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Design and Synthesis of a Prototype Scaffold for Five-Residue α-Helix Mimetics

Andrew R. Bayly,[a] Andrew J. P. White,[a] and Alan C. Spivey*[a]

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The development of structural mimetics of α-helices has traditionally focused on representation of the three residues that protrude from one face of the helical surface on three consecutive turns (i.e., *i*, *i*+3/*i*+4, and *i*+7). Despite the decisive contribution these residues make to the binding interaction with protein partners, peripheral residues can play important roles particularly with regard to imparting selectivity. Here, we describe the design and synthesis of a model azabicyclo[2.2.2]octane aryl amide scaffold designed to compactly present the *i*, *i*+1, *i*+2, *i*+4, and *i*+5 residues of an α-helix.

Introduction

α-Helices are the dominant secondary structure present in cellular proteins.[1] Within proteins, helices play a key role in stabilization of protein tertiary structure and are important structural features at protein–protein interfaces (PPIs). Recent analysis of the Protein Data Bank (PDB) by Arora indicates that about 60% of multiprotein complexes feature α-helices at the interface.[2] Moreover, although about 60% of these α-helix-mediated PPIs are implicated as featuring hot-spot residues on just one face of the helix, ca. 33% utilize helices with hot spots on two faces and about 10% require all three faces for interaction with the target protein partner.[3] The development of approaches to the stabilization of α-helical peptides and also the creation of small molecules capable of mimicking the presentation of α-helix amino acid side chains, therefore, constitute active current fields of research.[4]

A major focus of synthetic small-molecule approaches to disrupting PPIs to date has centered on creating three-residue α-helix mimetics that feature the *i*, *i*+3/*i*+4, and *i*+7 residues, which all protrude from one face of a helix (Figure 1).[4d,5]

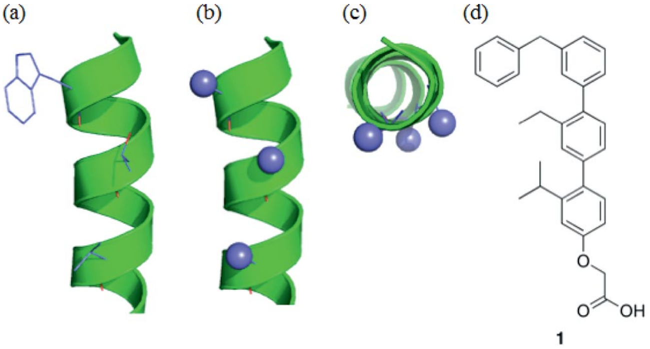

Figure 1. Design of the original Hamilton α-helix mimetic **1**.[5f] (a) Structure of smMLCK peptide binding partner with cAM with *i*, *i*+3, and *i*+7 residues displayed as blue sticks; (b) C_β of *i*, *i*+3, and *i*+7 residues displayed as blue spheres; (c) top view; (d) designed terphenyl mimetic **1**.

Despite some notable exceptions,[6] relatively few of this type of small-molecule three-residue α-helix mimetic have achieved sub-micromolar efficacies or been shown to display useful levels of target selectivity.[4a] Although these shortcomings are often attributed to the large binding interfaces that are characteristic of natural PPIs (ca. 800 Å^2 per protein on average),[7] they may also reflect a requirement for mimetics that project residues on additional faces of the relevant helices.

Synthesis of some α-helix mimetics that display more than one residue per turn (e.g., four-residue mimetics presenting *i*, *i*+3, *i*+4, and *i*+7 residues) have been described, but these have, with a few exceptions,[8] also thus far displayed limited potency and specificity.[9] Alternative small-molecule approaches that enable effective presentation of additional residues may offer the prospect of significantly extending and enhancing the current state-of-the-art.

Herein, we report the synthesis of an α-helix mimetic scaffold capable of presenting the five groups corresponding to the *i*, *i*+1, *i*+2, *i*+4, and *i*+5 helix side chains (i.e., a five-residue mimetic). It is hoped that this scaffold can provide a platform for mimetics with large 3D contact surfaces that can be tuned by independent variation at all five positions and display therapeutically useful levels of binding efficiency and selectivity in appropriate systems. This goal has

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[a] Department of Chemistry, Imperial College London,
South Kensington, London, SW7 2AZ UK
E-mail: a.c.spivey@imperial.ac.uk
Homepage: http://www.imperial.ac.uk/spiveygroup

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not yet been achieved, but the chemistry foundations that may facilitate this have been laid and are described.

Results and Discussion

α-Helix Mimetic Design

Identification of a molecular scaffold capable of representing five out of six consecutive amino acid residues began with analysis of the positions of the relevant α-stereogenic carbon atoms (C_α) in a representative α-helix. The XLXXLL motif within the α-helical "NR box 1" epitope of the nuclear receptor (NR) cofactor SHP was used for this purpose (PDB: 1YUC residues 20–25).[10] This structure revealed distances of about 3.8 and 5.3 Å between the $i{+}1 \leftrightarrow i{+}2$ C_αs and the $i{+}1 \leftrightarrow i{+}3$ C_αs, respectively (Figure 2).

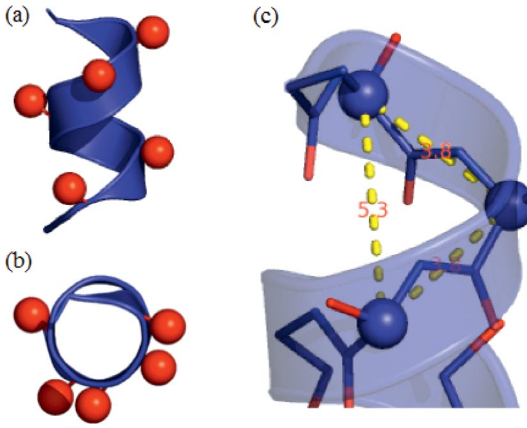


Figure 2. Analysis of consecutive amino acids in the SHP NR box 1 region (PDB: 1YUC residues 20–25). (a) α-Helix with i, $i{+}1$, $i{+}2$, $i{+}4$, and $i{+}5$ C_αs displayed with red spheres; (b) top view; (c) distances between $i{+}1 \leftrightarrow i{+}2$, $i{+}2 \leftrightarrow i{+}3$, and $i{+}1 \leftrightarrow i{+}3$ C_αs, with C_αs displayed by blue spheres.

Taking into account these distances and the desire to project the C_α–C_β bonds with the correct vectors, the mimetic design focused on bicyclic systems. Inspection of the geometry of many bicyclic systems identified bicyclo[2.2.2]octane scaffold **2** as capable of projecting three consecutive amino acid side chains (i, $i{+}1$, $i{+}2$) from its core with the appropriate C_α–C_β bond vectors. Analysis of the remaining bond vectors of bicyclic scaffold **2** suggested that an *endo*-2,5-disubstituted aryl amide could present the remaining $i{+}4$ and $i{+}5$ residues.[11] In the interests of devising a synthetic approach that would be modular and convergent whilst striving to minimize the lipophilicity of the scaffold, we selected azabicyclo[2.2.2]octane *endo* amide **3** as our initial target. This model scaffold was considered to embody all the required features for a model scaffold on which to develop the essential chemistry. It contains methyl groups at positions that correspond to where the C_α (i, $i{+}2$, $i{+}4$, and $i{+}5$) and C_β ($i{+}1$) atoms of side chains in viable mimetics of the SHP NR box 1 will be situated (Figure 3).

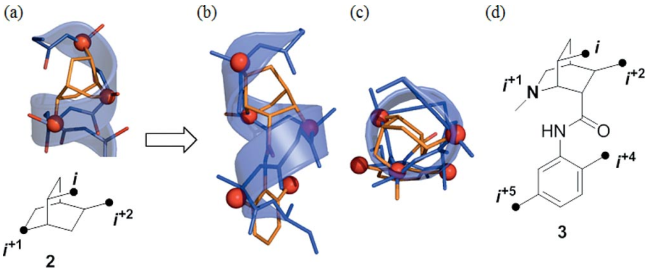


Figure 3. Design of α-helix mimetic scaffold **3**. (a) Overlay of bicyclo[2.2.2]octane scaffold **2** onto α-helix C_αs; (b) overlay of complete scaffold **3** onto α-helix;[11] (c) top view; (d) structure of **3**.

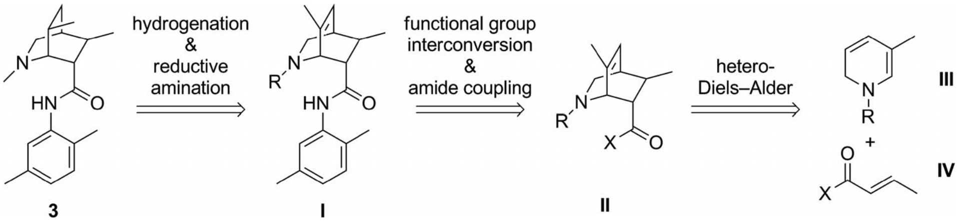
Herein, we describe a synthetic approach to compound **9a**, which is an isomer[12] of model compound **3**, through a synthetic route that should be amenable to the preparation of full α-helix mimetics based on this new scaffold.

Synthesis

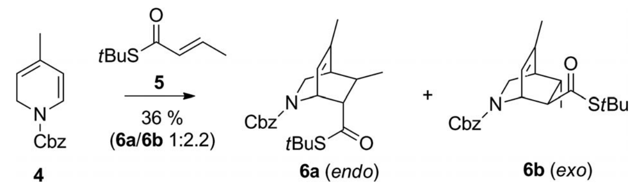
Retrosynthetic analysis of target molecule **3** identified a convergent strategy for its construction (Scheme 1).

For synthesis, these disconnections suggest a hetero-Diels–Alder reaction between 5-substituted-1,2-dihydropyridine **III** and (*E*)-α,β-unsaturated carboxylic acid derivative **IV** to establish *endo*-azabicyclo[2.2.2]octane core **II**. Aryl amide coupling (**II**→**I**) and then *sec*-amine deprotection (e.g., *N*-Cbz→*N*-H; Cbz = benzyloxycarbonyl), reductive alkylation (e.g., NH→N-Me), and diastereoselective alkene hydrogenation was anticipated to lead to target model compound **3**.

Many conditions were explored to reduce the Diels–Alder cycloaddition reaction to practice. It was during this phase of the work that *N*-Cbz-protected 4-methyl-1,2-dihydropyridine **4** was employed as the heterodiene rather than the less readily available[13] 5-methyl-1,2-dihydropyridine (cf. **III**).[12] Although crotonate esters were insufficiently reactive to participate in the required [4+2] cycloaddition, *tert*-butyl thioester **5** reacted successfully (Scheme 2).[14]



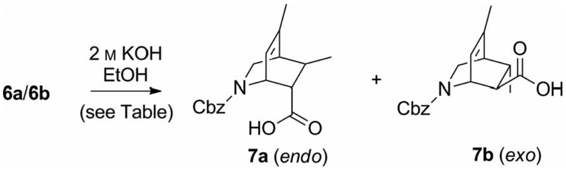
Scheme 1. Retrosynthetic analysis for α-helix mimetic model scaffold **3**.



Scheme 2. Synthesis of azabicyclo[2.2.2]octanes **6a** and **6b**.

Thermal conditions furnished an inseparable ca. 1:2.2 mixture of desired *endo*- and undesired *exo*-azabicyclo[2.2.2]octanes **6a** and **6b**, respectively, in 36% yield.[15] As attempts to effect direct amide coupling from thioesters **6a/6b** resulted in complete recovery (rec.) of starting material, hydrolysis to the corresponding carboxylic acids was performed (Table 1).

Table 1. Hydrolytic partial kinetic resolution of thioester **6**.

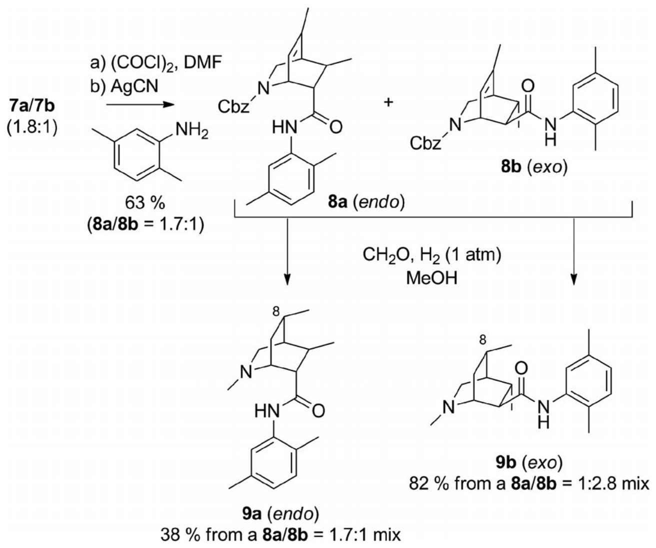


Entry	6a/6b	T [°C]	T [h]	Yield **7a** + **7b** (rec. **6a** + **6b**)	**7a/7b**[a]
1	1:2.2	20	3	5 (75)	1:5
2	1:2.2	80	16	10 (0)	1:1.5
3	1:2.2	45	3	78 (0)	1:2.8
4	1:2.2	35	3	66 (32)	1:2.5
5	2:1	45	3	74 (0)	1.8:1

[a] Ratio determined by ^{1}H NMR spectroscopy.

Attempted hydrolysis of thioesters **6a/6b** (ca. 1:2.2) by using 2 M KOH in EtOH at room temperature for 3 h allowed isolation of just 5% yield of acids **7a/7b** (ca. 1:5) along with 75% yield of recovered starting thioesters **6a/6b** (ca. 1:2; Table 1, entry 1). This suggested that hydrolysis of *exo* thioester **7b** proceeded at a greater rate than *endo* isomer **7a**, presumably due to its carbonyl being less sterically hindered. Heating the mixture at reflux at 80 °C for 16 h degraded **6a/6b** (Table 1, entry 2), whereas heating at 45 °C for 3 h effected complete hydrolysis of both isomers to give acids **7a/7b** in 78% yield (Table 1, entry 3). However, partial kinetic resolution could be achieved by conducting the reaction at 35 °C for 3 h, which allowed recovery of a mixture of thioesters **6a/6b** enriched in the desired *endo* isomer (ca. 2:1) in 32% yield (Table 1, entry 4). This mixture could then be further hydrolyzed at 45 °C to give *endo*-enriched acids **7a/7b** (ca. 1.8:1) in 74% yield (Table 1, entry 5).

Coupling of this *endo*-enriched acid mixture, via the acid chloride derivatives, with 2,5-dimethylaniline mediated by AgCN afforded inseparable amides **8a** and **8b** in a ratio of ca. 1.7:1 (63% combined yield, Scheme 3).



Scheme 3. Amide coupling and then "one-pot" *sec*-amine deprotection/reductive methylation/diastereoselective alkene hydrogenation to generate scaffold **9a** and isomer **9b**.

To complete the synthesis, one-pot *N*-Cbz hydrogenolysis, reductive methylation with formaldehyde, and fully diastereoselective[16] alkene hydrogenation was performed on amide mixture **8a/8b** (1.7:1) by using 20 wt.-% Pd/C in MeOH to afford complete α-helix model **9a** in 38% yield after purification by HPLC. This compound can be considered as corresponding to a Gly^{i}-Ala^{i+1}-Gly^{i+2}-X^{i+3}-Gly^{i+4}-Gly^{i+5} α-helical peptide.

An identical procedure was performed on a separate batch of amide mixture **8a/8b** (1:2.8) to give isomer **9b** in 82% yield, the identity of which was confirmed by single-crystal X-ray structure determination (Figure 4).[15]

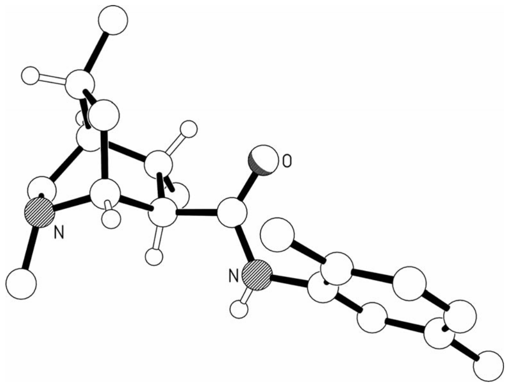


Figure 4. Molecular structure of compound **9b** as determined by single-crystal X-ray crystallography.

Conclusions

We have delineated a synthetic approach to the synthesis of azabicyclo[2.2.2]octane aryl amide **9a** that we believe comprises a promising scaffold for five-residue α-helix mimetic development. Although the reactions will require optimization, we anticipate that this route should prove amenable to the assembly of an array of azabicyclo[2.2.2]octane containing mimetics presenting combinations of hydrophobic groups corresponding to the *i*, *i*+1, *i*+2, *i*+4, and *i*+5 side chains found in numerous biologically significant PPIs. Work towards this end is ongoing in our laboratory and will be reported in due course.

Supporting Information (see footnote on the first page of this article): All experimental procedures and ^{1}H NMR and ^{13}C NMR spectra for compounds **4**–**9**.

Acknowledgments

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[11] The bond geometry of complete scaffold **3** was generated by using conformational analysis on Spartan molecular modeling software.

[12] Product **9a** differs from target compound **3** in that one methyl group on the azabicyclo[2.2.2]octane core is transposed to the adjacent carbon atom. This is because 4-methyl-1,2-dihydropyridine **4** was employed in place of 5-methyl-1,2-dihydropyridine, in: this exploratory work for ease of synthesis.

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[15] The stereochemical assignment of **6a** and **6b** follows from the X-ray structure determination of *exo* compound **9b** (Figure 4).

[16] For both **9a** and **9b**, a single peak in the ^{1}H NMR spectrum for the C-8 methyl substituent was observed, which indicates a completely face-selective hydrogenation.

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