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Synthesis and Structure of a Small Macrocyclic Hexapeptide Model for Antiparallel β-Sheet Containing Two Restrained 2-(3'-Aminopropynyl)-aniline Reverse-Turn Mimetics

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Abstract: A new reverse-turn mimetic containing a 2-(3'-aminopropynyl)-phenyl moiety in place of the natural *i* + *1* and *i* + *2* turn residues is described in which the key C-C bond was formed from an N-protected propargylamine and 2-iodoaniline using Heck chemistry. Nucleophilic displacement of triflate from activated (2S)-2-hydroxyalkanoate esters by the anilino N-atom, with inversion at C-2, gave homochiral tripeptide mimetics. Two variants of these were coupled and the product was deprotected and then cyclised to give a stable *cyclo*-hexapeptide analogue that possessed an anti-parallel β-sheet structure as determined by NMR spectroscopy.

Bends in which a polypeptide chain changes direction by 180° are common secondary structural elements in proteins and cyclic peptides. Bends participate in the formation of other types of secondary structure including 3_{10}-helices, α-helices and β-sheets and play a key role in stabilising intermediates that give rise to the long range interactions that form tertiary structure.[1] The design of molecules that induce extensive hydrogen bonding patterns in larger systems is currently an area of intense interest and can provide useful insight into the processes that determine protein folding and stabilisation.[1,2]

As part of a programme to accurately mimic protein structural motifs, a small unit that would emulate and constrain the geometry of reverse turns was required. Modelling and computer aided design indicated that peptides containing derivatives of the acetylenic amino acid, 5-amino-3-pentynoic acid **1**, would adopt the required conformation,[3] and provide the correct C^{α} to C^{α} interstrand distance of 4.5-5.3 Å, typical for β-turns. Oligopeptide systems based upon the parent compound **1** did display many of the required structural properties,[3] but it became evident that the inherent acidity of the C-2 protons limited their stabilities in the presence of excess base, during synthetic elaborations, and in aqueous solution, at high pH, in structural studies. Here we report on the synthesis of a new, stable, acetylenic peptide mimetic, 2-(3'-aminopropynyl)-phenylamine moiety **2**, which possesses the same potential to furnish a C^{α}-C^{α} interstrand distance of 4.5-5.3 Å in adjoining strands of β-sheet. We show that the moiety **2** can be elaborated to give oligopeptides through extension at the N-terminal primary amino group, *via* standard aminoacylation, and through alkylation of the anilino N-atom at the C-terminal. We also determine the solution structure of a cyclic derivative **3** which contains two 2-(3'-aminopropynyl)-phenylamino acid moieties.

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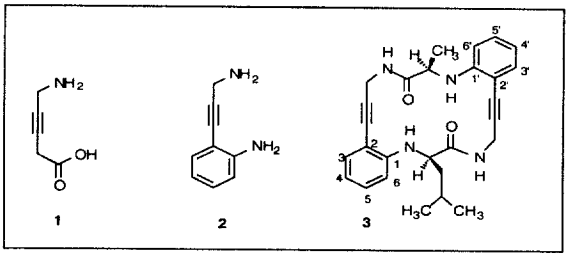
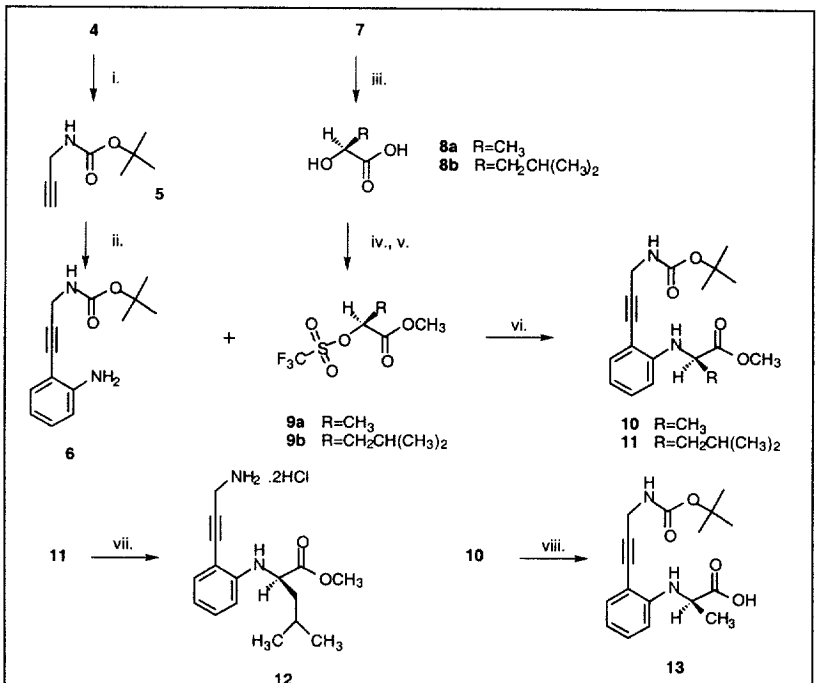


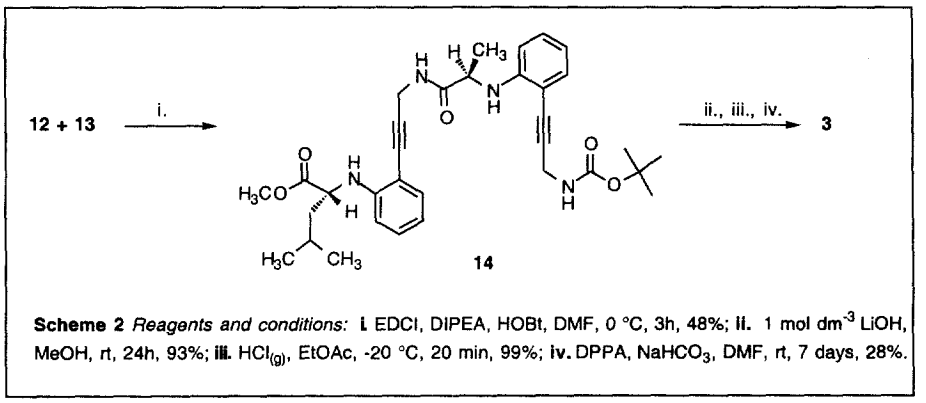
Propargylamine **4** was protected as its Boc ureathane derivative **5** and the product was coupled with 2-iodoaniline using a palladium catalysed Heck reaction to afford the 2-(3'-N-Boc-aminopropynyl)-phenylamine **6** as a yellow crystalline solid in 74% yield[4] (Scheme 1). Diazotisation of the (2S)-amino acids, alanine and leucine (**7**) involved a double inversion of configuration *via* 3-membered lactonium intermediates to produce the (2S)-hydroxy acids **8** with retention of stereochemistry. After esterification of compounds **8** and formation of the activated (2S)-2-hydroxyalkanoate esters **9**, tripeptide analogues **10** and **11** were formed by warming **6** and **9** together at 60 °C in dichloroethane with one equivalent of 2,6-lutidine.[5] The alkylation proceeded *via* an S_N2 mechanism to give the fully protected homochiral tripeptide mimetics **10** and **11**, respectively, in 81% yield.



Scheme 1 *Reagents and conditions:* **i.** $NaHCO_3$, Boc_2O, dioxane, H_2O, 0 °C, 5 h, 90%; **ii.** 2-iodoaniline, $(PPh_3)_4Pd$, CuI, Et_3N, DMF, rt, 12 h, 75%; **iii.** $NaNO_2$, H_2SO_4, H_2O, 0 °C, 12 h, 65%; **iv.** $SOCl_2$, MeOH, reflux, 2 h, 84%; **v.** Tf_2O, 2,6-lutidine, DCM, -78 °C, 91%; **vi.** 2,6-lutidine, dichloroethane, 60 °C, 18h, 82%; **vii.** $HCl_{(g)}$, EtOAc, -20 °C, 20 min, 95%; **viii.** $LiOH_{(aq)}$, MeOH, rt, 1 h, 90%.

Peptidomimetic **11**, containing the isobutyl side chain of leucine, was deprotected at the N-terminal through removal of the Boc group with dry HCl gas in ethyl acetate to give dihydrochloride salt **12**, while **10** was

unmasked at the C-terminal with LiOH in methanol to give, after aqueous work up, the free acid **13**. Components **12** and **13** were then coupled using standard solution phase peptide synthesis conditions, *i.e.* EDCI, DIPEA and HOBt in DMF to give the hexapeptide analogue **14,** which was subsequently deprotected at the C and N-termini with methanolic LiOH and dry HCl gas, respectively. Cyclisation was achieved using diphenylphosphoryl azide (DPPA) activation to give, after aqueous work up and column chromatography on silica gel, the desired cyclo-hexapeptide analogue **3** as a white crystalline solid in 28% yield (Scheme 2).



Scheme 2 *Reagents and conditions:* **i.** EDCI, DIPEA, HOBt, DMF, 0 °C, 3h, 48%; **ii.** 1 mol dm^{-3} LiOH, MeOH, rt, 24h, 93%; **iii.** $HCl_{(g)}$, EtOAc, -20 °C, 20 min, 99%; **iv.** DPPA, $NaHCO_3$, DMF, rt, 7 days, 28%.

^1H and ^{13}C NMR spectra were recorded in various solvents on a 500 MHz Varian Unity instrument at 30 °C and signals were fully assigned using TOCSY and HSQC techniques. The $^3J_{NH(amine)\text{-}C^{\alpha}H}$ coupling constants were 7.5 Hz in C^2HCl_3 and 7.9 Hz in 8% $C^2H_3O^2H/CH_3OH$ both of which are within the acceptable range for a β-sheet (7.0-10.0 Hz).[7] In C^2HCl_3, there were weak NH(amine)↔NH(amine) NOE cross-peaks, strong $C^{\alpha}H$↔NH(amide) crosspeaks and strong Ar-H6↔$C^{\alpha}H$, Ar-H6'↔$C^{\alpha}H$ NOE cross-peaks, all of which is consistent with **3** adopting a β-sheet conformation in C^2HCl_3 (Fig. 1).[7,8] Similar NOE cross-peaks were observed in methanol. Moreover, the amide NH resonances displayed a large downfield shift of 1.4 ppm upon changing the solvent from C^2HCl_3 to the more polar medium, 8% $C^2H_3O^2H/CH_3OH$, consistent with solvent interactions. Conversely, the anilino NH groups exhibited a very small down field shift (0.2 ppm) suggesting that they are solvent inaccessible and in accord with their involvement in intramolecular H-bond formation.

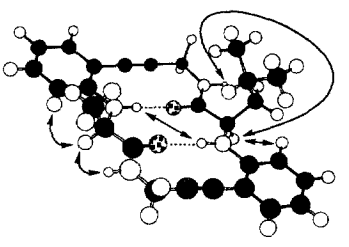


Figure 1 3D illustration of the H-bonding pattern of hexapeptide analogue **3** with key NOEs highlighted.

The highly constrained synthetic β-turn analogues reported by Kemp and Feigel contain aromatic groups as spacers for the peptide backbone and show a moderate tendency to form intramolecular interstrand H-bonds,[9]

but because of the extensive molecular scaffolding used, they are unlikely to be biologically compatible. Although peptidomimetic **2** is less constrained, it does show considerable promise in stabilising anti-parallel β-sheets in C^2HCl_3. This mimetic may also prove to be a useful replacement for the glycyl-proline dipeptide found in the *i*+1 and *i*+2 positions of the many sheet initiating β-hairpin bends in natural peptides,[10] and enable the synthesis of a family of non-peptidic therapeutic agents that are not vulnerable to proteolytic enzymes. The preparation of other longer cyclic and acyclic derivatives will show whether dipeptide analogues such as **2** can initiate and stabilise β-sheets in longer chains.

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References and Notes

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6. Selected data for **3**. *m/z* (HRMS: Found $[M + H]^+$ 443.2447. $C_{27}H_{31}N_4O_2$, requires 443.2451); δ_H(500 MHz; C^2HCl_3) 0.92 and 1.03 [6 H, 2 x d, *J* 6.2, $(CH_3)_2C$], 1.56 [3 H, d, *J* 6.6, CH_3 (Ala)], 1.86-1.90 [3 H, m, β-CH_2 and γ-CH (Leu)], 3.87 (1 H, m, 1 H of CH_2N), 3.90 (1 H, m, 1 H of CH_2N), 3.95 [1 H, m, α-H (Leu)], 4.04 [1 H, m, α-H (Ala)], 4.59 [1 H, m, 1 H of CH_2N], 4.66 [1 H, m, 1 H of CH_2N], 5.32 and 5.42 [2 H, m, Ar-NH], 6.52 [2 H, m, Ar-H6 and Ar-H6'], 6.66 [2 H, m, Ar-H4 and Ar-H4'], 6.80 [1 H, br. s, NHCO] and 7.17-7.27 [5 H, m, Ar-H3,5, Ar-H3',5' and NHCO]; δ_C(74.76 MHz; C^2HCl_3) 19.08 [CH_3 (Ala)], 22.05 and 22.88 [2 x δ-C (Leu)], 25.19 [γ-C (Leu)], 30.70 and 30.93 (2 x CH_2NH), 42.34 [β-C (Leu)], 52.90 [α-C (Leu)], 56.76 [α-C (Ala)], 80.79 and 80.87 [C≡$CCH_2NHCOCHCH_2C(CH_3)_2$ and C≡$CCH_2NHCOCH(CH_3)$], 91.25 and 91.32 [C≡$CCH_2NHCOCHCH_2C(CH_3)_2$ and C≡$CCH_2NHCOCH(CH_3)$], 107.37 and 107.68 [Ar-C2 and Ar-C2'], 109.55 and 109.66 (Ar-C6 and Ar-C6'), 117.15 (Ar-C4 and Ar-C4'), 130.13 and 130.43 (Ar-C5 and Ar-C5'), 131.22 and 131.40 (Ar-C3 and Ar-C3'), 147.40 and 148.48 (Ar-C1 and Ar-C1'), 173.49 and 174.70 [2 x CO (amide)].
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