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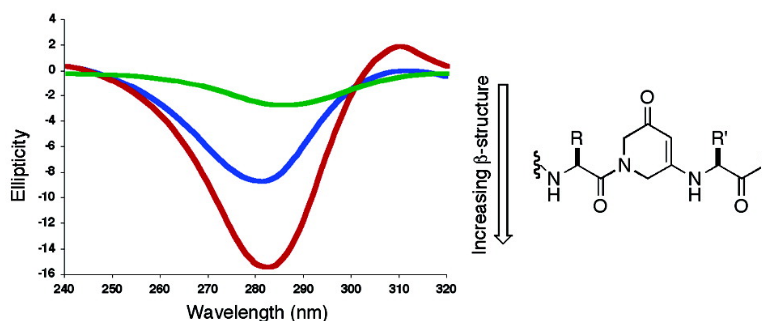


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# @-Tides as Reporters for Molecular Associations

Scott T. Phillips, Landy K. Blasdel, and Paul A. Bartlett*

*Contribution from the Center for New Directions in Organic Synthesis, Department of Chemistry, University of California, Berkeley, California 94720-1460*

Received August 12, 2004; E-mail: paul_bartlett@berkeley.edu

***Abstract:*** The 1,6-dihydro-3(2*H*)-pyridinone unit is an amino acid surrogate that favors the extended β-strand conformation when incorporated in an oligopeptide ("@-tide") strand. We now report that the circular dichroism (CD) signature of the vinylogous amide in the @-unit is sensitive to conformation in organic and aqueous solvents and, therefore, is useful as a quantitative measure of @-tide association and folding processes that involve this moiety. Moreover, this method can be employed in the micromolar concentration range, which is not readily accessible using other techniques. Measurements of @-tide dimerization and β-hairpin folding equilibria not only demonstrate the utility and generality of this approach but also provide a way to quantify amino acid side chain−side chain interactions relevant to β-sheet stability.

## Introduction

Reversible protein folding and protein−protein interactions are of critical importance in the biological sciences.[1−5] These intra- and intermolecular processes depend on the cumulative effects of many individual interactions involving the peptide backbones and the side chains of the component amino acids. As a consequence, a rapid and convenient method for detecting and characterizing molecular associations involving peptides would be of considerable utility in understanding the factors that stabilize peptide secondary and tertiary structures and in developing peptidomimetics. Current methods are labor-intensive (e.g., NMR),[6,7] suffer from limited generality (e.g., CD and FT-IR spectroscopy),[8−10] require appended chromophores (e.g., FRET),[11,12] or provide little structural information (e.g., surface plasmon resonance[13] and microcalorimetry[14]). Methods that are rapid and can be applied broadly, such as UV or fluorescence spectroscopy, are generally insensitive to peptide conformation or association state.[15,16] In the case of CD spectroscopy, the signal is usually a complex convolution of signatures from different regions of secondary structure.[9,17] Moreover, the range of affinities to which each method can be applied is restricted because of sensitivity, dynamic range, or competing effects; dissociation constants in the micromolar range are particularly difficult to quantify. For example, NMR is not sufficiently sensitive, and FRET-based methods suffer from saturation or quenching from nonradiative decay at these concentrations. Some recent approaches leverage biological diversity in enabling large numbers of different analogues and folding equilibria to be assessed, but they either rely on less direct methods for quantification[18] or are limited in throughput.[19] As a consequence, comprehensive comparisons of how individual amino acids or amino acid combinations perform in specific contexts have been difficult to obtain. The ideal spectroscopic system would involve a chromophore that is small and integral to the system studied and whose signal differs significantly between bound and unbound states, depends linearly on concentration, and is unaffected by solvent or peripheral structural elements.

In the course of our studies of the 1,6-dihydro-3(2*H*)-pyridinone moiety (the "@-unit") as a peptidomimetic, we observed that the CD spectra of @-tide oligomers contain strong signals attributable to the vinylogous amide absorbance near 280 nm.[20] The observation that these signals are often affected by solvent suggested that they might reflect the conformation of the oligomer in the vicinity of the chromophore. We now report that this relationship is indeed quantitative, and that the

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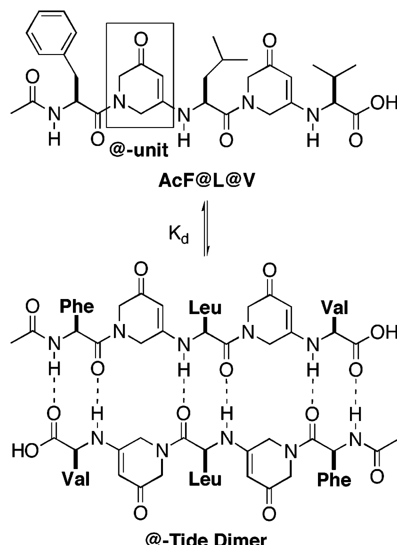


*Figure 1.* @-Tides show a strong propensity for the extended, $\beta$-strand conformation and dimerize in organic solvents.

@-unit provides a convenient and sensitive reporter of @-tide and oligopeptide structure. The utility of this spectroscopic probe is demonstrated in the context of intermolecular @-tide dimerizations and intramolecular oligopeptide folding. In addition, a quantitative assay is presented for $\beta$-hairpin folding, where measurement of weakly stabilizing interactions is required.[21] Implementation of this exceedingly straightforward assay is demonstrated by quantifying and comparing several amino acid interactions that contribute to hairpin stability, all within the same model $\beta$-hairpin framework.

The @-unit was devised as an amino acid surrogate that restricts the flexibility of the peptide backbone and stabilizes the extended conformation.[22] Peptide analogues in which alternating residues are replaced with the @-unit retain the natural pattern of hydrogen bond acceptors and donors along one edge and, thus, are predisposed to dimerize or to associate with another peptide strand (Figure 1). Intermolecular associations between @-tides in head-to-tail dimers[22] and intramolecular association of @-tides with peptides within $\beta$-hairpins in organic solvents have been characterized structurally by NMR.[20] By correlating these structures and their dependence on solvent and concentration with the CD signatures of the @-units, we show that the latter can be used as a convenient and quantitative measure of @-tide conformation. The @-unit thus serves as an intrinsic reporter group for peptide conformation, providing a signal at 280 nm which is independent of the normal peptide signature.

## Results and Discussion

**Effect of Dimerization on @-Tide Circular Dichroism Spectra.** @-Tide oligomers dimerize in chloroform but are dissociated in methanol.[22] Dissociation constants, determined from the concentration dependence of NMR chemical shifts, range from millimolar to micromolar depending on the length of the oligomer and the concentration of methanol. The $K_d$ value for AcF@L@V, for example, is 100 $\mu$M in 3% methanol−chloroform, increasing to 100 mM in 6% methanol−chloroform.


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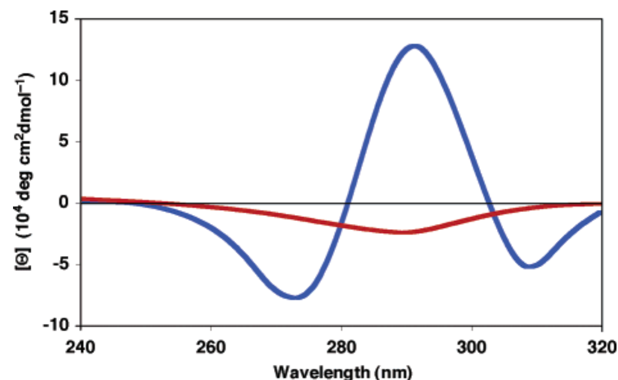


*Figure 2.* CD spectra of AcF@L@V in $CHCl_3$ and MeOH (0.22 mM, 25 °C), reflecting the dimeric ($CHCl_3$; blue) and monomeric species (MeOH; red); ellipticity is calculated on a per-@-unit basis.

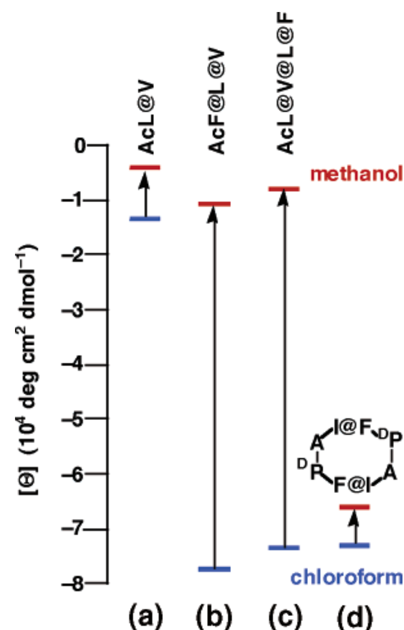


*Figure 3.* Solvent dependence ($CHCl_3$ blue → MeOH red) of the CD signals for a variety of @-tides at 25 °C (ellipticity calculated on a per-@-unit basis): (a) AcL@V (0.45 mM, 272 nm); (b) AcF@L@V (0.22 mM, 272 nm); (c) AcL@V@L@F (0.20 mM, 280 nm); (d) cyclo[I@F$^D$PAI@F$^D$PA] (0.12 mM, 284 nm).

The CD signal of this @-tide shows a similar dependence on solvent; the strong ellipticity observed in chloroform is almost completely attenuated in methanol (Figure 2).

The solvent dependence of the CD signals for other analogues provided further evidence of a correlation with @-tide structure (Figure 3). Large differences that are similar in magnitude on a per-@-unit basis are observed for the pentamer AcF@L@V and heptamer AcL@V@L@F between the dimeric forms in chloroform and the monomers in methanol. In contrast, the trimer AcL@V gives a weak signal in both solvents because it is monomeric even in chloroform. The CD spectrum of the cyclic analogue, cyclo[I@F$^D$PAI@F$^D$PA], which is covalently fixed in the "dimeric" conformation, has strong signals in both solvents.

The CD signature of the amide linkage in a peptide is relatively unaffected by the identity of the amino acid side chains.[17] Similarly, the CD signal from the @-unit is independent of amino acid composition. For example, the signal intensities in methanol of the monomeric tri-@-tides T@T,

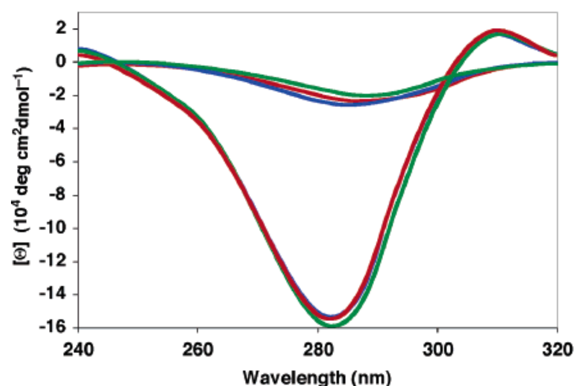


*Figure 4.* CD spectra in methanol for unstructured analogues at 25 °C (upper curves, AcL@V green, T@T red, V@T blue; 80−560 $\mu$M) and for cyclic $\beta$-sheet mimics (lower curves, cyclo[V@V$^{D}$PAVTV$^{D}$PA] green and cyclo[I@V$^{D}$PAVTT$^{D}$PA] red, superimposed on cyclo[V@V$^{D}$PAVVV$^{D}$PA] blue; 16−28 $\mu$M).

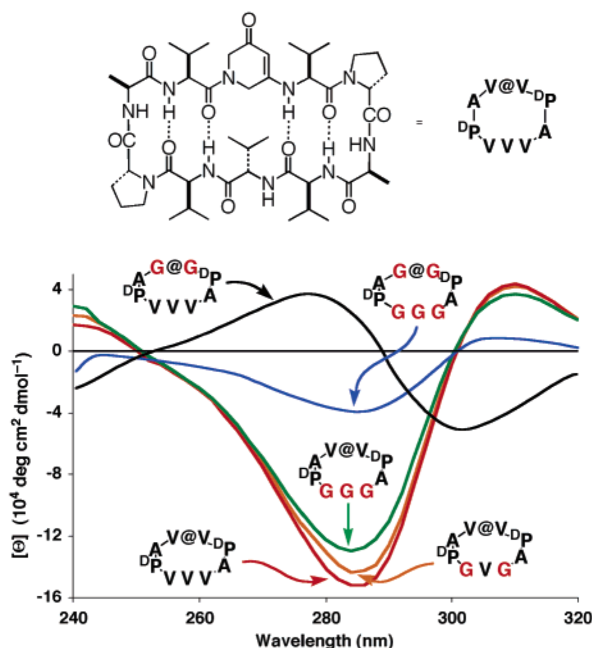


*Figure 5.* CD spectra for cyclo[V@V$^{D}$PAVVV$^{D}$PA] (red curve) and analogues with glycine substituted at various positions in the $\beta$-sheet region (12−44 $\mu$M in 3% methanol−$CHCl_3$ at 25 °C).

V@T, and AcL@V are consistently low, while the cyclic-@-tides cyclo[V@V$^{D}$PAVVV$^{D}$PA], cyclo[V@V$^{D}$PAVTV$^{D}$PA], and cyclo[I@V$^{D}$PAVTT$^{D}$PA] show strong and superimposable signals (Figure 4). However, exceptions to this uniform behavior are found when exciton transfer can occur between the @-unit and a nearby aromatic side chain absorbing in the same spectral region.[20]

**Origin of the @-Unit CD Signal.** The origin of the @-unit ellipticity was probed with a series of cyclic @-tides containing the achiral amino acid glycine at various positions. The cyclic structure and solvent (3% methanol in chloroform) were chosen to enforce the fully structured $\beta$-sheet conformation (Figure 5). The asymmetric environment of the @-unit is imparted by the flanking amino acids, the opposing hydrogen-bonded tripeptide, and the $^{D}$Pro-Ala $\beta$-turns.[23] The amino acids on either side of the @-unit clearly have the greatest influence on the CD signal;

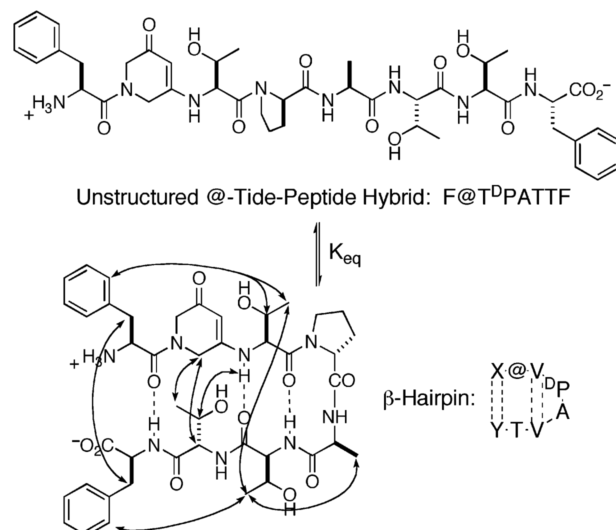


*Figure 6.* An @-tide−peptide hybrid as a model $\beta$-sheet system; key inter-residue and cross-strand NOEs are consistent with the $\beta$-hairpin conformation observed for F@T$^{D}$PATTF in aqueous solution (5.1 mM concentration in 10 mM phosphate buffer, pH 7.0, 10% $D_2O$−$H_2O$, 5 °C).

in contrast, replacement of the Val residues with Gly in the opposing tripeptide has only a small effect on the ellipticity. Substituting all of the $\beta$-sheet residues with Gly reduces the signal dramatically, indicating that the $\beta$-turn units themselves make only modest contributions to the signal. Interestingly, replacing the flanking residues with Gly while retaining Val in the opposing tripeptide leads to an inversion of the signal, suggesting that the dihydropyridinone ring may be forced into the opposite pucker for reasons that are not clear.

**Extension to $\beta$-Hairpin Structures.** While peptides containing fewer than 10 amino acids rarely adopt stable secondary structures in water,[8,19,24] @-tide−peptide hybrids in which the two strands are linked by a $\beta$-turn sequence ($^{D}$Pro-X)[23] show a strong propensity to fold as $\beta$-hairpins. In the sequence F@T-$^{D}$PATTF, hydrophobic interaction between the aromatic rings of the phenylalanines and electrostatic complementarity of the N- and C-termini help to stabilize the $\beta$-hairpin conformation of this hybrid. A series of cross-strand and inter-residue relationships observed in the NOESY NMR spectrum indicate that this conformation is populated to a significant extent in water (see Figure 6). Early work on autonomously folding $\beta$-sheet protein mimics was plagued by aggregation and insolubility since many such systems depended on hydrophobic interactions for stabilization.[8] With the discovery of residue pairs, such as $^{D}$Pro-Ala, that favor the $\beta$-turn conformation,[23] shorter mimics of $\beta$-structure have become accessible. These model systems, from 10 to 20 residues in size, have shown much less tendency to aggregate, remaining monomeric into the millimolar concentration range.[24−27] The reduction of "external" hydrogen-bonding ability through the incorporation of *N*-methyl amino acids also reduces the aggregation of $\beta$-sheet mimics.[28,29]

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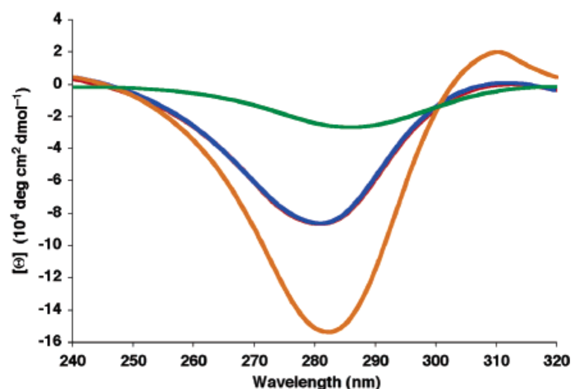


*Figure 7.* Superimposed CD signals for F@T$^{D}$PATTF at 19 $\mu$M (red) and 46 $\mu$M (blue) concentrations in 10 mM sodium phosphate buffer, pH 7, 25 °C; curves for V@T (84 $\mu$M, green) and cyclo[I@V$^{D}$PAVTT$^{D}$PA] (16 $\mu$M, orange) are included for comparison.

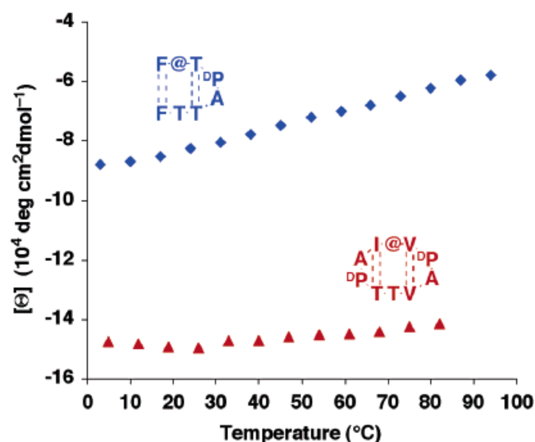


*Figure 8.* Temperature dependence of signal intensity at 282 nm for F@T-$^{D}$PATTF (blue, 45 $\mu$M) and cyclo[I@V$^{D}$PAVTT$^{D}$PA] (red, 16 $\mu$M) in 10 mM sodium phosphate buffer, pH 7.

The @-unit functions in a similar fashion in our $\beta$-hairpins, leaving only a single Thr residue in a position to participate in intermolecular hydrogen bonding. Consistent with this precedence, our short (8 residue) @-tide-stabilized hairpins proved to be readily soluble and showed no concentration-dependent changes in NMR chemical shifts (1−5 mM) or CD spectra (19−46 $\mu$M; see below).

In accord with a folded conformation, the CD spectrum of F@T$^{D}$PATTF shows a signal that is intermediate in strength to those of structured and unstructured @-tides (Figure 7), suggesting that the CD signal reflects the position of equilibrium between the folded and unfolded states. While the CD spectrum of this @-tide−peptide hybrid is unaffected by concentration between 19 and 46 $\mu$M, consistent with a monomeric state under these conditions, the ellipticity does decrease in magnitude with temperature as the hairpin unfolds (Figure 8). For comparison, cyclo[I@V$^{D}$PAVTT$^{D}$PA], which is fixed in the folded conformation, exhibits a much smaller change in signal over the same temperature range.

**Development of a Quantitative CD Assay.** The relationship between @-tide conformation and the ellipticity (mean-@-unit ellipticity) observed in the CD spectrum suggested that the @-unit not only can be used as a template for $\beta$-hairpin stabilization but also can serve as a spectroscopic reporter of conformation. In analogy to NMR methods for determining $\beta$-hairpin populations,[6,19] appropriate standards for 0 and 100%

*Table 1.* Mean-@-unit Ellipticities [Θ] ($10^4$ deg·cm$^2$·dmol$^{-1}$) at 282 nm for @-Tide Control Compounds Representing 0 and 100% $\beta$-Hairpin Conformations[a]

| control set | compound | [Θ] | average |
|---|---|---|---|
| 0% $\beta$-hairpin | V@T | −2.55 | $[\Theta]_0 = -2.51 \pm 0.24$ |
| | T@T | −2.26 | |
| | T@T$^{L}$PATTT | −2.73 | |
| 100% $\beta$-hairpin | Cyclo[I@V$^{D}$PAVTT$^{D}$PA] | −15.63 | $[\Theta]_{100} = -15.67 \pm 0.16$ |
| | Cyclo[V@V$^{D}$PAVTV$^{D}$PA] | −15.84 | |
| | Cyclo[V@V$^{D}$PAVVV$^{D}$PA] | −15.53 | |

[a] Determined in 10 mM phosphate buffer, pH 7, 25 °C; each value is the average of three independent measurements over the concentration range of 15−45 $\mu$M.

$\beta$-hairpins were used to determine the limiting ellipticities (Table 1), against which values observed for target hairpins can be evaluated. This quantitative assay is straightforward and rapid, and it is readily applied to a series of @-tide−peptide hybrids. It thus has several advantages over more labor-intensive and less generalizable NMR approaches, in which the signals observed are often dependent on sequence as well as conformation.[6,21,30,31] In addition, this assay requires only a single CD measurement to determine the folding equilibrium without the need for internal standards or HPLC separations such as those required in other systems.[32−34]

To sample a variety of structures and amino acid combinations, we synthesized two types of control compounds representative of unstructured @-tides (0% controls): tri-@-tides, which are monomeric in water,[22] and @-tide−peptide hybrids incorporating L-proline, which disfavors the $\beta$-hairpin structure.[35] A series of cyclic-@-tide−peptide hybrids serve as the 100% controls. The CD-derived mean-@-unit ellipticities for these analogues are summarized in Table 1. The $\beta$-hairpin populations for @-tide−peptide hybrids can be determined from eq 1, based on the average ellipticities ([Θ]) for each series, represented as $[\Theta]_0$ and $[\Theta]_{100}$. The equilibrium constants, $K_{eq}$, and standard free energies are also readily accessible (eq 2).

$$\%\ \beta\text{-hairpin} = \frac{[\Theta]_{obs} - [\Theta]_0}{[\Theta]_{100} - [\Theta]_0} \times 100 \qquad (1)$$

$$K_{eq} = \frac{[\Theta]_{obs} - [\Theta]_0}{[\Theta]_{100} - [\Theta]_{obs}};\ \Delta G^\circ = -RT\ln K_{eq} \qquad (2)$$

**Evaluation of β-Hairpin Folding.** The @-unit-based CD assay was used to investigate the influence of different amino acid pairs at the terminal positions of short $\beta$-hairpins and at the positions flanking the $^{D}$Pro-Ala $\beta$-turn, enabling hydrophobic, electrostatic, and hydrogen bonding side chain interactions to be compared. Quantitative studies probing the strengths of these interactions within the same $\beta$-hairpin model system, and thus in a common context, have been limited in the past for a variety of reasons.[8,21,26,27,32−34,36−44] Many models are relatively intolerant of significant alterations in amino acid composi-

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***Table 2.*** Percent $\beta$-Hairpin and Free Energies Associated with @-Tide–Peptide Hybrid Folding in 10 mM Phosphate Buffer (pH 7) at 25 °C

| compound | sequence | $\beta$-hairpin (%) | $\Delta G°$ (kcal·mol$^{-1}$) |
|---|---|---|---|
| 1 | T@**E**$^{D}$PA**K**TT | 8 | 1.48 |
| 2 | T@**T**$^{D}$PA**T**TT | 20 | 0.83 |
| 3 | T@**V**$^{D}$PA**V**TT | 31 | 0.47 |
| 4 | **AcT**@T$^{D}$PATT**T** | 8 | 1.50 |
| 5 | **E**@T$^{D}$PATT**K** | 15 | 1.03 |
| 2 | **T**@T$^{D}$PATT**T** | 20 | 0.83 |
| 6 | **V**@T$^{D}$PATT**V** | 23 | 0.73 |
| 7 | **F**@T$^{D}$PATT**F** | 46 | 0.10 |
| 8 | **G**@V$^{D}$PAVT**F** | 37 | 0.32 |
| 9 | **A**@V$^{D}$PAVT**F** | 38 | 0.28 |
| 10 | **V**@V$^{D}$PAVT**V** | 41 | 0.23 |
| 11 | **L**@V$^{D}$PAVT**F** | 49 | 0.03 |
| 12 | **I**@V$^{D}$PAVT**F** | 55 | −0.13 |
| 13 | **F**@V$^{D}$PAVT**F** | 75 | −0.64 |

tion,[21,26,31] and the methods for determining their folding equilibria have been difficult to apply to a large collection of analogues.[6,8,21,31] Against this background, use of the @-unit both to stabilize $\beta$-hairpin structures and to report on their conformations appeared to offer a unique opportunity. To demonstrate the potential of this approach, we measured the $\beta$-hairpin populations in water for a series of @-tide–peptide hybrids with varying side chain–side chain contacts (Table 2). In view of the small size of these hybrids, we assume a two-state folding equilibrium, consistent with previous observations with $\beta$-hairpin models.[26,27,38,39,43–46] Each hybrid was measured at three different concentrations from 15 to 60 $\mu$M; no concentration-dependent differences in the CD spectra were observed for any of the compounds. In Table 2, the free energies of folding for the @-tide $\beta$-hairpins are grouped according to the location of each substitution within the $\beta$-hairpin (e.g., adjacent to the $\beta$-turn or at the ends of the strand) and listed in order of increasing stability within each group. Even within this limited set of analogues, some clear trends can be discerned.

Three combinations of amino acids flanking the $^{D}$Pro-Ala $\beta$-turn were explored, with Thr at the terminal positions. The well-known stabilizing influence of hydrophobic side chains adjacent to the $\beta$-turn[47] is reflected in the present system in the greater stability of the Val–Val analogue **3** relative to that of the all-Thr derivative **2** and the Glu–Lys combination **1**. Despite the charge complementarity of the Glu and Lys side chains in **1**, alternative side chain–backbone interactions may interfere with the hairpin conformation for this sequence.

The stabilizing effect of hydrophobic side chains in preference to polar or charged residues is also observed at the terminal positions of the hairpins. In one related set of analogues


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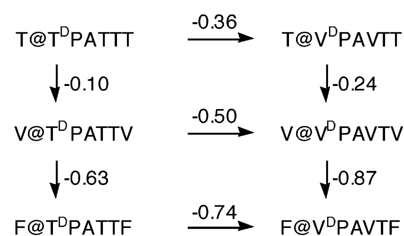


***Figure 9.*** Cooperative stabilization. Differences in folding energies ($\Delta\Delta G°$, kcal·mol$^{-1}$) for pairwise comparisons among selected hairpins.

(compounds **4–7**), threonines flank the $\beta$-turn and a variety of different amino acid pairs are presented at the N- and C-termini. Compared to the all-Thr analogue T@T$^{D}$PATTT (**2**), acetylation of the N-terminus (AcT@T$^{D}$PATTT, **4**) reduces the stability of the folded form by 0.67 kcal·mol$^{-1}$, reflecting a significant electrostatic stabilization from the opposing backbone charges in the zwitterionic structure. In comparison, electrostatic interactions between solvent-exposed amino acid side chains have been reported to stabilize a $\beta$-hairpin by ca. 0.30 kcal·mol$^{-1}$ in water.[39] The larger effect in the @-tide $\beta$-hairpin system likely reflects the closer proximity of charges along the backbone compared to the side chains. However, introducing charged but complementary side chains, as in E@T$^{D}$PATTK (**5**), results in a hairpin that is also less stable than the parent all-Thr analogue, albeit only slightly so. In this instance, the terminal ammonium and carboxylate charges are complemented internally by the respective side chains. These interactions reduce the significance of the cross-strand effects relative to the intrinsic conformational bias of amino acids with $\beta$-branched side chains, such as Thr and Val.

In contrast, introducing hydrophobic and aromatic side chains at the termini increases hairpin stability relative to the all-Thr reference [compare V@T$^{D}$PATTV (**6**) and F@T$^{D}$PATTF (**7**) with T@T$^{D}$PATTT (**2**)]. The Phe–Phe interaction is particularly stabilizing, reflecting the favorable desolvation and $\pi$-stacking effects of the aromatic side chains.[48] The Phe–Phe interaction at the terminal position of **7** is ca. 0.9 kcal·mol$^{-1}$ more favorable than the Glu–Lys interaction of **5**, an effect that is considerably greater than the 0.2–0.3 kcal·mol$^{-1}$ difference observed between similar pairings in a larger peptide.[41] In a third group of hairpins, **8–13**, Val residues flank the $\beta$-turn, the C-terminus is Phe (except for **10**), and hydrophobic side chains of varying size are explored at the N-terminus. This group of hairpins is inherently more stable, yet it still shows a clear, monotonic increase in stability with amino terminal side chain volume over a range of almost 1 kcal·mol$^{-1}$ in free energy. Indeed, the combination of valines flanking the $\beta$-turn with phenylalanines at the N- and C-termini in **13** results in a $\beta$-hairpin with an exceptional folded population (75% $\beta$-hairpin). This result is all the more remarkable in light of the small size of this oligomer in relation to that of other well-folded $\beta$-hairpins.[19,21,24,26,32,39,49]

An additional trend can be discerned from these data, namely, the synergistic effect of increasing stabilization at the two regions in the hairpin structure (Figure 9). That is, the effect of stabilizing interactions in one domain is enhanced by greater stabilization in the other. For example, comparison of F@T-$^{D}$PATTF (**7**) with V@T$^{D}$PATTV (**6**) indicates that the Phe–Phe pairing at the chain termini stabilizes the $\beta$-hairpin by 0.63 kcal·mol$^{-1}$ over the Val–Val analogue when Thr residues flank


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the $\beta$-turn. However, this difference increases to 0.87 kcal·mol$^{-1}$ when valines are adjacent to the turn [compare F@V$^{D}$PAVTF (**13**) with V@V$^{D}$PAVTV (**10**)]. Similarly, the enhanced stability of the V$^{D}$PAV turn over T$^{D}$PAT is more than twice as great when the terminal residues are Phe rather than Thr. Cooperative effects of this kind have been observed in $\beta$-hairpins,[21,38] as well as in other contexts, as clearly documented by Williams in analyzing contributions to the binding energies of peptide analogues with vancomycin antibiotics.[50−52] The synergy arises from the fact that stabilization in one region of the molecule leads to greater structure and, therefore, greater discrimination energetically in the other region. Thus, energy differences observed are strongly dependent on context, which underscores the need for systematic comparisons across an extensive series of analogues.

## Conclusion

The unusual properties of the @-unit as a cyclic amino acid surrogate are demonstrated by its ability to stabilize both linear oligomers as $\beta$-strands and very short peptides as $\beta$-hairpins in water, while simultaneously providing a spectroscopic probe for the conformations of these oligomers. In the case of $\beta$-hairpins, a set of control compounds has been developed that now enables rapid and quantitative measurement of $\beta$-hairpin folding for peptides containing the @-unit. This assay is particularly attractive because the circular dichroism intensities at 282 nm for @-tide $\beta$-hairpins correlate directly with their degrees of folding. Although the examples reported above constitute only a subset of all possible combinations of amino acids, they demonstrate an easily quantified system that will enable a wide variety of effects to be assessed systematically and quickly. This quantitative assay offers advantages over other approaches and may make it easier to probe the interactions that govern $\beta$-hairpin folding. Extension of this method to other models of $\beta$-structure can be readily envisaged.

## Experimental Section

**CD Methods.** Samples were prepared in aqueous buffer (10 mM sodium phosphate, pH 7), MeOH, or $CHCl_3$−MeOH mixtures. Compound concentrations were determined by UV absorbance at 284 nm ($\epsilon_{284}$ = 22 200 M$^{-1}$ cm$^{-1}$/@-unit). Data were collected on an Aviv 62DS spectropolarimeter at a scan speed of 20 nm·min$^{-1}$ with a bandwidth of 2 nm. The data were imported into Excel, and spectra were corrected for solvent contributions; mean-@-unit ellipticities [Θ] (10$^{4}$ deg·cm$^{2}$·dmol$^{-1}$) are reported on a per @-unit basis ([Θ] = 100·$\psi/lcn$, where $\psi$ = observable signal in millidegrees, $l$ = path length in cm, $c$ = concentration in mM, and $n$ = number of @-units). For variable temperature experiments, the samples were equilibrated for 5 min at each temperature.

**General Procedures for Solid Phase @-Tide and @-Tide−Peptide Hybrid Syntheses.** All new linear @-tides and @-tide−peptide hybrids were synthesized on solid phase using Wang resin in analogy to previously published procedures.[20,22,53]

**General Procedure for @-Tide Cyclization.** To a solution of an appropriate acyclic precursor (e.g., V$^{D}$PAV@V$^{D}$PAVV) in DMF (1 mM) was added HATU (3 equiv) and DIEA (3 equiv). The reaction mixture was stirred at room temperature under a $N_2$ atmosphere for 24 h, then evaporated, and the resulting oil was chromatographed using preparative reverse-phase HPLC. Isolated yields for the cyclic products ranged from 14 to 35%.

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**Supporting Information Available:** Synthesis and characterization of reported compounds, and NMR methods and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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