

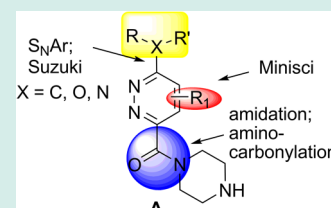
Synthesis of Pyridazine-Based  $\alpha$ -Helix Mimetics

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## S Supporting Information

**ABSTRACT:** A versatile synthesis of pyridazine-based small molecule  $\alpha$ -helix mimetics (**A**) is presented. Modular C–C, C–N, and C–O bond-forming reactions allow for the inclusion of a variety of aliphatic, basic, aromatic, and heteroaromatic side chain moieties. This robust synthesis is suitable for the preparation of small pyridazine-based libraries.

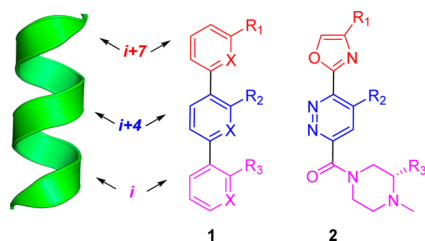


**KEYWORDS:** pyridazine,  $\alpha$ -helix mimetic, Minisci reaction, cross coupling

## INTRODUCTION

The  $\alpha$ -helix is one of the most common secondary protein structural motifs with roughly 3.6 amino acid residues per turn. Amino acid side chains that are adjacent in space, at positions  $i$ ,  $i + 3/i + 4$ ,  $i + 7$  of the helix, appear on the same face and define three distinct recognition elements. The key residues are often hydrophobic in nature and are frequently involved in crucial protein–protein interactions (PPI). The  $\alpha$ -helix conformation is stabilized by a combination of steric interactions and hydrogen bonding. Approximately 62% of the PPIs found in the Protein Data Bank have an  $\alpha$ -helix at the interface which makes this structural motif important in protein–protein recognition.<sup>1</sup> Several relevant biological targets utilize  $\alpha$ -helices as key recognition elements in PPIs.<sup>2</sup> Because of the nature of PPIs, it is probable that chemical matter targeting these interfaces can lie in beyond rule of five space.<sup>3</sup>

Several variants of nonpeptidic  $\alpha$ -helix mimetics based on terphenyl, terephthalamide, oligopyridine and pyridazine scaffolds are known and are believed to display side chains in a manner that closely resembles the positions  $i$ ,  $i + 3/i + 4$ , and  $i + 7$  of an  $\alpha$ -helix (Figure 1).<sup>4</sup> Some of these have been constructed



**Figure 1.** Representative examples of  $i$ ,  $i + 3/i + 4$ ,  $i + 7$   $\alpha$ -helix mimetics.

in a modular fashion allowing for the efficient synthesis of moderate-to-large libraries.<sup>4j–m</sup> Small molecule mimetics are expected to have fewer pharmacokinetic issues (e.g., permeability) than the corresponding peptides,<sup>5,6</sup> and thus could allow for discovery of chemical matter well-suited to small molecule drug discovery projects. More recently, amphiphilic helix mimet-

ics that have been designed to bind to multiple recognition surfaces have emerged.<sup>4e</sup> While multifaced helix mimetics are not directly addressed in this work, it is clear that the helix mimetic field could benefit from chemistry that is capable of introducing both polar and nonpolar substituents to various scaffolds.

Pyridazine-based mimetics offer the same conformational constraint and hydrophobic region as the related terpyridyl analogs (**1**)<sup>4a</sup> but also provide a hydrophilic face, rich in hydrogen bond donors and acceptors that can be directed toward the solvent. These mimetics offer several vectors to explore structure–activity relationships, but to date, the range of substituents at each of the pertinent vectors has been largely limited to side chains represented in naturally occurring amino acids. Expansion of substituents on helix mimetics to include non-natural side chains could be beneficial for discovery of new drug-like molecules. As shown in compound **2**,<sup>4c</sup> these vectors include (1) the 6-position amide (magenta), which can be modified via simple amidation chemistry from the corresponding acid, (2) the 3-position oxazole (red), which can be substituted with a variety of C, O, or N linked substituents using  $S_NAr$  or Suzuki reactions, and (3) the 4-position (blue) R group, which can be installed by either alkyl radical-based methods or lengthy de novo synthetic sequences. Since methods to address objectives 1 and 2 are well-established in the literature, the main focus of this work (objective 3) was to explore the addition of a wider variety of pyridazine 4-position substituents to mimic a range of amino acid side chains at the  $i + 3/i + 4$  position of the helix mimetic.

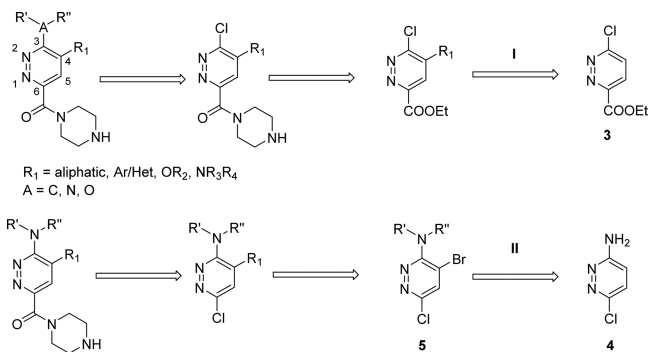
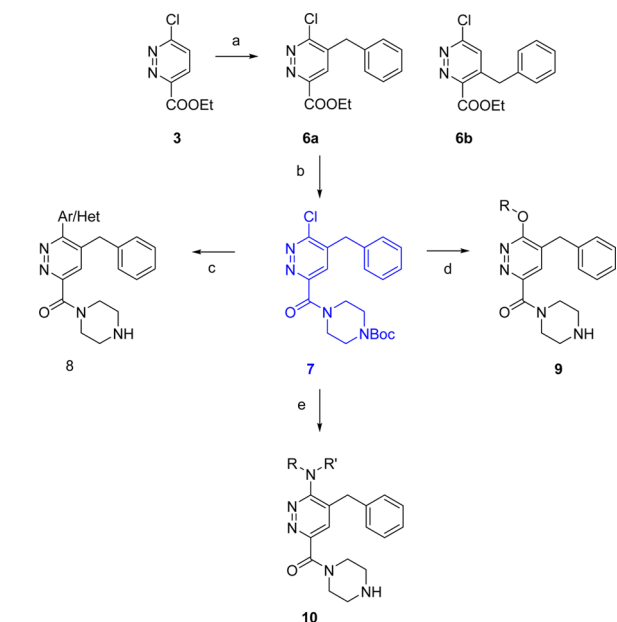
## RESULTS AND DISCUSSION

**Retrosynthesis.** Multiple routes to  $\alpha$ -helix mimetics based on compound **A** were devised starting from two readily available intermediates **3** and **4** (Scheme 1). Substitution of 3-chloropyridazines, such as **3**, via Suzuki coupling or  $S_NAr$  are well-established methods for diversification at this position and

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## Scheme 1. Retrosynthesis

Scheme 2. Expansion of C3 through Suzuki or  $S_NAr$  Reactions<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) phenylacetic acid,  $(NH_4)_2S_2O_8$ ,  $AgNO_3$ , conc.  $H_2SO_4$ ,  $H_2O$ , 70 °C, 37%; (b) (i) NaOH, MeOH, 30 °C, 100%, (ii) *N*-Boc-piperidine, HATU, DIPEA, DMF, 30 °C, 58%; (c) (i)  $ArB(OR)_2$ ,  $Pd(dtbpf)Cl_2$ ,  $K_3PO_4$ , dioxane,  $H_2O$ , 140 °C, (ii) HCl, MeOH, 30 °C; (d) (i) ROH,  $Cs_2CO_3$ , THF, 100 °C, (ii) TFA,  $CH_2Cl_2$ , 30 °C; (e) (i) amine, DIPEA, *t*AmOH, 110 °C, (ii) TFA,  $CH_2Cl_2$ , 30 °C.

were ultimately utilized. First, however, we aimed to diversify the 4-position of the pyridazine core with a variety of side chains including aliphatic, basic, aromatic, and heteroaromatic residues. As shown in Scheme 1, two general retrosynthetic approaches were utilized. Route I initiated with a C–C bond formation

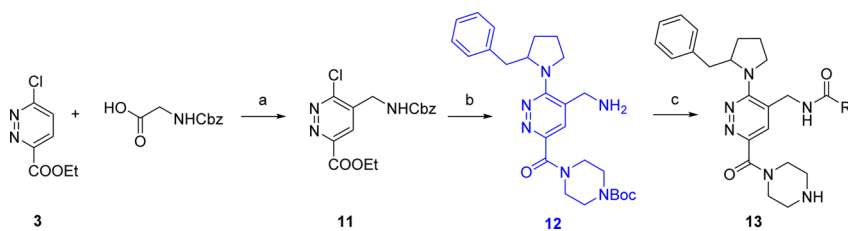
Table 1. Selected Analogues 8, 9, and 10<sup>a</sup>

8	9	10
8a (26%)	8b (26%)	8c (11%)
8d (20%)	8e (8%)	
9a (26%)	9b (23%)	9c (9%)
9d (21%)	9e (10%)	
10a (12%)	10b (26%)	10c (20%)
10d (7%)	10e (8%)	

<sup>a</sup>Isolated yield of purified product from two-step reaction sequence reported in parentheses.

between 3-chloro-6-carboxypyridazine ethyl ester 3<sup>5</sup> and various carbon radical sources via the Minisci reaction.<sup>7</sup> Route II employed 6-chloropyridazin-3-amine 4 as the starting material, which was subsequently brominated to afford 5 which allowed for 4-position substitution beyond carbon to include nitrogen and oxygen linkers.

**Synthesis.** Our major building block, pyridazine 3, was alkylated through Minisci coupling with a number of primary and secondary aliphatic acids. Unlike Negishi coupling with branched zincates, the Minisci reaction with branched acids, such as isobutyric acid, did not show any isomerization. In this well-known and versatile reaction, the purported mechanism involves a silver-catalyzed radical formation, via oxidative decarboxylation, and subsequent addition to the protonated pyridazine to generate a regioisomeric mixture of 4- and 5-alkylpyridazines.<sup>8,9</sup> Thus, the homolytic alkylation with 2-phenylacetic acid led to two regioisomers (Scheme 2). The major regioisomer 6a, which was readily separated from the minor isomer 6b by chromatography, was hydrolyzed with aq. NaOH and coupled with *N*-Boc-piperazine.<sup>10</sup> This first versatile synthetic intermediate 7 represented an  $i + 3/i + 4$ ,  $i + 7$  fragment with a Phe side-chain at  $i + 3/i + 4$  that allowed for exploration of the  $i$  position. The common intermediate 7 was applied

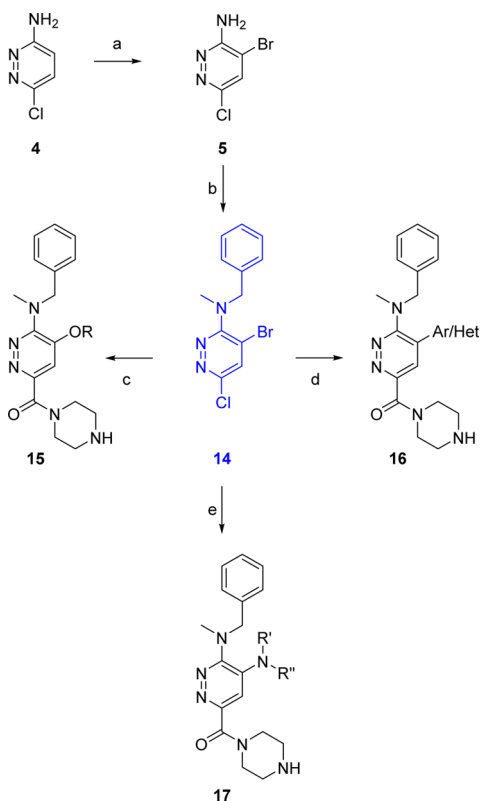
Scheme 3. Expansion of C4 through Minisci Reaction Followed by Acylation<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a)  $(NH_4)_2S_2O_8$ ,  $AgNO_3$ , TFA,  $H_2O$ , 70 °C, 30 min, 35%; (b) (i) 2-benzylpyrrolidine, DIPEA, NMP, 120 °C, 16 h, 94%, (ii) NaOH, MeOH, 97%, (iii) *N*-Boc-piperidine, HATU, DIPEA, DMF, 16 h, 74%, (iv)  $Pd/C$ ,  $H_2$ , MeOH, 90%; (c) (i) acid, HATU, DIPEA, DMF, 16 h, (ii) TFA,  $CH_2Cl_2$ .

Table 2. Selected Analogues 13<sup>a</sup>

 13				
 13a (22%)	 13b (18%)	 13c (19%)	 13d (24%)	 13e (26%)
 13f (26%)	 13g (16%)	 13h (11%)	 13i (26%)	 13j (22%)

<sup>a</sup>Isolated yield of purified product from two-step reaction sequence reported in parentheses.

Scheme 4. Expansion of C4 through C–C Bond Cross Coupling, S<sub>N</sub>Ar Reaction, Followed by C6 Amino Carbonylation<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) Br<sub>2</sub>, NaHCO<sub>3</sub>, MeOH, 57%; (b) (i) benzyl bromide, NaH, THF, 70%, (ii) iodomethane, NaH, THF, 65%; (c) (i) ROH, NaH, DMF, 30 °C, (ii) 1-Boc-piperazine, Pd(OAc)<sub>2</sub>, dppp, K<sub>3</sub>PO<sub>4</sub>, Et<sub>3</sub>N, DMF, CO (1 atm), 110 °C, (iii) TFA, CH<sub>2</sub>Cl<sub>2</sub>; (d) (i) ArB(OR)<sub>2</sub>, Pd(dppf)Cl<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub>, Cs<sub>2</sub>CO<sub>3</sub>, dioxane, H<sub>2</sub>O, 100 °C, (ii) 1-Boc-piperazine, Pd(OAc)<sub>2</sub>, dppp, K<sub>3</sub>PO<sub>4</sub>, Et<sub>3</sub>N, DMF, CO (1 atm), 110 °C, (iii) TFA, CH<sub>2</sub>Cl<sub>2</sub>; (e) amine, CsF, DIPEA, DMSO, 110 °C, (ii) 1-Boc-piperazine, Pd(OAc)<sub>2</sub>, dppp, K<sub>3</sub>PO<sub>4</sub>, Et<sub>3</sub>N, DMF, CO (1 atm), 110 °C, (iii) TFA, CH<sub>2</sub>Cl<sub>2</sub>.

to three libraries in a 2-step sequence (Table 1): (1) Suzuki coupling mediated arylation to give 8, after acidic deprotection, (2) S<sub>N</sub>Ar mediated etherification-deprotection to provide aryl ethers 9, and (3) amination-deprotection to generate aryl amines 10.<sup>11</sup> Among amine substituents on compounds 10, 2-benzylpyrrolidine and N-methylbenzylamine were favored for further exploration of the SAR (data not shown).

Using the same strategy, the Cbz-protected aminomethyl group was introduced to the C4 position by reaction of ethyl ester 3 with N-carbobenzyloxyglycine to provide 11 in 35% yield (Scheme 3). After S<sub>N</sub>Ar reaction with 2-benzylpyrrolidine, followed by ester hydrolysis, amide formation, and Cbz-deprotection, 11 was converted into intermediate 12. The C4 aminomethyl group of 12 was acylated followed by deprotection through parallel chemistry to provide 13 (Table 2).

In the second route, 6-chloropyridazin-3-amine 4 was employed as the starting material (Scheme 4). Substrate 4 allowed for sequential change of all three vectors on the pyridazine. Selective bromination of 4 gave 5 (R', R'' = H),<sup>12</sup> which was sequentially alkylated with benzyl bromide and methyl iodide to form intermediate 14. Pyridazine 14, with differentiated functional groups, allowed for selective manipulation of each position. The bromo on pyridazine 14 was used as the handle to produce three different libraries in a 3-step sequence: (1) etherification-amino carbonylation-deprotection to give 15, (2) Suzuki coupling-amino carbonylation-deprotection to provide 16, and (3) S<sub>N</sub>Ar-amino carbonylation-deprotection to offer 17 (Table 3).

Table 3. Selected Analogues 15, 16, and 17<sup>a</sup>

 15                      16                      17				
 15a (18%)	 15b (16%)	 15c (9%)	 15d (16%)	 15e (16%)
 16a (10%)	 16b (14%)	 16c (27%)	 16d (12%)	 16e (24%)
 17a (13%)	 17b (10%)	 17c (16%)	 17d (15%)	 17e (16%)

<sup>a</sup>Isolated yield of purified product from three-step reaction sequence reported in parentheses.

A comparison of the crystal structure of König's 1,4-dipiperazino benzene mimetic<sup>4d</sup> to 10d showed a similar display of the substituents in space. Likewise, superposition of a minimized version of mimetic 10b had a similar geometrical arrangement (see Supporting Information).

In summary, pyridazine-based  $\alpha$ -helix mimetics display side chains in a manner that closely resembles the  $i, i + 3/i + 4$ , and  $i + 7$  positions of peptide-derived  $\alpha$ -helices. Starting from a few readily accessible versatile synthetic intermediates, we developed several synthetic sequences that allow for inclusion of a variety of side chains including aliphatic, basic, aromatic, and heteroaromatic residues through a number of C–C, C–N, and C–O bond-forming reactions. The robust synthesis enabled the exploration of the three accessible vectors and was demonstrated by preparation of several small libraries. The application of this methodology to relevant medicinal chemistry targets will be reported in due course.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscmbosci.6b00111.

Experimental procedures, characterization data and X-ray data for the structure determination of **10d** (PDF)  
Crystallographic information file for compound **10d** (CIF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

PPI protein–protein interaction

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