



Synthesis and Properties of a Biocompatible Analogue for β -Turn Protein Structural Motifs Based on 5-Amino-3-pentynoic Acid.

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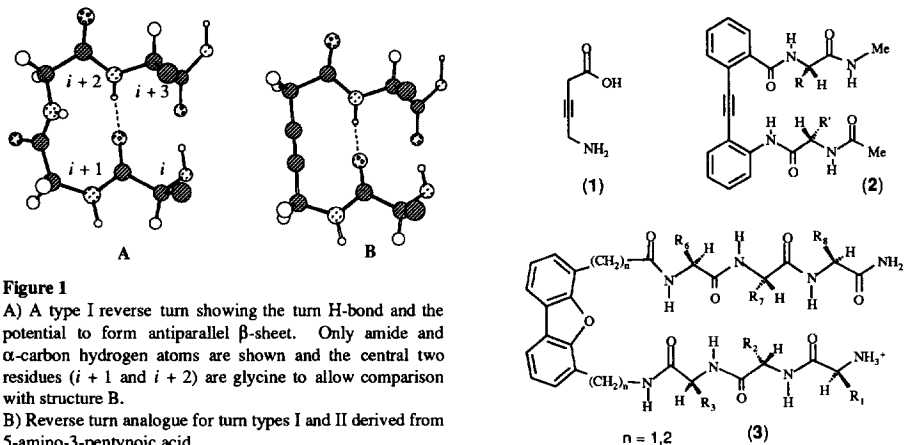
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Abstract. 5-Amino-3-pentynoic acid, a new 'lightly' constrained amino acid surrogate for glycylglycine and model for dipeptide homologues was prepared from propargylamine and methods were devised for its incorporation into linear oligopeptides through both C- and N-terminal extension.
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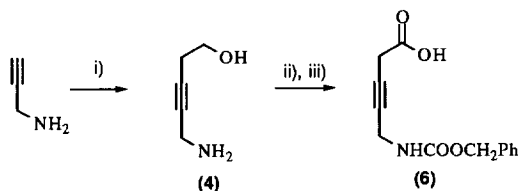
Reverse or β -turns in which a polypeptide chain changes direction by 180° , are common secondary structural elements in proteins and cyclic peptides.^{1,2} Turns exist in a number of different forms, designated type I to type VIII, and while reverse turns account for the several of these types (*e.g.* types I, II and VI), others are components of helices [see references 1a-d for reviews]. The conformations of turns are largely determined by their compositions although, as yet, the prediction of their existence and structures from sequence information alone is not possible and remains a major challenge. Usually one H-bond holds the turn together, and for type I, the H-bond connects the carbonyl group of the first residue (*i*) to the amide N-H of fourth residue (*i*+3), Fig. 1A. Frequently secondary interactions also occur between the side-chains of the turn residues and other parts of the protein.¹

As part of a programme to accurately mimic protein structural motifs,³ 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, Fig. 1B, and provide the correct C $^\alpha$ to C $^\alpha$ interstrand distance of 4.5-5.3 Å, typical for β -turns. Analysis indicated that such residues would fit within the space occupied by the various dipeptide moieties of natural turns and that appropriate side chains for the central residues could be introduced by the elaboration of one or both of the flanking methylene groups at C-2 and C-5 to confer biological specificity. These latter issues were of particular importance because previously reported constrained reverse turn analogues (**2** and **3**) had employed extensive molecular scaffolds^{4,5} and, therefore, were not biologically compatible.²

The parent amino acid (**1**) was expected to be unstable to conjugated allenic acid formation but had been previously tested as a GABA receptor agonist by Allan and coworkers.⁶ Their synthesis utilised the Schmidt reaction and gave the required compound as a polyhydrate in an optimised yield of 2.7% from 232 mg of hex-3-yne-1,6-dioic acid.⁶ To obtain useful amounts of material alternative routes were investigated and many of these also gave (**1**) in poor overall yield. The best route involved reacting the acetylide of unprotected propargylamine with ethylene oxide in liquid ammonia, Scheme 1, to give the amino alcohol (**4**) which was then converted to the benzylurethane (**5**) in 34% overall yield using benzyloxycarbonyl chloride (Cbz-Cl). Oxidation of the alcohol (**5**)



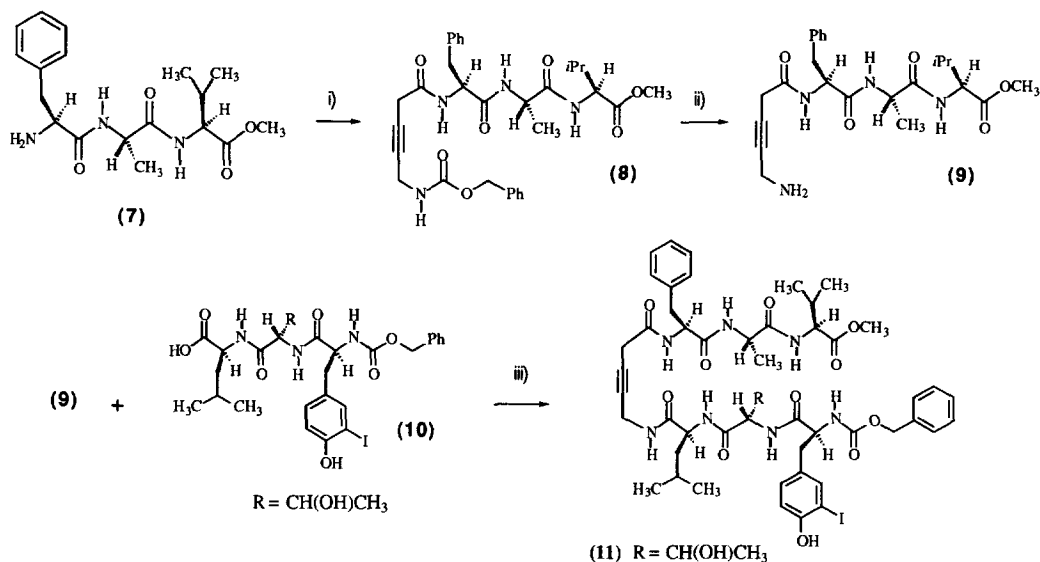
with potassium dichromate afforded the N-Cbz amino acid (**6**) in 85% yield, mp 76 °C. Compound (**6**) gave the expected spectroscopic and analytic data⁷ and could be converted to the parent amino acid (**1**) in 75% yield. Reactions were repeated on a large scale (~ 10 g) and gave similar yields.



Scheme 1 Reagents and Conditions. i) 1.2 eq. NaNH_2 , 2 eq. ethylene oxide, liquid NH_3 , -78 °C, ii) 1 eq. $\text{PhCH}_2\text{OCOCl}$, 2 eq. Na_2CO_3 , iii) 1.3 eq. $\text{K}_2\text{Cr}_2\text{O}_6$, H^+ , acetone, 29% over three steps.

The activation of the carboxy group of (**6**) for peptide coupling was investigated in detail using a variety of reagents but initial results were not encouraging. For example, reaction of the mixed isobutyl carbonic anhydride of (**6**) with various amines gave very low yields (5 - 20%) of the required amides. Under various conditions the major products did not contain an acetylenic moiety flanked by two methylene groups as judged by ^1H - and ^{13}C -NMR spectroscopy, although there was evidence of rearrangement to the allene. Presumably the increased acidity of the C-2 protons in the mixed anhydride, and the other activated esters, allowed base-catalysed rearrangement. Nevertheless, the addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI) to an equimolar solution of acid (**6**) and any of a number of amines gave the required amide products in good to excellent yield. For example, the reaction of *in situ* EDCI-activated (**6**) with (2S)-phenylalanyl-(2S)-alanyl-(2S)-valine methyl ester (**7**) afforded the required pure pentapeptide analogue (**8**) in 65% yield, Scheme 2. Note that the amide derivatives were markedly less prone to double bond migrations, in accord with expected effect of amide resonance; to decrease the acidity of the C-2 protons.

Deprotection of the N-Cbz group could be achieved in moderate yield using trimethylsilyl iodide in dry dichloromethane (DCM) although, again, the major side-products did not contain the acetylenic moiety. Moreover, the separation of these by-products from the desired pentapeptide analogue was difficult and tedious.



Scheme 2 Reagents and Conditions. i) (6), 1 eq. EDCl, DMF, 65%, ii) 30 eq. (CH₃)₂S, 10 eq. BF₃·Et₂O, 12 h, 75%, iii) NMM, *i*-BuOCOCl, THF, DMF, -15 °C, 67%.

[Compound (12) is the fully saturated derivative of (11)]

Treatment of compound (8) in DCM with excess BF₃ and Me₂S⁸ overnight gave the required amine (9) in 75% yield. Subsequently the amine (9) could be converted into a range of amide derivatives using standard peptide coupling techniques. The addition of amine (9) to a solution of the isobutyl carbonic anhydride of N-Cbz-(2S)-3-iodotyrosyl-(2S,3R)-threonyl-(2S)-leucine (10) (Scheme 2) gave the protected octapeptide analogue (11) in 67% yield as white crystals after an aqueous work-up and flash chromatographic purification on silica.⁹ The compound was completely stable below pH 9.5 in aqueous solution and thus the biocompatible 5-amino-3-pentynoic acid residue had been successfully incorporated into oligopeptides large enough to form β -turns and stable enough to perform structural analysis.

On the basis of the properties of the highly constrained synthetic β -turn analogues (2)⁴ and (3)⁵ which show no or only a moderate tendency to form intramolecular interstrand H-bonds, the unsubstituted analogue (11) was not expected to populate the β -turn structure to a significant extent. Indeed, turns containing glycine residues in the *i* + 1 and *i* + 2 positions are rare.¹ To verify these expectations the conformational preferences of the acetylenic peptide (11) and its fully saturated analogue (12) were assessed. The ¹H-, ¹³C- and ¹⁵N-NMR spectra of the compounds were recorded in various solvents on a 500 MHz Varian Unity instrument at 30 °C and were fully assigned using COSY, TOCSY, and HSQC techniques. One striking feature of the proton spectra for (11) and (12) in *d*₆-DMSO was that the chemical shifts and coupling constants for the similar moieties were almost identical. The values of *J*_{NH-H α} ranged from 7-8.5 Hz for each of the α -amino acid residues in each heptapeptide in accord with the expected values for extended peptide structures.^{5,10} Intrastrand NOESY and ROESY¹¹ correlations were consistent with those observed for β -sheet type conformations confirming that each strand existed in an extended conformation. In each case, no intramolecular interstrand NOEs or ROEs were detected from which we infer that intramolecular interstrand H-bonding conformations are not dominant. The observed small temperature coefficients¹² of 4-6 ppb K⁻¹ for each of the NH protons in (11) indicate that some H-bonding occurs and this may be derived from intermolecular H-bonding, see below. Similar results were obtained in other solvents including deuteriated chloroform-DMSO mixtures, C²H₃OH and CF₃C²H₂OH and at lower temperatures. Full details will be reported elsewhere. CD spectra for compounds (11) and (12) and for the precursor (8) recorded in MeCN, TFE and MeCN-TFE mixtures at ambient temperature confirmed that a β -turn

conformation was not significantly populated. In pure chloroform heptapeptide (**11**) formed solvated polymeric aggregates as expected.¹³ Such aggregates are thought to form, through H-bonding, upon the successive association of extra peptide strands to give an extended β -sheet arrangement.

The chemistry described here provides an entry to the synthesis and study of other lightly constrained oligopeptides derived from 5-amino-3-pentynoic acid. Those with C-2 and C-5 substitution patterns that correspond to the side-chain structures of the two central residues of natural β -turns are currently being pursued.

Acknowledgements: We thank the BBSRC for grant A01078, the Wellcome Trust for grant 040331, Dr. J. Wilkie, and Drs. M. Akhtar and A. Mehrotra for computational and scientific support and Dr. A. Drake for recording and interpreting CD spectra.

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7. All compounds were fully characterised and gave the expected spectral and analytic data. Selected data for compound **6**. *m/z* (Found: M^+ , 247.0838. $C_{13}H_{13}NO_4$ requires 247.0844); δ_H (300 MHz; C^2HCl_3) 3.35 (2H, s, 2- CH_2), 4.01 (2H, d, 5- CH_2), 5.15 (2H, s, - OCH_2), 6.60 (1H, brd s, -NH), 7.25 (5H, s, aromatics) and 8.95 (1H, brd s, - CO_2H); δ_C (75 MHz; C^2HCl_3) 25.73 (2-C), 31.18 (5-C), 67.25 (- OCH_2), 74.90 (3-C), 79.68 (4-C), 128.32, 128.40 and 128.69 (aromatics), 136.29 (ipso-C), 156.37 (NHCO) and 173.06 (- CO_2H).
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9. For compound **11**. *m/z* (Found: M^+ , 1081.3672. $C_{50}H_{64}N_7O_{12}I$ requires 1081.3659); δ_H (500 MHz; d_6 -DMSO) 0.84 and 0.87 (6H, 2d, 5 and 5'-Me of Leu), 0.88 & 0.89 (6H, 2d, 4 and 4'-Me of Val), 1.03 (3H, d, 4-Me of Thr), 1.23 (3H, d, 3-Me of Ala), 1.47 (2H, m, 3- CH_2 of Leu), 1.61 (1H, m, 4-CH of Leu), 2.04 (1H, m, 3-CH of Val), 2.59 and 2.91 (2H, ABX-multiplet, 2- CH_2 of Tyr), 2.63 and 2.76 (2H, AB-multiplet, 2- CH_2 of 5-aminopentyn.), 2.74 and 3.00 (2H, ABX-multiplet, 2- CH_2 of Phe), 3.07 and 3.83 (2H, AB-multiplet, 5- CH_2 of 5-aminopentyn.), 4.19 (1H, m, 2-CH of Val), 4.27 (1H, m, 2-CH of Tyr), 4.28 (1H, m, 2-CH of Thr), 4.30 (1H, m, 2-CH of Leu), 4.40 (1H, m, 2-CH of Ala), 4.53 (1H, m, 2-CH of Phe), 6.78-7.33 (13H, aromatics), 7.50 (1H, d, NH of Tyr), 7.81 (1H, d, NH of Leu), 7.94 (1H, d, NH of Thr), 8.03 (1H, d, NH of Val), 8.03 (1H, d, NH of Phe), 8.23 (1H, d, NH of Ala) and 8.27 (1H, t, NH of 5-aminopentyn.); δ_C (125 MHz; d_6 -DMSO, non-quaternary signals only) 21.8 (3-C of Ala), 21.8 and 22.4 (4-C and 4'-C of Val) 23.0 (4-C of Thr), 25.3 and 26.5 (5-C and 5'-C of Leu), 27.7 (4-C of Leu), 30.1 (2-C of 5-aminopentyn.), 31.9 (5-C of 5-aminopentyn.), 33.6 (3-C of Val), 39.5 (3-C of Tyr) 41.2 (3-C of Phe), 44.7 (3-C of Leu), 51.8 (2-C of Ala), 54.8 (2-C of Leu), 57.7 (2-C of Phe), 60.1 (2-C of Tyr), 61.2 (2-C of Thr) and 61.2 (2-C of Val); δ_N (50.7 MHz; d_6 -DMSO; referenced to external CH_3NO_2 @ 381.2 ppm from liquid ammonia) 86.7 (NH of Tyr), 108.9 (NH of 5-aminopentyn.), 109.3 (NH of Thr), 111.5 (NH of Phe), 118.2 (NH of Leu), 119.1 (NH of Val) and 119.8 (NH of Ala).
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(Received in UK 19 June 1996; revised 30 July 1996; accepted 1 August 1996)