schauer-Vukasinovic, V.; Agarwal, S.; Hess, S.; Dömling, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1740−1743. For reviews concerning α-helix mimetics, see: (j) Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4130−4163. (k) Fletcher, S.; Hamilton, A. D. *J. R. Soc. Interface* **2006**, *3*, 215−233. (l) Davis, J. M.; Tsou, L. K.; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326−334. See also: (m) Cummings, M. D.; Schubert, C.; Parks, D. J.; Calvo, R. R.; Lafrance, L. V.; Lattanze, J.; Milkiewicz, K. L.; Tianbao, L. *Chem. Biol. Drug Des.* **2006**, *67*, 201−205. (n) Ahn, J.-M.; Han, S.-Y. *Tetrahedron Lett.* **2007**, *48*, 3543−3547.

(8) (a) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382−5383. (b) Yin, H.; Lee, G.; Sedey, K. A.; Kutzki, O.; Park, H. S.; Orner, B. P.; Ernst, J. T.; Wang, H.-G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 10191−10196. (c) Yin, H.; Lee, G.; Park, H. S.; Payne, G. A.; Rodriguez, J. M.; Sebti, S. M.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 2704−2707.

(9) Ernst, J. T.; Becerill, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 535−539.

(10) Yin, H.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1375−1379.

(11) Due to the electronic properties of the substituents, the electrophilicity of C-4 and C-5 on **6** should not differ so much.

(12) Samaritoni, J. G. *Org. Prep. Proced. Int.* **1988**, *20*, 117−121.

(13) Morishita, M.; Kobayashi, J.; Yamada, H.; Yajima, T. *Chem. Pharm. Bull.* **1994**, *42*, 371−372.

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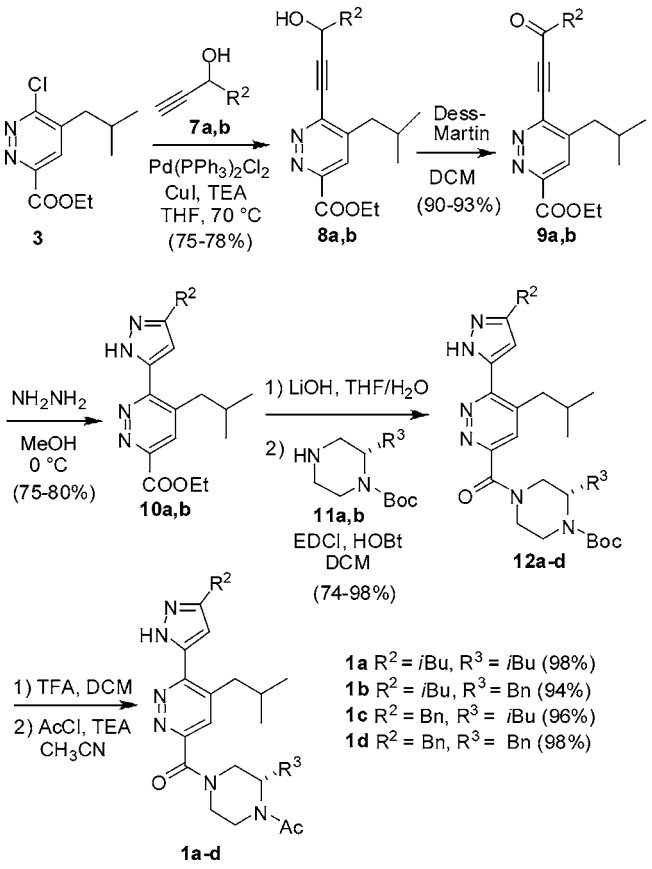
(15) For a very recent review on Sonogashira coupling see: Chinchilla, R.; Nájera, C. *Chem. Rev.* **2007**, *107*, 874−922 and references cited therein.

(16) Benzyl and isobutyl alkynyl alcohols **7a**,**b** were obtained in excellent yields by reacting the corresponding aldehydes with ethynylmagnesium bromide.

(17) Bagley, M. C.; Huges, D. D.; Taylor, P. H. *Synlett* **2003**, 259−262.

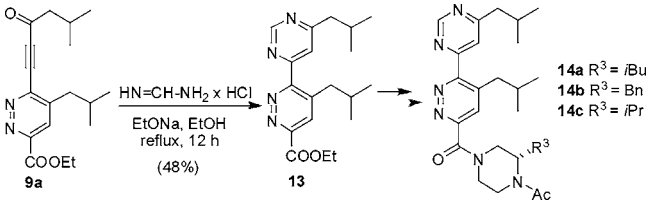
Scheme 2. Synthesis of the Pyrazole−Pyridazine−Piperazine Scaffold



followed by coupling with different commercially available *N*-Boc-protected piperazines **11a**,**b**, we obtained a small library of compounds **12a**−**d**. After deprotection, these were selectively acetylated at the free amine function of the piperazine ring leading to the target compounds **1a**−**d**. Such structures were recently shown to give good overlap of their protruding functions with the side chains of α-helices.[18]

The versatility of alkynyl ketone **9a** was further exploited in its reaction with formamidine in refluxing EtOH leading to the formation of the pyrimidine derivative **13** in moderate yield (Scheme 3). Following the same strategy depicted in

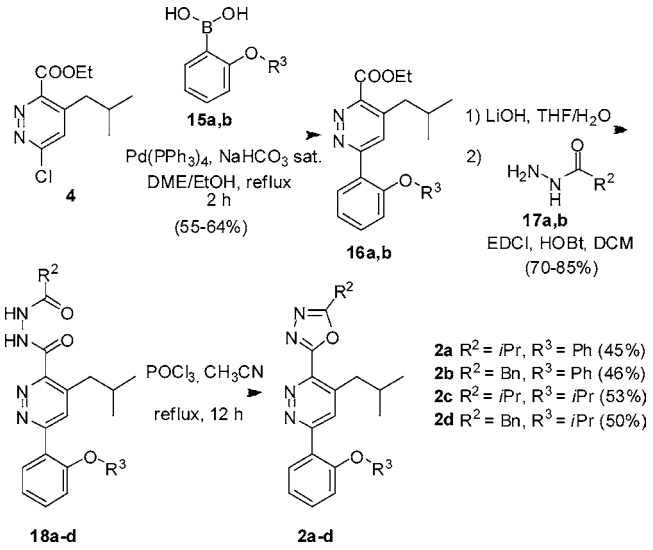
Scheme 3. Synthesis of the Pyrimidine−Pyridazine−Piperazine Scaffold



Scheme 2, we obtained the synthesis of a new class of α-helix mimetics, namely, the pyrimidine−pyridazine−piperazine scaffold (compounds **14a**−**c**).

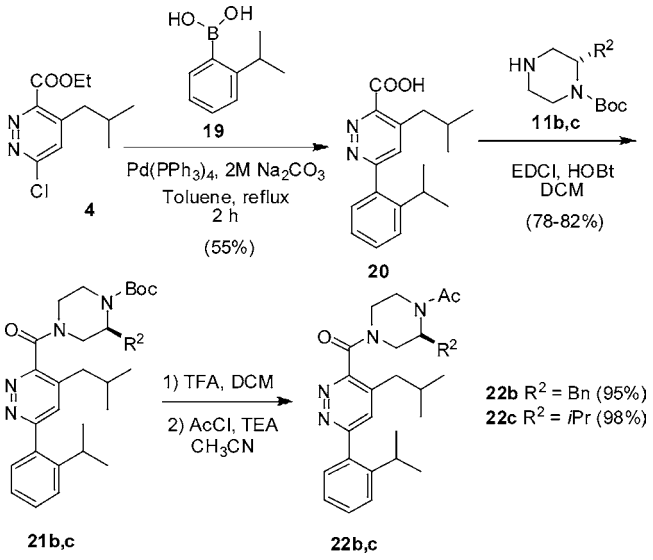
The minor regioisomer **4** was found to be sufficiently electron poor to undergo Suzuki coupling[19] with commercially available 2-alkoxyaryl boronic acids **15a**,**b** affording compounds **16a**,**b** in acceptable yields (Scheme 4). Both the

Scheme 4. Synthesis of the Oxadiazole−Pyridazine−Phenyl Scaffold



alkoxy side chains and alkyl side chains serve to mimic the key hydrophobic residues in protein−α-helix ligand interactions.[9] Hydrolysis of the ethyl ester with LiOH, followed by coupling with *N*-acyl hydrazides **17a**,**b** (easily obtained by reaction between hydrazine and the corresponding esters) mediated by EDCI/HOBt led to the formation of intermediates **18a**−**d** in good overall yields. Finally, *N*,*N*′-diacyl hydrazides **18a**−**d** were dehydrated by using $POCl_3$ in refluxing CH_3CN to achieve the synthesis of α-helix mimetic oxadiazole−pyridazine−phenyl scaffold **2**.

Scheme 5. Synthesis of the Piperazine−Pyridazine−Phenyl Scaffold



The Suzuki coupling with 2-isopropylphenyl boronic acid **19** with use of a 2 M aqueous solution of Na_2CO_3 (instead of a saturated aqueous solution of $NaHCO_3$) gave directly the free carboxylic acid **20**, which could be used as intermediate for the construction of other scaffolds (Scheme 5). For example, coupling **20** with *N*-Boc-piperazines **11b**,**c** gave, after the usual deprotection/acetylation sequence, the piperazine−pyridazine−phenyl scaffold represented by compounds **22b**,**c**.

In summary, the synthesis of new α-helix scaffolds mimicking *i*, $i + 3$ or $i + 4$, $i + 7$ residues was accomplished. The common pyridazine heterocycle originates from the easily available building block, **6**. These scaffolds may be thought of as synthetic counterparts of amphiphilic α-helices having a "wet face" along one side and a hydrophobic face along the other side of the helix. The combinatorial synthesis of an array of these compounds and their ability to recognize protein surfaces are currently under investigation.

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

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