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# Synthesis of $3_{10}$-Helix-Inducing Constrained Analogues of L-Proline

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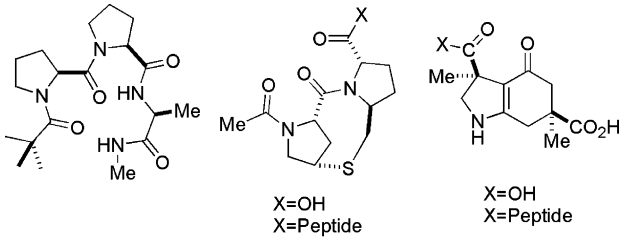
A bicyclic indolizidinone carboxylic acid and a tricyclic constrained analogue of L-proline were synthesized and evaluated for their ability to induce helix formation as L-Ala tetrapeptides. Variable-temperature NMR, DMSO titration, CD spectra, and X-ray structure analyses, in conjunction with molecular modeling, confirmed the existence of $3_{10}$-helical motifs with di- and tetrapeptides of L-Ala.

## Introduction

Studying the underlying principles of protein folding is crucial to our fundamental understanding of life processes. The pathogenesis of many diseases which originate as a result of misfolding of proteins may be viewed in a different context if a better knowledge of the relationship between structure and function was available.[1]

The ordered structural domains of proteins consist of α-helices and β-sheets.[2] In particular, α-helical motifs play a crucial role in a number of biological processes.[3] α-Helices can adopt interesting three-dimensional structures, as for example, in the four-helix bundle[4] and the coiled-coil.[5] A better understanding of the formation of α-helices could, in theory, be achieved by using low molecular weight molecules as models.[6a] However, this simulation is difficult because the small free energy difference necessary to stabilize the folded state over the unfolded one is difficult to achieve in practice. In addition, the nucleation of the helix is entropically disfavored because it requires the first four amino acids to adopt a helical conformation without being initially stabilized by hydrogen bonds with subsequent residues in the helix.[7,8]

Many approaches have been developed to date to induce folding in short peptides.[9] One of the most viable




1 2 3

**FIGURE 1.** Helix-inducing nucleating motifs.

methods[6a,b,10] is to propagate a desired conformation of a peptide chain from an ordered region starting with a conformationally restricted template (Figure 1). The entropy required to fold a linear peptide may thus be lowered relative to the existing conformational energy of the unfolded state. These templates normally direct the nucleation process by using hydrogen bonding or a mixture of hydrogen bonding and hydrophobic interactions. While L-Pro has a negative effect on helix stability when incorporated in an internal position of the peptide chain,[2,11] it is frequently found at the N-terminus of naturally occurring peptides.[12] In this latter case, L-Pro does not interfere with the hydrogen bonding network of the backbone. Another advantage is that the $\phi$ value for L-Pro (~60°) is close to the theoretical backbone $\phi$ value expected for a $3_{10}$ or α-helical conformation.[13] The idea of using simple L-Pro derivatives as potential α-helix nucleator templates for short peptides has already been exploited. Studies concerning the peptide Piv-L-Pro-L-Pro-

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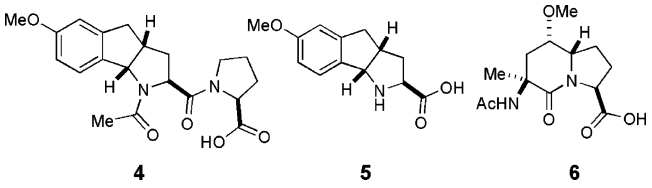


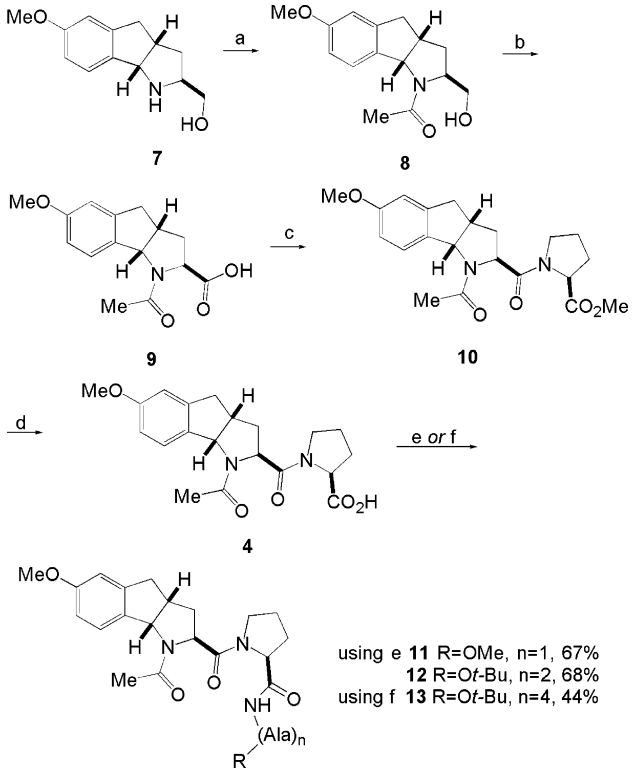
**4** **5** **6**

**FIGURE 2.** Constrained analogues of L-Pro.

NHMe **1** in organic solvents[14] suggested that $3_{10}$-helix formation is elicited by the presence of contiguous Pro residues. However, Xaa-L-Pro-L-Pro-OH derivatives that can be linked to the N-terminus of the desired peptide, while attractive because of the potential proper orientation of three amide carbonyls with the correct pitch and spacing for helix-type structures, are usually not sufficiently constrained to enforce stable and consistent helical folding. In fact, energetically similar cis/trans isomers in the Xaa-L-Pro as well as L-Pro-L-Pro amide bonds may create multiple conformers. Moreover, L-Pro-L-Pro is known to be a poor turn-constraining unit, because of the small energy difference between turn-like and extended structures.[15] Kemp and co-workers[16]devised a helical template based on a conformationally restricted analogue of the Ac-L-Pro-L-Pro sequence **2** (Figure 1). A stable helix formation in aqueous media for peptides 5−11 residues in length was observed. This template also allowed independent evaluation of helix propensities for the Ala residue.[6] Bartlett and co-workers[10] have studied the semirigid template **3** in inducing helical conformations of appended hexapeptides using CD and NMR.

We recently reported the synthesis of constrained analogues of L-proline, represented by the tricyclic motif **5** (Figure 2).[17] The synthesis of a precursor to the indolizinone **6**, which encompasses a constrained bicyclic L-proline, was also recently communicated.[18] In this paper, we describe the synthesis of the prolyl L-TcaP (tricyclic constrained proline) **4**, and the indolizidinone scaffold **6** with the objective to study their ability to induce L-Ala oligopeptides to adopt helical conformations. We first studied the solution conformation of **4**, its methyl ester, and its L-Ala oligopeptides. While unable to predict which could be the preferred rotational isomer of the amide bond between L-proline and the core structure, we surmized that if properly oriented, the tripeptide Xaa-L-TcaP-L-Pro derived from **4** could deploy the three carbonyl groups in the correct pitch and spacing for a right-handed or a $3_{10}$-helix. An acetyl group was selected as a mimic of the first amino acid (Xaa), and the choice of the methoxy substituent was based on synthetic precedent and convenience.[17] We planned to study L-Ala oligopeptides as an amide appendage since it is reported to have a high propensity for helix formation.[19] Furthermore, alanine base oligopeptides offer the advantage of more easily interpretable NMR spectra.

**SCHEME 1**[a]



[a] Reagents and conditions: (a) (i) $Ac_2O$, $K_2CO_3$, $CH_2Cl_2$, 0 °C, (ii) 2 N LiOH, THF/$H_2O$, rt, 86% two steps ;(b) TEMPO, NaOCl, $NaClO_2$, $CH_3CN$, 35 °C, 98%; (c) L-proline methyl ester hydrochloride, PyBOP, DIPEA, $CH_2Cl_2$, 0 °C then rt, 82%; (d) 2 N LiOH, THF/$H_2O$, rt, 91%; (e) H-(L-Ala)$_n$-R, EDC, HOBt, DIPEA, $CH_2Cl_2$, 0 °C then rt; (f) H-(L-Ala)$_4$-O*t*Bu, EDC, HOBt, DIPEA, DMF, 0 °C then rt.

## Synthesis

The previously reported (2*S*,3a*S*,8a*R*)-(6-methoxy-1,2,3,3a,8,8a-hexahydro-3-aza-cyclopenta[*a*]inden-2-yl)-methanol (**7**)[17] was used as the starting material for the synthesis of Ac-L-TcaP-L-Pro-OH (**4**) (Scheme 1). *N*-Acetylation of the nitrogen was accomplished at 0 °C in dichloromethane with use of potassium carbonate as the base. This reaction was also sometimes accompanied by *O*-acetylation. Selective hydrolysis of the acetate was then performed with lithium hydroxide in a mixture of THF/water at room temperature giving rise, after purification, to the desired *N*-acetyl derivative (**8**) in 86% yield. Subsequent oxidation of the alcohol to the corresponding acid, using a mixture of $NaClO_2$, commercial bleach, and catalytic TEMPO,[20] afforded the acid Ac-L-TcaP-OH (**9**) in quantitative yield. The acid was then coupled with L-proline methyl ester hydrochloride, using standard conditions (PyBOP, diisopropylethylamine), to afford Ac-L-TcaP-L-Pro-OMe (**10**) in 82% yield, which was subsequently treated with lithium hydroxide in a mixture of THF/water at room temperature to afford the desired Ac-L-TcaP-L-Pro-OH (**4**) in 91% yield. The L-alanine-based oligomers were prepared in solution by using conventional chemical manipulation with *N*-benzyloxycarbonyl and *tert*-butyl ester as the orthogonal protective groups.

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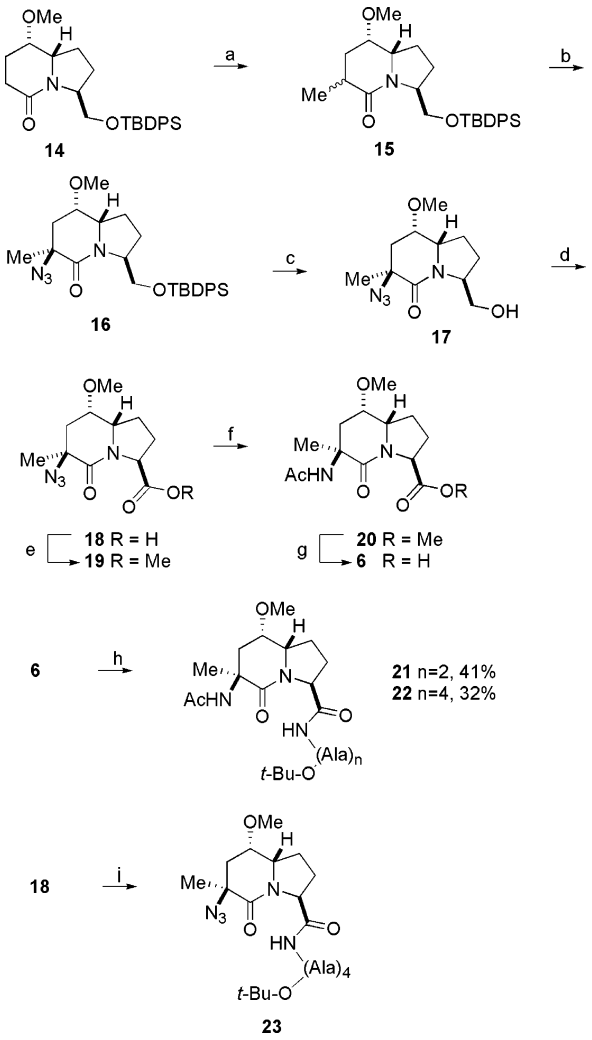
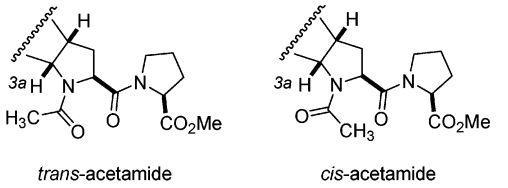
**SCHEME 2**[a]




[a] Reagents and conditions: (a) *t*-BuLi, $CH_3I$, THF, −78 °C, 76%; (b) *t*-BuLi, trisyl azide, THF, −78 °C, 63%; (c) $Bu_4NF$, THF, rt, 81%; (d) TEMPO, NaOCl, $NaClO_2$, $CH_3CN$, 35 °C, 95%; (e) $CH_2N_2$, $CH_3OH$, 100%; (f) (i) $H_2$, Pd/C, $CH_3OH$, (ii) $Ac_2O$, $CH_3OH$, rt, 85%, 2 steps; (g) 2 N LiOH, THF/$H_2O$, rt, 99% crude; (h) H-(L-Ala)$_n$-O*t*Bu, EDC, HOBt, DIPEA, DMF, 0 °C then rt; (i) H-(L-Ala)$_4$-O*t*Bu, EDC, HOBt, DIPEA, DMF, 0 °C to rt, 50%.

Ac-L-TcaP-L-Pro-L-Ala-OMe (**11**) was prepared by coupling template **4** with the commercially available L-Ala methyl ester hydrochloride (Scheme 1), using a conventional coupling protocol (EDC/ or HOBT, diisopropylethylamine, 67% yield). The same reaction conditions were employed to obtain Ac-L-TcaP-L-Pro-L-(Ala)$_2$-O*t*Bu (**12**) in 68% yield. In the case of the longer peptide Ac-L-TcaP-L-Pro-L-(Ala)$_4$-O*t*Bu (**13**), the use of DMF instead of $CH_2Cl_2$ afforded the desired product in 44% yield.

The synthesis of the second scaffold **6** follows a recently published protocol,[18] starting with *S*-pyroglutamic acid (Scheme 2). Intermediate **14** was transformed to the α-C-methyl derivative **15** via alkylation of the lithium enolate with *tert*-butyllithium as the most suitable base. Reformation of the Li enolate under the same reaction conditions and treatment with trisyl azide[21] gave the desired tertiary azide **16** as the major product. The pseudoaxial methoxy group at C-8 appears to exert a stereocontrolling influence in the approach of the electrophilic azidating reagent as in related cases.[21] Subsequent steps involved functional group manipulations to give the alcohol **17**, followed by oxidation to **18**, reduction of the azide, and *N*-acetylation to give eventually **6**. Peptide coupling with H-(L-Ala)$_n$-O*t*Bu, using HOBT, gave the di- and tetrapetides **21** and **22**, respectively. We also prepared the tetrapeptide **23** by the same general procedure as a control.




*trans*-acetamide *cis*-acetamide

| Solvent | Ratio trans/cis[a] |
|---|---|
| $CDCl_3$ | 3:1 |
| $D_2O$ | 1:1 |
| Pyridine-$d_5$ | 1:1.5 |

[a] See supporting information

**FIGURE 3.** Cis/trans acetamide conformational isomers.

## Results

Proton NMR spectra of **10** in $CDCl_3$ showed a 3:1 ratio of two major conformational isomers, as determined by the ratio of the cleanly resolved pair of doublets at 5.4 and 5.7 ppm, attributed to the C-3a proton of the trans and cis isomers, respectively (Figure 3). This ratio changes to 1:1 in $D_2O$, and inverts to 1:1.5 in pyridine-$d_5$. NOESY experiments in $CDCl_3$ and in pyridine-$d_5$ allowed us to assign the upfield doublet to the *trans*-acetamido isomer and the downfield doublet to the cis isomer. The data are in agreement with literature precedents showing that the *trans*-acetamide isomer is usually predominant in small proline-containing peptides, and that the proportion of the cis isomer increases in more polar solvents.[22,23] The prevalence for the trans orientation of **10** in solvents of low polarity could be due to the proximity of the electron-rich phenyl ring and the so-called "benzene−oxygen repulsion". [24]

To further establish the geometry of the amide bond between L-TcaP and the L-Pro unit in **10** we relied on $^{13}C$ chemical shifts as a diagnostic tool. The $^{13}C$ resonances of $\beta$- and $\gamma$-carbon atoms of the proline ring are known to be strongly influenced by the proximity effect of the carbonyl group of the amide function.[25] Comparison

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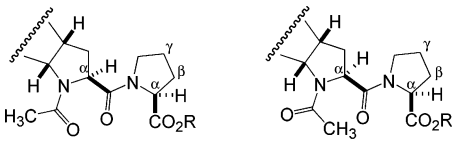
**TABLE 1. $^{13}$C NMR Chemical Shift of the $C_\beta$ and $C_\gamma$ of the Proline for Compounds 4 and 10 ($CDCl_3$)**



*trans*-acetamide *cis*-acetamide

| compd | $C_\beta$ | $C_\gamma$ |
|---|---|---|
| R = Me (**10**) | 29.2 ppm (trans)[a] | 25.3 ppm (trans)[a] |
| | 29.0 ppm (cis)[a] | 25.4 ppm (cis)[a] |
| R = H (**4**) | 28.2 ppm (trans)[a] | 25.4 ppm (trans)[a] |
| | 28.7 ppm (cis)[a] | |

[a] Indicated isomer corresponds to acetamido cis/trans orientation.

between the resonances of the $\beta$- and $\gamma$-carbon atoms in $CDCl_3$ led us to conclude that a trans orientation of the proline amide is preferred in **10**, regardless of the geometry of the acetamido group on the L-TcaP unit as indicated in the perspective drawings (Figure 3). These results were also valid in pyridine-$d_5$ (data not shown). The lack of NOE between the two α-protons in L-TcaP and L-Pro in $CDCl_3$ further confirmed the trans orientation of the inter-Pro bond.

One- and two-dimensional NMR experiments in $CDCl_3$ of the acid **4** showed a trans/cis ratio of 7:1 for the acetamide bond. $^{13}$C resonances of $\beta$- and $\gamma$-carbons of the major isomer in $CDCl_3$ conform to the reported range for a trans-amide bond[26] (Table 1). The increase in the ratio of the trans vs cis isomer in the acid **4** compared to the ester **10** can be explained on the basis of an increased difficulty in aligning three carbonyl dipoles. In fact, the 7:1 trans−cis ratio observed in $CDCl_3$ for **4** drops to 1:1 in $D_2O$ showing a strong solvent dependence.

**L-TcaP L-Ala Oligopeptides.** A qualitative assessment of the presence of intramolecular H-bonding in oligomeric peptides **11**−**13** was done by analyzing the amide N−H stretching in the region of 3200−3500 cm$^{-1}$ by FT-IR[27] at a concentration of 1 mM in $CDCl_3$. An enhancement of intramolecular H-bonding was observed at 3320−3340 cm$^{-1}$ compared to that of the solvent-exposed free N−H amide at 3412−3418 cm$^{-1}$.[27] Analysis of Cbz-L-(Ala)$_4$-O*t*Bu used as a control at the same concentration in $CDCl_3$ showed only a small hydrogen bond stretching band, probably due to an irregular $\gamma$-turn-like structure.[28,29]

Ac-L-TcaP-L-Pro-L-Ala-OMe (**11**) was analyzed by one- and two-dimensional NMR at a concentration of 5.8 mM (Figure 4A). Three conformers can be seen in a ratio of 1:1.6:1.4, as measured from the ratio of the clearly resolved doublets of C-3a at 5.66, 5.50, and 5.47 ppm, as well as by the ratios of the NH resonances. NOESY experiments in the same solvent confirm that the doublet peaks at 5.50 and 5.47 pm correspond to the major trans

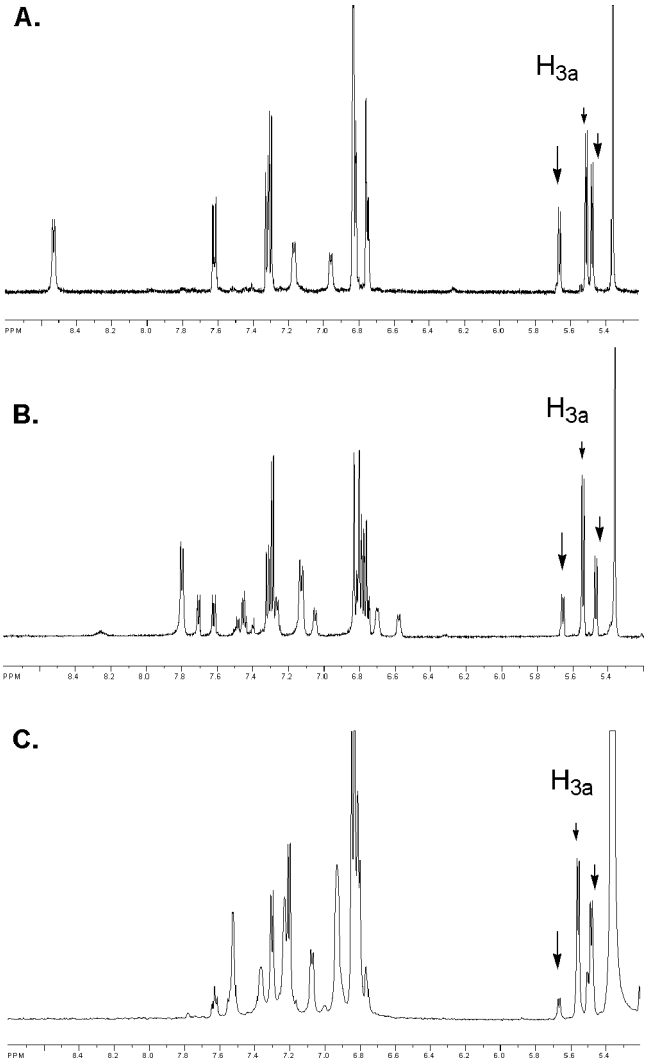



**FIGURE 4.** $^1$H NMR (600 MHz) spectra in $CD_2Cl_2$ of compounds **11**, **12**, and **13** (A, B, and C, respectively). The shoulder at 5.49 ppm is a $CH_2Cl_2$ satellite peak.

isomer. The appearance of a second trans-isomer peak at 5.50 ppm corresponds to a minor component in **4** and **10**, which is amplified in **11** after attachment of the first Ala unit. This may be related to a H-bond resulting in an enthalpic advantage over the two other isomers. To gain some insight, we performed titration experiments[30] with increasing amounts of DMSO-$d_6$ (0−15% v/v) and temperature dependence of the NH chemical shifts[31] in the range 210−290 K in $CD_2Cl_2$.[29] The presence of one intramolecularly H-bonded NH was obvious, which corresponded to the *trans*-acetamido isomer. A $\gamma$-turn H-bonded array was ruled out since it is known to have little or no enthalpic advantage over the non-H-bonded state.[32]

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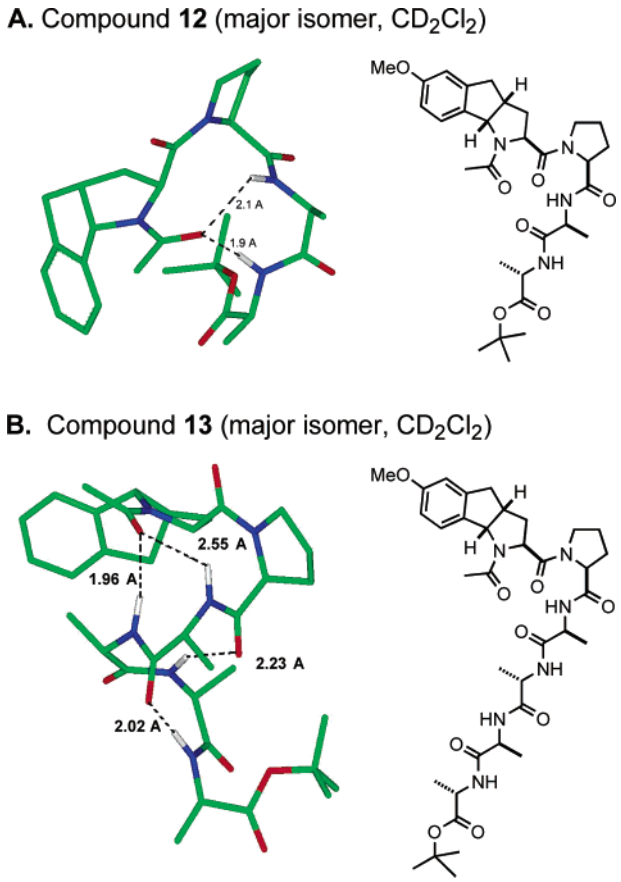


**FIGURE 5.** Lowest energy structures obtained by restrained conformational calculations from 2D NOESY data.

NMR studies on Ac-L-TcaP-L-Pro-L-$(Ala)_2$-O*t*Bu (**12**) in $CD_2Cl_2$ at a concentration of 8.9 mM showed three conformers in a ratio of 1:3.4:1.8, as measured from the C-3a doublets at 5.65, 5.54, and 5.47 ppm (Figure 4B). As expected, COSY experiments showed that the two downfield doublets attributed to H-bonded NH groups belong to two different alanine units corresponding to the major *trans*-acetamido isomer. NOE contacts from 2D NOESY data allowed us to obtain a lowest energy structure using restrained conformational calculation (HyperChem) (Figure 5A). As depicted, the structure suggests a bifurcated H-bonding motif involving the *trans*-acetamido carbonyl group.

NMR studies on Ac-L-TcaP-L-Pro-L-$(Ala)_4$-O*t*Bu (**13**) in $CDCl_3$ at a concentration of 5.6 mM showed the presence of three conformers in a ratio of 1:8.5:6.5 at 5.66, 5.56, and 5.48 ppm (Figure 4C). COSY experiments showed that the four doublets of the NH groups belong to four different alanine units, corresponding to the major *trans*-acetamido isomer. NOE contacts and 2D NOESY data with restrained conformational calculation (HyperChem) generated a motif encompassing an intramolecular H-bonding network (Figure 5B). The bifurcated H-bonded unit observed for **12** is now complemented by further contacts leading to a $3_{10}$-helical folding in **13**. CD spectra[29] for **12** and **13** in MeOH at a concentration of $1.9 \times 10^{-4}$ and $1.4 \times 10^{-4}$ M, respectively, showed bands at 205 and 222 nm indicating a propensity for an $3_{10}$-helix,[32] rather than an α-helix. Unfortunately, DMSO-$d_6$ titrations and temperature-dependent NH shifts were difficult to quantitate due to overlapping signals.

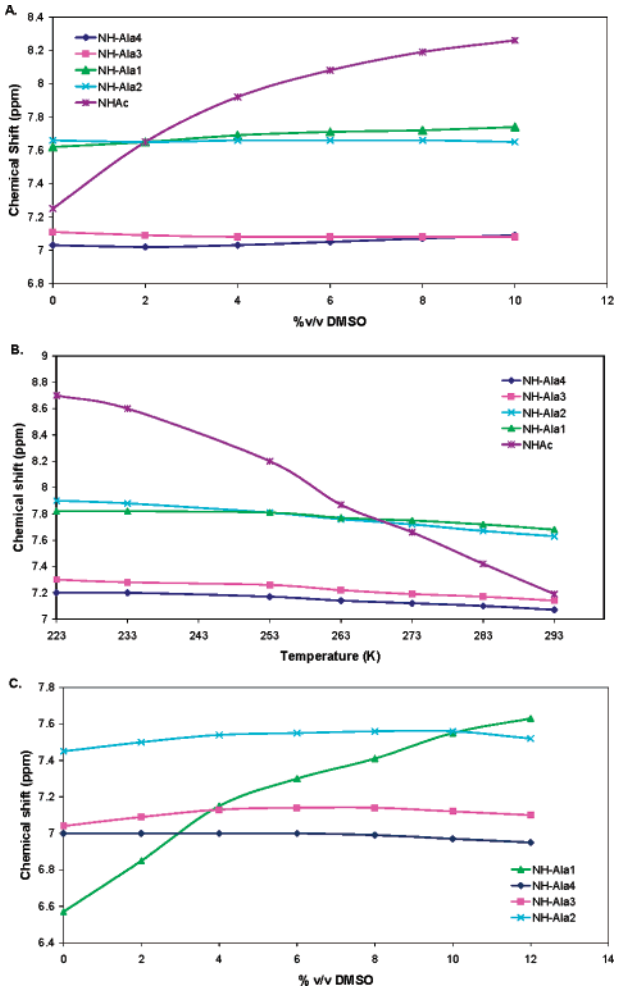


**FIGURE 6.** (A) DMSO titration experiment for compound **22**; (B) temperature variation experiment for compound **22**; and (C) % DMSO variation experiment for compound **23**.

**Indolizidinone L-Ala Oligopeptides.** We had anticipated that the L-Ala oligopeptides **21** and **22** attached to the bicyclic scaffold would adopt a helical folding shape, starting with one or more β-turn-type H-bonding arrays, with the axially disposed *N*-acetyl carbonyl and the first L-Ala amide. The prospects for such folding and structural organization were substantiated by NMR experiments. Thus, titration of **22** in $CDCl_3$ with increasing amounts of DMSO-$d_6$ clearly showed the prevalence of four NH signals that were unaffected up to 10% v/v of DMSO (Figure 6 A).

The only NH chemical shift change was due to the free N-Ac group. This behavior was corroborated by temperature variation studies for **22**, where the same four N-H signals were not influenced in the range 223−293 K (Figure 6B). As a control, the DMSO titration of the azido tetrapeptide **23** showed a similar pattern compared to the *N*-acetyl analogue **22**, except for the absence of a second β-turn H bond and distinctly different chemical shifts. The CD spectra of **21**−**23** in $CDCl_3$ or MeOH showed a similar pattern as for **11**−**13** with bands at ca. 208 and 222 nm characteristic of a $3_{10}$-helix.[29] 2D NOESY experiments with **22** corroborated the proposed helical

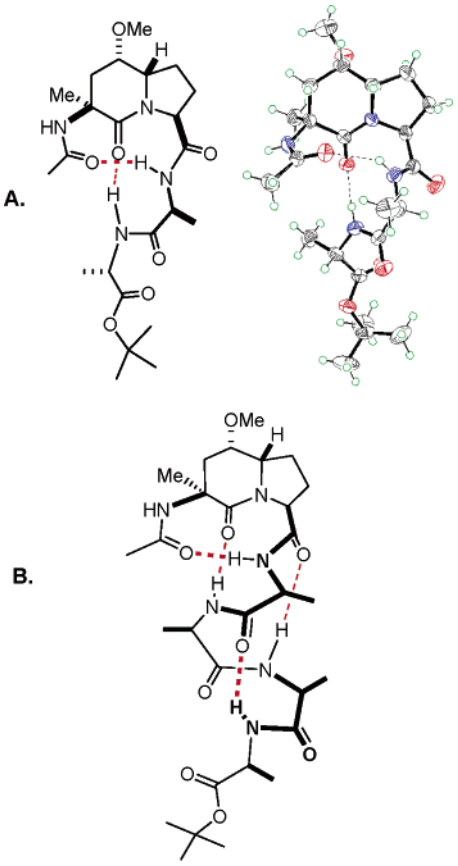


**FIGURE 7.** Top (A): Ortep diagram representation of **21**. Bottom (B): Modeled tetrapeptide **22**.

structure based on the NH correlations. We were gratified to obtain X-ray quality crystals of the dipeptide **21**.[29] As anticipated from the titration experiments we observed two characteristic intramolecular $\beta$-turn-type H bonds linking the lactam carbonyl and acetamide carbonyl oxygen atoms of the bicyclic scaffolds to NH amide groups of $N_i$ and $N_i + 1$ Ala units (Figure 7A). On the basis of the H-bonding pattern seen in the X-ray structure of **21**, the titration and temperature variation experiments, as well as the CD spectra characteristics, and 2D NOESY data,[29] we propose that the tetrapeptide **22** adopts a $3_{10}$-helical shape as depicted in Figure 7B.

## Conclusion

We have described the synthesis of two novel constrained amino acid core structures that induce the formation of $3_{10}$-helices from appended tetraalanine peptides in organic solvents. The first motif, consisting of Ac-L-TcaP-L-Pro tetrapeptide, exists in different conformations around the Pro-Pro amide bond with respect to the *N*-acetyl. The *trans*-acetamide conformation is favored in nonpolar solvents, which corroborates the observed bifurcated intramolecular H bond of two NHs on different Ala residues with the acetamide carbonyl.

The second motif utilized an indolizidinone as a potential nucleator for a di- and tetraalanine peptidic appendage. A $3_{10}$-helical arrangement was observed by X-ray crystallography of the dipeptide **21**. Titration and variable-temperature experiments revealed that only one NH (NHAc) was affected under the NMR conditions of measurement, suggesting a $3_{10}$-type folding, as well. Goodman and Saltman[28] have already demonstrated the importance of a nucleating template based on the observation that linear homooligopeptides showed unfolded or irregular $\gamma$-turn-like structures in a variety of solvents.

Studies are ongoing in our laboratory to uncover other types of core structures capable of conferring stable secondary structures to appended polypeptides.

## Experimental Section

The synthesis of compounds **8**, **9**, and **10** is given in the Supporting Information.[29]

**Ac-L-TcaP-L-Pro-OH (4).** To a stirred solution of Ac-L-TcaP-L-Pro-OMe (**10**; 65 mg, 0.168 mmol) in 1.3 mL of THF, at room temperature, was added a 2 N aqueous solution of LiOH (0.17 mL, 0.336 mmol) dropwise and the biphasic system was stirred at room temperature for 3 h. The solvent was evaporated; the resulting mixture was diluted with water (5 mL) and then washed with diethyl ether (2 × 5 mL). The organic layers were discarded while the aqueous one was acidified to pH 3−4 with 1 N HCl, then extracted with dichloromethane (3 × 10 mL) and dichloromethane/methanol 9:1 (3 × 10 mL). The combined organic phases were dried ($Na_2SO_4$) and evaporated to dryness to afford a white solid (57 mg, 91%): mp 134−136 °C, 153−154 °C dec; $[\alpha]_D$ −209.0 (*c* 0.5, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz) for a mixture approximately 1:7 of major rotamers $\delta$ 7.68, 7.26 (0.12 H, d, $J$ = 8.1 Hz; 0.88 H, d, $J$ = 5.5 Hz), 6.81−6.72 (2 H, m), 5.75, 5.46 (0.12 H, d, $J$ = 7.0 Hz; 0.88 H, d, $J$ = 7.0 Hz), 4.68 (1 H, d, 5.8 Hz), 4.58, 4.52 (0.88 H, d, $J$ = 9.0 Hz; 0.12 H, d, $J$ = 9.0 Hz), 3.81, 3.78 (2.64 H, s; 0.36 H, s), 3.76−3.68 (1.76 H, m), 3.54−3.47 (2.24 H, m), 3.12−3.07 (1 H, dd, $J$ = 16.4, 6.5 Hz), 2.71−2.67 (1 H, d, $J$ = 16.4 Hz), 2.42 (3.52 H, m), 2.33−2.29 (0.12 H, m), 2.14−2.06 (2.12 H, m), 2.05 (1.24 H, m), 1.87−1.78 (1 H, m); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ (174.0), 173.7, 173.0, (171.1), 170.7, 160.5, (160.2), 143.6, (142.7), (136.4), 134.2, (128.6), 125.7, 113.6, 111.5, (110.5), 68.2, (67.9), 59.9, (59.6), 59.5, 55.8, (55.7), 47.6, 42.9, (39.6), 35.2, 33.9, (28.7), 28.2, 25.4, 22.9, (22.8); MS (EI+) *m/e* 372 $(M)^+$; HRMS (EI+) calcd for $C_{20}H_{24}N_2O_5$ 372.16852, found 372.16870.

**Ac-L-TcaP-L-Pro-L-Ala-OMe (11).** To a chilled solution of L-alanine methyl ester hydrochloride (3 mg, 0.0156 mmol) in 2 mL of dichloromethane was added Ac-L-TcaP-L-Pro-OH (**4**; 5 mg, 0.013 mmol), HOBt (3 mg, 0.02 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (4 mg, 0.02 mmol), and diisopropylethylamine (3 $\mu$L, 2.5 equiv). The reaction mixture was stirred for 1 h at 0 °C and 48 h at room temperature. Water (2 mL) was then added to the reaction mixture and the phases were separated. The organic layer was washed with saturated $NaHCO_3$ aqueous solution (2 × 2 mL) and brine (1 × 2 mL), dried ($Na_2SO_4$), and evaporated to dryness. The resulting solid was purified via silica gel flash column chromatography (AcOEt/MeOH, 85:15). Compound **11** was obtained as a white solid (4 mg, 67%): $[\alpha]_D$ −158 (*c* 0.1, $CHCl_3$); $^1$H NMR ($CD_2Cl_2$, 600 MHz) for a mixture of approximately 1:1.4:1.6 of major rotamers $\delta$ 8.53−8.52 (0.4 H, d, $J$ = 7.8 Hz), 7.62−7.61 (0.25 H, d, $J$ = 9.2 Hz), 7.32−7.31 (0.35 H, d, $J$ = 8.1 Hz), 7.30−7.29 (0.4 H, d, $J$ = 8.1 Hz), 7.16−7.15 (0.35 H, d, $J$ = 6.5 Hz), 6.95−6.94 (0.25 H, d, $J$ = 6.0 Hz), 6.83−6.81 (1.2 H, m), 6.75−6.74 (0.8 H, m), 5.67−5.65 (0.25 H, d, $J$ = 7.1 Hz), 5.51−5.50 (0.4 H, d, $J$ = 7.1 Hz), 5.48−5.47 (0.35 H, d, $J$ = 7.1 Hz), 4.61−4.60 (1 H, d, $J$ = 9.1 Hz), 4.57−4.54 (0.6 H, m), 4.53−4.48 (0.5 H, m), 4.45−4.40 (0.35 H, quint, $J$ = 7.3 Hz), 4.32−4.29 (0.15 H, m), 4.25−4.24 (0.4 H, d, $J$ = 8.1 Hz), 3.82 (2.2 H, s), 3.80 (0.8 H, s), 3.76 (1.5 H, s), 3.74−3.71 (0.5 H, m), 3.59−3.50 (1.4 H, m), 3.55 (0.6 H, m), 3.42−3.37 (0.8 H, m), 3.13−3.03 (1.2 H, m), 2.73−2.69 (1 H, m), 2.53−2.50 (0.5 H, dd, $J$ = 12.4, 6.3 Hz), 2.40 (1 H, s), 2.38 (1.25 H, s), 2.32−2.23 (1.25 H, m), 2.19−1.87 (2.4 H, m), 1.94 (1 H, s), 1.86−1.80 (1.2 H, m), 1.77−1.69 (1 H, m), 1.68−

1.62 (0.4 H, m), 1.50−1.48 (1.2 H, d, $J$ = 7.3 Hz), 1.44−1.42 (0.75 H, d, 7.1 Hz), 1.41−1.40 (1.05 H, d, $J$ = 7.1 Hz); $^{13}$C NMR (pyridine-$d_5$, 125 MHz) $\delta$ 174.5, 173.2, 171.3, 170.9, 160.9, (144.5), 143.2, 137.7, 130.4, (127.7), 116.0, (115.9), (113.2), 112.3, (69.6), 69.5, (67.7), 63.2, 61.8, (61.4), (56.9), 56.8, 53.2, 49.5, (49.4), (48.3), 48.2, (43.6), 40.4, 36.8, 36.3, (35.9), (34.5), 30.4, (30.0), 26.3, (25.9), (23.4), 23.2, 17.7, (16.3); MS (EI+) *m/e* 458 $(M+H)^+$; HRMS (EI+) calcd for $C_{24}H_{32}N_3O_6$ (M + H) 458.22911, found 458.22770.

**Ac-L-TcaP-L-Pro-L-Ala-L-Ala-O*t*Bu (12).** To a chilled solution of L-alanyl-L-alanine *tert*-butyl ester (13 mg, 0.06 mmol) in 5 mL of dichloromethane was added Ac-L-TcaP-L-Pro-OH (**4**; 19 mg, 0.05 mmol), HOBt (11 mg, 0.08 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (15 mg, 0.08 mmol), and diisopropylethylamine (23 $\mu$L, 0.13 mmol). The reaction mixture was stirred for 1 h at 0 °C and 40 h at room temperature. Water (5 mL) was then added to the reaction mixture and the phases were separated. The organic layer was washed with saturated $NaHCO_3$ aqueous solution (2 × 5 mL) and brine (1 × 5 mL), dried ($Na_2SO_4$), and evaporated to dryness. The resulting solid was purified via silica gel flash column chromatography (AcOEt/MeOH, 85:15). Compound **12** was obtained as a light-yellow solid (19 mg, 68%): $[\alpha]_D$ −110 (*c* 0.2, $CHCl_3$); $^1$H NMR ($CD_2Cl_2$, 600 MHz) for a mixture approximately 1:1.8:3.4 of major rotamers $\delta$ 7.82−7.77 (0.55H, d, $J$ = 7.3 Hz), 7.63−7.60 (0.16H, d, $J$ = 8.8 Hz), 7.33−7.30 (0.29H, d, $J$ = 8.0 Hz), 7.30−7.27 (0.55H, d, $J$ = 8.0 Hz), 7.26−7.22 (0.29H, d, $J$ = 7.5 Hz), 7.14−7.10 (0.55H, d, $J$ = 8.0 Hz), 7.03−7.00 (0.16H, d, $J$ = 7.1 Hz), 6.84−6.74 (2H, m), 6.70−6.66 (0.29H, d, $J$ = 6.6 Hz), 6.57−6.54 (0.16H, d, $J$ = 7.1 Hz), 5.67−5.64 (0.16H, d, $J$ = 7.5 Hz), 5.55−5.53 (0.55H, d, $J$ = 7.5 Hz), 5.48−5.45 (0.29H, d, $J$ = 6.6 Hz), 4.69−4.65 (0.55H, d, $J$ = 8.8 Hz), 4.62−4.59 (0.16H, dd, $J$ = 8.4, 3.5 Hz), 4.59−4.57 (0.29H, d, $J$ = 9.6 Hz), 4.57−4.54 (0.29H, d, $J$ = 8.3 Hz), 4.42−4.27 (2.71H, m), 3.82 (0.87H, s), 3.80 (1.65H, s), 3.79 (0.48H, s), 3.76−3.72 (0.16H, m), 3.60−3.56 (1.68H, m), 3.55−3.35 (1.16H, m), 3.14−3.08 (1H, dd, $J$ = 16.6, 7.5 Hz), 2.75−2.69 (1H, dd, $J$ = 16.6, 6.6 Hz), 2.52−2.47 (0.58H, m), 2.44−2.42 (1.65H, s), 2.40−2.38 (0.87H, s), 2.34−2.27 (0.32H, m), 2.17−2.10 (1.1H, m), 2.09−2.00 (2.29H, m), 1.98−1.90 (1.19H, m + s), 1.89−1.80 (0.84H, m), 1.80−1.73 (0.16H, m), 1.49 (1.44H, s), 1.48−1.46 (2.52H, d, $J$ = 7.4 Hz), 1.42−1.40 (0.48H, d, $J$ = 7.2 Hz), 1.40−1.38 (0.48H, d, $J$ = 7.2 Hz), 1.31 (2.61H, s), 1.25 (4.95H, s), 1.23−1.21 (2.52H, d, $J$ = 7.3 Hz); $^{13}$C NMR (pyridine-$d_5$, 125 MHz) $\delta$ 173.3, 172.8, 172.4, 171.6, 170.3, (170.1), 160.6, 143.2, 137.4, 128.8, (126.1), 114.1, 110.5, 81.1, (68.2), 68.0, 61.8, 60.7, (60.5), (60.1), (55.5), 55.4, 49.5, (47.4), 47.3, (42.8), 39.8, 36.2, 35.7, (34.0), 30.1, 29.6, (29.3), 28.0, 25.5, (25.3), (23.1), 22.8, (18.9), 18.8, 17.8; MS (FAB+) *m/e* 593 $(M + Na)^+$, 571 $(M + H)^+$; HRMS (FAB+) calcd for $C_{30}H_{42}N_4O_7Na$ 593.29512, found 593.29260.

**Ac-TcaP-L-Pro-L-Ala-L-Ala-L-Ala-L-Ala-O*t*Bu (13).** To a chilled solution of L-alanyl-L-alanyl-L-alanyl-L-alanine *tert*-butyl ester (15 mg, 0.04 mmol) in 2 mL of dry DMF was added Ac-L-TcaP-L-Pro-OH (**4**; 17 mg, 0.045 mmol), HOBt (10 mg, 0.075 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (15 mg, 0.075 mmol), and diisopropylethylamine (22 $\mu$L, 0.125 mmol). The reaction mixture was stirred for 1 h at 0 °C and 48 h at room temperature. DMF was evaporated under high vacuum and the orange residue was dissolved in dichloromethane (50 mL). The solution was washed with water (1 × 20 mL), saturated $NaHCO_3$ aqueous solution (2 × 20 mL), and brine (1 × 20 mL), dried ($Na_2SO_4$), and evaporated to dryness. The resulting orange solid was purified via silica gel flash column chromatography (AcOEt/MeOH, 80:20). Compound **13** was obtained as a light yellow solid (16 mg, 44%): $[\alpha]_D$ −90 (*c* 0.2, $CHCl_3$); $^1$H NMR ($CD_2Cl_2$, 600 MHz) for a mixture of approximately 1:6.5:8.5 of major rotamers $\delta$ 7.62−7.60 (0.06H, d, $J$ = 8.1 Hz), 7.62−7.60 (0.06H, d, $J$ = 8.8 Hz), 7.56−7.54 (0.53H, d, $J$ = 4.5 Hz), 7.40 (0.41H, br s), 7.30−7.29 (0.41H, d, $J$ = 8.1 Hz), 7.26−7.25 (0.53H, d, $J$ = 5.4 Hz), 7.21−7.19 (0.53H, d, $J$ = 8.3 Hz), 7.12−7.10 (0.41H, d, $J$ = 8.0 Hz), 7.03 (0.18H, m), 6.97−6.96 (0.94H, d, $J$ = 7.0 Hz), 6.86−6.76 (2.94H, m), 5.66 (0.06H, d, $J$ = 7.7 Hz), 5.56 (0.53H, d, $J$ = 7.1 Hz), 5.49−5.47 (0.41H, d, $J$ = 6.9 Hz), 4.66−4.64 (0.06H, d, $J$ = 9.8 Hz), 4.61−4.59 (0.06H, d, $J$ = 8.7 Hz), 4.57−4.55 (0.12H, m), 4.51−4.47 (0.41H, br s), 4.45−4.43 (0.06H, m), 4.40 (0.41H, m), 4.39 (0.41H, m), 4.35−4.33 (0.53H, d, $J$ = 9.0 Hz), 4.30−4.26 (1.94H, m), 4.24−4.21 (0.53H, m), 4.16−4.12 (1.47H, m), 3.82 (1.23H, s), 3.81 (1.59H, s), 3.80 (0.18H, s), 3.74−3.71 (1H, m), 3.61−3.57 (1H, m), 3.49−3.44 (0.59H, m), 3.43−3.38 (0.41H, m), 3.18−3.14 (0.41H, dd, $J$ = 16.4, 6.6 Hz), 3.14−3.10 (0.59H, dd, $J$ = 16.5, 6.9 Hz), 2.81−2.78 (0.41H, d, $J$ = 16.4 Hz), 2.75−2.71 (0.06H, d, $J$ = 16.5 Hz), 2.73−2.70 (0.53H, d, $J$ = 16.5 Hz), 2.46 (1.59H, s), 2.44 (1.23H, s), 2.36−2.12 (2H, m), 2.07−2.03 (1H, m), 2.02−1.95 (1H, m), 1.93 (0.18H, s), 1.88−1.81 (2H, m), 1.56−1.54 (1.77H, d, $J$ = 7.3 Hz), 1.48−1.46 (1.23H, m), 1.48 (3.69H, s), 1.47 (4.77H, s), 1.45−1.43 (1.23H, d, $J$ = 7.4 Hz), 1.41−1.39 (1.23H, d, $J$ = 8.1 Hz), 1.39−1.38 (1.77H, d, $J$ = 7.4 Hz), 1.30 (0.54H, s), 1.23−1.21 (1.77H, d, $J$ = 7.3 Hz), 1.20 (1.77H, d, $J$ = 7.3 Hz), 1.20−1.19 (1.23H, m); $^{13}$C NMR (pyridine-$d_5$, 125 MHz) $\delta$ 173.5, 173.0, 172.9, 172.7, 171.6, 171.0, 170.8, 160.6, 143.3, 137.3, 128.8, 114.1, (111.5), 110.5, 81.1, (68.1), 69.7, 61.7, 60.8, (55.6), 55.5, 50.0, 49.7, 49.5, 49.1, (47.5), 47.4, 36.1, 35.7, (30.0), 29.7, 28.1, 25.5, (23.1), 22.9, 19.0, 18.9, 18.8, 18.6, 17.7; MS (FAB+) *m/e* 735 $(M + Na)^+$, 713 $(M + H)^+$, 657 $(M - (CH_3)_2C{=}CH_2)$; HRMS (FAB+) calcd for $C_{36}H_{53}N_6O_9$ 713.38740, found 713.38600.

**(3*S*,8*S*,9*S*)-3-(*tert*-Butyldiphenylsilanyloxymethyl)-8-methoxy-6-methylhexahydroindolizin-5-one (15).** To a stirred solution of lactam **14**[18] (1.18 g, 2.694 mmol) in 10.8 mL of THF at −78°C was added dropwise a 1.5 M solution of *t*-BuLi (1.98 mL, 2.239 mmol) in THF. The mixture was stirred for 30 min at this temperature then methyl iodide (0.24 mL, 3.853 mmol) was added. The reaction mixture was stirred for 30 min at −78 °C, and then the reaction was neutralized by adding slowly a saturated solution of $NaHCO_3$. The mixture was extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was purified via flash column chromatography on silica gel (30% AcOEt /hexanes) to give the mixture of diastereoisomers as a yellow oil (921 mg, 76%). A sample of the major isomer (α-Me) was separated via chromatography for spectroscopic data: $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.65−7.58 (4H, m), 7.43−7.34 (6H, m), 4.23−4.20 (1H, m), 4.12−4.08 (1H, dd, $J$ = 10.0, 4.0 Hz), 3.76−3.72 (1H, m), 3.70−3.66 (1H, dd, $J$ = 10.0, 2.2 Hz), 3.56 (1H, m), 3.34 (3H, s), 2.47−2.32 (2H, m), 2.05−1.96 (3H, m), 1.85 (1H, m), 1.50−1.42 (1H, t, $J$ = 13.1 Hz), 1.30−1.27 (3H, d, $J$ = 7.0 Hz), 1.04 (9H, s); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 172.0, 135.9, 134.1, 133.9, 130.0, 129.9, 128.1, 128.0, 72.8, 64.6, 64.2, 58.4, 56.7, 33.2, 31.4, 27.3, 26.9, 26.8, 25.1, 19.8, 18.4; MS (FAB) *m/e* 452 $(M + H)^+$; HRMS (EI+) calcd for $C_{27}H_{38}NO_3Si$ 452.26209, found 452.26169.

**(3*S*,6*S*,8*S*,9*S*)-6-Azido-3-(*tert*-butyldiphenylsilanyloxymethyl)-8-methoxy-6-methylhexahydroindolizin-5-one (16).** To a stirred solution of lactam **15** (920 mg, 2.035 mmol) (mixture of diastereoisomers) in 8.2 mL of THF at −78 °C was added dropwise a 1.5 M solution of *t*-BuLi (1.49 mL, 2.239 mmol) in THF. The mixture was stirred for 1 h at this temperature then a solution of trisyl azide (756 mg, 2.442 mmol) in THF was added. The reaction mixture was stirred for 3 h at −78 °C and neutralized with a saturated solution of $NaHCO_3$; the mixture was then extracted with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The mixture of diastereoisomers was purified via flash column chromatography on silica gel (20% AcOEt /hexanes) to give the major isomer **16** as a yellow oil (628 mg, 63%): $[\alpha]_D$ −120 (*c* 0.6, $CHCl_3$); FT-IR ($\nu$, $cm^{-1}$) 2957, 2103, 1645, 1428, 1109; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.67−7.63 (4H, m), 7.43−7.39 (6H, m), 4.25−4.19 (2H, m), 3.85−3.82 (1H, m), 3.66−3.63 (1H, dd, $J$ = 9.7, 1.6 Hz), 3.55−3.53 (1H, m), 3.36 (3H, s), 2.39−2.35 (1H, dd, $J$ = 14.7, 3.8 Hz), 2.09−2.01 (3H, m), 1.94−1.92 (1H, m), 1.79−1.75 (1H, dd, $J$ = 14.7, 2.1 Hz), 1.69 (3H, s), 1.06 (9H,

s); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 168.4, 135.9, 133.9, 133.6, 130.1, 130.0, 128.2, 128.1, 73.8, 64.2, 63.5, 60.6, 59.2, 57.3, 37.3, 27.2, 27.0, 25.2, 25.1, 19.7; MS (EI) *m/e* 492 $(M)^+$, 435; HRMS (EI+) calcd for $C_{27}H_{36}N_4O_3Si$ 492.25567, found 492.25560.

**(3*S*,6*S*,8*S*,9*S*)-6-Azido-3-hydroxymethyl-8-methoxy-6-methylhexahydroindolizin-5-one (17).** To a stirred solution of protected alcohol **16** (50 mg, 0.101 mmol) in THF was added a 1 M solution of tetrabutylammonium fluoride in THF (203 $\mu$L, 0.203 mmol) dropwise. The reaction mixture was subsequently stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure and was loaded directly onto a column of silica gel for purification (AcOEt/hexanes, 1:1). The desired compound **17** was obtained as a yellow oil (21 mg, 81%): $[\alpha]_D$ −199.8 (*c* 1.0, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 4.75 (1H, br s), 4.12−4.17 (1H, quad, $J$ = 8.0 Hz), 3.69−3.72 (2H, m), 3.55−3.61 (2H, m), 3.36 (3H, s), 2.35−2.39 (1H, dd, $J$ = 14.8, 3.6 Hz), 2.07−2.17 (1H, m), 1.97−2.04 (1H, m), 1.79−1.85 (1H, m), 1.73−1.77 (1H, dd, $J$ = 14.8, 2.0 Hz), 1.66 (3H, s), 1.39−1.48 (1H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 171.4, 73.5, 67.6, 63.5, 63.0, 60.9, 57.3, 37.1, 26.8, 26.4, 24.9; MS (MAB) *m/e* 254 $(M)^+$; HRMS (EI+) calcd for $C_{11}H_{18}N_4O_3$ 254.13789, found 234.13597.

**(3*S*,6*S*,8*S*,9*S*)-6-Azido-8-methoxy-6-methyl-5-oxo-octahydroindolizine-3-carboxylic Acid (18).** To a stirred solution of **17** (20 mg, 0.079 mmol) in acetonitrile (0.4 mL) was added a sodium phosphate buffer (0.3 mL of a 0.67 M aqueous solution of $NaH_2PO_4 \cdot H_2O$ buffered at pH 6.8 with 2 N aqueous sodium hydroxide) and the mixture was vigorously stirred at room temperature for 5 min; TEMPO (1.0 mg, 0.005 mmol), a solution of sodium chlorite in water (18 mg dissolved in 0.1 mL of water, 0.158 mmol), and a commercial bleach solution, previously diluted with water (2 $\mu$L of a 5.25% solution diluted in 0.4 mL of water, 0.025 mmol), were subsequently added. The resulting red-brown solution was stirred and heated at 35 °C for 24 h, cooled to room temperature, and diluted with water (2.0 mL) and the pH was adjusted to pH 8 by adding 5 drops of 2 N aqueous sodium hydroxide. The solution was then cooled to 0 °C and quenched with aqueous sodium sulfite solution (64 mg dissolved in 1.21 mL of water), stirred for 30 min at 0 °C, then extracted with ether (2 × 10 mL). The organic phases were discarded, while the aqueous one was acidified (pH 2−3) with 1 N HCl then extracted with ether (2 × 10 mL) and then with $CH_2Cl_2$ (5 × 10 mL). The combined organic phases were dried over sodium sulfate, filtered, and evaporated to dryness. The resulting crude acid was obtained as a white foam and was used in the next step without any further purification (20 mg, 95%): $[\alpha]_D$ −241 (*c* 1.0, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 4.57−4.60 (1H, t, $J$ = 8.4 Hz), 3.80−3.82 (1H, m), 3.58−3.61 (1H, m), 3.37 (3H, s), 2.36−2.40 (1H, dd, $J$ = 14.8, 3.8 Hz), 2.27−2.33 (2H, m), 2.03−2.13 (1H, m), 1.98−2.01 (1H, m), 1.78−1.82 (1H, dd, $J$ = 14.8, 2.4 Hz), 1.69 (3H, s); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 176.0, 168.8, 73.7, 62.8, 60.5, 60.7, 57.2, 37.2, 27.8, 27.0, 25.3; MS (MAB) *m/e* 268 $(M)^+$; HRMS (EI+) calcd for $C_{11}H_{16}N_4O_4$ 268.11715, found 268.11722.

**(3*S*,6*S*,8*S*,9*S*)-6-Azido-8-methoxy-6-methyl-5-oxo-octahydroindolizine-3-carboxylic Acid Methyl Ester (19).** To a stirred solution of **18** (20 mg, 0.074 mmol) in methanol was added diazomethane dropwise until the reaction was complete. The reaction mixture was subsequently quenched with acetic acid and the mixture was concentrated under reduced pressure to give the desired compound as a yellow foam (21 mg, 100%): $[\alpha]_D$ −198.0 (*c* 1.0, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 4.47−4.50 (1H, t, $J$ = 8.2 Hz), 3.91−3.93 (1H, m), 3.75 (3H, s), 3.58 (1H, d, $J$ = 2.8 Hz), 3.36 (3H, s), 2.31−2.37 (2H, m), 2.07−2.16 (1H, m), 1.82−1.95 (3H, m), 1.64 (3H, s); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 172.8, 168.8, 73.7, 62.6, 60.5, 59.1, 57.2, 52.8, 37.5, 28.1, 27.2, 25.1; MS (MAB) *m/e* 282 $(M)^+$; HRMS (EI+) calcd for $C_{12}H_{18}N_4O_4$ 282.13280, found 282.13342.

**(3*S*,6*S*,8*S*,9*S*)-6-Acetylamino-8-methoxy-6-methyl-5-oxo-octahydroindolizine-3-carboxylic Acid Methyl Ester (20).** Compound **19** (20 mg, 0.071 mmol) was dissolved in methanol (2 mL) and hydrogenated (1 atm) with Pd/C 10% (10 mg, 10% mol) for 16 h at room temperature. After removal of the catalyst via filtration through Celite, the solvent was removed in vacuo to give 25 mg (quantitative) of amine as a yellow oil. To a solution of the amine (18 mg, 0.07 mmol) in methanol (500 $\mu$L) was added acetic anhydride (17 $\mu$L, 0.18 mmol). The reaction mixture was stirred at room temperature for 16 h, the solvents were removed under reduced pressure, and the residue was purified via flash column chromatography on silica gel (AcOEt/MeOH 8:2) to give the desired compound **20** as a yellow oil (17 mg, 85%): $[\alpha]_D$ −148.7 (*c* 1.0, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 6.20 (1H, s), 4.39−4.43 (1H, t, $J$ = 8.1 Hz), 4.06−4.10 (1H, m), 3.73 (3H, s), 3.58−3.59 (1H, d, $J$ = 2.8 Hz), 3.37 (3H, s), 2.61−2.66 (1H, dd, $J$ = 14.7, 3.3 Hz), 2.40−2.44 (1H, dd, $J$ = 14.7, 1.7 Hz), 2.30−2.33 (1H, m), 2.12−2.17 (1H, s), 1.93 (3H, s), 1.87−1.93 (2H, m), 1.54 (3H, s); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 173.0, 171.0, 170.2, 74.1, 62.5, 59.5, 57.0, 55.5, 52.6, 35.6, 27.9, 27.3, 27.0, 23.9; MS (FAB+) *m/e* 299 $(M)^+$; HRMS (FAB+) calcd for $C_{14}H_{23}N_2O_5$ 299.16069, found 299.16108.

**(3*S*,6*S*,8*S*,9*S*)-6-Acetylamino-8-methoxy-6-methyl-5-oxo-octahydroindolizine-3-carboxylic Acid (6).** To a stirred solution of methyl ester **20** (17 mg, 0.057 mmol) in 500 $\mu$L of THF was added dropwise a 2 N aqueous solution of LiOH (60 $\mu$L, 0.114 mmol) and the biphasic system was stirred at room temperature for 24 h. The solvent was evaporated and the resulting mixture was diluted with water (5 mL) then washed with ethyl acetate (2 × 5 mL). The organic layers were discarded while the aqueous one was acidified to pH 2 with 2 N HCl. The solution was then concentrated to dryness and the residue was used in the next step without any further purification.

**L-BcaP-L-Ala-L-Ala-O*t*Bu (21).** To a chilled solution of $H_2$N-L-Ala-L-Ala-O*t*Bu hydrochloride (9 mg, 0.042 mmol) in 500 $\mu$L of DMF was added **6** (10 mg, 0.035 mmol), HOBt (7 mg, 0.053 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (10 mg, 0.053 mmol), and diisopropylethylamine (18 $\mu$L, 0.106 mmol). The reaction mixture was stirred for 1 h at 0 °C and 48 h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (2 mL). Water (2 mL) was then added and the phases were separated. The organic layer was washed with saturated $NaHCO_3$ aqueous solution (2 × 2 mL) and brine (1 × 2 mL), dried ($Na_2SO_4$), and evaporated to dryness. The resulting solid was purified via silica gel flash column chromatography (AcOEt to AcOEt/MeOH, 4:1). The desired compound **21** was obtained as a white solid (7 mg, 41%): $[\alpha]_D$ −102.5 (*c* 0.2, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.50−7.52 (1H, d, $J$ = 7.4 Hz), 7.46−7.48 (1H, d, $J$ = 8.9 Hz), 6.18 (1H, s), 4.48−4.53 (1H, quint, $J$ = 7.4 Hz), 4.36−4.47 (1H, quint, $J$ = 8.9 Hz), 4.31−4.35 (1H, quint, $J$ = 7.3 Hz), 3.99−4.04 (1H, m), 3.61 (1H, d, $J$ = 3.2 Hz), 3.38 (3H, s), 2.49−2.56 (1H, dt, $J$ = 12.6, 7.7 Hz), 2.33−2.37 (1H, dd, $J$ = 14.2, 2.1 Hz), 2.27−2.31 (1H, dd, $J$ = 14.2, 3.4 Hz), 2.10−2.16 (1H, m), 1.91 (3H, s), 1.80−1.95 (3H, m), 1.51 (3H, s), 1.44 (9H, s), 1.42 (3H, d, $J$ = 6.1 Hz), 1.35 (3H, d, $J$ = 7.2 Hz); $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 172.9, 172.0, 171.3, 171.0, 81.6, 73.9, 63.2, 61.9, 57.3, 54.9, 49.2, 48.9, 36.4, 28.7, 28.3, 27.3, 27.0, 23.5, 17.8, 17.4; MS (FAB+) *m/e* 483 $(M + H)^+$; HRMS (FAB+) calcd for $C_{23}H_{39}N_4O_7$ 483.28187, found 483.28038.

**L-BcaP-L-Ala-L-Ala-L-Ala-L-Ala-O*t*Bu (22).** To a chilled solution of $H_2$N-L-Ala-L-Ala-L-Ala-L-Ala-O*t*Bu hydrochloride (16 mg, 0.042 mmol) in 500 $\mu$L of DMF was added **6** (10 mg, 0.035 mmol), HOBt (7 mg, 0.053 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (10 mg, 0.053 mmol), and diisopropylethylamine (18 $\mu$L, 0.106 mmol). The reaction mixture was stirred for 1 h at 0 °C and 48 h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (2 mL). Water (2 mL) was then added and the phases were separated. The organic layer was washed with saturated $NaHCO_3$ aqueous solution (2 × 2 mL) and brine (1 × 2 mL), dried ($Na_2SO_4$), and

evaporated to dryness. The resulting solid was purified via silica gel flash column chromatography (AcOEt/MeOH, 4:1). The desired compound **22** was obtained as a yellow foam (7 mg, 32%): $[\alpha]_D$ −65.0 (*c* 0.7, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.66 (1H, d, $J$ = 7.1 Hz), 7.62 (1H, d, $J$ = 6.7 Hz), 7.25 (1H, s), 7.11 (1H, d, $J$ = 7.7 Hz), 7.03 (1H, d, $J$ = 7.2 Hz), 4.41−4.43 (1H, quint, $J$ = 7.7 Hz), 4.30−4.35 (2H, m), 4.27−4.30 (1H, t, $J$ = 8.2 Hz), 4.16−4.18 (1H, quint, $J$ = 7.2 Hz), 4.05−4.07 (1H, m), 3.63 (1H, br s), 3.37 (3H, s), 2.50−2.54 (1H, m), 2.28−2.36 (2H, m), 2.14−2.16 (1H, m), 1.96 (3H, s), 1.94−1.98 (1H, m), 1.82−1.84 (1H, m), 1.50 (3H, s), 1.44 (3H, d, $J$ = 7.4 Hz), 1.43 (9H, s), 1.42 (3H, d, $J$ = 8.0 Hz), 1.40 (3H, d, $J$ = 7.3 Hz), 1.38 (3H, d, $J$ = 7.2 Hz); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 174.0, 173.4, 172.8, 172.1, 171.8, 171.7, 170.8, 81.2, 73.1, 63.0, 61.8, 56.7, 54.2, 50.9, 49.7, 49.0, 48.8, 36.1, 28.4, 27.8, 26.7, 26.3, 22.8, 17.3, 17.1, 16.6, 16.1; MS (FAB+) *m*/*e* 647 (M + Na)$^+$, 625 (M + H)$^+$; HRMS (FAB+) calcd for $C_{29}H_{49}N_6O_9$ 625.35610, found 625.35623.

**Azide-L-BcaP-L-Ala-L-Ala-L-Ala-L-Ala-O*t*Bu (23).** To a chilled solution of $H_2$N-L-Ala-L-Ala-L-Ala-L-Ala-O*t*Bu hydrochloride (17 mg, 0.045 mmol) in 500 $\mu$L of DMF was added **18** (10 mg, 0.037 mmol), HOBt (8 mg, 0.056 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (11 mg, 0.056 mmol), and diisopropylethylamine (26 $\mu$L, 0.149 mmol). The reaction mixture was stirred for 1 h at 0 °C and 48 h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (2 mL). Water (2 mL) was then added and the phases were separated. The organic layer was washed with saturated $NaHCO_3$ aqueous solution (2 × 2 mL) and brine (1 × 2 mL), dried ($Na_2SO_4$), and evaporated to dryness. The resulting solid was purified via silica gel flash column chromatography (AcOEt/MeOH, 9:1). The desired compound **23** was obtained as a yellow foam (11 mg, 50%): $[\alpha]_D$ −155 (*c* 0.25, MeOH); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.46 (1H, d, $J$ = 6.6 Hz), 7.04 (1H, d, $J$ = 8.2 Hz), 7.00 (1H, d, $J$ = 7.3 Hz), 6.54 (1H, d, $J$ = 5.2 Hz), 4.54−4.50 (1H, quint, $J$ = 7.5 Hz), 4.39−4.31 (3H, m), 4.25−4.16 (1H, dq, $J$ = 8.1, 7.0 Hz), 4.00−3.93 (1H, m), 3.61−3.60 (1H, m), 3.37 (3H, s), 2.47−2.42 (2H, m), 2.22−2.07 (1H, m), 2.05−1.92 (2H, m), 1.87−1.83 (1H, dd, $J$ = 15.1, 2.1 Hz), 1.66 (3H, s), 1.49 (3H, d, $J$ = 7.0 Hz), 1.46 (9H, s), 1.44 (3H, d, $J$ = 7.5 Hz), 1.43 (3H, d, $J$ = 7.0 Hz), 1.39 (3H, d, $J$ = 7.5 Hz); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 173.1, 172.7, 172.4, 172.2, 172.1, 171.5, 81.8, 73.4, 63.9, 62.5, 60.6, 57.3, 51.3, 50.6, 49.3, 49.2, 37.7, 28.3, 28.1. 27.2, 24.7, 18.2, 17.7, 17.6, 17.2; MS (FAB+) *m*/*e* 609 (M + H)$^+$; HRMS (FAB+) calcd for $C_{27}H_{45}N_8O_8$ 609.33603, found 609.33549.

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**Supporting Information Available:** Selected experimental procedures, $^1$H, $^{13}$C NMR, NOESY, CD, and FT-IR spectra, and DMSO-$d_6$ and variable-temperature titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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