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COMMUNICATION

Amphiphilic α-helix mimetics based on a benzoylurea scaffold†‡

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The design and synthesis of amphiphilic benzoylurea α-helix mimetics is described. These conformationally constrained molecules allow for the correct angular and spatial projection of hydrophobic and hydrophilic groups and thus the reproduction of side-chains on *both* faces of an α-helix.

The inhibition of aberrant protein–protein interactions using small molecules is an attractive approach for the treatment of a range of pathological conditions.[1] One strategy for the design of such agents involves the mimicry of common intrafacial domains such as α-helices where key side-chain residues (often the *i*, *i* + 4, *i* + 7 positions) play an important role.[2] Exploitation of this approach led to the discovery of a compound based on the benzoylurea scaffold **I** that has shown 2.4 μM inhibition of the Bcl-xL–Bak interaction in a fluorescence polarization assay.[3]

However, there are few examples of non-peptidic molecules able to reproduce the position and angular projection of side-chains on two faces of an α-helix.[4] The side-chains projecting from the exterior face of an α-helix have been widely implicated in the binding of multiple proteins, bacterial cell wall sensing and membrane penetration.[5]

The benzoylurea scaffold offered a logical extension to an amphiphilic mimic **II** of the *i*, *i* + 1, *i* + 4, *i* + 6 and *i* + 8 positions of a peptide (Fig. 1). To the best of our knowledge there are currently no mimics of this selection of amino acid side-chains. We rationalised that commercially available dihydroxylated aromatics would serve as good starting materials for our syntheses. These molecules allow for a range of straight-forward alkylation reactions and thus the creation of bespoke mimics in which both lipophilic and hydrophilic amino acid side-chains are reproduced. As targets for mimicry we selected aspartic acid, leucine and methionine as representative examples of amino acids with polar and non-polar side-chains.

Synthesis of the *i*, *i* + 1, *i* + 4 component commenced with protection of 3,5-dihydroxybenzoic acid as the benzyl ester[7] and alkylation with *iso*-propyl iodide and *tert*-butyl bromoacetate to give **4**. The allyl **6** and 3-butenyl **7** amides were prepared in excellent yields *via* hydrogenolysis of the benzyl ester and carboxyl activation through acid chloride formation (Scheme 1).

To simplify the synthesis we initially designed a route to an *i* + 6, *i* + 7 component (rather than *i* + 6, *i* + 8). Dialkylation of 2-nitroresorcinol, followed by reduction of the nitro group and isocyanate formation, afforded a precursor of the *i* + 6, *i* + 7 component **11** in an overall yield of 52% (Scheme 2).

Under our standard conditions of benzoylurea formation with lithium bis(trimethylsilyl)amide (LiHMDS) in tetrahydrofuran at

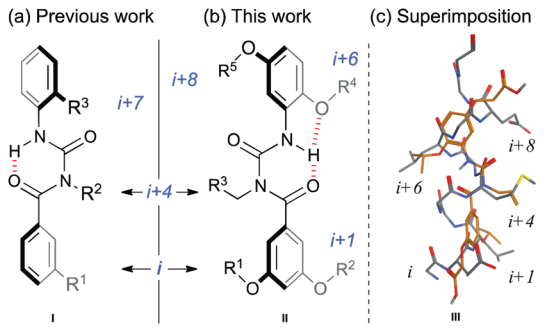
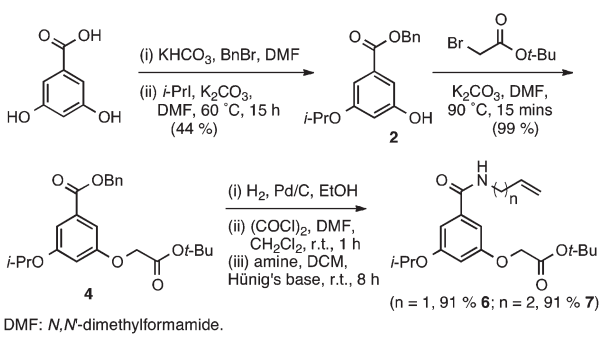


Fig. 1 A synthetic scaffold for mimicry of an α-helix. (a) **I** benzoylurea scaffold with R^1, R^2, R^3 mimicking the *i*, *i* + 4, *i* + 7 side-chains on one face of an α-helix; (b) **II** amphiphilic mimic of the *i*, *i* + 1, *i* + 4, *i* + 6 and *i* + 8 side-chains on *both* faces of an α-helix. (c) Superimposition of the calculated lowest energy conformer of **II** (orange, $R^1 = R^5 = CH_2CO_2H$, $R^2 = R^4 = i$-Pr, $R^3 = CH_2CHCH_2$) with an α-helix (grey bonds) showing good spatial and angular agreement of substituents with side-chains.[6]



Scheme 1 Synthesis of the *i*, *i* + 1, *i* + 4 component.

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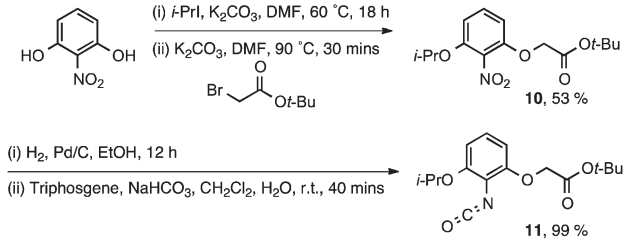
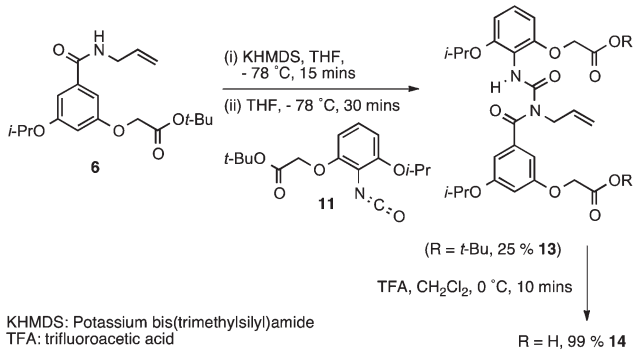
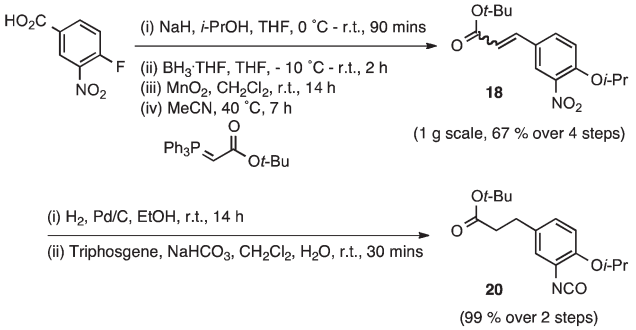


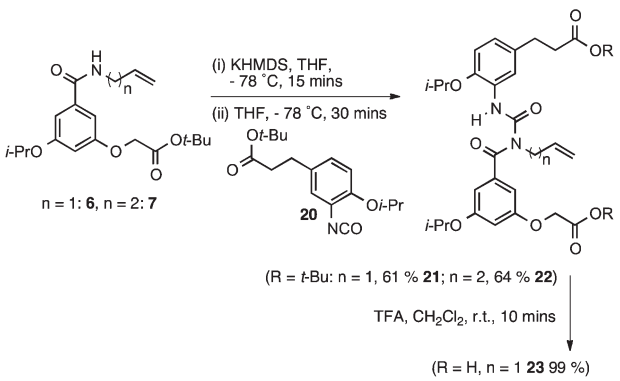
Scheme 2 Synthesis of the $i + 6$, $i + 7$ component.



Scheme 3 Fragment union giving a benzoylurea mimic of the i, $i + 1$, $i + 4$, $i + 7$ and $i + 8$ side-chain residues.



Scheme 4 Synthesis of the $i + 6$, $i + 8$ component.



Scheme 5 Fragment union giving a benzoylurea mimic of the i, $i + 1$, $i + 4$, $i + 6$ and $i + 8$ side-chain residues.

−78 °C,[8] there was little reaction between amide **6** and isocyanate **11**. Since we were able to recover significant amounts of the starting amide and inseparable mixtures of compounds apparently derived from the isocyanate, we reasoned that the extra steric bulk of the 6-substituent was hindering nucleophilic approach of the lithiated amide. Raising the temperature of the reaction mixture during addition of the isocyanate failed to give desired product, however generating a more reactive amide with potassium bis(trimethylsilyl)amide (KHMDS) allowed isolation of **13** in low yield. It was important to maintain the reaction at −78 °C and to quench the mixture with acetic acid. Removal of the *t*-butyl protecting groups with trifluoroacetic acid proceeded in excellent yield (Scheme 3).

In order to form an $i + 6$, $i + 8$ component we synthesised a 2,5-disubstituted isocyanate **20** from 4-fluoro-3-nitrobenzoic acid in 6 steps and 66% overall yield. Whilst more direct approaches from 4-chloro-3-nitrobenzaldehyde and 4-hydroxy-3-chlorobenzaldehyde were unsuccessful, we were able to synthesise **18** on a gram scale with a single chromatographic step in 67% yield (Scheme 4).

When treated with KHMDS in tetrahydrofuran at −78 °C, *N*-allyl **6** and *N*-butenyl **7** amides reacted with 2,5-disubstituted isocyanate **20** to give benzoylureas **21** and **22** in 61% and 64% yields respectively. Acidic deprotection of the *t*-butyl esters gave di-carboxylic acid **23** (Scheme 5).

A single crystal X-ray structure of **22**‡ confirmed the connectivity and the presence of N–H···O=C (1.9 Å) and N–H···O*i*-Pr (2.2 Å) hydrogen bonds (Fig. 2a).[9] Superimposition of this structure with a model peptide shows good overlap of substituents with the i, $i + 1$, $i + 4$, $i + 6$ and $i + 8$ side-chain residues (Fig. 2b), and is in accordance with our computational modelling

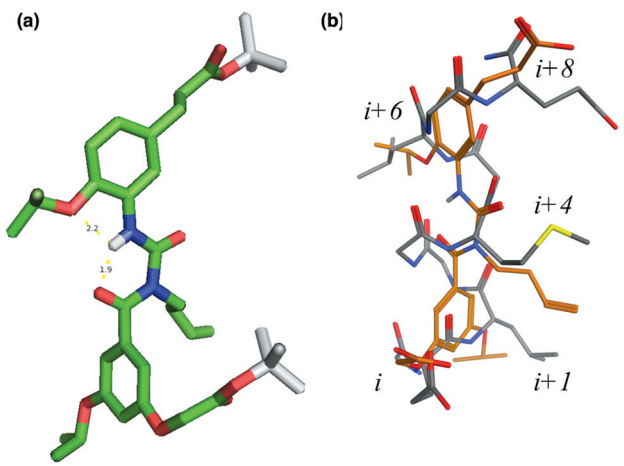


Fig. 2 (a) X-ray crystal structure of benzoylurea **22** with two intra-molecular hydrogen bonds providing a constrained conformation (values in Å, *t*-butyl groups shown in grey). (b) Superimposition of X-ray structure **22** (orange) with an α-helix (grey bonds). There is good agreement between the side-chain angles and positions of the i, $i + 1$, $i + 4$, $i + 6$ and $i + 8$ residues (*t*-butyl groups omitted for clarity).

work (Fig. 1c). The root mean square deviation (RMSD) for the five peptide α-carbon atoms and the corresponding scaffold positions was calculated as 1.25 Å.[10] While this value is larger than those often reported for peptidomimetics it is important to

consider that the RMSD value will not scale linearly with the number of positions considered. Previous reports have generally measured the 'goodness-of-fit' of only three groups and not the five substituents of molecule **22**. In this scaffold the RMSD value for the groups on one face of the molecule (i, $i + 4$ and $i + 8$) is 0.84 Å, which compares comparably to the previously reported benzoylurea mimic of the i, $i + 4$ and $i + 7$ side-chains (0.67 Å).[3]

^{1}H NMR of the deprotected molecule **23** shows the benzoylurea N–H resonance as a sharp singlet at 11.38 ($CDCl_3$) and 11.46 (DMSO-d_6) consistent with previous studies,[8] and supports the existence of an intra-molecular hydrogen bond and thus a linear scaffold conformation in both of these solvent systems.

In summary, we have designed a route to amphiphilic α-helix mimetics based on the well-established benzoylurea scaffold, and have implemented this in a modular and scalable synthesis of molecules accurately reproducing the spatial and angular projection of five amino acid side-chains on both sides of an α-helical strand. This represents a significant improvement in the scope of non-peptidic peptidomimetics, allowing for mimicry of hydrophobic and hydrophilic side-chains on two faces of an α-helix.

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