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2-Amino-2′-carboxydiphenylacetylenes as β-Turn Mimetics. Synthesis and Conformational Properties

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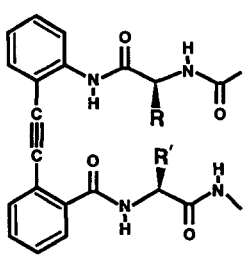
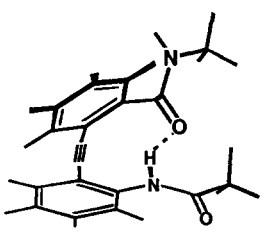
Abstract: Peptide-linked 2-amino-2′-carboxydiphenylacetylenes **1** by ^{1}H NMR spectroscopy are shown to assume short sheet structures with distinctive CD signatures.

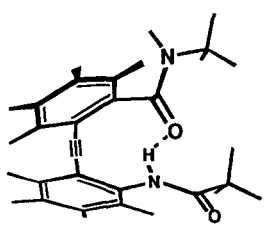
Conformationally restricted templates can enforce helix or sheet conformations on a linked polypeptide.[1] Mimetics of the β-turn conformation were first devised to enhance drug-receptor binding and specificity,[2] and many have been subsequently reported.[3] In protein structures the β-turn can cap a pair of antiparallel β-sheet strands, and recently Kelly and coworkers have shown that a properly functionalized dibenzofuran spacer can act as a sheet-capping turn mimetic.[4] Replacement of Ala by sheet-stabilizing amino acid residues in an immunoglobin-derived domain increases the stability of a sheet region by *ca.* 1 kcal/mol.[5] These results suggest that a properly constituted turn mimetic should induce sheet structure in a pair of linked polypeptides that contain amino acids with strong sheet stabilizing propensities. We here report synthesis and study of 2,2′-functionalized diphenylacetylenes **1**, a new class of sheet-initiating turn mimetic.



1



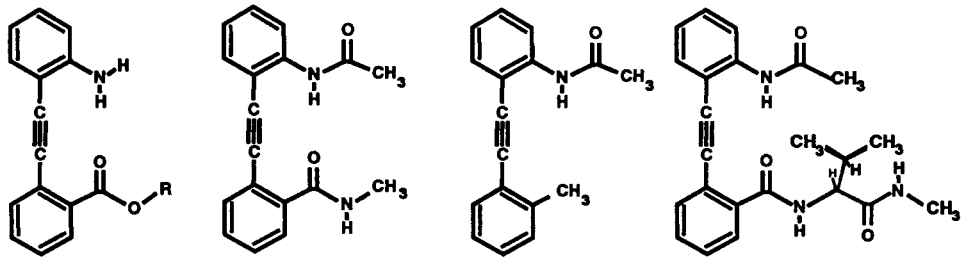
1a



x

A sheet-initiating turn mimetic should be tailored to the geometry of the antiparallel sheet. It should be easily synthesized and structurally versatile, permitting introduction of a range of functionalities at sites not required for the turn structure itself. It should be maximally rigid, yet it should contain a built-in spectroscopic reporter function that allows energetic characterization.[1] The previously reported turn mimetics fail to meet these conditions, but the tolan derivative **1** has the potential of doing so. The C-α to C-α interstrand antiparallel sheet spacing is 5.3 ± 0.1 Å[6] but is only 4.9 ± 0.4 Å for β-turns.[7] The analogous C-2 to C-2′ separation of the tolan function of a *syn*, planar **1** is 5.45 ± 0.02 Å.[8] Synthesis of **1** is expected to proceed readily via Pd(0)-catalyzed cuprate ligations, allowing ready synthesis of analogs. The small resonance interactions between the aryl functions themselves as well as their 2 and 2′ substituents are expected to favor planar orientations of the two amide functions, while the tolan function itself has only one degree of rotational

freedom that could be constrained by introducing interacting 6,6′ substituents.



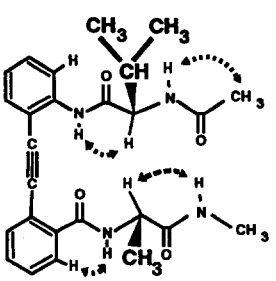
2 3 4 5

Following adaptations of known procedures,[9] **2** (R = Me) was synthesized in 84% yield from 2-ethynylaniline and methyl 2-iodobenzoate. Coupling with N-blocked amino acids yielded N-acyl derivatives of **2**, and ester saponification followed by standard acyl coupling with peptide or amino acid amines yielded species **1**. Alternatively, N-(2-iodobenzoyl)-blocked amino acid or peptide derivatives were coupled with 2-ethynyl aniline, then subjected to N-acylation to yield **1**. The amino group of **2** is inductively and sterically deactivated with respect to a typical peptide amine, with the practical consequence that **2** (R = H) can be coupled with H-L-Val-NH-Et using dicyclohexylcarbodiimide and N-hydroxybenzotriazole to form N-(2-(2′-aminophenylethynyl)-benzoyl)-L-Val-NH-Et in 55% isolated yield,[10] suitable for further elaboration to **1**.

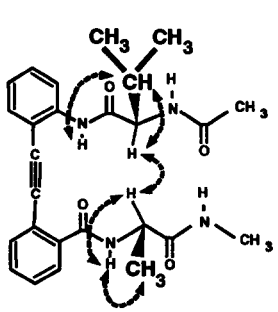
An N-acylaniline function has been successfully used as a reporter function for an ^{1}H NMR peptide-epindolidione conjugates[1b] in which the grouping is flanked by two hydrogen atoms at the *o,o′*-positions. Although derivatives **1**, **3**, and **4** contain an N-acylaniline, it can only assume reporter capacity if in the absence of intramolecular hydrogen bonding, the amide carbonyl assumes a *syn*-orientation with respect to the ethynyl function. NOE studies in DMSO-d_6 of derivatives **1** consistently show only a weak interaction between the 2-aryl NH proton resonance and the resonance for H-3, consistent with a high fractional population of the indicated conformation at the aryl-N bond. Strikingly, a relatively weak interaction of this type is also seen for **4**, which cannot form an intramolecular hydrogen bond. From the behavior of other N-acylaniline derivatives that exhibit reorientation of the acylamido group with intramolecular hydrogen bond formation, derivatives **1**, **3**, **4**, & **5** would be expected to exhibit a large change in H-3 chemical shift with change in structure and solvent, corresponding to a change in fractional population that assumes an *anti*-orientation with respect to the ethynyl function. Species **3**, **5**, and fourteen derivatives **1** with R and R′ derived from Gly, Ala, Val, Ile, Leu, Phe, Met, Gln, & Asn, show chemical shifts for H-3 in the narrow range of δ 8.23-8.40 ppm in DMSO-d_6. Thus as expected, the amido function at C-2′ is invariably oriented with the amide NH *syn* to H-3 and cannot be used as an NMR conformational reporter. Of the four potential nearly planar conformations at the C2-N and C2′-CO single bonds, only one appears to be significantly populated, as expected from simple steric arguments. A stereodiagram of **3** generated by molecular mechanics modeling is shown in **1a**. Resonance effects are not included in this vacuum simulation, and the energy difference between **1a** and members of a structural class in which the C2′-CO torsional angle is constrained to 0° is calculated to be only 1.3 kcal/mol. Energetically the tolan framework thus appears to be very tolerant of small variations in structure.

NOESY[11] spectra of **1** R = Gly, R′ = L-Ala in CD_2Cl_2 and in DMSO-d_6, ROESY[12] spectra of **1** R = L-Val, R′ = L-Ala and other derivatives examined in DMSO-d_6 show the signature CH-α to CH-α′ and amide MeNH to CH-α crosspeaks expected for a sheet structure, in addition to all the expected short-range intrachain interactions. Sheet characterizing crosspeaks between Gly CH-α and Ala CH-β and between Ala CH-α and Val CH-γ are also observed. All NOEs observed for **1** R = L-Val, R′ = L-Ala are shown in **6abc**. The NH-C^{α}H coupling constants were also consistent with sheet structures. Variable temperature studies of the amide NHs in DMSO-d_6 indicate that the aryl NH is well shielded from the solvent whereas MeNH is partially

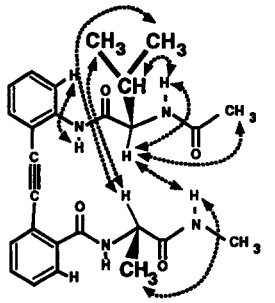
exposed to the solvent.[13] When the strongly hydrogen bonding solvent DMSO was replaced by CD_2Cl_2 or $CDCl_3$ a significant shift to higher field chemical shifts (> 1.5 ppm) was observed only for the solvent-exposed amino acid amide NHs.[13] All NMR results are therefore consistent with **1** as the major conformation in solution with the solvents studied.



6a (strong NOEs)



6b (medium NOEs)



6c (weak NOEs)

Circular dichroism (CD) spectra of a total of eighteen derivatives were examined in EtOH, selected for its UV transparency. All show the characteristic features of the representative examples shown in Fig. 1. The CD spectra of derivatives **1** exhibit four major bands of unusually high molar ellipticity; the long wavelength bands at 255 and 320 nm are attributable solely to chiral perturbations of the 2-amido-2′-carboxamidotolan chromophore. Tolan derivatives that lack sheet-forming capacity show minimal ellipticity at these wavelengths. Thus the molar ellipticity of **1** R = Ala, R′ = Val is four-fold larger than that of **5** at 320 nm and thirteen fold at 255 nm. In general, the largest long wavelength ellipticities are found for pairs of amino acid side chains that are strongly sheet stabilizing.[5] As seen in Fig. 1, the Gly cases show a dramatic loss of CD intensity which may reflect an alternative sheet conformation that lacks pleating at the Gly CH_2..

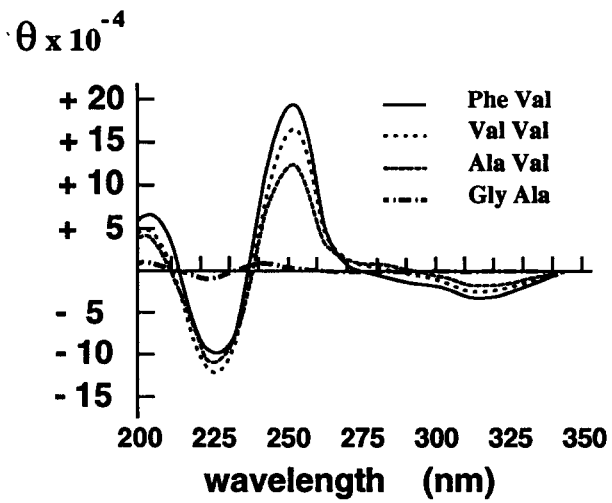


Fig. 1

Two significant questions remain unanswered. Although the observed NOE interactions and other NMR evidence is consistent with the proposed sheet structure, its precise conformation remains to be determined. Sheets are flexibile structures and intrinsically show significant structural variability. When suitable crystals are available, a combination of X-ray structral data with CD studies on cyclic peptide analogs of **1** is expected to

establish the precise structural dependence of the observed CD regularities. Studies on solvents of different propensity for stabilizing sheet structure are expected to reveal the extent of fraying and test the simple hypothesis that the observed large variation of long wavelength ellipticity can be used to monitor the fractional sheet character in solution. These studies are in process. The likelihood of a sensitive CD reporter of local conformation combined with a structure that may permit evaluation of the sheet-stabilizing effects of inter-chain hydrophobic interactions renders **1** a very promising new conformational template for turn and sheet structure.

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10. All the tolan systems were synthesized by the Pd(0)-catalyzed cuprate coupling[9] and standard peptide coupling reactions. All derivatives **1** were further purified by reversed phase HPLC before they were subject to high field NMR and CD studies. All data are consistent with each structure. For example:

    2 R = Me. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.917 (s, 3H), 4.9 (br, s, 2H), 6.67 (m, 1H), 6.74 (m, 1H), 7.14 (m, 1H), 7.31-7.38 (m, 2H), 7.50 (m, 1H), 7.64 (m, 1H), 8.01 (m, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 52.296, 92.423, 93.679, 107.459, 114.340, 117.372, 124.610, 127.379, 130.167, 130.218, 130.549, 132.021, 133.695, 149.241, 166.286; HRMS (EI): calc'd for $C_{16}H_{13}NO_2$ 251.0946294, found 251.09480.

    1 R = Leu, R′ =Val. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.770 (d, J=6.57 Hz, 3H), 0.813 (d, J=6.58 Hz, 3H), 0.868 (d, J=6.67 Hz, 3H), 0.914 (d, J=6.66 Hz, 3H), 1.561 (m, 2H), 1.65 (m, 1H), 1.861 (s, 3H), 2.047 (m, 1H), 2.479 (d, J=4.69 Hz, 3H), 4.306 (dd, J=8.55, 8.64 Hz; 1H), 4.968 (m, 1H), 7.140 (td, J=1.1, 7.5 Hz; 1H), 7.401 (m, 1H), 7.493-7.529 (m, 2H), 7.558 (m, 1H), 7.709-7.739 (m, 2H), 8.103 (br, quart, J=4.7 Hz, 1H), 8.228 (d, J=8.36 Hz, 1H), 8.357 (d, J=7.89 Hz, 1H), 8.672 (d, J=8.64 Hz, 1H), 9.502 (s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 18.695, 19.171, 22.749, 23.317, 23.470, 24.960, 25.850, 32.254, 42.388, 52.677, 58.082, 89.699, 94.674, 112.272, 119.735, 122.238, 123.436, 127.259, 128.318, 129.777, 130.837, 132.219, 133.816, 135.751, 140.342, 166.969, 170.116, 172.097, 172.497; HRMS (EI): calcd for $C_{29}H_{36}N_4O_4$ 504.273657, found 504.27250.
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