H-Bonded Foldamers

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Benzoylurea Oligomers: Synthetic Foldamers That Mimic Extended α Helices**

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A key role of α-helical domains within proteins is to act as rigidifying functionalized scaffolds that define a functional architecture.[1] Projection of side chains from the surface of the α helix allows it to contact other residues intramolecularly in a folded protein or intermolecularly in mediating protein–protein or protein–DNA interactions. In some cases, long stretches of α-helical domains form extended protein structures through homo- or heterodimerization, as seen in proteins ranging from transmembrane protein channels to four-helix bundles.[2] These interactions are crucial for both the structural stability and overall function of the protein.

In recent years an idea has taken hold that non-natural oligomers might take up helical conformations,[3] and in doing so, mimic the structure and function of α helices. Particular progress has been shown with β-amino acid homologues[4] of natural peptides, which can be used to inhibit helix–protein contacts, such as those found in p53/MDM2 (murine double minute)[5] and Bak/Bcl-x_L.[6] Helical conformations have also been observed in other oligomers based on peptoids,[7] γ-amino acids,[8] δ-amino acids,[9] pyridine dicarboxamides,[10] quinoline oligoamides,[11] and anthranilamides.[12] An alternative approach to α-helix mimicry ignores the helical conformation and in its place seeks scaffolds that project functional side chains in an analogous fashion[13–15] or mimic the organizational capabilities of helices.[16] In this regard, oligophenylene derivatives have attracted attention owing to their rigidity and functional stability.[10,13]

We have previously shown that trisubstituted terphenyl derivatives can mimic the side-chain projection of two turns of an α helix and can disrupt various helix–protein interactions.[14,15,17] The further extension of these mimetics might involve, for example, a nine-ring differentially substituted oligophenylene, such as **1**, reproducing eight turns of an α helix and long enough to span a bilayer membrane (Figure 1). However, the synthesis of **1** would be challenging and result in a highly hydrophobic product. In searching for alternatives, we were struck that an acylurea group might replace certain aryl rings in this scaffold through intramolecular hydrogen bonding.[18] Thus, an oligomer composed of *para*-aminobenzoic acid derivatives could form an alternating aromatic ring–hydrogen-bonded acylurea structure, such as **2**, with a similar overall shape to oligophenylene **1** (Figure 1).

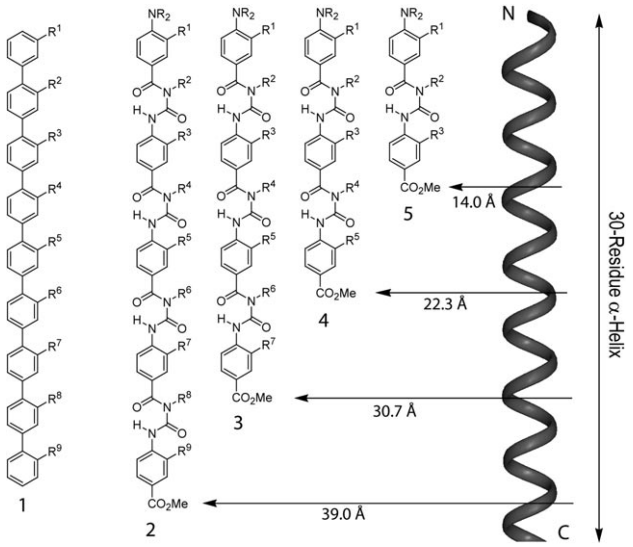
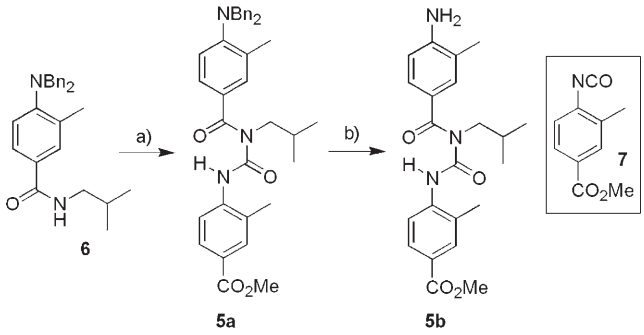


Figure 1. Structures of oligophenylene **1** and benzoylureas **2–5** in comparison to an ideal α helix. N and C refer to the N and to the C terminus, respectively. R = Bn or H, $R^1 = R^3 = R^5 = R^7 = R^9 = CH_3$, $R^2 = R^4 = R^6 = R^8 = i$Bu.

An iterative synthesis was developed for the benzoylurea oligomers using two basic monomer units, a secondary amide and an isocyanate derived from 4-aminobenzoic acid (see Supporting Information). A representative cycle of the synthetic procedure is outlined in Scheme 1. Deprotonation of amide **6** with LiHMDS followed by nucleophilic attack on



Scheme 1. Synthesis of **5b**. Reagents and conditions: a) LiHMDS, THF, −78 °C, 5 min, then **7**, −78 °C, 15 min; b) H_2, Pd/C, EtOAc/MeOH. Bn = benzyl, HMDS = hexamethyldisilazide.

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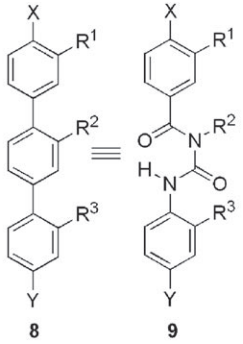
isocyanate **7** gives a bis(benzyl)-protected benzoylurea **5a** that is subsequently deprotected to afford the functionalized benzoylurea **5b** in good yield. ^{1}H NMR spectroscopy experiments performed on a model benzoylurea compound **9a** (Figure 2a) show little change in the amide-NH resonance of

a)



Compound	X	Y	R^1	R^2	R^3
8a	OCH_2CO_2H	$(CH_2)_2CO_2H$	*i*Bu	CH_2-2-naphthyl	*i*Bu
9a	H	H	CH_3	CH_3	CH_3
9b	NH_2	CO_2CH_3	CH_3	CH_3	CH_3
9c	OCH_2CO_2H	$(CH_2)_2CO_2H$	*i*Bu	CH_2-2-naphthyl	*i*Bu

b)

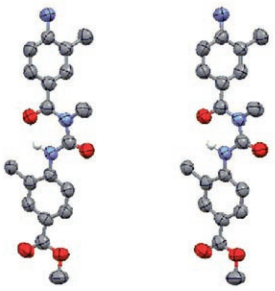
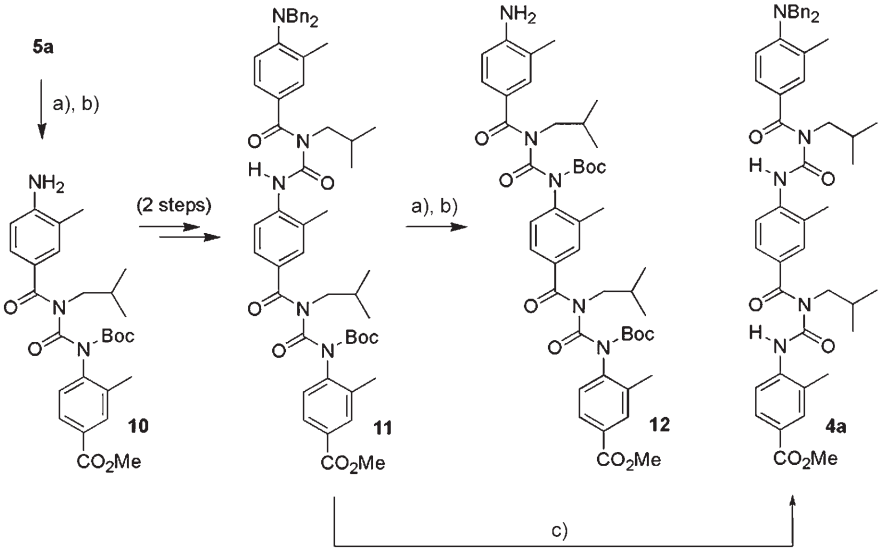


Figure 2. a) Structural comparison of terphenyl **8** to benzoylurea scaffold **9**. b) X-ray structure of **9b** in stereoview. Non-NH hydrogen atoms omitted for clarity. Red O, blue N, gray C, white H.

the urea in DMSO ($\Delta\delta = 0.126$ ppm) and in $CDCl_3$ ($\Delta\delta = 0.015$ ppm) when the concentration of the sample was varied between 0.005 and 0.5 M. In addition, the temperature-dependence coefficient of **9a** in DMSO was determined from variable temperature (VT) NMR spectroscopy to be $\Delta\delta/\Delta T = -2.9$ ppb K^{-1}. Taken together, these data confirm the presence of an intramolecular hydrogen bond in the benzoylurea group of **9a** in solution. Additionally, X-ray structures of **9a** and **9b**[20] (**9b** only is shown in Figure 2b) show that in the solid state the benzoylurea adopts a staggered conformation with an intramolecular hydrogen bond between the urea NH groups and carbonyl oxygen atoms, with interatomic distances of 1.87 and 1.78 Å and N–H···O angles of 141.2° and 136.2°, respectively.

The mimicry of longer helices requires straightforward and stepwise elongation of the scaffold. To achieve this goal, amino ester benzoylurea **5a** can be treated to further cycles of the synthetic procedure represented in Scheme 2. However, prior to hydrogenolytic debenzylation, the benzoylurea group is protected with Boc to preclude any instability under the strongly basic conditions. Subsequent reaction of **10** with triphosgene gave the new isocyanate which could be treated with secondary amide **6** to give, after full deprotection, the bis(benzoylurea) **4b** containing five substituted ring systems (see Supporting Information, Scheme S1, for further experimental details of the transformation from **10** to **4b**).

The X-ray structure of **4a**[20] (Figure 3a) shows intramolecular hydrogen bonds between the urea NH groups and carbonyl oxygen atoms, with interatomic distances of 1.84 and 1.78 Å and N–H···O angles of 141.9° and 136.8° for the benzoylurea groups nearest to the NBn_2 and to the CO_2Me groups, respectively. Importantly, the five substituents project in a staggered arrangement from the scaffold in a similar manner to an α helix. Computational modeling, based on the X-ray structures of **9b** (Figure 2b) and **4a** (Figure 3a), shows that considerable spacer length can be generated through the elongation of the urea oligomer. Figure 1 shows the variation of length and helix mimicry. Compound **5**, corresponding to two turns of an α helix, extends a distance of about 14.0 Å (measured between the aryl-*N*H and the ester -*C*O_2Me atoms). Pentasubstituted **4** adds a further 7.7 Å (approx. 5 residues, 1.4 turns of a helix) to its length and can potentially mimic four turns of the helix (length 22.3 Å). Further cycles of Boc-protection, debenzylation, isocyanate formation, reaction with a secondary amide and deprotection have been carried out to give the homologous hepta- and nonasubstituted mimetics **3** and **2**, respectively. These highly elongated structures, extending 29.4 and 37.1 Å, correspond to approx-



Scheme 2. Synthesis of **4a**. Reagents and conditions: a) Boc_2O, DMAP, THF; b) H_2, Pd/C, EtOAc/MeOH; c) TFA, CH_2Cl_2. Boc = *tert*-butoxycarbonyl, DMAP = 4-(dimethylamino)pyridine, TFA = trifluoroacetic acid.

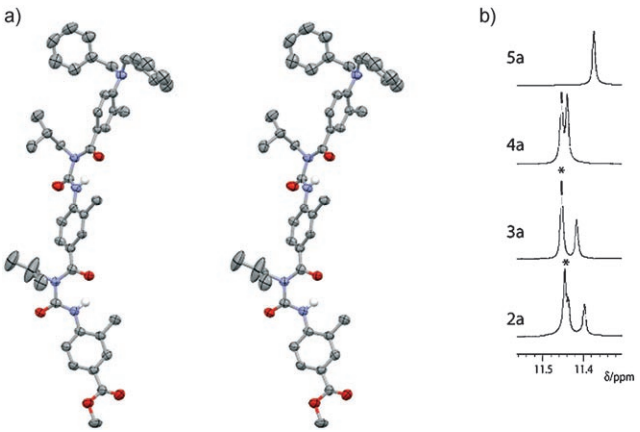


Figure 3. a) X-ray structure of **4a** in stereoview. Non-NH hydrogen atoms omitted for clarity. Red O, blue N, gray C, white H. b) Selected region of the ^{1}H NMR spectra of **5a**, **4a**, **3a**, and **2a** in $CDCl_3$. An asterisk indicates the presence of two overlapping singlets.

imately 5.4 and 6.8 turns of an α helix, respectively, and permit the projection of seven and nine substituents in a well-defined manner from one face of a molecule. Importantly, the linear synthetic route, by appropriate choice of secondary amide and aminobenzoate components, allows for each of these substituents to be chosen individually such that $R^1 \neq R^2 \neq R^3$, and so on, and parallels the efficacy of solid-phase synthesis. In this way, highly asymmetrically substituted scaffolds, in direct analogy to α helices, can be generated that span the length of many naturally occurring α-helical domains.

The rigidity of the oligomeric benzoylureas allows for their ready characterization. Sharp singlets are observed in the ^{1}H NMR spectrum for every intramolecular hydrogen-bonded NH group present in the scaffold (observed around $\delta = 11.4$ ppm, Figure 3b). Similar to **5a**, the NMR spectra of derivatives **4a**, **3a**, and **2a** in $CDCl_3$ confirm that the intramolecular hydrogen-bond-stabilized elongated conformation is maintained in solution.

To test the validity of these acylureas as α-helix mimetics and to assess their compatibility in aqueous biological systems, we synthesized a benzoylurea derivative **9c** (Figure 2a) as an exact isostere of our best terphenyl inhibitor[14] of the Bcl-x_L/Bak interaction (**8a**), which has an inhibition constant K_i of 114 nM. Benzoylurea **9c** was tested for its ability to displace a fluorescently labeled Bak peptide from Bcl-x_L in a fluorescence polarization competition assay.[14,19] A plot of polarization (mP) against log[**9c**] gave a sigmoidal displacement curve from which a K_i value of 2.4 μM could be calculated (see Supporting Information). This value is comparable to those of various terphenyl derivatives (0.114–13.6 μM) that are known to be effective mimics of the helical Bak peptide binding to Bcl-x_L.[15,17]

In conclusion, a new foldamer family based on benzoylurea oligomers has been designed and synthesized. Intramolecular hydrogen bonding favors a linear conformation that allows for derivatives of **2** to spatially mimic residues of an α helix. Preliminary assay results confirm that benzoylurea derivatives are compatible with aqueous conditions and can function as α-helix mimetics in disrupting the Bcl–x_L/Bak interaction. These molecules can be readily elongated to produce extended scaffolds with variable side chain composition making them attractive for potential applications in protein recognition and materials design.

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