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## Substituted Imidazo[1,2-*a*]pyridines as $\beta$ -strand Peptidomimetics

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### Abstract



New conformationally extended dipeptide surrogates based on an imidazo[1,2-*a*]pyridine scaffold are described. Efficient synthesis and incorporation into host peptides affords structures with native side-chain functionality and hydrogen bonding elements on one face of the backbone. Structural analysis by NMR suggests that model peptidomimetics adopt a  $\beta$ -strand-like conformation in solution.

$\beta$ -Strands are key structural motifs in a number of protein-protein interfaces relevant to human disease.<sup>1</sup> For example, the Akt-GSK3 $\beta$ ,<sup>2</sup> Ras-Raf1,<sup>3</sup> and KRas-FTase<sup>4</sup> interactions are all implicated in oncogenesis and involve the recognition of an extended or  $\beta$ -strand domain. Isolated  $\beta$ -strand peptides are also substrates for various proteolytic enzymes<sup>5</sup> and major histocompatibility complex (MHC) proteins.<sup>6</sup> Conformational mimicry of  $\beta$ -strands has thus gained increasing attention as a strategy toward new chemical probes and therapeutics.<sup>7</sup>

Synthetic templated  $\beta$ -strands/sheets often feature  $\beta$ -hairpin or macrocyclic motifs to impart conformational rigidity.<sup>7</sup> In these cases, enhanced stability may require large scaffolding elements or complementary strands that are auxiliary to a sequence of interest. The incorporation of constrained residues or backbone isosteres within extended host peptides represents a more ‘minimalist’ approach toward peptidomimetic drug candidates. The utility of hybrid peptides featuring  $\beta$ -strand prosthetics such as the Hao subunit,<sup>8</sup> @-tide residues,<sup>9</sup> and other dipeptide surrogates<sup>10</sup> have been demonstrated in various applications. The development of artificial  $\beta$ -strands comprised entirely of non-peptide subunits has also emerged as an approach toward novel proteomimetic foldamers.<sup>11</sup>

Our interest in peptidomimetics targeting the Akt-GSK3 $\beta$  interaction<sup>12</sup> led us to explore scaffolds that could be easily prepared and incorporated into host sequences, enforce an extended backbone conformation, and impart more ‘drug-like’ character onto short peptides. Here, we describe the design and synthesis of peptidomimetics incorporating a novel imidazo[1,2-*a*]pyridine scaffold. Structural analysis of model compounds by NMR suggests that hybrid imidazo[1,2-*a*]pyridine-based peptides adopt an extended conformation in

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**Supporting Information Available.** Experimental procedures and structural characterization of compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

solution. These studies lay the groundwork for the development of imidazo[1,2-*a*]pyridines as new core motifs for peptido- and proteomimetic drug design.

In our search for constrained templates with favorable pharmacokinetic properties, we were particularly attracted to the imidazo[1,2-*a*]pyridine core structure due to its presence in therapeutic agents such as the hypnotics zolpidem (Ambien®) and alpidem,<sup>13</sup> and the vasodilator olprinone.<sup>14</sup> We envisioned that imidazo[1,2-*a*]pyridines appropriately substituted with terminal amine and carboxy groups, and with side-chain diversity at the putative  $\beta$  carbon, could serve as ‘drug-like’ extended dipeptide surrogates. Figure 1 depicts the design of our scaffold as well as an overlay of a model tetrapeptide mimic with an idealized strand from an antiparallel  $\beta$ -sheet. Although the planarity of the aromatic core results in some deviation in main chain and side chain geometry, the imidazole nitrogen (H-bond acceptor) overlays reasonably well with the carbonyl oxygen in the native peptide strand. We thus pursued this scaffold as a potential backbone replacement for highly extended dipeptides. Despite a wealth of literature on the chemistry and pharmacology of imidazo[1,2-*a*]pyridines,<sup>15</sup> to the best of our knowledge, these scaffolds have not previously been synthesized or studied in the context of peptide mimicry.

Scheme 1 depicts the synthesis of imidazo[1,2-*a*]pyridine (IP) dipeptide surrogates via monobromination of acetoacetate derivatives and condensation with 2,3-diaminopyridine. Although various methods exist for the  $\alpha$ -bromination of  $\beta$ -ketoesters, we found reaction of **1** with 1.1 equiv of N-bromosuccinimide in PEG-400 to be the most convenient. Optimal yields of IP scaffolds **2** were obtained by directly heating the crude  $\alpha$ -bromo- $\beta$ -ketoesters with 1.0 equiv 2,3-diaminopyridine in the presence of NaHCO<sub>3</sub>. We found that the yields of imidazopyridine formation could be increased by using mono-Cbz-protected 2,3-diaminopyridine<sup>16</sup> in the condensation reaction. To show that a wider array of substituents could be similarly incorporated, we also prepared orthogonally-protected dipeptide surrogates featuring butenylglycine (**3c**), homoserine (**3d**), tyrosine (**3e**), ornithine (**3f**), and arginine (**3g**) side chains.

We next studied the incorporation of our IP building blocks into short peptides using conventional coupling reactions. Condensation of **2a** with Cbz-Leu-OH proved challenging, presumably due to the low nucleophilicity of the IP aryl amine. As shown in Table 1, a variety of standard conditions afforded poor yields of the desired amide (entries 1-3). The use of EDC with catalytic DMAP (entries 4 and 5) led to an improvement in yield, but was attended by significant racemization of the Leu residue.<sup>17</sup> Surprisingly, we found that omission of an auxiliary base resulted in higher conversions. Optimal results were obtained by using 2.0 eq. of both EDC and Cbz-Leu-OH without any added base to give **4** in 91% yield and >98:2 er (entry 7). Other coupling reagents such as PyBOP, COMU, DEPBT, and DCC consistently gave inferior results (entries 8-11).

Hydrolysis of the ester in **4** required extended reaction times in the presence of hydroxide. Prolonged exposure to aqueous LiOH also resulted in an unacceptable degree of amide bond scission. We found that this issue could be circumvented by using Cbz-protected building block **3a** for peptide assembly in the C–N direction (Scheme 2). The urethane bond in **3a** proved stable to hydrolysis with LiOH. Condensation of the resulting crude acid with HPh-OME was followed by hydrogenolysis and coupling to Cbz-Leu-OH to give tetrapeptide mimic **6** in 45% yield over 4 steps.

The observed lack of aryl amine reactivity in the presence of various standard amidation reagents (Table 1, entries 1-3) also led us to explore the direct condensation of a fully unprotected IP derivative. We found that hydrolysis of **2a** followed by neutralization afforded the crude amino acid zwitterion, which could be used directly in the subsequent

coupling. Reaction with H-Phe-OMe in the presence of HBTU, HOBT, and NEt<sub>3</sub> gave the desired amide exclusively, without any detectable homocoupling of the IP scaffold. The phenylalanyl intermediate was then reacted with Cbz-Leu-OH and EDC to provide **6** in an improved 59% overall yield from **2a**.

With a short model peptide in hand, we used NMR to determine whether **6** adopts an extended conformation in solution. The <sup>1</sup>H NMR of **6** in CDCl<sub>3</sub> was well resolved and showed no evidence of rotational isomers. The CH<sub>α</sub> proton resonances of both the Leu and Phe residues also appeared 0.3–0.5 ppm downfield of their expected values in an unstructured peptide.<sup>18</sup> This is consistent with the chemical shift of β-strand/sheet CH<sub>α</sub> protons relative to those in random coil peptides. Most important, the 2D ROESY spectrum of **6** exhibited a correlation between the IP<sub>Me</sub> and Phe<sub>NH</sub> protons, as well as between Leu<sub>CH<sub>α</sub></sub> and IP<sub>NH</sub> (Scheme 2). These correlations strongly support trans amide bond configurations across tetrapeptide mimic **6**.

We then synthesized heptapeptide mimic **8** featuring two IP scaffolds embedded within a larger strand (Scheme 3). Compound **8** again exhibited a well-resolved <sup>1</sup>H NMR spectrum that could be readily assigned using 2D COSY. The absence of NMR signals corresponding to minor rotational isomers was particularly notable due to the presence of 1 urethane and 4 amide bonds. Each of the amino acid CH<sub>α</sub> signals in **8** showed pronounced downfield shifts in CDCl<sub>3</sub> relative to their corresponding random-coil values.<sup>18</sup> In addition, the NH-CH<sub>α</sub> coupling constants obtained for the Ile and Leu residues were in the 8–9 Hz range, consistent with the values typical of a β-strand conformation. These coupling constants were used to calculate possible φ dihedral angles of the Ile, Leu, and Phe residues in **8** using the Pardi modification to the Karplus equation.<sup>19</sup> Amino acid residues in idealized parallel and antiparallel β-sheets exhibit φ values of approximately –119° and –139°, respectively (see Scheme 3).<sup>7</sup> The corresponding torsions in **8** are thus in good agreement with a β-strand-like conformation.

The ROESY data obtained earlier for **6** suggested that the IP<sub>Me</sub> <sup>1</sup>H NMR signal could serve as a useful reporter of local conformation. As with **6**, we observed ROESY correlations between the IP<sub>1Me</sub>-Leu<sub>NH</sub> and IP<sub>2Me</sub>-Phe<sub>NH</sub> protons in heptapeptide mimic **8**, indicating the presence of trans amide bonds between these residues (Figure 2). Additional correlations between Ile<sub>CH<sub>α</sub></sub>-IP<sub>1NH</sub> and Leu<sub>CH<sub>α</sub></sub>-IP<sub>2NH</sub> further supported an extended structure for **8** in solution. Dilution studies with **8** indicated a negligible chemical shift dependence on concentration, ruling out the presence of dimeric species that may give rise to intermolecular correlations (see Supporting Information).

Conformers with IP scaffold φ' torsions at or near 0°, exemplified by **A** in Figure 3, would be expected to exhibit a strong Overhauser correlation between IP<sub>1NH</sub> and H<sub>a</sub>. The absence of (non-vicinal) ROESY cross-peaks involving either protons H<sub>a/a'</sub> or H<sub>c/c'</sub> suggests that stable turn conformations are not significantly populated on the NMR time-scale. While 2D NMR does not rule out the possibility of acute Ile, Leu, or Phe φ angles, the J<sub>HNCH</sub> coupling constants observed for **8** (see Scheme 3) are not consistent with structures such as **B** (Figure 3). Moreover, the position of the Ile, Leu, and Phe NH resonances in the <sup>1</sup>H NMR spectrum of **8** (< 7.0 ppm) argue against their participation in intramolecular H-bonds resulting from tight turns.<sup>20</sup> We also carried out Δδ/ΔT experiments in DMSO-d<sub>6</sub> and found that each of the 5 NH protons in **8** exhibited a high (> 4.0 ppb/K) temperature dependence on chemical shift consistent with solvent accessibility (see Scheme 3 and Supporting Information).<sup>21</sup> Taken together, these data provide compelling evidence that heptapeptide mimic **8** adopts an extended conformation in CDCl<sub>3</sub>.

In summary, we have described the design and synthesis of novel extended dipeptide surrogates based on substituted imidazo[1,2-*a*]pyridine scaffolds. These building blocks can be readily prepared from  $\beta$ -ketoesters and incorporated into host peptides in either protected or unprotected forms. The efficient synthesis of model peptidomimetics harboring these scaffolds affords structures that adopt  $\beta$ -strand-like conformations in solution. We anticipate that the imidazo[1,2-*a*]pyridine subunit will find utility as a probe of peptide conformation, and as a dipeptide surrogate with enhanced drug-like character. Efforts toward IP-based  $\beta$ -strand mimics capable of disrupting protein-protein interactions are currently underway in our laboratory and will be reported in due course.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

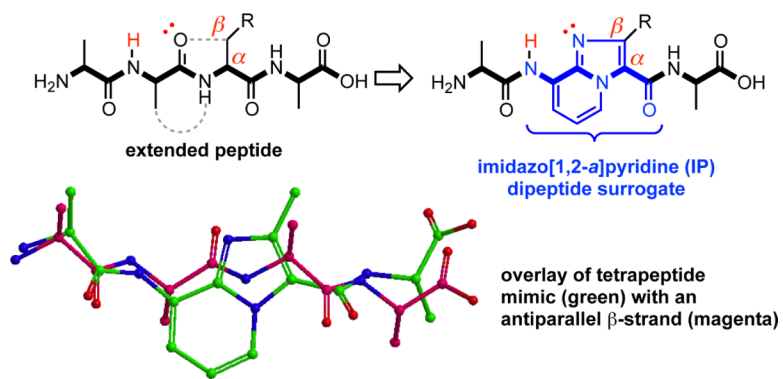
## Acknowledgments

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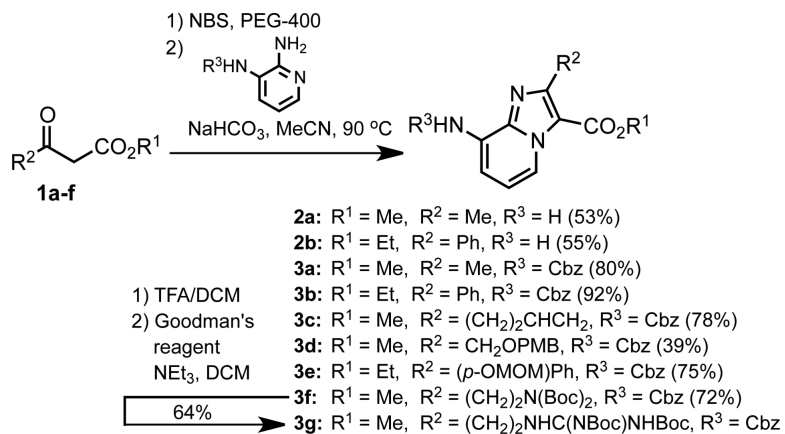
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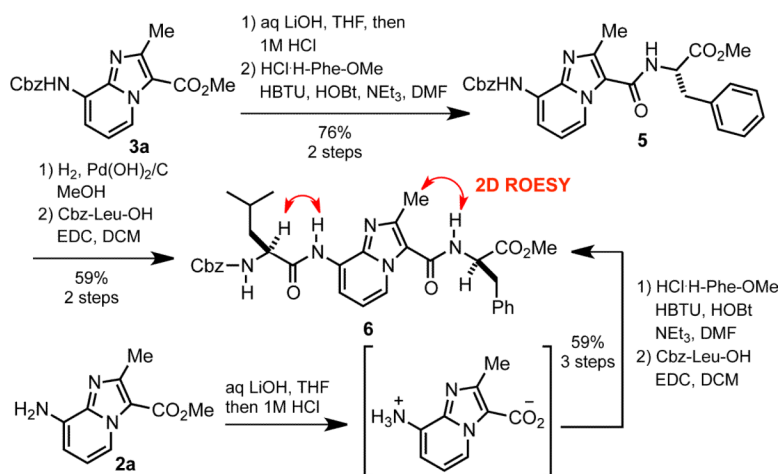
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**Figure 1.**  
Design of IP-based  $\beta$ -strand mimics.

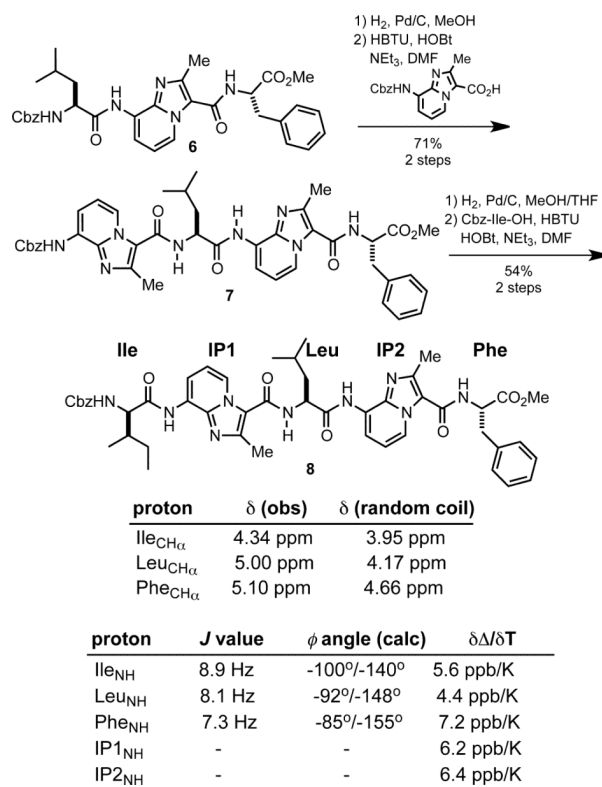
**Scheme 1.**

Synthesis of orthogonally-protected IP-based extended dipeptide surrogates.

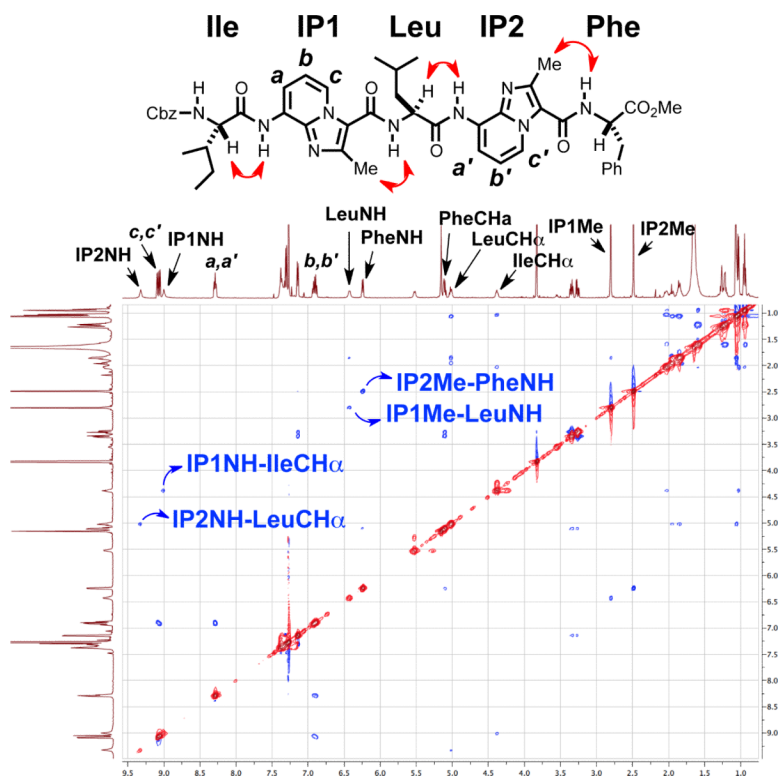


**Scheme 2.**  
Synthesis and selected ROESY correlations (in red) of tetrapeptide mimic **6**.

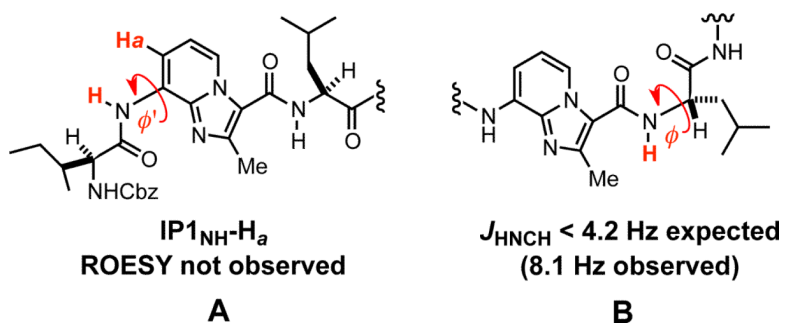


**Scheme 3.**

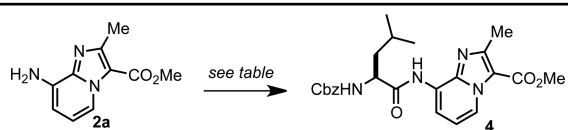
Synthesis of heptapeptide mimic **8** and conformational analysis by <sup>1</sup>H NMR.



**Figure 2.** ROESY spectrum of **8** (500 MHz in CDCl<sub>3</sub>) highlighting key Overhauser correlations.



**Figure 3.**  
Structures of possible turn conformations of **8**.

**Table 1**N-terminal coupling of IP scaffold **2a**.

entry	conditions <sup>a</sup>	yield <sup>b</sup>
1	1.2 equiv Z-Leu-OH, 1.2 equiv HBTU, HOBt, NEt <sub>3</sub> , MeCN	trace
2	1.2 equiv Z-Leu-OH, 1.2 equiv PyBOP, NEt <sub>3</sub> , DMF	trace
3	1.2 equiv Z-Leu-OH, 1.2 equiv EDC, HOBt, NEt <sub>3</sub> , DCM	trace
4	1.2 equiv Z-Leu-OH, 1.2 equiv EDC, 0.2 eq. DMAP, DCM	33 <sup>c</sup>
5	2.0 equiv Z-Leu-OH, 2.0 equiv EDC, 0.2 eq. DMAP, DCM	58 <sup>c</sup>
6	1.2 equiv Z-Leu-OH, 1.2 equiv EDC, DCM	59
7	<b>2.0 equiv Z-Leu-OH, 2.0 equiv EDC, DCM</b>	<b>91</b>
8	2.0 equiv Z-Leu-OH, 2.0 equiv PyBOP, DCM	33
9	2.0 equiv Z-Leu-OH, 2.0 equiv COMU, DCM	44
10	2.0 equiv Z-Leu-OH, 2.0 equiv DEPBT, DCM	26
11	2.0 equiv Z-Leu-OH, 2.0 equiv DCC, DCM	77

<sup>a</sup>All reactions carried out for 24 h at rt.<sup>b</sup>Isolated yields.<sup>c</sup>HPLC analysis of a phenylalanyl derivative showed significant epimerization of the Leu chiral center.