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"@-Tides": The 1,2-Dihydro-3(6*H*)-pyridinone Unit as a *β*-Strand Mimic

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Abstract: The cyclic amino acid surrogate **1** was designed to mimic the extended conformation of a peptide unit and to provide hydrogen bond donor and acceptor functions conducive to *β*-sheet formation. A convenient synthesis of this unit and solution and solid-phase methods for its incorporation into an oligomer alternating with peptide units have been devised. The resulting "@-tides", as these oligomers have been designated, show a high propensity for self-association in comparison to oligopeptides; insights into the structure and dynamical properties of their antiparallel dimers have been obtained by NMR.

Introduction

Local conformation within proteins and peptides is largely described by secondary structural elements, such as α-helices, *β*-turns, and *β*-strands, which determine the three-dimensional orientation of the amino acid side chains and thereby the longer range interstrand and intermolecular interactions. *β*-Strands, and *β*-sheets derived from them, play important roles in protein−protein interactions and in the association of proteins with other biopolymers such as nucleic acids.[1,2] The *β*-sheetlike association and precipitation of hydrophobic protein fragments in amyloid plaques is strongly implicated in neurodegenerative diseases.[3,4] Despite its ubiquity, the fully extended *β*-strand conformation is typically a minor component of the dynamic equilibrium for an oligopeptide outside the context of a folded protein structure, in which the hydrogen-bonded network of a *β*-sheet provides a stabilizing template.[5,6] Short *β*-sheets are known only as insoluble aggregates, and peptides designed to exist in monomeric, all-*β*-sheet form contain at least 20 amino acids.[5b] A variety of small-molecule *β*-sheet templates have been described which either juxtapose two peptide strands in the antiparallel orientation[7] or provide an extended array of hydrogen-bonding sites via a *β*-turn linkage.[8] A single-strand mimic was introduced by Hirschmann, Smith, and their co-workers, based on the pyrrolinone scaffold, which mimics both the functionality and the side chain orientation of a peptide in the extended conformation.[9]

β-Sheet templates that could act *inter*molecularly would be useful tools for studying *β*-sheet structure and dynamics, in devising peptide host−guest systems based on a *β*-sheet motif, or for disrupting macromolecular interactions that involve *β*-strands at the recognition domain. Oligomeric structures

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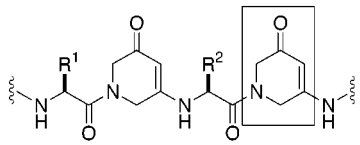
represented by **1** were devised for this purpose, with the cyclic



1 "Azacyclohexenone" unit (Ach or @)

amino acid replacement providing some conformational restriction and the tertiary amide limiting hydrogen bonding to a single edge of the strand. In its simplest form, the design of **1** suffers from the lack of an α-substituent, and from the possibility of *cis*−*trans* isomerism about the tertiary amide bond. However, the substitution of only one amino acid as well as the opportunity for ready incorporation of such a unit in a modified peptide synthesis made it an attractive target for investigation. For convenience, we use "Ach" as a 3-letter abbreviation (from the trivial "azacyclohexenone") and the "@" symbol as a 1-letter code for the cyclic unit; alternating oligomers with amino acids we refer to as "@-tides".

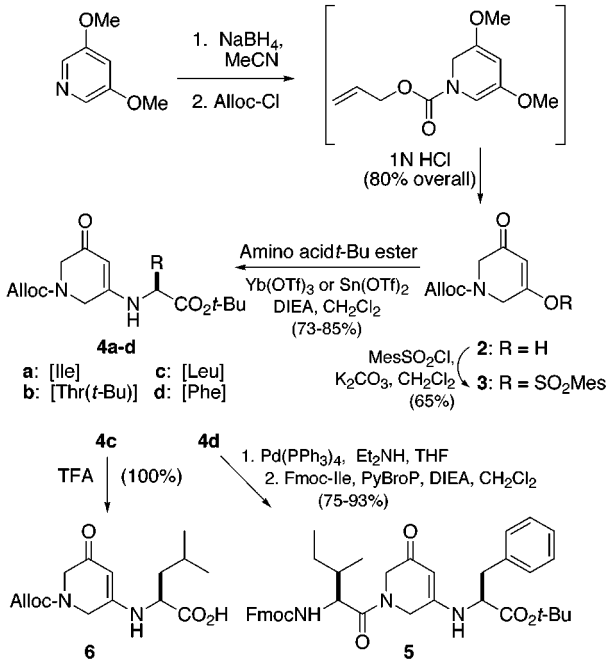
Synthesis of the Ach Building Block and Incorporation in Oligomeric @-Tides

We envisaged a synthetic approach to @-tides involving the incorporation of an *N*-protected, *O*-activated intermediate in analogy to normal peptide synthesis. Our initial attempts to prepare such a synthon began with *N*-benzylpiperidine-3,5-dione, reported by Ziegler and Bennett.[10] However, an alternative, shorter synthesis leading directly to a suitably *N*-protected precursor was developed from 3,5-dimethoxypyridine (Scheme 1). Addition of sodium borohydride to an acetonitrile solution of this material at −45 °C, followed by addition of allyl chloroformate, affords an intermediate *N*-acyl dihydropyridine. This material is not isolated but is hydrolyzed directly to the protected enolic dione **2**. This one-pot sequence affords the key intermediate in multigram quantities in good yields (75−85%).

Activation of the vinylogous acid **2** for coupling to a peptide proved to be more of a challenge than we had anticipated; traditional coupling methods led to complex mixtures apparently resulting from initial reaction of the amine nucleophile in a 1,2- rather than a 1,4-manner. However, these problems were overcome by activation of the hydroxyl group as the mixed anhydride **3**, formed with mesitylenesulfonyl chloride, and catalysis of the coupling reaction with either ytterbium triflate or tin triflate in THF.[11,12]

The adduct **4** can be *N*-deprotected and coupled to an amino acid to afford the tertiary amide (e.g., **5**) in good yield (75−93%) following normal protocols.[13,14] Low molecular weight @-tides such as **5** typically show multiple resonances in the NMR spectrum in chloroform as a result of *cis*−*trans* isomerism

Scheme 1



at the tertiary amide linkage. As the chain is elongated, this multiplicity disappears because dimerization shifts the conformational equilibrium to the extended form. We have not carried out an exhaustive investigation of the stereochemical course of the amino acid acylation reactions. However, a sample of D-Phe-Ach-L-Ile prepared from Boc-D-Phe and Ach-L-Ile-O*t*Bu under the standard coupling conditions (PyBroP, DIEA, and DMAP in methylene chloride for 24 h) was shown to be contaminated with less than 1% of the L−L diastereomer, which is readily resolved on HPLC.

Alternatively, the ester can be deprotected (e.g., to **6**) and the acid then coupled as a unit for more rapid chain elongation. The convergent synthetic strategy was employed in the synthesis of compound **9** (Scheme 2). The Alloc group was removed from adduct **4a** and the amine was coupled to acid **6** to give tetra-@-tide **7**, which was converted in a straightforward fashion to penta-@-tide **9.**

Solid-Phase Synthesis

This coupling process can be translated to solid phase as demonstrated by the synthesis of tri-@-tide **13** and penta-@-tide **15** in 75% and 43% overall yields, respectively (Scheme 3). The solid-phase procedure is similar to that in solution, but it requires a few significant modifications. The mixed solvent DMF/methylene chloride is more effective than CH_2Cl_2 alone in promoting complete reaction of the resin-bound intermediates. As a consequence, we found that tin triflate is more effective than ytterbium triflate in coupling the activated Ach unit, **3**, to the support-bound substrate. For example, the ytterbium-catalyzed reaction between **3** and isoleucine on resin resulted in only a 78% yield of di-@-tide **11**, while better than 95% conversion was achieved with tin triflate. We attribute this difference in reactivity to the greater solubility of $Sn(OTf)_2$ in the mixed solvent system.

It also proved to be necessary to modify the scavenging reagent for the palladium-catalyzed Alloc deprotection of the resin-bound @-tides. A significant quantity of the *N*-allylated

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(12) The corresponding chloroenone (**3**, with Cl in place of OSO_2Mes) can also be coupled to an amino acid ester with catalysis by ytterbium; however, a major byproduct arises from loss of the Alloc protecting group. This side reaction is not seen with the mesitylene sulfonate **3** or in the absence of ytterbium, so it presumably reflects an Yb-catalyzed nucleophilic dealkylation of the Alloc group by chloride ion.
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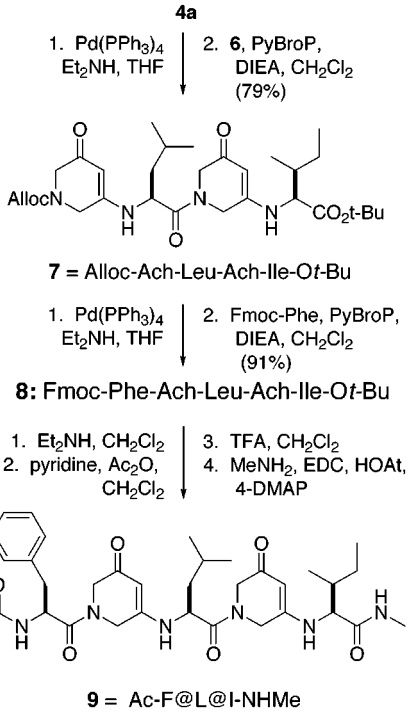
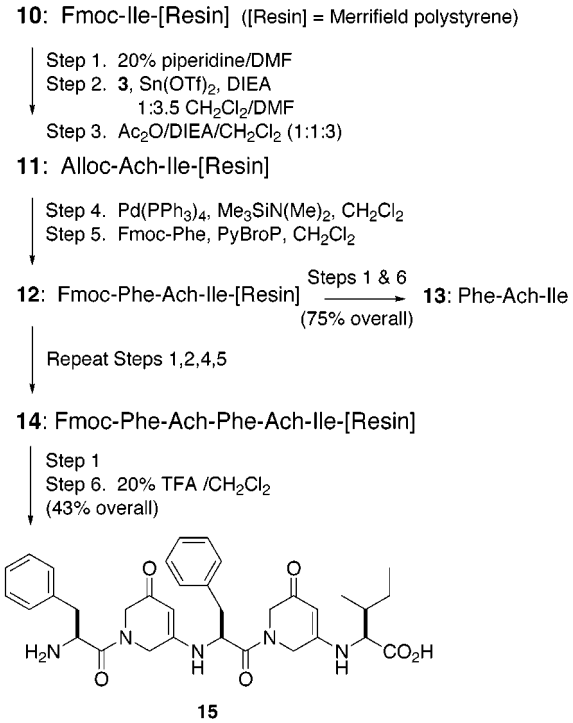
Scheme 2



Scheme 3



@-tides was observed with *N*-methylmorpholine (NMM) in acetic acid/chloroform (37:1:2 $CHCl_3$/NMM/AcOH) for the deprotection.[15] However, when $Me_3SiN(Me)_2$ is employed as the scavenger, formation of this byproduct is completely suppressed.[16]

Characterization of @-Tide Association

We anticipated that penta-@-tide **9** would be self-complementary, so we looked for evidence of dimerization as an indication of its ability to mimic a β-strand. Complete assignments of the ^{13}C and ^{1}H spectra were obtained as follows. Broadband ^{1}H-decoupled ^{13}C spectra were assigned via DEPT subspectra and comparison of observed chemical shifts with those predicted by an NMR simulation program.[17] Two-dimensional HMQC experiments then led directly from the assigned ^{13}C chemical shifts to the corresponding ^{1}H signals. The methylene hydrogens of the two Ach units in **9**, which show almost identical shifts, could be distinguished by their short-range NOE cross-peaks to nearby aliphatic side chains.[18] Amide hydrogens were assigned from 2D TOCSY spectra acquired in 1% $CD_3OH/CDCl_3$.[19] The TOCSY spectra also confirmed the other ^{1}H assignments.

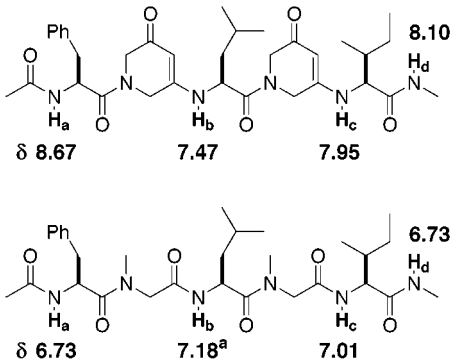


Figure 1. NH chemical shifts of @-tide **9** and peptide **16** in 1% $CD_3OH/CDCl_3$ at 20 °C. The superscript a indicates the resonance for amide rotamer observed at δ 8.12 ppm.

The NH chemical shifts of **9** in $CDCl_3$ provided the first indication that the @-tide hydrogen-bonds like a β-sheet. Hydrogen-bonded NH protons in peptides typically resonate around 8 ppm, which is ca. 2 ppm downfield of their chemical shifts when not hydrogen-bonded.[8b] In penta-@-tide **9**, NH protons resonate from 7.5 to 8.7 ppm (Figure 1), significantly downfield from the corresponding resonances (6.7−7.2 ppm) observed for a control pentapeptide, **16**, in which the Ach units have been replaced with sarcosine. These data suggest that @-tide **9** participates in hydrogen-bonding interactions more extensively than does peptide **16**. However, since two of the NH resonances in @-tide **9** are vinylogous amides, the downfield shifts should be considered in the context of other experimental data supporting a β-sheet model of dimerization.

The $C_\alpha H$ chemical shifts for penta-@-tide **9** provided additional evidence for the β-sheet conformation. Relative to the chemical shifts observed for the α-hydrogens of a peptide in an unstructured, random-coil conformation, those of an α-helix are shifted upfield and those of a β-strand (or extended) conformation are downfield.[20] The chemical shifts for the α-hydrogens of penta-@-tide **9** are well downfield of those expected for a random-coil model (Figure 2), which provides further evidence for the extended conformation expected in a hydrogen-bonded dimer.

$^{3}J_{HN\alpha}$ Coupling Constants

The magnitude of the $^{3}J_{HN\alpha}$ coupling constant for a peptide residue is dependent on the ϕ-angle and therefore on the local

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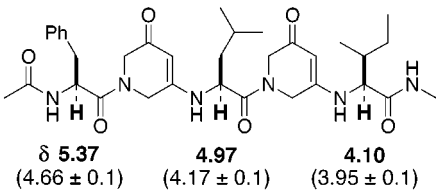

Figure 2. C_α−H chemical shifts of 26 mM @-tide **9** in 1% $CD_3OH/CDCl_3$ at 20 °C (random coil values in parentheses).

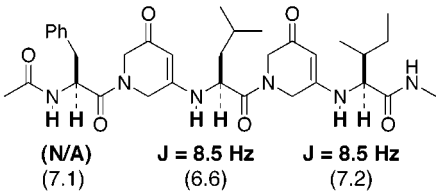

Figure 3. $^3J_{HN\alpha}$ coupling constants of 20 mM @-tide **9** in 5% $CD_3OH/CDCl_3$ at 20 °C (random coil values in parentheses).

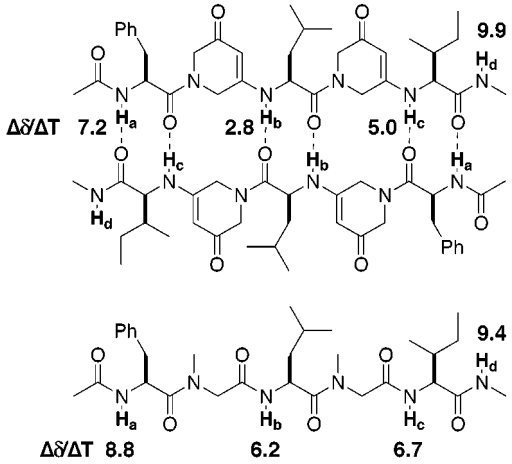

Figure 4. Temperature dependence of NH chemical shifts ($\Delta\delta/\Delta T$, ppb/K) for @-tide **9** and peptide **16** in 1% $CD_3OH/CDCl_3$ (16−22 mM).

conformation of the polypeptide backbone.[21] $^3J_{HN\alpha}$ values for β-sheet conformations fall in the range from 8 to 10 Hz, while $^3J_{HN\alpha}$ values for an unstructured random coil range from 5.8 to 7.3 Hz. NH−C_αH coupling constants for the Leu and Ile residues of penta-@-tide **9**, shown in Figure 3, are within the range for a β-sheet structure and are significantly higher than those predicted for a random coil. Although the differences in coupling constants give an indication of β-sheet conformation for the mimics, they do not provide an indication of the ϕ-angle directly, since the Karplus equation was derived for peptide amides. Direct comparison of @-tide **9** with peptide **16** was not possible, since an NH−C_αH coupling constant could only be resolved for the Phe residue in the peptide, which in turn was not resolved for the @-tide.

Temperature Dependence of NH Chemical Shifts

Whether an NH group is hydrogen bonded intermolecularly or is exposed to solvent can be revealed by the temperature dependence of the chemical shift: low values for $\Delta\delta/\Delta T$ reflect persistent, intermolecular hydrogen bonds, and high values indicate an equilibrium between hydrogen-bonded and non-bonded states.[22,23] In 1% $CD_3OH/CDCl_3$, dramatic differences are observed between the peptide **16** and the penta-@-tide **9** (Figure 4), with the former exhibiting much higher $\Delta\delta/\Delta T$ values than the latter. More revealingly, there are significant

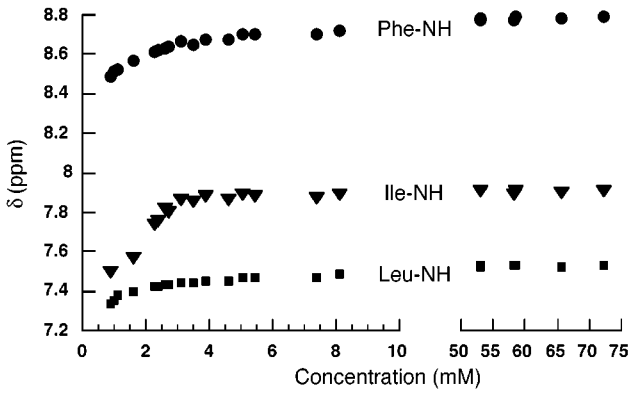

Figure 5. Concentration dependence of NH chemical shifts for @-tide **9** in $CDCl_3$ at 20 °C.

differences among the various NH groups of the penta-@-tide, with lower values for those in the center of the strand than those at the ends. This behavior is consistent with an antiparallel dimer structure in which the least solvent exposed NH exhibits the smallest $\Delta\delta/\Delta T$ value.

Concentration Dependence of NH Chemical Shifts

@-Tide dimerization can be detected by observing changes in NH chemical shifts as a function of concentration. For a dimerization process with dissociation constant K_d and NMR chemical shifts δ_{mono} and δ_{di}, respectively, the observed chemical shift, δ_{obs}, as a function of concentration is expressed by eq 1.

$$\delta_{obs} = \delta_{di} + (\delta_{mono} - \delta_{di})\frac{1}{2c}\left(\frac{-K_d}{2} + \sqrt{\frac{K_d^2}{4} + 2K_d c}\right) \quad (c = \text{concentration}) \quad (1)$$

Experimental data were obtained for several @-tides and peptide **16** in $CDCl_3$ at 25 °C and were fitted to this equation, as shown for penta-@-tide **9** in Figure 5, to give the dissociation constants listed in Table 1. Whereas the dimerization constant determined for the peptide **16** is greater than 150 mM, that for penta-@-tide **9** is 0.4 mM in pure $CDCl_3$,[24] demonstrating quantitatively the profound effects that the Ach unit has on the conformation and hydrogen-bonding ability of the @-tide. Increasing the length of the @-tides dramatically increases the affinity of the homodimer, so much so that the dissociation constants for related tri-, penta-, and hepta-@-tides cannot be measured by NMR under the same conditions. Methanol promotes dissociation, so the @-tides **17**, **18**, and **19** were measured at increasing methanol concentrations (Table 1). Although direct comparison under identical conditions is not possible, the trend is quite apparent. Interestingly, the data suggest that the C-terminal carboxylic acid moiety promotes dimerization more strongly than the *N*-methyl amide (compare Entry 2 with Entries 4 and 5).

The dissociative effect of methanol was explored with penta-@-tide **18** (Figure 6). The dependence of K_d on methanol

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(24) The K_d for @-tide **9** was estimated to be 0.4 ± 0.1 mM based on multiple independent titrations. δ_{free} and δ_{bound} determined for the NH protons were respectively as follows: Phe, 7.90 & 8.84 ppm; Ile, 7.21 & 7.97 ppm; Leu, 7.03 & 7.55 ppm.

Table 1. @-Tide Dimerization in $CD_3OH/CDCl_3$ Mixtures

entry	oligomer	solvent (% $CD_3OH/CDCl_3$)	K_d (mM)
1	Ac-Phe-Sar-Leu-Sar-Ile-NHMe (**16**)	0	>150
2	Ac-Phe-Ach-Leu-Ach-Ile-NHMe (**9**)	0	0.4
3	Ac-Leu-Ach-Val-OH (**17**)	1	35, 71[a]
4	Ac-Phe-Ach-Leu-Ach-Val-OH (**18**)	2.5	0.09
5	Ac-Phe-Ach-Leu-Ach-Val-OH (**18**)	5	8
6	Ac-Leu-Ach-Val-Ach-Leu-Ach-Phe-OH (**19**)	15[b]	1.5

[a] Amide rotamers with different K_d values were observed for tri-@-tide **17**. [b] 15% CD_3OH in $CDCl_3$ was necessary to observe changes in chemical shift of **19** with concentration; at lower percentages of CD_3OH, no change was observed down to 0.2 mM.

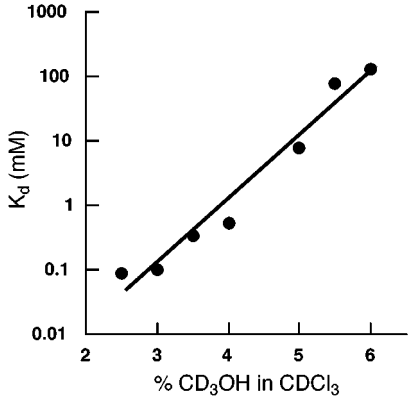

Figure 6. Dependence of dimer dissociation constant for penta-@-tide **18** on the percentage of CD_3OH in $CDCl_3$ (R^2 for the exponential line fit is 0.96).

concentration is dramatic, increasing more than 3 orders of magnitude between 3% and 6% methanol. The effect is roughly exponential, as would be expected at low concentrations of the dissociating agent, where incremental effects on the free energy of association are additive. Because of sensitivity limitations in the NMR method used to determine the dissociation constants, K_d values below 100 μM cannot be determined accurately; however, extrapolation of the line in Figure 6 suggests that the dissociation constant of **18** in pure chloroform could be as low as 0.13 μM.

NOE Spectroscopy

In an antiparallel β-sheet structure, interstrand NOE effects are generally observed between the side chains and between the amide hydrogens of opposing residues. Additional evidence for @-tide dimer formation was thus sought by acquiring NOESY spectra in 1% $CD_3OH/CDCl_3$. At a concentration of 20 mM, the spectrum of the penta-@-tide **9** shows cross-peaks between hydrogens at opposite ends of the molecule, which would not be expected to arise intramolecularly (Table 2). These NOE interactions are also identified in Figure 7. Spectra obtained at increasing CD_3OH concentrations demonstrated that these cross-peaks are intermolecular; they are weaker in 2.5% $CD_3OH/CDCl_3$ and absent entirely in 10% $CD_3OH/CDCl_3$. For comparison, the NOESY spectrum for peptide **16** was obtained with the same parameters, solvent, and concentration as those for penta-@-tide **9**; however, no cross-peaks between hydrogens at opposite ends of the molecule were observed for peptide **16**.

Further evidence for the β-strand conformation of mimic **9** is provided by the *intra*molecular cross-peaks in the NOE spectrum (Figure 8). Cross-peaks between the C_α hydrogens of the amino acids and the C2 methylene and C4 vinyl hydrogens of the Ach units are consistent with a conformation in which

Table 2. Intermolecular NOE Crosspeaks Observed for Penta-@-tide **9** at Varying Concentrations of $CD_3OH/CDCl_3$[a,b]

NOE no.	protons involved	1% CD_3OH	2.5% CD_3OH
1	Phe-aryl—Ile-δ	S	W
2	Phe-aryl—Ile-γ	W	n/o
3	Ac-Me—NH-CH_3	M	n/o
4	Phe-β—Ile-NH	W	W
5	Ach-I-γ—Ach-II-γ	M	M
6	Ach-II-γ—Leu-NH	W	n/o
7	Ach-II-γ—Phe-aryl	W	n/o
8	Ile-δ—Phe-β	S	n/o
9	Ile-β—Phe-β	S	M
10	NH—CH_3—Ac	S	M
11	Ile-γ—Ac	S	n/o
12	Ile-β—Phe-aryl	M	n/o

[a] Spectra were recorded at 20−35 mM concentrations at 20 °C; mixing times were optimized to minimize spin-diffusion.[25, 26] [b] S = strong, M = medium, W = weak, n/o = not observed.

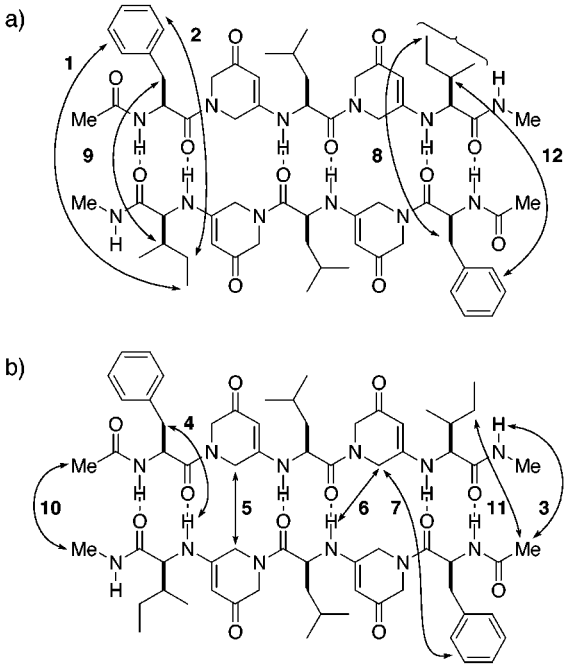

Figure 7. Intermolecular NOE cross-peaks observed for penta-@-tide **9** in 1% $CD_3OH/CDCl_3$ at 20 mM concentration: (a) side chain−side chain cross-peaks; (b) cross-peaks involving backbone hydrogens.

these atoms lie close to each other in the pleated conformation. Similarly, cross-peaks are observed between the C6 methylene hydrogens of the Ach units and the Leu and Ile amide hydrogens. Equally telling are the cross-peaks that are not observed, for example between the amide hydrogens and the C2 and C4 positions, or the C_α hydrogens and the C6 position.[27]

(25) Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: New York, 1987.

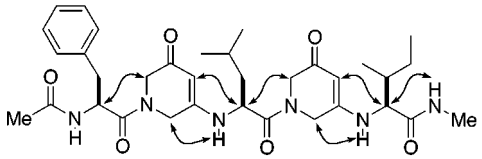

Figure 8. Intramolecular NOE interactions consistent with a β-strand conformation for penta-@-tide **9**. NOE measurements were obtained in 1% $CD_3OH/CDCl_3$ at 20 mM and 20 °C.

Conclusion

It is apparent that replacing amino acids at alternate positions in a peptide with the 1,2-dihydro-3(6*H*)-pyridinone ("Ach") unit affords an oligomeric molecule that exhibits many of the NMR and hydrogen-bonding characteristics of a peptide in the extended, β-strand conformation in chloroform and chloroform/methanol. This behavior is revealed by an enhanced propensity to dimerize in comparison to a related peptide, by reduced exposure of the central NH groups to solvent, and by a pattern of solvent-dependent NOE interactions that are consistent with an antiparallel hydrogen-bonded dimer. This amino acid surrogate thus offers promise as a β-strand mimetic and in model studies of β-sheet formation.

Experimental Section

General. Reagents were obtained from commercial suppliers and used as received. Solvents were purchased from commercial suppliers in the anhydrous form or were dried via distillation. Flash chromatography was performed according to the method of Still[28] with 60-mesh silica gel from E. Merck & Co.

Abbreviations: @ and Ach, the 1,2-dihydro-3(6*H*)-pyridinyl unit; PyBroP, bromotris(pyrrolidino)phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; 4-DMAP, 4-(dimethylamino)pyridine.

Prop-2-enyl 5-Hydroxy-3-oxo-1,2,6-trihydropyridine-1-carboxylate (2). To a solution of 3,5-dimethoxypyridine[29] (8.5 g, 61 mmol) in dry MeCN (230 mL) at −45 °C was added $NaBH_4$ (4.16 g, 110 mmol) in portions over 10 min, and the resulting mixture was stirred for an additional 10 min. Allyl chloroformate (7.79 mL, 73.4 mmol) was added over 45 min while the temperature (internal thermometer) was kept at −45 to −40 °C. The reaction was allowed to proceed for an additional 15 min at −40 °C, and then 1 N HCl (150 mL) was added at −40 °C. The HCl addition was followed immediately by addition of saturated $NaHCO_3$ (100 mL) until the pH was basic. The aqueous layer was extracted with EtOAc (3 × 50 mL) and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was dissolved in THF (200 mL) and 1 N HCl (200 mL). The reaction mixture was stirred for 30 min at room temperature and then made basic with solid NaOH at 0 °C. The aqueous layer was washed once with EtOAc (50 mL), and the organic layer was subsequently washed with 1 N NaOH until the aqueous layer was no longer yellow. The combined aqueous layers were acidified with 6 N HCl at 0 °C, saturated with NaCl, and extracted three times with EtOAc (50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to a thick oil. The enolic diketone tended to decompose on standing, so the crude product (9.5 g, 48 mmol, ca. 79%) was used immediately in the following step. An analytical sample was purified by flash chromatography using a gradient of petroleum ether/EtOAc to give the enolic diketone **2** as an oil. ^{1}H NMR δ 4.20 (s, 4), 4.64 (d, 2, J = 5.3), 5.27 (d, 1, J = 19), 5.31 (d, 1, J = 25), 5.63 (s, 1), 5.87−6.00 (m, 1), 9.90 (br s, 1); ^{13}C NMR δ 46.56, 47.44, 66.89, 102.84, 118.31, 131.95, 154.73, 184.93, 186.86; HRMS (FAB, *m*/*z*) Calcd. for $C_9H_{11}NO_4$ ($M + H^+$), 198.0766; found, 198.0767.

Prop-2-enyl 3-Oxo-5-[(2,4,6-trimethylphenyl)sulfonyloxy]-1,2,6-trihydropyridine-1-carboxylate (3). To a stirring solution of dione **2** (9.5 g, 48 mmol) in anhydrous CH_2Cl_2 (150 mL) under a nitrogen atmosphere was added powdered anhydrous K_2CO_3 (10.97 g, 79.50 mmol) and mesitylenesulfonyl chloride (15.8 g, 72.3 mmol). After 4 h, excess reagent was quenched by addition of saturated NH_4Cl (100 mL). The aqueous phase was washed three times with CH_2Cl_2 (100 mL), and the combined organic phases were washed with brine, dried over (Na_2SO_4), and concentrated under vacuum. The crude product was chromatographed (EtOAc/hexanes 1:2) to yield the mixed anhydride **3** (9.1 g, 24 mmol, 69%) as a pale yellow oil. Anhydride **3** was found to be stable at room temperature in a 0.1 M CH_2Cl_2 solution, but for prolonged storage the compound was dissolved in CH_2Cl_2 (1 M) and kept at −78 °C. ^{1}H NMR δ 2.35 (s, 3), 2.63 (s, 6), 4.09 (s, 2), 4.32 (s, 2), 4.62 (d, 2, J = 5.5), 5.24 (d,1, J = 10.6), 5.30 (d, 1, J = 17.5), 5.83−5.98 (m, 2), 7.05 (s, 2); ^{13}C NMR δ 20.92, 22.49, 44.02, 50.30, 66.69, 113.78, 118.12, 129.93, 131.86, 132.03, 139.94, 144.94, 154.15, 191.96; MS (FAB) *m*/*z* (%) 144 (100), 323 (70), 380 (30, $M + H^+$).

***tert*-Butyl (*2S,3S*)-3-Methyl-2-{[5-oxo-1-(prop-2-enyloxycarbonyl)-1,2,6-trihydropyridyl]amino}pentanoate (Alloc-Ach-Ile *t*-Butyl Ester, 4a).** To a solution of anhydride **3** (1.0 g, 2.6 mmol) in dry THF (11 mL) were added isoleucine *tert*-butyl ester hydrochloride (0.5 g, 2.7 mmol), anhydrous ytterbium(III) triflate (1.64 g, 2.65 mmol), and DIEA (1.38 mL, 7.92 mmol) under a nitrogen atmosphere. After 24 h, saturated NH_4Cl was added (10 mL), and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated. Purification of the crude product by flash chromatography (hexanes/EtOAc, 1:1) gave vinylogous amide **4a** (0.70 g, 1.9 mmol, 73%) as a light yellow oil. Proton and carbon spectra show peak doubling due to amide bond rotamers. ^{1}H NMR δ 0.89−0.98 (m, 6), 1.47−1.49 (s, 9, rot), 1.49−1.63 (m, 1); 1.65−1.78 (m, 1); 1.83−1.93 (m, 1); 3.88 (dd, 1, J = 4.9, 7.7), 4.02 (d, 1, J = 17.9), 4.10 (d, 1), 4.27 (d, 1, J = 16.1), 4.38 (d, 1, J = 16.6); 4.63 (d, 2, J = 5.5), 5.18 (s, 1), 5.23 (d, 1, J = 10.4), 5.31 (m, 1, J = 17.3, 1.6, 3.1), 5.84 (d, 1, J = 6.9), 5.83−5.99 (m, 1); ^{13}C NMR δ 11.55, 11.67, 14.06, 14.83, 15.53, 24.69, 25.96, 27.94, 27.97, 37.31, 39.18, 44.22, 50.57, 59.28, 59.46, 66.53, 80.67, 82.95, 95.64, 117.83, 174.71; MS (FAB) *m*/*z* (%) 450 (100), 367 ($M^+ + H$), 338 (42), 311 (84), 292 (22), 265 (20), 244 (28), 225 (20), 198 (32), 179 (10), 154 (18); HRMS (FAB, *m*/*z*) Calcd. for $C_{19}H_{30}N_2O_5$ (MH^+), 367.2233; found, 367.2232.

***tert*-Butyl (*2S,3S*)-3-Methyl-2-[(5-oxo-1,2,6-trihydro-3-pyridyl)-amino]pentanoate (Ach-Ile *t*-Butyl Ester).** To a solution of Alloc-protected dimer **4a** (0.46 g, 1.3 mmol) in a 1:1 mixture of THF/diethylamine (4.8 mL) at room temperature was added tetrakis-(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$; 0.12 g, 0.11 mmol), and the mixture was stirred for 1 h. The solvent was evaporated, 1 N HCl (25 mL) was added, and the solution was washed three times with EtOAc. The aqueous solution was brought to pH >14 with solid NaOH and extracted with three portions of EtOAc. The pH was readjusted to >14 and the extraction was repeated. The combined organic extracts were washed with brine and with brine containing diethyl dithiocarbamic acid, dried over Na_2SO_4, and evaporated to afford the crude amine, which was used immediately in the next step. An analytical sample was purified by flash chromatography with CH_2Cl_2/MeOH (9:1) containing 3% Et_3N. ^{1}H NMR δ 0.91 (d, 3, J = 6.6), 0.96 (t, 3, J = 7.5), 1.31−1.38 (m, 1H), 1.49 (s, 9), 1.51−1.61 (m, 1), 1.81−1.91 (m, 1), 3.39 (s, 2), 3.60 (s, 2), 3.86 (dd, 1, J = 4.6, J = 7.5), 5.11 (s, 1), 5.59 (d, 1, J = 7.7); ^{13}C NMR δ 11.54, 14.84, 25.94, 27.94, 37.28, 47.20, 53.23, 59.20, 82.93, 95.39, 162.73, 170.33, 195.98; MS (FAB) *m*/*z* (%) 338 (48), 292 (30), 283 (74, $M+H^+$), 227 (66); the mass spectrum also showed aggregates with masses higher than M^+.

(26) A very weak interaction was observed between the Leu-NH and the Ach vinyl hydrogen, which may reflect some conformational heterogeneity.
(27) Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy*; Oxford University Press: Oxford, 1994.
(28) Still, W. C.; Kahn, M.; Mitra, S. *J. Org. Chem.* **1978**, *43*, 2923.
(29) Testaferri, L.; Tiecco, M.; Tingoli, M.; Bartoli, D.; Massoli, A. *Tetrahedron* **1985**, *41*, 1373−84.

Fmoc-Ile-Ach-Phe *tert*-Butyl Ester (5). To a solution of the Alloc-protected Ach-Phe ester **4d** (100 mg, 0.25 mmol) in a 1:1 mixture of THF/diethylamine (2 mL) at room temperature was added $Pd(PPh_3)_4$ (28 mg, 0.03 mmol), and the mixture was stirred for 1 h. The solvent was evaporated under reduced pressure, then coevaporated under reduced pressure from dioxane (2 × 2 mL) to afford the crude amine, which was used immediately in the next step. To this product (80 mg, 0.25 mmol) in CH_2Cl_2 (3.5 mL) was added Fmoc-isoleucine (0.18 g, 0.51 mmol), PyBroP (0.24 g, 0.52 mmol), and DIEA (0.44 mL, 2.5 mmol). The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 26 h, then evaporated under reduced pressure. The residue was redissolved in EtOAc and the solution was washed with 1 M HCl (3 × 3 mL), $NaHCO_3$ (3 mL), and brine (3 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes (2:1)) to afford tri-@-tide **5** (0.13 g, 0.21 mmol, 82%) as a light yellow oil. ^{1}H NMR δ 0.59 (bs, 0.3), 0.68 (bs, 0.3), 0.88 (m, 6), 1.15 (m, 1), 1.37 (s, 9), 1.50 (bm, 1), 1.60 (bm, 1), 3.13 (m, 2), 4.06 (m, 2), 4.18 (m, 2), 4.32 (m, 3), 4.56 (m, 1), 5.06 (s, 0.5), 5.09 (s, 0.5), 5.23 (s, 0.2), 5.28 (s, 1), 5.62 (d, 0.2), 5.71 (d, 0.2), 6.23 (d, 1), 6.54 (bs, 1), 7.12 (m, 2), 7.24 (m, 6), 7.36 (m, 1), 7.46 (m, 1), 7.55 (m, 1), 7.67 (m, 1), 7.74 (d, 1); ^{13}C NMR δ 11.19, 15.67, 24.25, 37.33, 37.60, 42.86, 47.11, 52.66, 54.87, 56.43, 83.64, 95.20, 119.92, 125.08, 125.14, 126.89, 127.08, 127.30, 127.63, 128.42, 128.47, 128.52, 129.40, 131.90, 131.92, 132.02, 132.10, 132.88, 135.10, 141.22, 143.76, 143.91, 156.44, 159.96, 169.76, 171.74, 189.53; MS (FAB) *m*/*z* (%) 652 (55, $M + H^+$), 596 (20), 400 (10); HRMS (FAB, *m*/*z*) Calcd. for $C_{39}H_{46}N_3O_6$ ($M + H^+$), 652.3387; found, 652.3394.

(2*S*)-4-Methyl-2-[[5-oxo-1-(prop-2-enyloxycarbonyl)(3-oxo-1,2,6-trihydropyridyl)]amino]pentanoic Acid (Alloc-Ach-Leu, 6). *tert*-Butyl ester **4c** (2.75 g, 7.48 mmol) was dissolved in neat TFA (25 mL) under argon and stirred for 2 h. After evaporation of the solvent, EtOAc was added, and the solution was washed with two portions of saturated NaH_2PO_4 and brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography using a gradient of petroleum ether/EtOAc/AcOH (79:20:1, then 0:99:1); traces of acetic acid were removed by coevaporation with three portions of toluene to give the pure acid **6** as a yellow oil in quantitative yield (2.32 g, 7.48 mol). ^{1}H NMR δ 0.92 (d, 3, J = 5.0), 0.96 (d, 3, J = 5.2), 1.68−1.79 (m, 3), 4.01−4.20 (m, 2), 4.12 (dd, 1, J = 7.2, 7.2), 4.34 (d, 1, J = 17.4), 4.40 (d, 1, J = 16.8), 4.61 (s, 2), 5.23 (d, 1, J = 10.5), 5.30 (d, 1, J = 16.9), 5.37 (s, 1), 5.86−5.94 (m, 1), 6.80 (bs, 1); ^{13}C NMR δ 21.74, 22.57, 24.86, 40.46, 44.01, 54.52, 60.49, 67.01, 94.42, 118.35, 131.91, 154.88, 164.02, 171.40, 174.21; MS (FAB) *m*/*z* (%) 311 (M^+, 100), 265 (20), 225 (33), 154 (86), 136 (74), 107 (34); HRMS (FAB, *m*/*z*) Calcd. for $C_{15}H_{22}N_2O_5$ ($M + H^+$), 311.1607; found, 311.1615.

Alloc-Ach-Leu-Ach-Ile *tert*-Butyl Ester (7). To solution of acid **6** (1.66 g, 5.35 mmol) and Ach-Ile *tert*-butyl ester **4a** (1.51 g, 5.35 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C was added DIEA (1.67 mL, 9.63 mmol), 4-DMAP (63 mg, 535 μmol), and PyBroP (3.24 g, 6.96 mmol). After 30 min, the ice bath was removed, and the mixture was stirred for 14 h at room temperature. After dilution with CH_2Cl_2, the solution was extracted with four portions of 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography using a gradient of CH_2Cl_2/MeOH (97:3, 95:5) to give a fraction of pure peptide **7** (1.28 g, 2.33 mmol, 42%) and a fraction (2.65 g) contaminated with tris(pyrrolidino)phosphoramide. The NMR spectra are complicated by peak doubling due to amide rotamers. ^{1}H NMR δ 0.86−0.98 (m, 12), 1.45−1.49 (s, 9), 1.64−2.08 (m, 4), 3.88 (dd, 1, J = 4.9, 7.6), 4.00−4.38 (m, 6), 4.44 (dd, 1, J = 4.9, 8.2), 4.46 (d, 1, J = 16.9), 4.57 (d, 1, J = 16.9), 4.63 (d, 2, J = 5.2), 5.20 (s, 1), 5.22 (ddd, 1, J = 1.4, 2.5, 10.5), 5.30 (ddd, 1, J = 1.5, 3.1, 17.2), 5.39 (s, 1), 5.91 (ddt, 1, J = 10.5, 17.2, 5.5), 6.08−6.17 (d, 1, J = 7.8), 6.43 (s, 1); ^{13}C NMR δ 12.31, 12.35, 15.67, 16.09, 23.45, 25.40, 25.93, 26.73, 28.68, 28.73, 38.12, 38.54, 42.34, 44.82, 52.40, 55.83, 57.73, 60.55, 61.07, 67.38, 82.65, 83.97, 95.80, 96.23, 118.52, 158.28, 170.39, 188.52; MS (FAB) *m*/*z* (%) no M^+, 480 (78), 424 (100), 323 (22), 265 (30), 225 (14), 179 (19).

Fmoc-Phe-Ach-Leu-Ach-Ile *tert*-Butyl Ester (8). To a solution of tetramer **7** (1.10 g, 1.91 mmol, contaminated with tris(pyrrolidino)-phosphoramide) in a 1:1 mixture of dry THF/Et_2NH (20 mL) was added $Pd(PPh_3)_4$ (20 μmol, 23 mg) under argon. After 4 h, 1 N HCl was added to pH <1, and the mixture was extracted with three portions of EtOAc. The aqueous layer was brought to pH >14 with 5 N NaOH and extracted with four portions of EtOAc. Washing with brine (containing ca. 200 mg of sodium diethyldithiocarbamate), drying over Na_2SO_4, and evaporation of the solvent gave a crude product, which was purified by flash chromatography (gradient of CH_2Cl_2/MeOH/Et_3N 90:10:0 → 78:19:3) to give the *N*-deprotected Ach-Leu-Ach-Ile *tert*-butyl ester (721 mg, 1.74 mmol, 77%) as a yellow solid. ^{1}H NMR δ 0.87 (d, 3, J = 5.6), 0.91 (d, 3, J = 2.8), 0.92 (d, 3, J = 3.2), 0.95 (t, 3, J = 7.6), 1.30−1.34 (m, 1), 1.50 (s, 9), 1.52−1.69 (3), 1.71−1.79 (m 1), 1.84−1.92 (m, 1), 3.35 (s, 2), 3.58 (d, 1, J = 16.9), 3.64 (d, 1, J = 16.6), 3.91 (dd, 1, J = 5.0, 7.8), 4.00 (d, 1, J = 17.1), 4.10 (d, 1, J = 16.9), 4.26 (d, 1, J = 17.1), 4.46−4.52 (m, 1), 5.14−5.16 (s, 1), 5.19−5.22 (s, 1), 7.02 (d, 1, J = 8.1), 7.18 (d, 1, J = 7.3); ^{13}C NMR δ 12.23, 15.70, 22.05, 23.84, 25.37, 26.67, 28.65, 38.40, 41.63, 43.54, 46.56, 47.81, 51.17, 52.83, 54.15, 60.61, 83.68, 94.93, 95.11, 162.04, 165.38, 170.54, 171.78, 189.71, 196.36; MS (FAB) *m*/*z* (%) 491 (M^+, 100), 435 (44), 340 (6), 319 (10), 281 (8), 225 (22), 179 (30), 154 (18), 136 (14), 111 (20); HRMS (FAB, *m*/*z*) Calcd. for $C_{26}H_{42}N_4O_5$ ($M^+ + H$), 491.3233; found, 491.3233.

To a solution of the *N*-deprotected compound (510 mg, 1.04 mmol) in dry CH_2Cl_2 (5 mL) were added Fmoc-phenylalanine (603 mg, 1.56 mmol), PyBroP (726 mg, 1.56 mmol), 4-DMAP (6 mg, 52 μmol), and DIEA (723 μl, 4.16 mmol). The reaction mixture was stirred under argon at room temperature for 16 h; EtOAc was added, and the solution was washed with 1 N HCl, saturated NH_4Cl, and brine, dried over Na_2SO_4, and evaporated. Purification by flash chromatography (EtOAc/MeOH 95:5) gave pentamer **8** (812 mg, 944 μmol, 91%) as a white solid. ^{1}H NMR δ 0.80−0.92 (m, 12), 1.23−1.32 (m, 1), 1.40 (s, 9), 1.44−1.63 (m, 4), 1.77−1.86 (m, 1), 2.85−2.93 (m, 2), 2.99 (s, 2), 3.69−4.48 (m, 10), 4.74−5.18 (m, 4), 6.96−7.24 (m, 7), 7.25−7.35 (m, 2), 7.38−7.51 (m, 2), 7.60−7.73 (m, 2); ^{13}C NMR δ 11.50, 13.94, 14.91, 20.82, 22.10, 23.06, 24.57, 26.11, 27.81, 27.86, 37.93, 38.83, 41.14, 42.06, 46.86, 51.68, 51.85, 59.64, 60.41, 67.02, 83.58, 93.70, 94.16, 119.80, 124.91, 124.99, 126.77, 127.09, 127.55, 128.53, 129.00, 135.60, 141.16, 143.53, 143.68, 156.15, 160.95, 161.17, 169.93, 170.50, 171.31, 171.47, 188.97, 189.65; MS (FAB) *m*/*z* (%) 861 (M^+, 48), 179 (100), 154 (84), 137 (58); HRMS (FAB, *m*/*z*) Calcd. for $C_{50}H_{61}N_5O_8$ ($M^+ + H$), 860.4598; found, 860.4611.

Ac-Phe-Ach-Leu-Ach-Ile *N*-Methylamide (9). A solution of Fmoc-pentamer **8** (749 mg, 871 μmol) in dry CH_2Cl_2 (5 mL) was treated with Et_2NH (5 mL) at room temperature under argon for 3 h. The solution was evaporated under reduced pressure and the residue was coevaporated with three portions of dichloroethane (5 mL) and dried under high vacuum. The crude amine was redissolved in dry CH_2Cl_2 (5 mL), and dry pyridine (1.41 mL, 17.5 mmol) and acetic anhydride (831 μL, 8.71 mmol) were added. After 50 min, the volatile materials were removed under vacuum and the residue was coevaporated with three portions of $C_2H_4Cl_2$ (5 mL). Purification of the crude product by flash chromatography (gradient of CH_2Cl_2/MeOH 95:5−9:1) as eluant gave the acetyl derivative (505 mg, 743 μmol, 85%) as a yellowish solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85−1.02 (m, 12), 1.28−1.43 (m, 1), 1.49−1.53 (s, 9), 1.64−1.74 (m, 1), 1.84−1.95 (m, 1), 2.01−2.06 (s, 3), 2.93 (d, 2, J = 6.6), 3.80−4.72 (m, 10), 5.09−5.47 (m, 3), 6.94−7.29 (m, 5); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.38, 14.96, 20.80, 22.02, 22.56, 23.11, 24.43, 25.92, 27.81, 38.02, 39.01, 41.95, 42.49, 42.69, 49.68, 50.37, 51.58, 51.78, 59.63, 82.50, 83.13, 93.64, 94.04, 126.89, 128.29, 128.89, 135.38, 161.06, 161.26, 169.91, 170.12, 170.47, 170.90, 188.91, 189.59; MS (FAB) *m*/*z* (%) 680 (M^+, 100), 624 (30),

435 (30), 225 (36), 179 (54), 120 (62); HRMS (FAB, *m*/*z*) Calcd. for $C_{37}H_{53}N_5O_7$ ($M^+ + H$), 680.4023; found, 680.4012.

The above material (388 mg, 571 μmol) was dissolved in dichloroethane (3.5 mL) and treated with TFA (1.5 mL) for 5 h. The volatile materials were evaporated under reduced pressure, and the residue was coevaporated with three portions of dichloroethane (5 mL) and dissolved in CH_2Cl_2. The solution was washed with saturated NaH_2PO_4, dried over Na_2SO_4, and evaporated to yield the crude acid (347 mg, 556 μmol, 97%) as a yellowish foam.

A solution of the crude acid and 1-hydroxy-7-azabenzotriazole (108 mg, 799 μmol), EDC (137 mg, 714 μmol), 4-DMAP (3.5 mg, 29 μmol), and methylamine (2.0 M in THF, 570 μL, 1.14 mmol) in dry CH_2Cl_2 (5 mL) was stirred under argon at 0 °C for 20 h at room temperature. CH_2Cl_2 was added, the solution was washed twice with 10% $KHSO_4$ and saturated $NaHCO_3$, and with brine, dried over Na_2SO_4, and evaporated to give 240 mg of crude methylamide. Purification by flash chromatography (gradient of CH_2Cl_2/MeOH 9:1−8:2) gave 154 mg (242 μmol, 42% over 2 steps) of the amide **9** as a colorless solid: mp 210−215 °C dec. ^{1}H NMR ($CDCl_3$−CD_3OH 10:1) δ 0.80−0.86 (m, 6), 0.87−0.94 (m, 6), 1.06−1.16 (m, 1), 1.54 (bs, 2), 1.57−1.64 (m, 1), 1.75−1.87 (m, 1), 1.89 (d, 0.5, J = 6.4, rot), 1.95 (s, 3, rot), 2.71 (s, 3), 2.89 (dd, 1, J = 8.9, 12.5); 2.96 (dd, 1, J = 6.9, 13.3), 3.71 (d, 1, J = 16.8), 3.82 (d, 1, J = 16.8), 3.82−3.86 (m, 1), 3.98−4.07 (m, 1), 4.07−4.11 (m, 2), 4.27−4.40 (m, 1), 4.53 (dd, 1, J = 7.6, 2.2); 4.82 (d, 1, J = 17.6), 4.83 (d, 1, J = 17.6), 5.01−5.09 (m, 0.3, rot), 5.21−5.25 (m, 1, rot), 5.19 (s, 1), 5.37 (s, 1), 7.08 (d, 2, J = 6.4), 7.11−7.18 (m, 3), 7.35 (d, 1, J = 9.0), 7.91 (d, 1, J = 8.4), 8.05 (d, 1, J = 4.4), 8.35 (bs, 1); ^{13}C NMR δ 10.89, 14.93, 22.19, 22.47, 23.18, 24.34, 25.01, 25.78, 37.89, 39.19, 42.08, 42.45, 42.63, 49.58, 50.32, 51.29, 51.60, 60.40, 93.66, 126.79, 128.31, 128.86, 135.76, 160.60, 161.61, 170.79, 170.82, 170.93, 171.00, 189.13, 189.38; MS (FAB) *m*/*z* (%) 637 (M^+, 100), 179 (64), 154 (32), 137 (28), 120 (68); HRMS (FAB, *m*/*z*) Calcd. for $C_{54}H_{48}N_6O_6$ ($M^+ + H$), 637.3714; found, 637.3723.

General Procedures for Solid-Phase Synthesis. Merrifield polystyrene resins were obtained from NovaBiochem loaded with Fmoc-amino acids (ca. 0.7−0.9 mmol/g). Solid-phase syntheses were carried out in silylated glass reaction vessels fitted with a frit. The resin was washed in the following manner: DMF (3×), alternating MeOH and CH_2Cl_2 (3× each), and CH_2Cl_2 (3×). When palladium was used in the reaction, the washing included MeOH (1×) prior to the normal washing procedure. During washings, the resin was agitated with nitrogen bubbling for 2 min before the solvent was removed. The presence or absence of free amine was detected by the Kaiser test.[31] Fmoc quantitation analysis was performed by using the method of MilliGen[32] with a Uvikon 860 spectrometer. Reactions were agitated either with a Burrell Wrist Action Shaker or a Labquake rotator. Deprotection of Fmoc was accomplished by shaking the resin in 20% piperidine in DMF for 20 min, followed by the washing procedure and drying of the resin in vacuo for 16−20 h. Resins were stored dry at 0 °C.

Ach Addition. Tin(II) triflate (0.04 g, 0.09 mmol) was added to resin (0.1 g, 0.91 mmol/g) followed by DIEA (0.08 mL, 0.46 mmol), activated Ach unit, **3** (1M in CH_2Cl_2, 0.36 mL, 0.36 mmol), and DMF (2.5 mL). The reaction vessel was rotated for 16 h at room temperature, and workup involved the washing procedure described above and drying of the resin in vacuo for 2 h.

Capping. To cap free amines remaining after an acylation or coupling procedure, the resin (0.1 g, 0.91 mmol/g) was prewashed once with dry CH_2Cl_2. The drained resin (0.1 g, 0.91 mmol/g) was swollen in 3:1:1 CH_2Cl_2/DIEA/Ac_2O (5 mL total volume), and the reaction was allowed to proceed for 2 h prior to washing and drying of the resin.

Alloc Deprotection. The resin (0.1 g, 0.91 mmol/g) was prewashed once with dry CH_2Cl_2, and then suspended in 3 mL of dry CH_2Cl_2. $Me_3SiN(Me)_2$ (0.29 mL, 1.8 mmol) was added to the resin followed by $Pd(PPh_3)_4$ (0.11 g, 0.09 mmol). The resin was quickly shaken for even mixing, followed by rotating for 40 min. The resin was washed and then dried in vacuo for 2 h.

Fmoc-Amino Acid Addition. The resin (0.1 g, 0.91 mmol/g) was prewashed once with dry CH_2Cl_2, and then suspended in 3 mL of dry CH_2Cl_2. The desired Fmoc-protected amino acid (5 equiv in relation to the resin) was added to the resin, followed by PyBroP (5 equiv) and DIEA (10 equiv). The reaction vial was vigorously shaken, followed by rotating at room temperature for 24 h. The resin was washed and immediately Fmoc-deprotected as previously described.

Cleavage from Resin. The product was cleaved from resin after Fmoc-deprotection without drying the resin prior to cleavage. The resin was suspended in 1:1 CH_2Cl_2/TFA (3 mL) and rotated in a glass vial for 2 h. The solvent was removed under reduced pressure, and the residue was redissolved in MeOH, filtered, and washed (4 × 2 mL MeOH). This solution was combined and the solvent was removed under reduced pressure; the crude product was immediately purified by preparative HPLC.

Phe-Ach-Ile (13). Resin-bound tri-@-tide **12** (0.71 mmol/g) was assembled from Fmoc-Ile resin (NovaBiochem) according to the general procedures described above. This material (0.46 g resin) was deprotected and cleaved from the resin and purified by preparative reverse-phase HPLC to afford tri-@-tide **13** (0.09 g, 0.25 mmol, 75% overall) as a light yellow foam. The NMR spectra are complicated due to the presence of rotamers. ^{1}H NMR ($(CD_3)_2CO$) δ 0.93 (m, 35.9), 1.00 (d, 15.3, J = 6.5), 1.04 (d, 1.6, J = 7.0), 1.24 (br m, 1.4), 1.34 (m, 7.6), 1.51 (m, 1.9), 1.64 (br m, 7), 1.95 (s, 4.8), 2.06−2.07 (m, 47.4), 2.08 (s, 3.8), 3.10 (t, 1.4, J = 9.5), 3.21 (m, 6), 3.30 (m, 4.2), 3.38 (m, 5.6), 3.52 (m, 0.46), 3.92 (br m, 17.4), 4.23 (d, 3.9, J = 17), 4.33 (m, 1.4), 4.36 (d, 0.4, J = 5.5), 4.44 (d, 1, J = 6.0), 4.49 (m, 1.6), 4.73−4.81 (m, 5.4), 5.06 (m, 3.8), 5.29 (q, 0.3, J = 5.0), 5.68 (q, 1.2, J = 5.5, 8.5), 5.82 (q, 1), 6.87 (m, 0.3), 7.30 (m, 28), 7.38 (m, 9), 7.83 (s, 0.2), 7.93 (s, 0.2); ^{13}C NMR (CD_3OD) δ 10.19, 10.29, 10.4 (rot), 14.17 (rot), 14.31, 14.59 (rot), 20.89 (rot), 24.87, 25.15, 25.21 (rot), 35.54 (rot), 36.69, 36.79 (rot), 36.88 (rot), 37.05, 41.69, 44.68 (rot), 48.42, 50.86, 50.99 (rot), 51.11 (rot), 60.08 (rot), 60.26, 127.6 (rot), 127.67, 128.45 (rot), 128.64 (rot), 128.94, 129.13 (rot), 129.24, 129.34 (rot), 129.46 (rot), 133.32 (rot), 133.52, 166.81 (rot), 167.01, 171.79 (rot), 171.99, 190.11, 191.37 (rot); IR (film) ν_{max} 3264, 2956, 2916, 1672 cm^{-1}; MS (FAB) *m*/*z* (%) 374 (100, $M + H^+$), 227 (45), 120 (85); HRMS (FAB, *m*/*z*) Calcd. for $C_{20}H_{28}N_3O_4$ ($M + H^+$), 374.2080; found, 374.2083.

Phe-Ach-Phe-Ach-Ile (15). In a similar manner, penta-@-tide resin **14** (0.91 mmol/g) was synthesized and a sample (0.06 g) was deprotected and cleaved as described above. The crude material was purified by preparative reverse-phase HPLC to afford penta-@-tide **15** (15 mg, 0.03 mmol, 45% overall) as a light yellow foam. The proton spectrum is complicated due to the presence of rotamers; ^{1}H NMR (CD_3OD) δ 0.99 (br m, 5.57), 1.31 (br m, 1.6), 1.61 (br m, 0.8), 1.94 (br m, 1), 3.07 (br m, 3.3), 3.86 (br m, 1.5), 4.02 (br m, 0.7), 4.07−4.12 (br m, 0.8), 4.22 (br m, 1.0), 4.36 (br m, 0.4), 4.47 (br m, 1), 4.54 (br m, 1), 4.70 (t, 0.5), 4.95 (s, 0.3), 4.99 (d, 0.2), 5.10 (br m, 0.9), 7.17−7.31 (br m, 8); IR (film) ν_{max} 3318, 2952, 2915, 2847, 1648 cm^{-1}; MS (FAB) *m*/*z* (%) 616 (70, $M + H^+$), 340 (60), 312 (90), 284 (100); HRMS (FAB, *m*/*z*) Calcd. for $C_{34}H_{42}N_5O_6$ ($M + H^+$), 616.3135; found, 616.3118.

NMR Analysis. NMR spectra were obtained with a Bruker 500 MHz spectrometer in $CDCl_3$ solution unless otherwise indicated. Spectral data are reported as chemical shifts (multiplicity, number of hydrogens, coupling constants in Hz). ^{1}H NMR chemical shifts are referenced to TMS (0 ppm) in $CDCl_3$, CD_3OD (3.31 ppm), or $(CD_3)_2CO$ (2.05 ppm); ^{13}C NMR spectra were proton decoupled and referenced to $CDCl_3$ (77.16 ppm) or CD_3OD (49.00 ppm). Resonance assignments were obtained by the method of Wüthrich[18] using TOCSY and NOESY

(30) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Company: Rockford, 1984; p 105.
(31) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.
(32) Procedure given on p P4 of the NovaBiochem 2000 catalog.

spectra. Samples were analyzed at approximately 20 mM in CD_3OH/$CDCl_3$ solutions. Rigorous degassing was performed prior to the NOESY experiments using three freeze−pump−thaw cycles.[25] NOESY experiments were performed by the method of Ananikov[30] with mixing times optimized to minimize spin-diffusion (0.7 s).[26] NOESY data were collected with 2048 data points in F2 and 512 data points in F1. For VT experiments, the sample was allowed to adapt to the adjusted temperature for at least 10 min prior to acquisition.

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Supporting Information Available: Experimental procedures and characterization of the compounds not described above; full TOCSY and NOESY spectra, VT data, and data on concentration dependence of NH shifts for @-tide **9** as well as peptide **16** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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