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Super-secondary structure peptidomimetics: design and synthesis of an α-α hairpin analogue

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Abstract

The α-α helix motif presents key recognition domains in protein-protein and protein-oligonucleotide binding, and is one of the most common super-secondary structures. Herein we describe the design, synthesis and structural characterization of an α-α hairpin analogue based on a tetra-coordinated Pd(II) *bis*-(iminoisoquinoline) complex as a template for the display of two α-helix mimics. This approach is exemplified by the attachment of two biphenyl peptidomimetics to reproduce the side-chains of the *i* and *i+4* residues of two helices.

Keywords

structural mimicry; protein-protein interaction; alpha helix, side chain, GCN4

Introduction

The design of non-peptidic small-molecules to reproduce the recognition properties of therapeutically relevant secondary structural protein elements is now an established field.[1] With a particular emphasis on the moderation of protein-protein interactions (PPIs), there are many examples of peptidomimetics of α-helices[2,3,4] and a growing number that mimic some of the properties of β-strands and β-sheets.[5,6] However, there are few non-peptidic mimics of more complex super-secondary structures.[7] The majority of approaches are based on the modification of peptidic folding preferences *via* manipulation of the primary sequence.[8,9] Examples include careful positioning of non-natural α,β-dehydrophenylalanine to promote hydrophobic interactions,[10] or the incorporation of reactive natural amino acids, including cysteine,[11] that are responsive to an oxidative stimulus.[12] Strategies relying on the templating effect of cyclic peptides or synthetic molecules, such as norbornene, have also been explored.[13,14]

One of the most common super-secondary structures is the α-α hairpin, in which a sequence of amino acids links two α-helices, usually in an antiparallel arrangement.[15,16] Others

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include the β-α-β motif,[17,18] α-α corner,[19] β-β corner,[20] and β-β hairpin (Figure 1).[21] Various models for α-helix packing emphasise the importance of helix complementarity,[22,23,24] in which the nature of the side-chains determines the packing (or torsion) angle **ω**.[25,26] For the α-α hairpin De Grado found a strong correlation between the number of linking residues and the inter-helical distance (the average of the orthogonal space between the axis of the two helices).[27,28] Distances range between 5 Å for short linkers of one or two amino acids, with many examples of 10 Å where the turn is formed of several amino acids.

Results and discussion

Herein we report the design and synthesis of a tetra-coordinated Pd(II) *bis*-(iminoisoquinoline) complex that templates the display of a range of α-helical peptidomimetics to reproduce the structural characteristics of an α-α hairpin. To replicate the inter-helical loop angle ω, of approximately 0° for the parallel disposition and 180° for the anti-parallel hairpin, we sought a metal complex with a square-planar coordination geometry.[29,30] Inspired by previously reported Pd(II)-2-imino-pyridine complexes,[31,32] and other metals with a N_4 coordination geometry,[33] we designed and synthesized *bis*-(imino-isoquinoline) ligand **4** (Scheme 1).

The synthesis commenced with nitration of 1-methyl-isoquinoline to give exclusively the 5-nitro regioisomer **2**,[34] followed by selenium dioxide-mediated oxidation of the methyl group to aldehyde **3**.[35] Condensation with 1,3-diaminopropane provided di-imine **4** as a reactive intermediate. Addition of commercially available tetrakis(acetonitrile)palladium(II) tetrafluoroborate gave complex **5**, with an immediate chromatic change of the reaction mixture from yellow to dark orange-brown. Purification was achieved by precipitation from acetonitrile with chloroform (Scheme 1).

Upon chelation, the ^{1}H-NMR signals corresponding to the methylenes adjacent to the imine nitrogens, and the central methylene of the propyl linker, show a change in multiplicity attributable to the introduction of cyclic rigidity. In addition, resonances corresponding to the aromatic and imine hydrogens undergo marked downfield shifts consistent with the withdrawal of electron density (Table 1, compound **5**). Further characterization confirms the presence of the complex, with mass spectrometry showing ions consistent with the free di-cationic complex, and that of the mono-tetrafluoroborate anion. Elemental analysis is in good agreement with the hypothesized structure: found (calculated) C, 38.06 (38.24); H, 2.56 (2.51); N, 11.51 (11.63).

Single-crystal X-ray diffraction was performed on samples grown at room temperature through slow evaporation of diethyl ether into a solution of complex **5** in acetonitrile.[36] A slightly distorted square-planar geometry at palladium was observed with a mean planar deviation of 0.053 Å for the four Pd-N contacts. N-Pd-N *cis*- and *trans*-bite angles ranged from 79.67 to 105.16 and 174.91 to 172.94° respectively. The distance between the nitrogen atoms of the nitro groups, which provide attachment points for helix-mimetics, is 10.76 Å and thus congruent with the properties of common α-α hairpin motifs in proteins (Figure 2, see also supporting information Chapter 3).

In order to adapt scaffold **5** for the display of helix mimetics, the synthesis required modification to allow nitro group reduction and coupling prior to imine formation and complexation. Accordingly, aldehyde **3** was protected as a dimethyl acetal and the nitro group reduced to give amine **7** in excellent yield (86 – 90 % over two steps). As proof of principle we chose commercially available 3-methyl-4-nitrobenzoic acid **8**, as a mimic of the alanine side-chain and previously synthesised biphenyl carboxylic acid **9**[37] as a mimic of two leucine residues in the *i* and *i+4* positions. These acids were activated with thionyl chloride before coupling to give amides **10** and **11** respectively. The aldehyde was un-masked in good yield after treatment with a mixture of acetic- and hydrochloric acids. Di-imines were synthesized with 1,3-diaminopropane and used for complexation without further purification. Following the procedure for the synthesis of complex **5**, precipitation gave two-amino acid mimetic **16** as a dark yellow powder in 60 % yield (Scheme 2).

In accordance with model system **5**, the ^{1}H-NMR spectra of complexes **16** and **17** show a similar downfield shift of resonances when compared to those of the free ligands **14** and **15** (Table 1). Electrospray mass spectrometry and elemental analysis are also in close agreement with calculated isotope patterns (see supporting information).

Whilst we were unable to obtain diffraction-quality crystals of complexes **16** or **17**, a computationally derived[38,39] low energy conformation of four amino acid residue mimic **17** gave encouraging results when superimposed on the dimer conformation of the leucine zipper transcription factor GCN4.[40] Given that coiled-coil α-helical transcription factors control the expression of myriad genes, it is an appealing strategy to use a synthetic agent to regulate transcription, and thus influence the translation of proteins implicated in human disease. The C_α positions of the peptide show good agreement with those of the oxygen atoms of the biphenyl helix (Figure 3).

Conclusions

We have designed, synthesized and structurally characterized a simple linker based on a Pd(II) *bis*-(iminoisoquinoline) complex that reproduces the geometric characteristics of a peptidic hairpin motif. Two amino groups allow for the attachment of a diverse array of peptidomimetics *via* amide bond formation. The approach was exemplified by the linking of α-helical mimics of the *i* and *i+4* positions to replicate the structure of an α-α hairpin. However, the synthetic strategy is flexible and convergent, and in principle allows for the display of homo- or hetero-dimeric combinations of helical peptidomimetics to produce a wide range of super-secondary structural mimics. Improved aqueous solubility may be aided by the use of recently reported helix mimics that contain a greater number of heteroatoms in the scaffold, or the inclusion of side-chain mimics bearing polar groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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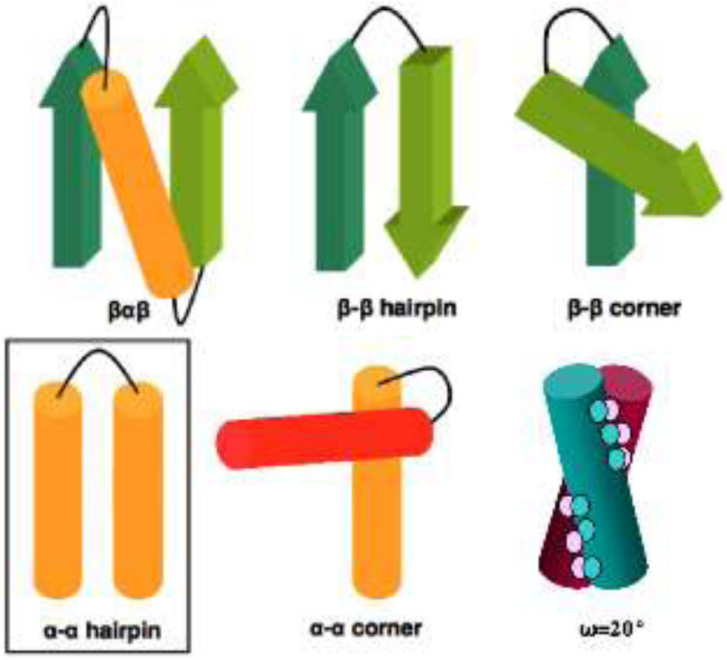


Figure 1.
Common super-secondary structural motifs, with the focus of this work – the α-α hairpin, highlighted. Bottom right: an omega angle of 20° between two α-helices.

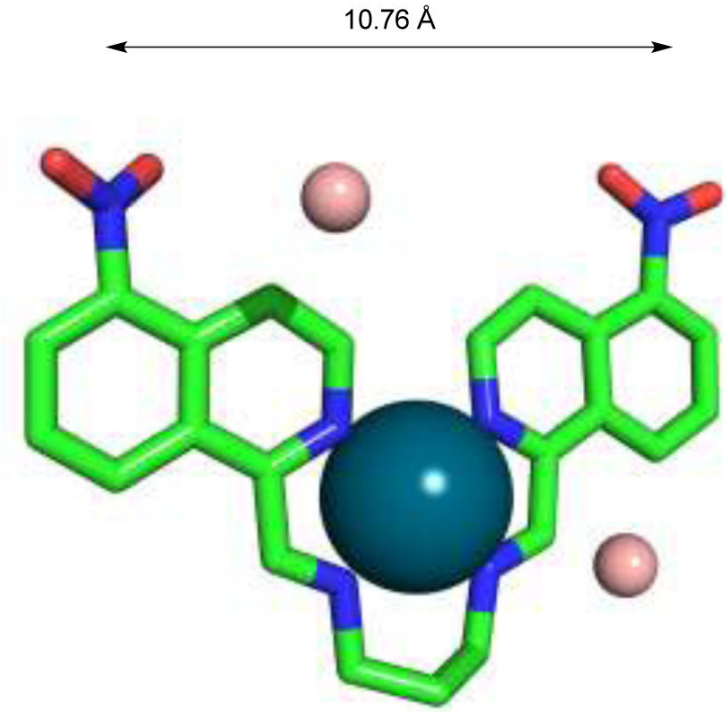


Figure 2.

Single-crystal X-ray structure of Pd(II) *bis*(iminoisoquinoline) complex **5** (palladium grey, tetrafluoroborate brown). The position of the tetrafluoroborate ions is characterized by close intermolecular contacts: Pd···F 2.85 and 3.91 Å.

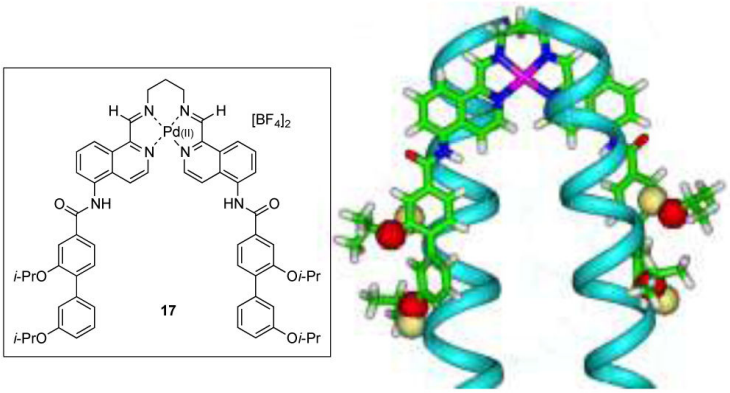
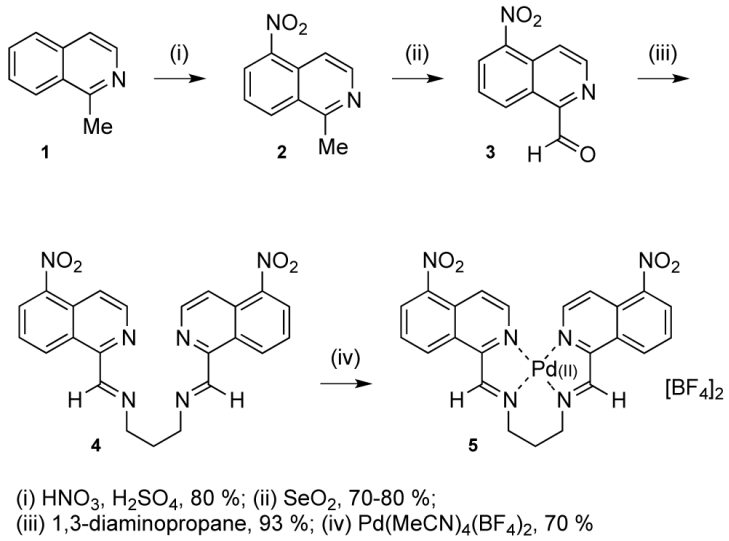
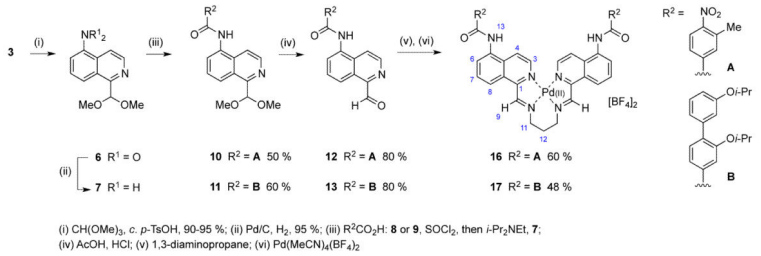


Figure 3.

Superimposition of a low-energy conformation of four-residue *bis*-helix mimetic **17** (green sticks and red spheres) and the GCN4 dimer (pdb: 2ZTA; blue ribbon and yellow spheres).



Scheme 1.
Synthesis of a model linker **5**.



Scheme 2.

Synthesis of α-α hairpin mimetics bearing two **16**, and four **17**, side-chain groups. The methyl and *iso*-propoxy groups may be considered as mimics of alanine and leucine amino acid side-chains.

Table 1

Selected ^{1}H-NMR chemical shifts of the free ligand and the corresponding Pd(II) complex (**5, 16** and **17**). Values in ppm, multiplicities in brackets: (s) singlet, (d) doublet, (t) triplet, (qn) quintet, (m) multiplet, (br) broad singlet. [a] The chemical shift of this signal is overlapped with those of the biphenyl group.

Compound	^{1}H Signal	Free Ligand	Complex
5	3	8.70 (d)	9.11 (d)
5	4	8.24 (d)	8.91 (m)
5	6	8.38 (d)	8.91 (m)
5	7	7.62 (t)	8.21 (m)
5	8	9.88 (d)	8.94 (d)
5	9	8.77 (s)	9.60 (br)
5	11	4.02 (t)	4.11 (m)
5	12	2.37 (q)	2.44 (m)
16	3	8.27 (d)	8.62 (d)
16	4	7.36 (d)	8.50 (d)
16	6	7.48 (d)	7.99 (d)
16	7	7.09 (t)	8.07 (m)
16	8	7.84 (d)	8.25 (d)
16	9	8.72 (s)	9.54 (s)
16	11	4.00 (t)	4.09 (m)
16	12	2.40 (q)	2.45 (m)
16	13	7.80 (m)	9.40 (s)
17	3	[a]	8.65 (d)
17	4	[a]	8.50 (d)
17	6	[a]	8.21 (d)
17	7	[a]	8.09 (m)
17	8	[a]	8.46 (d)
17	9	8.77 (br)	9.54 (s)
17	11	4.03 (m)	4.11 (m)
17	12	2.43 (m)	2.44 (m)
17	13	[a]	9.31 (s)