($CDCl_3$) δ 203.9, 128.7, 128.5, 128.3, 127.96, 127.6, 83.45, 72.50, 29.69, 26.83, 22.45, 13.76; IR (neat, NaCl) 3000–3100, 2850–3000, 1745, 1510, 1475 cm^{-1}.

2-(Benzyloxy)-3-methylbutanal (1b): ^1H NMR ($CDCl_3$) δ 9.60 (d, J = 2.4 Hz, 1-H), 7.30 (s, 5-H), 4.67 (d, 1-H), 4.44 (d, 1-H), 3.43 (dd, J = 2.4, 5.8 Hz,1-H), 2.03 (m, 1-H), 0.94 (dd, 6-H), ^{13}C NMR ($CDCl_3$) δ 204.4, 128.3, 127.8, 127.7, 127.5, 88.0, 72.7, 29.9, 18.3, 17.5; IR (neat, NaCl) 3000–3100, 2880–3000, 1740, 1605, 1595, 1500, 1455, 1375 cm^{-1}.

2-(Benzyloxy)-3,3-dimethylbutanal (1c): ^1H NMR ($CDCl_3$) δ 9.68 (d, J = 3.6 Hz, 1-H), 7.31 (s, 5-H), 4.60 (d, J = 11.6 Hz, 1-H), 4.38 (d, J = 11.6 Hz, 1-H); 3.24 (d, J = 3.6 Hz, 1-H), 0.97 (s, 9-H), ^{13}C NMR ($CDCl_3$) δ 204.95, 128.3, 127.8, 90.4, 72.9, 35.3, 26.0; IR (neat, NaCl) 3000–3100, 2880–3000, 1735, 1605, 1595, 1505, 1460 cm^{-1}.

Typical Procedure for the Lanthanide-Induced Shift Experiments. The gradient method[20] was employed for all the substrates. An analytically prepared sample of substrate (typical concentration was 0.040–0.050 M) in $CDCl_3$ was titrated with a solution of $Eu(hfc)_3$ (typical concentration was 0.033–0.060 M in $CDCl_3$) via a Hamilton microliter syringe. The sample was allowed to equilibrate for 5 min before acquisition. The change in chemical shift (Δδ) for each increment was calculated and plotted versus the concentration of LSR/substrate to give the data depicted in Tables II and III.

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Synthesis and Conformational Analysis of Epindolidione-Derived Peptide Models for β-Sheet Formation

D. S. Kemp,* Benjamin R. Bowen, and Christopher C. Muendel

Department of Chemistry Room 18-584, Massachusetts Institute of Technology, Cambridge Massachusetts 02139

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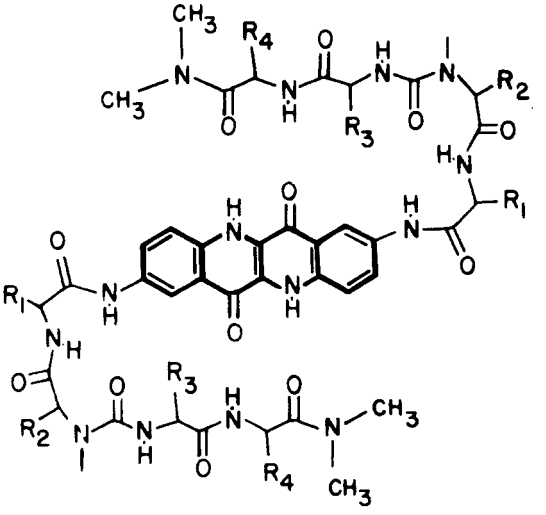
Synthesis of 2,8-diaminoepindolidione (2,8-diaminodibenzo[*b*,*g*][1,5]naphthyridine-6,12(5,11*H*)-dione) in 19% yield from *p*-nitroaniline is reported, as well as further conversion to 2,8-bis(Boc-L-Pro-Xxx)epindolidione (Xxx = Gly, D-Ala) and then to 2,8-bis(OC(Yyy-Zzz-NMe_2)-L-Pro-Xxx)epindolidione (Yyy = Gly, L-Ala, D-Ala; Zzz = Gly, L-Phe, D-Phe). The β-turn-forming tendencies of the series 2,8-bis(X-L-Pro-D-Ala)epindolidione, where X = Ac, Boc, and COGlyOEt, are assigned from ^1H NMR evidence.

The study of the secondary structures of polypeptides is impeded by the high cooperativity of the folding process for peptide chains, which ensures that most short linear peptides under normal conditions have no detectible conformational preferences,[1] although recently important exceptions have have reported.[2] In an effort to determine the role of hydrogen bonding in stabilizing secondary structure and to develop means of predictably enhancing the tendency of functionalized peptides to assume sheet or helical conformations, we have prepared conjugates of short peptides with rigid functionalities that mimic the hydrogen-bonding patterns of β-sheets[3] and α-helices[4] and that may therefore act as nucleation sites or templates for the folding of the linked peptide chain. Study of such template–peptide conjugates is expected to permit at least qualitative assignment of the relative importance of the factors that direct formation of secondary structure, to provide new, low molecular weight models for determining the individual and correlated biases of amino acids toward particular secondary structures, and ultimately to allow rational design of chimeric proteins in which the template structures are introduced in particular nucleation regions.

In preliminary reports[3] we have described synthesis and ^1H NMR study of antiparallel β-sheet formation with conjugates 1 of urea derivatives of short polypeptides and



1

the epindolidione function, which was first synthesized by Robert Robinson as a structural isomer of indigo.[5] Doubtless owing to intermolecular hydrogen bonding within the crystalline phase, simple epindolidione derivatives are exceptionally insoluble substances and their

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(3) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 4970, 4975. Kemp, D. S.; Bowen, B. R. *AAAS monograph on protein folding*; AAAS: Washington, DC, 1990; pp 293–303. Kemp, D. S.; Muendel, C. C.; Blanchard, D. E.; Bowen, B. R. In *Peptides—Chemistry, Structure, and Biology*; Rivier, J. E., Marshall, G. R., Eds.; ESCOM: Leiden, 1990; p 674.

(4) Kemp, D. S.; Curran, R. C. *Tetrahedron Lett.* **1988**, *29*, 4931, 4935.

(5) Ainley, A. D.; Robinson, R. *J. Chem. Soc.* **1938**, 1538.

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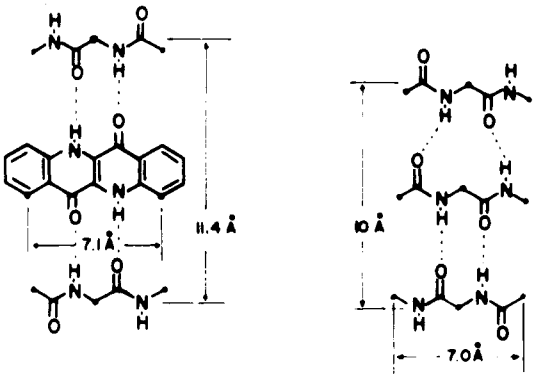
synthetic manipulation is often difficult. This paper reports experimental procedures for the synthesis of urea-functionalized peptide conjugates of 2,8-diaminoepindolidione of general structure 1 as well as evidence concerning the formation of β-turns with dipeptide-acylated 2,8-diaminoepindolidiones.

Structural and Spectroscopic Properties of 2,8-Bis(acylamino)epindolidiones. Classical Pauling–Corey parallel and antiparallel β-sheets are characterized by the following structural features: (1) The polypeptide backbones assume a nearly extended conformation, with alternating orientations of consecutive amide dipoles and nearly maximal separation between the α-carbons C_{α_i} and $C_{\alpha_{i+2}}$ (7.0 ± 0.1 Å for six short antiparallel sheets by X-ray structure).[6] (2) Adjacent peptide strands are linked by hydrogen bonds that lie parallel to and near the plane that bisects the global sheet structure. (3) Backbone atoms of linked peptide residues lie in pairs of planes that define pleats, with a dihedral angle of roughly 118°. (4) The C_α–C_β bonds of amino acid side chains are nearly perpendicular to, and alternately above and below, the global plane that bisects the sheet structure. Sheet structures found in proteins always bear a chiral twist that can be ignored in considering the short sheet structures of this model study.

A comparison of the spacing and dimensions of the 2,8-bis(acylamino)epindolidione function and the classical antiparallel pleated sheet conformation of polypeptide strands is given in part in structures **2** and **3**, which compare literature distances for β-sheets[6] with values calculated for the planar epindolidione from standard bond distances by molecular mechanics.



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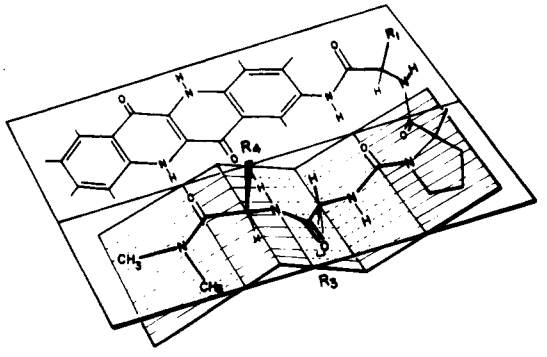
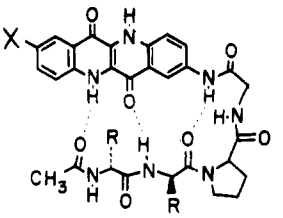


3

The bis(acylamino)epindolidione function preserves the in-plane hydrogen-bonding characteristics of a β-sheet but presents a minimal steric bulk above and below the plane defined by the hydrogen bonds. As a nucleation site, this function therefore tests the capacity of three oriented hydrogen bonds to enforce extended structure on a linked polypeptide chain. The effect of nonplanar peptide sheet pleating on the C_{α_i}–$C_{\alpha_{i+2}}$ distance is almost exactly compensated by the shorter bonds of the heteroaryl function, and this distance in the sheet is thus nearly identical with the C_1–C_{10} distance of the epindolidione, as noted in **2**. The correspondence shown in **3** of the hydrogen-bonding patterns of an extended peptide chain and a 2-(acylamino)epindolidione shows that although the hydrogen-bonding sites are equivalent, a sequence reversal occurs with the epindolidione. As a result, linkage of the epindolidione function to a β-turn and a normal polypeptide chain can only generate the pattern of hydrogen bonds characteristic of a parallel β-sheet structure, as shown in 4. (Preliminary results of an examination of the conformational properties of conjugates of simple peptides with epindolidiones have been reported.[3]) Formation of an antiparallel structure requires introduction within the peptide chain of a sequence-reversing element, and we have chosen a urea functionality as shown in **1** and **3** for the present studies. A C_2 symmetric bis(peptide) functionalization of the epindolidione was chosen for synthetic convenience and for the simplicity of interpretation of the ^{1}H NMR spectra of these derivatives in the heteroaryl region.



4

The extended conjugation of the epindolidione function generates a complex electronic absorption spectrum, as indicated in Table I, and all derivatives of structure **1** are highly fluorescent (emission maxima in DMSO, 480, 520 nm; excitation maximum, 306 nm). The heterocyclic NH functions can be deprotonated above pH 9, the carbonyls can be protonated below pH 2, and fluorescence is abolished on protonation or deprotonation. It was anticipated and found[3] that the epindolidione function is a sensitive reporter of chiral environment above or below the plane of the rings as seen by intense circular dichroism in the visible and long-wavelength UV regions.

Design and Synthesis of Peptide-Functionalized 2,8-Bis(acylamino)epindolidiones. The first practical syntheses of epindolidiones were reported in 1968 by Jaffe and Matrick[7] who described 2,8- and 3,10-substituted fluoro, chloro, methyl, and methoxy derivatives as very sparingly soluble, greenish yellow to orange polymorphic, microcrystalline powders with melting or decomposition points >400 °C. Purification of these potentially useful pigments was carried out by solution in 100% sulfuric acid followed by the addition of 4–6% water, which results in nearly complete precipitation of the above derivatives. This intractability, which is attributable in part to efficient intermolecular hydrogen bonding in the solid state, limits the usefulness of simple functionalized epindolidiones as starting materials for synthesis. Thus, we have been unsuccessful in carrying out electrophilic substitution on epindolidione itself. Nitration fails, owing apparently to

(6) Pauling, L.; Corey, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1953**, *39*, 253. Ashida, T.; Tanaka, I.; Yamane, T. *Int. J. Peptide Protein Res.* **1981**, *17*, 322.

(7) Jaffe, E. E.; Matrick, H. *J. Org. Chem.* **1968**, *33*, 4004.

Table I. Visible and Ultraviolet Absorption Maxima (nm) of 2,8-Bis(acylamino)epindolidiones

compound	solvent	λ_{max} (ϵ)		
9a (free acid)	H_2O	299 (67 000)		468 (6300)
	0.1 M HCl	297 (50 000)		467 (5100)
	0.1 M NaOH	300 (71 000)		472 (5500)
		498 (4700)		
	EtOH	298 (62 000)	437 (5900)	463 (7200)
	dioxane	294 (40 000)		468 (5500)
9b	DMSO	308 (72 000)	342 (19 000)	380 (1600)
		416 (4300)	441 (9700)	468 (14 000)

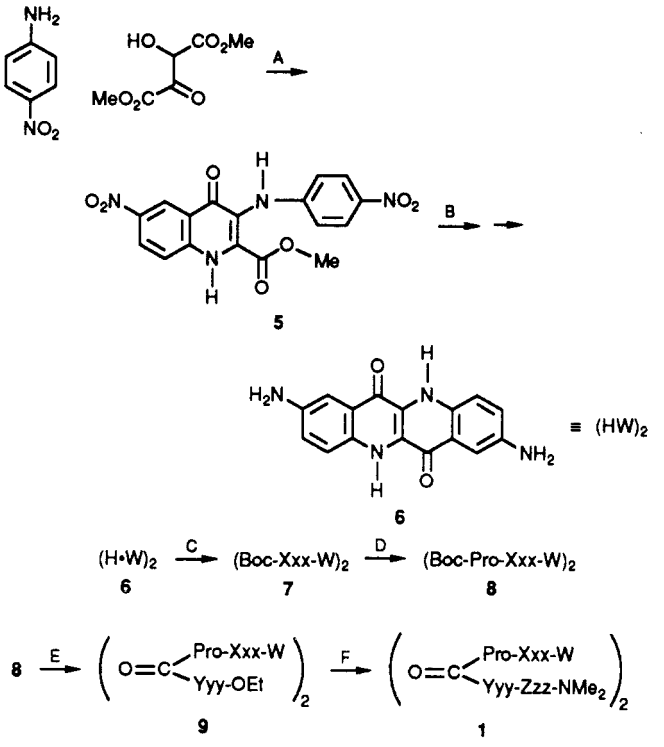
insolubility and oxidative degradation. N-Alkylation with sodium hydride/alkyl halides in DMSO can be carried out on simple epindolidiones, although mixtures of products are formed in low yield.

Intermolecular association is also a common property of polypeptides that form β-structure,[8] but it was hoped that by proper choices of amino acids and chain terminating function the hydrogen-bonding affinities of peptide and epindolidione could be satisfied mutually and intramolecularly, with reduced intermolecular affinity and a correspondingly increased ease of manipulation, purification, and spectroscopic characterization. For this first exercise in template-directed generation of β-structure, we planned a study of urea-linked dipeptide derivatives in which the first two amino acids (1 = Gly, D-Ala; 2 = L-Pro) were selected for their disposition toward forming a type II β-turn conformation.[9] For molecules that assume the proposed folding of **1**, the steric shielding of the (acylamino)epindolidione carbonyls, the presence of the imino acid proline at site b, and the addition of an *N,N*-dimethylamide as the chain-terminating function at site d leave only two NH functions and two carbonyls free to take part in intermolecular hydrogen bonding, one each in the proposed β-turn and the β-sheet regions.

The synthesis of compounds **1** was carried out as shown in Scheme I. A modification of the general synthetic protocol of Jaffe and Matrick[7] was used to generate 2,8-diaminoepindolidione **6** in an overall yield of 19% from *p*-nitroaniline. The uncatalyzed high-temperature cyclization to form the carbomethoxyquinolone **5** of Scheme I raises an interesting mechanistic point. We have noted in this and in similar cases that N-methylation completely blocks cyclization, and therefore a likely mechanism is prior elimination of methanol to form an iminoketene intermediate that undergoes electrocyclic closure. Attempts to cyclize the dinitroquinolone **5** without catalysis in hot Dowtherm failed, suggesting that strongly electron-withdrawing substituents inhibit the thermal mechanism. Cyclization of **5** is achieved with harsh electrophilic catalysis and presumably proceeds via an acylium ion. Other examples of this difference were noted by Jaffe and Mattrick.[7]

Diamine **6** can be best purified by reprecipitation as the bisulfate salt by addition of water to a solution in sulfuric acid; however, the solubilizing effect of the two ammonium ions is evident in that precipitation occurs only when an equal volume of water has been added. Acylation of the exceptionally insoluble diamine itself can be effected by solution of its bisulfate salt in DMSO, addition of triethylamine, and reaction of the resulting slurry with an active acylating agent. The peptide products of Scheme

Scheme I[a]



[a] Key: A, HCl/MeOH and then Dowtherm, reflux 15 min, 34%; B, $SnCl_2$ and then $NaAlCl_4$, 160 °C, 1 h, 60%; C, $(Boc\text{-}Xxx)_2O$, DMSO, 97%; D, TFA and then TEA/Boc-Pro-OC_6F_5 in DMF, 92%; E, TFA and then TEA/OCN-CH(R)-CO_2Et in DMF, 95%; F, LiOH/THF/H_2O and then H-Zzz-NMe_2/DCC-HOBt in DMF, 70%.

I are obtained as red or orange powders with melting points >300 °C. Generally speaking, an increase in size of the peptide chain results in a progressive increase in solubility and ease of synthetic and chromatographic manipulation. Thus, **6** has been successfully acylated only in DMSO solution. Either DMSO or DMF can be used for acylation of the amines formed by Boc removal from derivatives **7**, while a variety of solvents are suitable for acylation of the amines derived from **8** or the carboxylic acid derived from **9**. A practical demonstration of the association-breaking efficacy of substitution at site 2 of **1** by the tertiary amide forming amino acids proline and sarcosine is provided by the behavior of derivative **1** (1 = 2 = Gly; 3 = L-Ala; 4 = L-Phe), which lacks this substitution. Attempts to prepare this intractable substance were thwarted by strong binding to all chromatographic media. One derivative, **1f** (1 = D-Ala; 2 = L-Pro; 3 = Gly; 4 = L-Phe) forms large crystals that appear to be suitable for X-ray analysis.

Derivatives **1** listed in Table II were purified to >99% purity as judged by ^1H NMR and analytical HPLC by chromatography on a Sephadex LH-20 column, followed by preparative HPLC on a reversed-phase Vydac column. Yields reported in Table II are for chromatographically pure material and, owing to the difunctional character of the starting materials and products, correspond to the square of the yields for the individual acylation steps.

Conformational Preferences of the Dipeptide-Functionalized 2,8-Bis(acylamino)epindolidiones. Elsewhere we will report the spectroscopic evidence that permits conformational assignments for a subclass of the 13 derivatives of structure **1** that are reported in Table II. In our preliminary reports[3] we have noted that three classes of ^1H NMR evidence, temperature dependence of chemical shifts, $J_{C_\alpha H\text{-}NH}$ values, and NOE effects support

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Table II. Peptide-Functionalized 2,8-Bis(acylamino)epindolidiones

deriv	1	2	3	4	yield, %
7a	Boc-Gly				97 (from **6**)
7b	Boc-D-Ala				76
8a	Gly	Boc-L-Pro			92 (from **7a**)
8b	D-Ala	Boc-L-Pro			50 (from **7b**)
8c	Gly	Boc-Sar[a]			15 (from **7a**)
8d	D-Ala	Ac-L-Pro			
9a	Gly	L-Pro	Gly-OEt		67 (from **8a**)
9b	D-Ala	L-Pro	Gly-OEt		95 (from **8b**)
9c	D-Ala	L-Pro	L-Ala-OEt		67 (from **8b**)
9d	D-Ala	L-Pro	D-Ala-OEt		64 (from **8b**)
9e	D-Ala	L-Pro	Aib-OEt[b]		
9f	Gly	Sar[a]	L-Ala-OEt		60 (from **8c**)
1a	Gly	L-Pro	Gly	L-Phe	73 (from **9a**)
1b	Gly	L-Pro	Gly	L-Ala	73
1c	Gly	L-Pro	Gly	Gly	77
1d	D-Ala	L-Pro	Gly	L-Phe	75 (from **9b**)
1e	D-Ala	L-Pro	Gly	L-Ala	60
1f	D-Ala	L-Pro	Gly	L-Phe	76
1g	D-Ala	L-Pro	Gly	D-Phe	77
1h	D-Ala	L-Pro	L-Ala	L-Phe	44
1i	D-Ala	L-Pro	L-Ala	D-Phe	31
1j	D-Ala	L-Pro	D-Ala	L-Phe	60
1k	D-Ala	L-Pro	D-Ala	D-Phe	58
1l	Gly	Sar[a]	L-Ala	L-Phe	17 (from **9f**)
1m	D-Ala	L-Pro	Aib[b]	L-Phe	42 (from **9b**)

[a] Sar = $-N(CH_3)-CH_2-CO-$. [b] Aib = $-NH-C(CH_3)_2-CO-$.

the assignment of conformation **3** for derivative **1f** in DMSO-d_6 and in mixtures of DMSO-d_6 with $DCCl_3$ and D_2O. A natural question concerns the degree to which conjugates of epindolidiones with shorter peptides also show detectable major conformations. In this section we address the question of β-turn formation with peptide–epindolidione conjugates of structure **8**.

The chemical shift of the epindolidione 2(8)-NH in DMSO-d_6 provides a measure of its local structure.[3] For derivatives such as **1f** in which a type II β-turn has been demonstrated, values in DMSO-d_6 of δ 9.8 ± 0.1 are seen, while values of δ 10.2 ± 0.1 are characteristic of solvent-exposed arylamido NH groups. The data of Table III are consistent with this generalization. Thus, the simple derivatives **7a** and **7b** that are incapable of forming β-structure or intramolecular H bonds exhibit aryl NH resonances at δ 10.2, as does the sarcosine dipeptide **8c**. The aryl NH of the urea derivative **9b** shows a δ that is characteristic of the β-turn function of structured tetrapeptide conjugates such as **1f**, while the three dipeptide derivatives **8a**, **8b**, and **8d** exhibit pairs of aryl NH resonances of fractional area, one at δ 10.3 and one in the solvent-shielded range of δ 9.8–9.9. Although only single proline α-CH resonances appear in the spectra of **8ab**, peak doubling with relative areas proportional to those of the pair of aryl NH resonances is seen for the Gly or Ala NH resonances of **8a,b,d** and for the *tert*-butyl resonances of the urethanes **8a,b**. Broadening consistent with peak doubling is seen in the aryl 3(9)-CH and Ala CH_3 of **8b**.

N-Acylproline derivatives usually exhibit pairs of resonances attributable to s-cis and s-trans CO–N conformers that equilibrate slowly on the NMR time scale.[10] These can be distinguished by ^{13}C chemical shift differences for the pyrrolidine β- and γ-carbons; a larger chemical shift difference is seen between the resonances of the β- and γ-carbons of the cis conformer.[10,11] The ^{13}C NMR spectrum of **8b** in DMSO-d_6 shows a pair of resonances at δ 23.2 and 31.1 ($\Delta\delta$ 7.9) and a pair of somewhat weaker, broader resonances at δ 24.1 and 29.8 ($\Delta\delta$ 5.7). The former corresponds to C_β and C_γ values of δ 21.7 and 30.7 ($\Delta\delta$ 9.0) reported for *s-cis*-H_2-D-Ala-L-Pro-OH in DMSO,[10] and the latter, to C_β and C_γ values of δ 24.1 and 28.6 ($\Delta\delta$ 4.5) reported for the s-trans conformer. The minor resonance therefore corresponds to the trans conformer, which exhibits the upfield aryl NH resonance at δ 9.82, consistent with the previous assignment by ^{13}C NMR of the *trans*-acylproline conformation to **1f**.[3] The cis:trans area ratios for urethanes **8a** and **8b** thus are 1:1 and 3:2, respectively, and under the assumption of a consistent correlation between δ values of the aryl NH and acyl proline resonances, amide **8d** can be assigned a cis:trans ratio of 3:1. Urea **9b**, like tetrapeptide urea **1f**, contains an undetectible fraction of the s-cis conformer.

Table III. ^1H NMR Spectral Data in DMSO-d_6, 300 MHz

deriv	aryl NH: δ (rel area)	$-\Delta\delta/\Delta T$, 10^{-3} ppm/K	Ala or Gly NH: δ (rel area)	$-\Delta\delta/\Delta T$, 10^{-3} ppm/K
7a Boc-Gly	10.18	(1.0)	7.10	(1.0)
7b Boc-D-Ala	10.18	(1.0)	7.12	(1.0)
8a Boc-Pro-Gly	10.27 (0.52) 9.89 (0.48)	3.58 2.86	8.24 (0.55) 8.44 (0.45)	4.24 3.71
8b Boc-Pro-D-Ala	10.31 (0.59) 9.82 (0.41)	4.20 2.76	8.14 (0.58) 8.46 (0.42)	1.83 3.95
8c Boc-Sar-Gly	10.19 (1.0)		8.27 (1.0)	
8d Ac-Pro-D-Ala	10.30 (0.24) 9.77 (0.76)	3.40 1.8	8.40 (0.2) 8.67 (0.8)	4.6 3.9
9b $EtO_2C-CH_2-NH-CO-Pro-D-Ala$	9.79 (1.0)		8.67 (1.0)	
1f $Me_2NCO-CH(Bzl)-NHCO-CH_2-NHCO-Pro-D-Ala$	9.83 (1.0)	1.5	8.57 (1.0)	3.7

Low values for the vicinal NH(i+3)–CH(i+3) vicinal coupling constant and for the temperature dependence of the (i + 4) NH resonance, together with characteristic NOE effects, are the three NMR spectroscopic signatures that have been shown to characterize β-turns in small L-amino acid derived cyclic and acyclic peptides.[12,13] For **8a,b,d** the D-Ala $J_{C_\alpha H-NH}$ values lie in the range 6.5–7.5 Hz, close to the random-coil value of 7.5 Hz. These values are consistent with but do not establish the CH–NH dihedral angle (120–150°, predicted J_{vic} = 4.0–7.5 Hz[14]) that is expected for a D-amino acid at site (i + 3) of a type II β-turn. More compelling structural evidence is available from studies of NH temperature dependence and from NOE experiments.

In relatively polar solvents such as DMSO, values for $-\Delta\delta/\Delta T$ of $>5 \times 10^{-3}$ ppm/K are typical of an amide NH that is exposed to solvent, and values $<3 \times 10^{-3}$ ppm/K are typical of an amide NH that is shielded from solvent through intramolecular hydrogen bonding or through steric constraint.[15] As seen in Table III for **8a,b,d**, three structures that show peak splitting owing to detectable amide rotamers, only one of each pair of aryl NH resonances shows a $-\Delta\delta/\Delta T$ value that is less than 3×10^{-3} ppm/K. Invariably this is the upfield resonance attrib-

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(11) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379. Siemion, I. Z.; Wieland, T.; Pook, K.-H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702.
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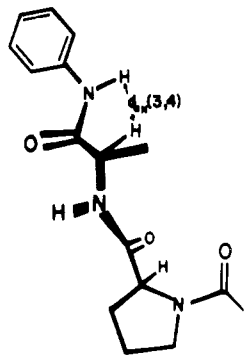
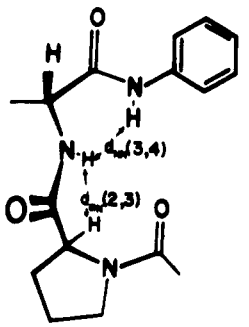
utable to the *s-trans*-acylproline conformer. Amide **8d** shows a $\Delta\delta/\Delta T$ value of -1.8×10^{-3} ppm/K, close to the value of -1.5×10^{-3} ppm/K seen for **1f** and in the range of values expected for solvent-shielded amides. The values of $-\Delta\delta/\Delta T$ seen for the urethanes **8a,b** approach the structurally ambiguous value of 3×10^{-3} ppm and therefore provide less compelling evidence for a significant population of β-turn conformers. All of the *s-trans*-acylproline conformers exhibit $-\Delta\delta/\Delta T$ values for the Ala or Gly NH resonances that are consistent with solvent exposure as do most of the values for the NH resonances of the s-cis conformations; the $-\Delta\delta/\Delta T$ value for the Ala NH of the s-cis conformer of **8b** is low and consistent with shielding from solvent.

As applied by Wuethrich and co-workers[16] the nuclear Overhauser effect can provide a series of distance parameters useful for defining local peptide conformation. Drawing on a large data base of structures, Dyson et al.[17] have applied these to β-turns formed by acyclic peptides in water. Because random-coil structures are expected to lie in the global energy minimum of backbone conformational space, they are approximated by an extended conformation (e.g., **10a**), which is characterized by a maximal



10a



10b

separation between protons of adjacent backbone amide NH groups; thus, $d_{NN}(i,i+1)$ is typically 4.1–4.3 Å in such a structure. Extended structures also are characterized by a minimal separation between an α-CH proton and the proton of the succeeding NH; thus, $d_{N_\alpha}(i,i+1)$ is typically 2.2 Å. A large NOE is expected for the latter interaction, but a negligable NOE is expected for the former. Judged by proton–proton separation, a type II β-turn **10b** is structurally distinguishable from a random coil by its short $d_{NN}(3,4)$ (2.3–2.4 Å), by its lengthened $d_{N_\alpha}(3,4)$ (3.1–3.3 Å), and by its unusually short $d_{N_\alpha}(2,4)$ (3.3–3.5 Å).

Examining a data base that contained favorable examples in which turns were estimated to consist of up to 50% of the conformational population, Dyson et al.[17] noted that for these examples a turn signature consists of strong NOE signals corresponding to the $d_{NN}(3,4)$ and $d_{N_\alpha}(2,3)$ connectivities. Although in one case the $d_{N_\alpha}(3,4)$ connectivity was reduced to medium, usually this NOE connectivity was also found to be strong. Thus, the inverse correlation that might be expected between strengths of $d_{NN}(3,4)$ and $d_{N_\alpha}(3,4)$ interactions was not seen, doubtless owing to the presence of random-coil conformational states as significant contributors to all structures. Only the most favorable cases generated a weak signal corresponding to the $d_{N_\alpha}(2,4)$ connectivity.

(16) Billeter, M.; Braun, W.; Wuethrich, K. *J. Mol. Biol.* **1982**, *155*, 321.

(17) Dyson, H. J.; Rance, M.; Houghten, R. A.; Lerner, R. A.; Wright, P. E. *J. Mol. Biol.* **1988**, *201*, 161.

NOESY 2D NMR spectra were obtained in DMSO for the three derivatives **8a**, **8b**, and **8d**. Relative estimates of NOE intensities were obtained by comparing peak heights of the observed NOE interactions with the corresponding peak heights for the NOE interactions within the bis(acylamido)epindolidione nucleus between the aryl NH and H-1 and between H-4 and the heteroaryl NH, as described in the Experimental Section. Within our error limits, no derivative showed significant $d_{N_\alpha}(2,4)$ NOE connectivity. The dominant s-trans conformation of acetamide derivative **8d** showed $d_{NN}(3,4)$, $d_{N_\alpha}(3,4)$, and $d_{N_\alpha}(2,3)$ NOE interactions that were all strong. By contrast, the s-trans states of both urethanes **8a,b** showed medium to weak $d_{NN}(3,4)$ and $d_{N_\alpha}(2,3)$ connectivities, together with strong $d_{N_\alpha}(3,4)$ NOE connectivities.

All data support the conclusion that amide derivative **8d** is substantially in a type II β-turn conformation in DMSO solution. The evidence supporting turn conformations for the urethanes **8a,b** is less persuasive. The borderline value of the temperature dependences for the aryl NH δ values together with the observable $d_{NN}(3,4)$ NOE interactions can be taken as evidence in favor of a conformational average of structurally diverse backbone geometries to which a turn structure makes a modest to moderate contribution.

The available evidence[3] concerning the nucleation of sheet structure with peptide–epindolidione conjugates points to the indispensibility of a strong turn-forming signal at the epindolidione–peptide junction for the formation of either antiparallel or parallel sheet structures. The results of this study demonstrate that simple amide but not simple urethane derivatives of Pro-D-Ala or Pro-Gly can sustain turn conformations in DMSO solution when attached to the epindolidione nucleus. Experiments designed to refine the structural requirements for stabilized turn formation in these systems are in progress and will be reported subsequently.

Experimental Section

Microanalyses were performed by Multichem Laboratories, Inc., Lowell, MA. Melting points were taken on a Thomas-Hoover Unimelt apparatus and are reported uncorrected. High-resolution ^{1}H NMR spectra were taken either on a Bruker WM-250 spectrometer or on a Varian XL-300 spectrometer, as indicated for each spectrum reported. Chemical shifts are reported (δ) relative to tetramethyl silane (TMS), and splitting patterns are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. UV and visible spectra were determined on a Perkin-Elmer Model 330 spectrophotometer, and circular dichroism (CD) spectra were obtained with a Jasco J-500 spectropolarimeter. Mass spectra were determined on a Finnigan MAT 8200 in either the electron impact or fast atom bombardment mode as indicated for each mass spectum reported.

Analytical high-pressure liquid chromatography (HPLC) was performed on a Waters Associates system consisting of two Model 501 pumps, a Model 441 single-channel UV detector (208, 254, 280, and 313 nm), a Model U6-K injector, a Model 660 solvent gradient programmer, a Model 740 data module, and a Vydac 218TP54 reversed-phase, C_{18} column. Preparative HPLC was performed on a system including a Waters Associates Model 590 pump fitted with preparative heads, a Rheodyne injector, an Autochrom OPG/S prepump solvent mixer, and a Waters Associates variable-wavelength UV detector.

Tetrahydrofuran (THF) was obtained dry and peroxide free by distillation from sodium benzophenone ketyl. Dimethylformamide, where referred to as dry, was distilled from ninhydrin under high vacuum and was stored over activated 4-Å molecular sieves. Dimethyl sulfoxide, where referred to as dry, was distilled under high vacuum and stored over 4-Å molecular sieves. Diisopropylethylamine (DIEA) was distilled from KOH. All other solvents used were of reagent grade and were used without further purification unless otherwise noted.

Volatile solvents, for example, acetonitrile, ether, and ethyl acetate, were evaporated at 40 °C under reduced pressure with a Buchi Rotovapor R rotory evaporator operating at water aspirator pressure (ca. 18 mmHg). Water, DMF, and DMSO were evaporated at full vacuum, ca. 0.1 mmHg, with the stripping flask immersed in a 40 °C water bath. The phrase "evaporated in vacuo" refers to the latter process.

Symmetrical anhydrides of urethane-blocked amino acids were prepared by addition of 0.5 equiv of dicyclohexylcarbodiimide to a 5 °C solution of 1 equiv of 1 M *N*-urethanoamino acid in dichloromethane. After 30 min, the urea was removed by filtration, and the solvent was removed in vacuum to yield a crude solid or oil that was used immediately without purification.

As reported in the following procedures the bis(urea)–tetrakis(amino acid) conjugates of epindolidione were purified by chromatography on columns and characterized by high-field ^{1}H NMR, HPLC, and FAB mass spectrometry. Except in the case of the master derivative **1f**, which can be induced to crystallize, combustion analysis was not attempted on these high molecular weight species. Proof of homogeneity can be established by the simple and clean resonances of ^{1}H NMR spectra that are seen for all derivatives here reported, except as otherwise noted.

Preparation of Dimethyl Dihydroxyfumarate. Dihydroxyfumaric acid (21.45 g, 145 mmol) was dissolved in anhydrous methanol (100 mL) to afford a clear, brown solution. The flask was chilled in ice, and the contents were stirred with the aid of a magnetic stirrer. Thionyl chloride (21.0 mL, 288 mmol) was slowly introduced under the surface of the methanolic solution through a syringe. The ice bath was removed 5 min after the addition was complete. The mixture was allowed to stir 2 days at room temperature, and the product precipitated as an off-white solid during that time. The solid was collected on a glass frit and was washed first with 20 mL of cold methanol and then with 80 mL of water. The filter cake was broken with a spatula and set aside to dry in air: 21.09 g, 83%; mp 177–179 °C (lit.[7] mp 168–172 °C).

The material was used in the next preparation without further purification.

Preparation of Dimethyl Bis(*p*-nitroanilino)maleate. The procedure mimics that reported by Jaffe and Matrick for their preparation of bis(anilino)maleates.[7] Dimethyl dihydroxyfumarate (9.85 g, 56 mmol) and *p*-nitroaniline (15.3 g, 111 mmol) were slurried in 42 mL of methanol, ca. 1.0 mL of concentrated HCl was added, and the mixture was brought to reflux. One hour was required for complete solution; shortly thereafter, precipitation of a colored solid began. After 4 h, the reaction was cooled slowly, chilled in ice, and then filtered through a glass frit. The collected orange solid was rinsed with methanol several times and then dried under high vacuum: 14.9 g, 65%; mp 238–244 °C; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 8.08 (2 H, d), 6.73 (2 H, d), 3.77 (3 H, s); MS (70 eV) *m/e* (relative intensity) 417 (M + 1$^+$, 39), 416 (100), 356 (44), 297 (53), 207 (41), 149 (66), 117 (95), 92 (41), 76 (62), 59 (67).

Preparation of 2-(Methoxycarbonyl)-3-(*p*-nitroanilino)-6-nitro-4-quinolone (5). Dowtherm A (150 mL) was brought to a vigorous reflux in a three-neck round-bottomed flask fitted with two stoppers, a Friedrichs condensor, and a heating mantle. One of the stoppers was replaced by a powder funnel, and dimethyl bis(*p*-nitroanilino)maleate (11.93 g, 28.7 mmol) was added in small portions over 5 min. After the addition was complete, the funnel was washed with 10 mL of Dowtherm A and the stopper was replaced. After 15 min of vigorous reflux, the heating mantle was removed and the flask was allowed to cool slowly to room temperature as a precipitate formed. The resulting orange powder was collected on a large glass frit and was washed with petroleum ether (200 mL). The filter cake was crumbled and was allowed to dry in air: 5.88 g, 53%; mp 265–270 °C dec.

Note: Higher yields (up to 87%) can be obtained by adding the starting material as a hot Dowtherm A solution to the refluxing Dowtherm A. The addition funnel should be jacketed with a heating element to keep the solution hot enough to avoid precipitation of the dimethyl bis(*p*-nitroanilino)maleate: ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.72 (1 H, br s), 8.87 (1 H, d, J = 2.6 Hz), 8.83 (1 H, s), 8.50 (1 H, dd, J = 2.6, 9.2 Hz), 8.07 (1 H, d, J = 9.2 Hz), 8.01 (2 H, d, J = 9.2 Hz), 6.74 (2 H, d, J = 9.6 Hz), 3.84 (3 H, s); MS (70 eV) *m/e* (relative intensity) 384 (M$^+$, 100), 306 (46), 278 (31), 232 (25), 204 (20).

Preparation of 2-(Methoxycarbonyl)-3-[*N*-(*p*-aminophenyl)amino]-6-aminoquinolone. A solution of the above-prepared **5** (1.50 g, 3.9 mmol) in aqueous HCl (12 M, 100 mL) was warmed on the steam bath, filtered, and treated with stannous chloride dihydrate (7.5 g, 36 mmol) in one portion. The resulting solution was heated for 10 min on a steam bath, filtered, and then cooled and evaporated in vacuum. A solution of the resulting brown gum in MeCN (30 mL) was treated with ether (200 mL) to precipitate a bright yellow solid that was collected on a glass frit, washed with ether, and dried under vacuum to yield 1.96 g of the title compound as its dihydrochloride salt. This substance was used in the next preparation without purification: FAB MS (glycerol) *m/e* (relative intensity) 326 (M + 2$^+$, 39), 325 (75), 324 (26), 293 (51); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.90 (4 H, br s), 7.99 (1 H, s), 7.97 (1 H, d, J = 9.5 Hz), 7.11 (2 H, d, J = 8.8 Hz), 6.73 (2 H, d, J = 8.8 Hz), 3.78 (3 H, s).

Preparation of 2,8-Diaminoepindolidione (6). A mixture of anhydrous $AlCl_3$ (17 g) and finely ground NaCl (1.7 g) was heated in small round-bottomed flask with magnetic stirring until the mixture fused at ca. 100 °C as measured with an internal thermometer. To this was added the above-prepared dihydrochloride salt of 2-(methoxycarbonyl)-3-[*N*-(*p*-aminophenyl)-amino]-6-quinolone (1.96 g, 4.9 mmol) as a slow, steady stream. The resulting viscous purple melt was heated to 160 °C for 1 h and then was carefully poured over ice and concentrated HCl (25 g and 3 mL). The flask was rinsed with a minimum volume of water, and the combined aqueous phases were heated to near-boiling for 10 min. The resulting suspension was transferred to 30-mL Corex centrifuge tubes and centrifuged for 20 min. The resulting clear supernatant phase was discarded, and the solid was resuspended in water (20 mL). After recentrifugation, the resulting solid was subjected to a modification of the purification procedure for epindolidiones of Jaffe and Matrick.[7] The precipitate from the second centrifugation was dissolved in a minimum volume of concentrated sulfuric acid (ca. 8 mL); the solution was chilled in ice and treated dropwise with an equal volume of water. The resulting solid was collected by centrifugation, resuspended in water, and again collected by centrifugation to give a yellow-green solid. Repetition of the suspension–centrifugation cycle with MeCN as solvent followed by evaporation of an MeCN suspension and drying under vacuum gave the bis(bisulfate) salt of 2,8-diaminoepindolidione (**6**) as a microcrystalline yellow-green solid from **5**: 1.00 g, 60%; mp >300 °C dec; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.37 (1 H, s), 8.27 (1 H, d, J = 1.5 Hz), 8.18 (1 H, d, J = 8.9 Hz), 7.75 (1 H, dd, J = 8.4, 1.7 Hz), 6.10 (large broad resonance); MS (70 eV) *m/e* (relative intensity) 293 (M + 1$^+$, 35), 292 (100). Anal. Calcd for $C_{16}H_{12}N_4O_2 \cdot 1.2H_2SO_4 \cdot H_2O$: C, 44.90; H, 3.86; N, 13.09; S, 8.90. Found: C, 45.09; H, 3.85; N, 12.96; S, 8.86.

Preparation of 2,8-Bis(Boc-D-Ala-NH)epindolidione (7b). To a solution of 2,8-diaminoepindolidione bisulfate salt (0.47 g, 0.96 mmol) in DMSO (30 mL) was added with stirring triethylamine (270 μL, 1.93 mmol), which generated a pink suspension of the bis(diamine). Freshly recrystallized (Boc-D-Ala)$_2$O (0.76 g, 2.1 mmol; mp 116–117 °C; prepared from Boc-D-Ala-OH and DCCI) was added immediately in one portion, and the reaction was stirred for 1 h. Filtration of the resulting clear solution through Celite and evaporation in vacuum gave a red crust that was suspended in MeOH (60 mL) with the help of a sonicator. The suspension was centrifuged, and the solid was collected as an ether slurry to give 0.46 g (76%) of the title compound as a red powder: mp >300 °C dec; ^{1}H NMR (DMSO d_6, 250 MHz) δ 12.03 (1 H, s), 10.18 (1 H, s), 8.66 (1 H, s), 8.04 (1 H, d, J = 9.1 Hz), 7.87 (1 H, br d, J = 8.5 Hz), 7.12 (1 H, br d, J = 6.0 Hz), 4.15 (1 H, m), 1.40 (9 H, s), 1.30 (3 H, J = 6.8 Hz); FAB MS (glyerol) *m/e* (relative intensity) 636 (100), 635 (M+1$^+$, 31).

Preparation of 2,8-Bis(Boc-Pro-D-Ala-NH)epindolidione (8b). A slurry of 2,8-bis(Boc-D-Ala-NH)epindolidione (**7b**) (254 mg, 0.40 mmol) in CH_2Cl_2 (10 mL) was chilled to 0 °C and treated with trifluoroacetic acid (10 mL). After 10 min, the deep red solution was evaporated and the resulting solid was triturated with ether (10 mL), then dried under vacuum for 1 h, and dissolved in dry DMF (40 mL). To this solution was added triethylamine (97 μL, 0.71 mmol), followed by the pentafluorophenyl ester of Boc-ProOH (305 mg, 0.90 mmol; prepared from C_6F_5OH, Boc-

Pro-OH, and DCC). After 4 h, the solution was filtered through Celite and evaporated. The residue was triturated with cold MeOH and then collected as a red powder, 126 mg, 50%. The material was sufficiently pure for succeeding steps in the synthesis. For NMR studies, a portion (28 mg) of the red powder was purified by gel filtration on an LH-20 column (2.5 cm × 50 cm, 0.5 mL/min) with DMF as eluant. The purified material is yellow: ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.03 (1 H, s), 10.31 and 9.82 (1 H, s), 8.66 (1 H, s), 8.46 and 8.14 (1 H, d and d, J = 6.9 Hz and J = 6.3 Hz), 8.04 (1 H, d, J = 9.2 Hz), 7.90 (2 H, m), 4.52 (1 H, m), 4.18 (1 H, m), 3.40 (obscured by HOD), 2.22 (1 H, m), 1.83 (3 H, m), 1.38 and 1.30 (9 H, s); FAB MS (glycerol) m/e (relative intensity) 831 (19), 830 ($M+1^+$, 29), 829 (25), 828 (20), 631 (25), 630 (57), 629 (100), 562 (21), 560 (23).

2,8-Bis[(*N*-carbonyl-Gly-OEt)-Pro-D-Ala-NH]epindolidione (9b). A slurry of **8b** (98 mg, 0.12 mmol) in CH_2Cl_2 (6 mL) at 0 °C under N_2 was treated with trifluoroacetic acid (6 mL). After 20 min the red solution was evaporated under vacuum, and the resulting red crust was dried for 2 h under vaccum. A solution of this substance in dry DMF (18 mL) under N_2 was treated with triethylamine (50 μL, 0.36 mmol), followed by ethyl isocyanatoacetate[18] (50 μL, 0.4 mmol). After 4 h, HPLC analysis indicated absence of starting material. Aliquots applied to a Vydac 218TP54 column (gradient, 20–50% MeCN/0.1% TFA over 10 min; 254-nm detection) showed loss of a peak, R_t < 5 min, appearance and disappearance of a peak corresponding to half-reaction, and appearance of the product peak, R_t 9.95 min. Application of the solution to a Sephadex LH-20 column (2.5 cm × 50 cm) and elution with DMF, 0.5 mL/min, gave purification to 98+% homogeneity. Evaporation of appropriate fractions gave the title compound as an orange crust: 103 mg,95%; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.06 (1 H, s), 9.79 (1 H, s), 8.89 (1 H, d, J = 2.0 Hz), 8.67 (1 H, d, J = 6 Hz), 8.04 (1 H, d, J = 6 Hz), 6), 7.97 (1 H, dd, J = 2.1, 5.7 Hz), 6.97 (1 H, t), 4.38 (1 H, p), 4.21 (1 H, t), 4.04 (2 H, m), 3.79 (2 H, d J = 5.7 Hz), 3.40 (2 H, m), 2.05 (2 H, m), 1.87 (2 H, m), 1.38 (3 H, d, J = 7.2 Hz), 1.14 (3 H, t, J = 7.1 Hz); HPLC, R_t 9.95 min, Vydac 218TP54 column, 1.0 mL/min, 254-nm detector, 20–50% acetonitrile/0.1% TFA over 10 min.

2,8-Bis[(*N*-carbonyl-Gly-OH)-Pro-D-Ala-NH]epindolidione. To a solution of **9b** (103 mg, 0.02 mmol) in THF/H_2O (1:1, 20 mL) was added with stirring LiOH (160 μL of 2.1 M, 0.33 mmol). The reaction was monitored by HPLC and was complete after 36 h. The solution was neutralized with HCl to pH 3, and the solvent was removed under vacuum. The resulting suspension in water was centrifuged, and the residue was collected and dried under vacuum to yield a dark green solid of suitable purity for the next synthetic step: 96 mg, 99%; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 12.03 (1 H, s) 9.82 (1 H, s), 8.85 (1 H, s), 8.57 (1 H, d, J = 7.4 Hz), 8.06 (1 H, d, J = 9.2 Hz), 7.97 (1 H, dd, J = 1.4, 9.2), 6.83 (1 H, m), 4.38 (1 H, m), 4.21 (1 H, m), 3.75 (2 H, m), 3.39 (2 H, m, overlapping with HOD), 2.05 (2 H, m), 1.94 (2 H, m), 1.38 (3 H, d, J = 7.1); HPLC, R_t 7.70 min, Vydac 218TP54 column, 1.0 mL/min, 254-nm detector, 20–50% acetonitrile/0.1% TFA over 10 min; FAB MS (glycerol) m/e (relative intensity) 832 (17), 830 (M + 1^+, 27), 828 (50, coincident with glycerol peak).

L-Phenylalanine Dimethylamide Hydrobromide, H-Phe-NMe$_2$·HBr. To a solution in CH_2Cl_2 (10 mL) of the *p*-nitrophenyl ester of Z-L-Phe-OH (1.77 g, 4.21 mmol; prepared by reaction of Z-L-Phe-OH with HONp and DCC) was added at 0 °C freshly distilled dimethylamine (1 mL at 0 °C). After 5 min, yellow needles were removed by filtration, and the filtrate was diluted with CH_2Cl_2 and extracted (saturated $NaHCO_3$, 2 × 10 mL; 0.1 M HCl, 10 mL; brine, 10 mL), then filtered, dried, and evaporated to a white glass, 1.28 g, 93%. A solution of this glass in 40% HBr/HOAc (15 mL) was maintained with swirling at 23 °C for 15 min and then was treated with dry ether (150 mL). The resulting precipitate was washed with ether, collected, and then dried to give 0.88 g of white solid that was recrystallized from MeOH/ether to give white plates: 0.81 g, 75%; mp 255–257 °C; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 8.22 (3 H, br s, exchanges with D_2O), 7.28 (5 H, m), 4.60 (1 H, t), 3.00 (2 H, m), 1.82 (3 H, s), 1.63 (3 H, s); MS (70 eV) m/e (relative intensity) 193 (M^+, 1.3), 121 (22), 120 (100), 103 (29), 101 (100), 91 (28), 82 (20), 80 (22), 77 (17), 73 (29). Anal. Calcd for $C_{12}H_{16}BrN_2O$: C, 48.54; H, 5.93; Br, 29.36; N, 10.29. Found: C, 48.30; H, 6.15; Br, 29.33; N, 10.23.

2,8-Bis[(*N*-carbonyl-Gly-Phe-NMe$_2$)-Pro-D-Ala-NH]epindolidione (1f). To a solution in dry DMF (5 mL) of 2,8-bis[(*N*-carbonyl-Gly-OH)-Pro-D-Ala-NH]epindolidione (84.4 mg, 0.102 mmol) under N_2 was added HBr·H-Phe-NMe$_2$ (108 mg, 0.40 mmol), *N*-hydroxybenzotriazole (54 mg, 0.40 mmol), diisopropylethylamine (70 mL, 0.40 mmol), and diisopropylcarbodiimide (63 μL, 0.40 mmol). The conversion of starting material to its amide was monitored by HPLC. Test samples of the reaction mixture were analyzed on a Vydac 218TP54 column, showing disappearance of a peak with R_t 7.70 min, appearance and disappearance of a peak with R_t 11.20 min, and appearance of a product peak at 14.26 min (gradient, 20–50% acetonitrile/0.1% TFA over 10 min; detection at 254 nm).

After 22 h, the reaction was complete, and the reaction mixture was applied to a Sephadex LH-20 column (2.5 cm × 50 cm, flow rate 0.5 mL/min) and eluted with DMF. The fractions of appropriate purity were combined and evaporated under vacuum to yield an orange crust, 85.5 mg, 71%, which was further purified by preparative HPLC. A sample (20 mg) was dissolved in MeCN (0.7 mL), and the solution was injected into a preparative HPLC system consisting of a Vydac 218TP1022 column (isocratic elution, 45% MeCN/0.1% TFA at 18.0 mL/min, 254-nm detection). Highly purified product, as judged by HPLC, was recovered from the appropriate fractions. As the column was washed with pure MeCN, a second band containing only the desired compound eluted and was found to contain additional pure product, total yield 10.5 mg + 4.5 mg = 15 mg, or 75%. Additional product could be recovered by recycling impure fractions. By HPLC and NMR, the recovered product was estimated to be 99% homogeneous.

The title compound can be crystallized from anhydrous MeCN to form compact, orange rhombs that convert to fine red needles over a longer period or in the presence of moisture. The former were subject to preliminary X-ray diffraction analysis. The unit cell is tetragonal with $a = b = 22.5865$ Å and $c = 29.451$ Å. The space group is $P42_12$. HPLC, R_t 14.26 min, Vydac 218TP54 column, 1.0 mL/min, 254-nm detector, 20–50% acetonitrile/0.1% TFA over 10 min; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.85 (1 H, s), 9.82 (1 H, s), 8.74 (1 H, d, J = 2.8 Hz), 8.59 (1 H, d, J = 7.2 Hz), 8.29 (1 H, d, J = 8.7 Hz), 8.19 (1 H, dd, J = 2.8, 10.1 Hz), 8.06 (1 H, d, J = 9.3 Hz), 7.09 (5 H, m), 6.65 (1 H, m), 4.93 (1 H, q, J = 7.9 Hz), 4.39 (1 H, p), 4.24 (1 H, m), 4.10 (1 H, dd, J = 6.0, 16.7 Hz), 3.73 (1 H, dd, J = 4.4, 16.6 Hz), 3.40 (obscured by HOD), 3.33 (HOD), 2.81, (2 H, br m), 2.78 (3 H, s), 2.75 (3 H, s), 2.06 (2 H, m), 1.90 (2 H, m), 1.39 (3 H, d, J = 7.3 Hz); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ 172.82, 171.62, 171.17, 170.43, 169.9, 156.57, 136.87, 135.80, 133.51, 129.11 (CH), 127.84 (CH), 126.52 (CH), 126.18 (CH), 125.23 (CH), 120.93, 119.42 (CH), 113.80 (CH), 60.16 (CH), 49.73 (CH), 48.69 (CH), 46.01 (CH_2), 43.22 (CH_2), 38.15 (CH_2), 36.49 (CH_3), 35.22 (CH_3), 30.35 (DMF), 29.32 (CH_2), 24.53 (CH_2), 14.41 (CH_3); FAB MS (glycerol) m/e (relative intensity) 1181 (44), 1180 (M + 1^+, 100), 1179 (52), 1178 (66). Anal. Calcd for $C_{60}H_{70}N_{14}O_{12}·4H_2O$: C, 57.59; H, 6.28; N, 15.67. Found: C, 57.59; H, 6.18; N, 15.67.

NMR Temperature Dependence Experiments. A solution of several milligrams of the sample in 0.50 mL of DMSO-d_6 is transferred to a clean NMR tube, frozen, and then thawed under reduced pressure a few times to remove dissolved O_2. TMS is added as an internal standard, and the tube is purged with N_2 and sealed with parafilm. The $\Delta\delta/\Delta T$ experiments were run on a Bruker 250M instrument using default setup parameters, except for those that affect digital resolution, which were modified to achieve digitization >0.4 Hz/point, ensuring that δ changes >0.0016 ppm are significant. Spectra were acquired from 295 to 340 K at 5 K intervals, using the T control feature of the instrument. After a T change, data acquisition was delayed for >3 min. After a cycle to 340 K, the sample was cooled and the cycle repeated to confirm the absence of sample degradation. The $\Delta\delta/\Delta T$ values were obtained by a least-squares analysis; linear correlations were obtained, and correlation coefficients R were generally greater than 0.99.

NOESY Experiments. Two-dimensional NOESY experiments were performed on a Varian VXR-500S instrument at 22 or 25 °C in DMSO-d_6 at ca. 15 mM sample concentration in the

(18) Goldschmidt, S.; Wick, M. *Justus Leibigs Ann. Chem.* **1950**, *575*, 217.

magnitude mode. Generally 32 transients were acquired for each of 256 increments in t_1, which were zero-filled to a 2K × 1K matrix and transformed with pseudoecho weighting. The mixing time was set to 350 ms, and the recycle delay, to 3.0 s. NOESY cross-peak intensities as estimated by peak height were divided by peak intensities for the epindolidione aryl NH to H-1 and the heteroaryl NH to H-4 cross peaks in order to establish relative strengths of signals. The following rating system was used for these ratios: vs, >0.5; s, 0.15–0.5; m, 0.03–0.14; w, <0.03. For the derivatives discussed in the text, the following intensities were observed (heteroaryl NH to H-4 comparison appears in parentheses). For $d_{NN}(3,4)$: **8a**, m (w); **8b**, m (w); **8d**, s (s). For $d_{N_\alpha}(3,4)$: **8a**, vs (m); **8b**, s (m); **8d**, s (s). For $d_{N_\alpha}(2,3)$: **8a**, m (w); **8b**, m (w); **8d**, s (m).

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Supplementary Material Available: Experimental procedures for the preparation of substances **1a–m** and their precursors (19 pages). Ordering information is given on any current masthead page.

General Synthesis of β,γ-Alkynylglycine Derivatives

Robert M. Williams,*,† David J. Aldous, and Suzanne C. Aldous

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

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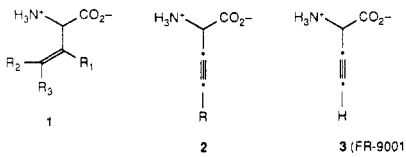
The coupling of α-haloglycinates **8** with alkynyltin reagents produces the fully protected β,γ-alkynylglycines **9**. Subsequent deprotection of the amino or carboxyl groups generates differentially protected β,γ-alkynylglycine derivatives **10–14**. The free amino acids were found to be too labile to isolate but can be generated in situ and trapped.

Introduction

β,γ-Unsaturated α-amino acids **1** have recently attracted considerable attention[1] due to their known ability[2] to pose as suicide inhibitors of pyridoxal-linked enzyme systems. The rarer β,γ-alkynyl α-amino acids **2** are represented by a single known[3] natural antibiotic, ethynylglycine (**3**, FR-900130), which is a suicide substrate for alanine racemase.



1 **2** **3** (FR-900130)

Both the vinyl (**1**) and ethynyl (**2**) amino acids are very challenging structures to prepare due to their chemical lability to racemization and tautomerization to unstable α,β-dehydro amino acids. While methodology to construct vinylglycines has been emerging,[4] comparable technology to construct the alkynyl systems has lagged[5] due to the greater relative chemical lability of these substances. As part of a general program aimed at embracing the most labile classes of amino acids, we have developed a mild alkynylation[6] of electrophilic glycinates[7] via organotin acetylides. We have recently communicated[8] the application of this methodology to construct *N*-acetylethynylglycine (*N*-acetyl-FR-900130) in racemic form. In this article, we disclose a full account of this general approach to differentially protected alkynylglycines.

Results and Discussion

The general approach that has been deployed is illustrated in Scheme I. Three different haloglycinates **8** were prepared according to the method of Ben-Ishai.[9] Both the carboxyl and amine protecting groups were varied in hopes of identifying suitable combinations for selective unmasking of the amine and carboxyl, respectively. We also hoped to be able to obtain the free amino acids, a task that has not yet been achieved due to the lability of the free amino acids themselves. The coupling reactions were

Scheme I

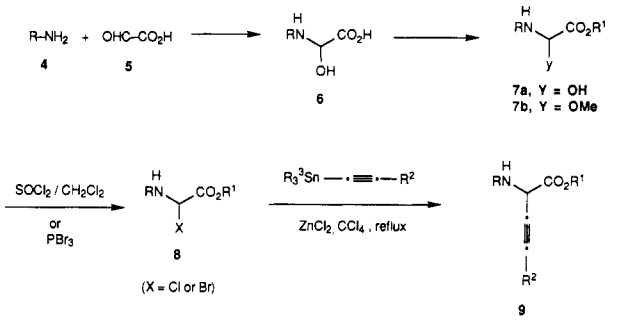


Table I

R	R^1	R^2	R^3	yield, % 7	8	9
CBz	Me			~quant.	~quant.	
		$SiMe_3$	Me			88
		Me	*n*-Bu			84
		n-C_3H_7	*n*-Bu			62
		n-C_4H_9	*n*-Bu			50
		n-C_6H_{13}	*n*-Bu			56
		Ph	*n*-Bu			64
		$CH_2CH_2OSiMe_2$-*t*-Bu	*n*-Bu			44
CBz	$CH(Ph)_2$			~quant.	~quant.	
		$SiMe_3$	Me			86
		Me	*n*-Bu			68
		n-C_3H_7	*n*-Bu			62
		n-C_4H_9	*n*-Bu			60
		n-C_6H_{13}	*n*-Bu			55
		Ph	*n*-Bu			80
		$CH_2CH_2OSiMe_2$-*t*-Bu	*n*-Bu			66
Ac	$CH(Ph)_2$			~quant.	~quant.	
		$SiMe_3$	Me			63
		Me	*n*-Bu			54
		n-C_3H_7	*n*-Bu			55
		n-C_4H_9	*n*-Bu			44
		n-C_6H_{13}	*n*-Bu			50
		Ph	*n*-Bu			58
		$CH_2CH_2OSiMe_2$-*t*-Bu	*n*-Bu			55

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