Pseudo-A(1,3) Strain as a Key Conformational Control Element in the Design of Poly-L-proline Type II Peptide Mimics

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Abstract: A strategy for the synthesis of peptide mimics of the poly-L-proline type II secondary structure from 4-substituted prolines is presented. Dimeric and trimeric oligomers composed of 4-substituted prolines are shown by NMR to preferentially populate the poly-L-proline type II secondary structure in both $CDCl_3$ and D_2O. Oligomers composed of 4-substituted prolines thus imitate the desired backbone conformation and are able to incorporate non-prolyl side chains on the proline backbone.

Introduction

Recent years have seen the emergence of a wealth of NMR and X-ray crystal structure data which demonstrates that kinases, SH3 domains, and MHC class II molecules as well as other proteins bind peptide ligands in the poly-L-proline type II (PPII) conformation or in extended conformations closely resembling this geometry.[1] In addition, a survey of protein X-ray crystal structures has shown that short spans of poly-L-proline type II helices occur regularly in globular proteins where they are predominantly located on the protein surface,[2] a position where their extended structure makes them ideal for interacting with binding clefts on other proteins. The PPII secondary structure thus emerges as a critical recognition element in mediating protein−protein interactions and therefore regulating cellular signaling, a role which makes the design of PPII mimics highly desirable as potential chemotherapeutic agents.

The poly-L-proline type II secondary structure is defined as an extended left-handed helix with 3.3 amino acid residues per turn and $\phi = -75°$, $\psi = 145°$, and $\omega = 180°$. While this conformation was first identified in polyproline, a peptide strand possessing no prolines but described by these ϕ, ψ, and ω angles is defined as a PPII helix. Furthermore, while many PPII helices contain proline amino acids which serve to stabilize the secondary structure, often it is the side chains of the non-proline amino acids in the PPII helix which are critical for receptor recognition of the PPII motif. Thus, the challenge in the development of mimics of the PPII secondary structure is the design of a framework which imitates the correct peptide backbone conformation and can incorporate non-prolyl side chains required for recognition of the PPII mimic by the receptor site. This paper describes work in our laboratory toward this goal by the design and synthesis of oligomers containing proline-templated amino acids.

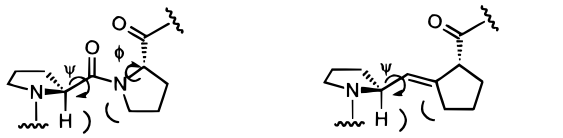

Figure 1. Minimization of pseudo-A(1,3) strain defines $\psi \sim 145°$.

We have recently reported that appropriately protected all-proline oligomers ($n = 2-5$) adopt the *all-trans* amide bond conformation in solution, as required for oligopeptides in the PPII secondary structure.[3] This work further implies that proline-containing oligomers are conformationally constrained given (1) the ϕ angle is defined as $\sim -75°$ by the constraints of the proline pyrrolidine ring, (2) the ψ angle ($\sim 145°$) is defined by minimization of pseudo-A(1,3) strain in which the amide bond represents a double-bond surrogate (Figure 1), and (3) the amide *s-trans* conformation ($\omega = 180°$) is favored by 2.3−5 kcal mol.[4] Due to the presence of these conformational control elements, in an all-proline oligomer, the PPII secondary structure is non-cooperative (i.e., a critical chain length is not required to conformationally bias the peptide); therefore, an oligomer unit as small as an appropriately protected proline dimer is conformationally constrained.[3]

Having established the stability of the amide *trans* conformation in proline-containing oligomers ($n = 2-5$), we now report the design and synthesis of novel oligopeptides which mimic the PPII secondary structure. The basic strategy involves the synthesis of peptides from substituted proline analogues (proline-templated amino acids, PTAAs, see Figure 2) which possess a non-prolyl side chain functionality (natural or unnatural) on the proline backbone. These PTAA-derived oligopeptides therefore furnish molecules which not only preferentially populate the PPII conformation in solution but also possess the amino acid side chain functionality which corresponds to that found on hydrophobic, basic, and acidic amino acids (for a representative example, see Figure 3). An additional advantage of this

(1) (a) For a recent review, see: Siligardi, G.; Drake, A. F. *Peptide Sci.* **1995**, *37*, 281. (b) For recent examples of proteins which bind PPII helices, see: Feng, S.; Chen, J. K.; Yu, H.; Simon, J. A.; Schreiber, S. L. *Science* **1994**, *266*, 1241. (c) Yu, H.; Chen, J. K.; Feng, S.; Dalgarno, D. C.; Brauer, A. W.; Schreiber, S. L. *Cell* **1994**, *76*, 933. (d) Gorina, S.; Pavletich, N. P. *Science* **1996**, *274*, 1001. (e) Raj, P. A.; Marcus, E.; Edgerton, M. *Biochemistry* **1996**, *35*, 4314. (f) Lee, C.-H.; Saksela, K.; Mirza, U. A.; Chait, B. T.; Kuriyan, J. *Cell* **1996**, *85*, 931. (g) Feng, S.; Kasahara, C.; Rickles, R. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12408. (h) Jardetzky, T. S.; Brown, J. H.; Gorga, J. C.; Stern, L. J.; Urban, R. G.; Strominger, J. L.; Wiley, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 734. (i) Zeile, W. L.; Purich, D. L.; Southwick, F. S. *J. Cell Biol.* **1996**, *133*, 49.

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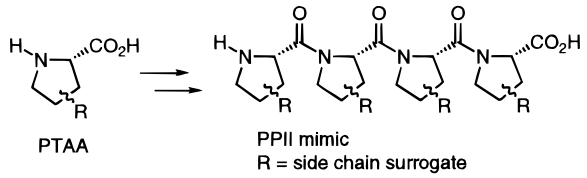


Figure 2. PTAA and oligoPTAA as PPII mimic.

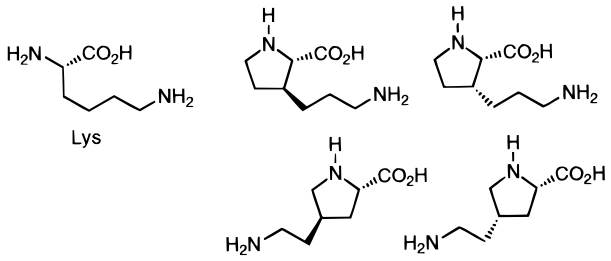


Figure 3. Representative amino acid and corresponding PTAA analogues.

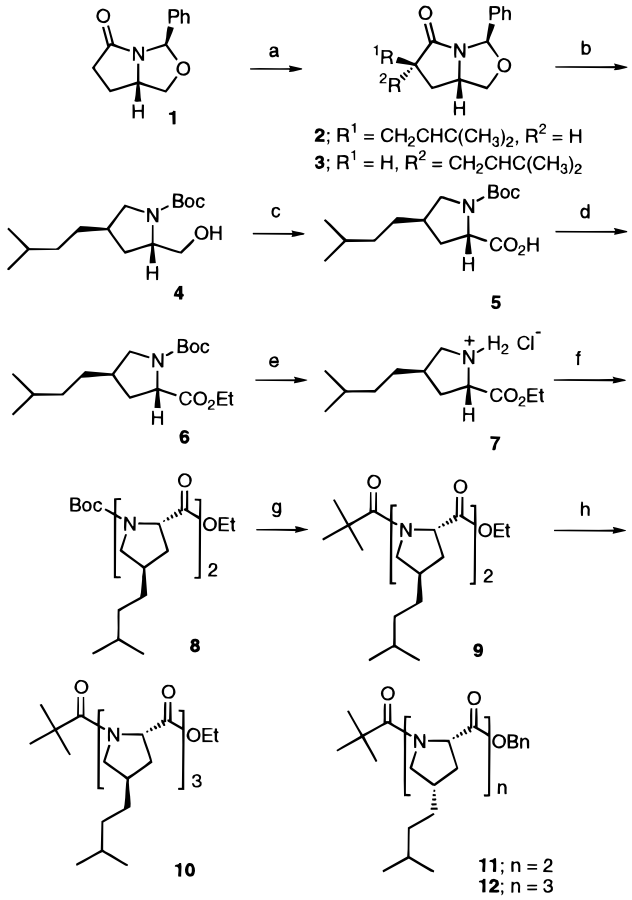
approach is that the introduction of the pyrrolidine ring creates a new stereocenter which allows definition of the side chain orientation. Therefore, oligopeptides composed of PTAAs should possess not only well-defined ϕ, ψ, and ω angles but also defined side chain angles (χ_1 and χ_2) useful in mapping the side chain disposition required for occupancy of receptor sites. While some substituted prolines (PTAAs) have been synthesized and incorporated into polypeptides, the idea that conformational control of the peptide backbone can be accomplished through coupling these modified prolines together has thus far not been explored.[5]

The focus of these initial studies involves an evaluation of the conformational properties of oligopeptides composed of PTAAs. In particular, we sought to establish that the fidelity of the PPII conformation is maintained in oligomers possessing alternate side chain orientations and that this fidelity is also maintained in various solvents, including water, in which these agents would serve as bioactive agents. To this end, we have synthesized and studied a series of oligomers (n = 2, 3) composed from 2,4-*trans*- and 2,4-*cis*-3-methylbutyl-L-proline as well as 2,4-*trans*-carboxymethyl-L-proline. Although these proline substitutions do not exactly correspond to any of the side chains of the natural amino acids, they serve to evaluate the effect of a proline substituent on the conformational properties of these peptides. Furthermore, the PPII mimics composed of 2,4-*trans*-carboxymethylproline are designed to provide water-soluble peptides for evaluation in aqueous medium. In addition, although conformational evaluation of a dimer will provide evidence of the PPII forming potential of the PTAAs, a trimer was chosen as a key unit to study since it represents one turn of a PPII helix. Finally, the N-terminus was protected as the pivalamide (or was deblocked) to avoid any of the spectral complications which would arise from the use of other protecting groups capable of existing as the *cis*/*trans* N-terminal amide bond rotamers.[3,6]

Results and Discussion

PPII Mimic Synthesis. The synthesis of the hydrophobic PTAAs was achieved by a modified procedure for the synthesis of 4-cyclohexyl-L-proline (Scheme 1).[7] Functionalization of the *O*,*N*-acetal **1** was accomplished through enolization (LDA) and alkylation with 4-bromo-2-methyl-2-butene to give the diastereomers **2** and **3** in 57% and 21% yields, respectively. Reduction of the 2,4-*trans*-substituted acetal **2** with LAH gave the prolinol in 88% yield. Hydrogenolysis of the benzyl group and reduction of the double bond (H_2, Pd−C) was achieved simultaneously to afford the deprotected amine which was reprotected with Boc_2O to give the alcohol **4**. Jones oxidation of the alcohol then afforded the PTAA **5** in 63% yield. The acid was converted to the ethyl ester **6** with DIC/DMAP in 94% yield. Boc deprotection (1 M HCl/HOAc) followed by coupling with the PTAA **5** furnished the Boc dimer **8**. To avoid diketopiperazine formation which is especially troublesome with proline dimers, the oligomers were synthesized in an N-terminal to C-terminal sense. Thus, the N-terminus was Boc-deprotected (1 M HCl/HOAc) and reprotected as the pivalamide with trimethylacetyl chloride/Et_3N to give the dimer **9**, which exhibited a single conformation by ^1H NMR.[8] Finally, hydrolysis of the ester (LiOH, dioxane/H_2O) and coupling with the PTAA salt **7** (DIC, HOBt, DMAP, Et_3N) afforded the trimer

Scheme 1[a]



[a] (a) LDA, THF, −78 °C; prenyl bromide; (b) (i) LAH; (ii) H_2, Pd−C; (iii) Boc_2O; (c) Jones oxidation; (d) DIC, DMAP, EtOH; (e) HCl/HOAc; (f) **5**, Et_3N, DIC, HOBt, DMAP, CH_2Cl_2; (g) (i) HCl/HOAc; (ii) pivaloyl choride, Et_3N; (h) (i) LiOH, dioxane/H_2O; (ii) **7**, Et_3N, DIC, HOBt, DMAP, CH_2Cl_2.

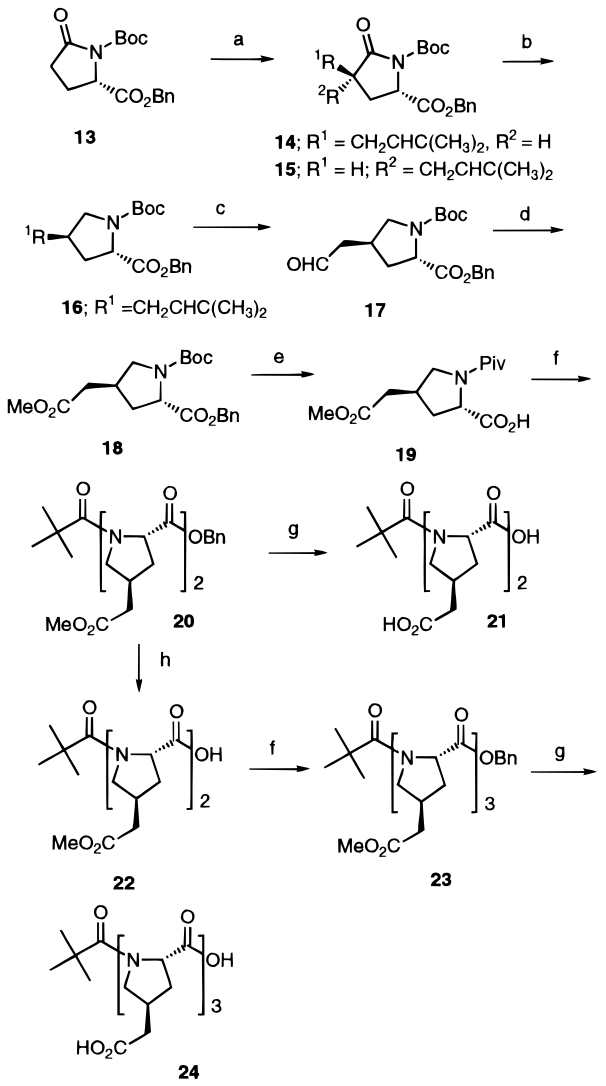
(5) (a) Mosberg, H. I.; Lomize, A. L.; Wang, C.; Kroona, H.; Heyl, D. L.; Sobczyc-Kojiro, K.; Ma, W.; Mousigian, C.; Porreca, F. *J. Med. Chem.* **1994**, *37*, 4371−83. (b) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnyak, G.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Neubeck, R.; Atwal, K. S.; Petrillo, E. W. *J. Med. Chem.* **1988**, *31*, 1148−60.

(6) Liang, G.-B.; Rito, C. J.; Gellman, S. H. *Biopolymers* **1992**, *32*, 293.

(7) Thottahil, J. K.; Moniot, J. L.; Mueller, R. H.; Wong, M. K. Y.; Kissick, T. P. *J. Org. Chem.* **1986**, *51*, 3140.

(8) Whereas reaction of $Cl^-H_2^+$-PTAA-PTAA-OEt with PivCl/Et_3N afforded Piv-PTAA-PTAA-OEt, reaction of $Cl^-H_2^+$-PTAA-PTAA-OEt with Boc-PTAA-OH/DIC/HOBt/Et_3N was slow relative to diketopiperazine formation. Some PTAA-trimer was formed via reaction with Boc-PTAA-F/Et_3N; however, this transformation remains unoptimized.

Scheme 2[a]



[a] (a) LDA, THF, −78 °C; prenyl bromide; (b) (i) Et_3BHLi, THF, −78 °C; (ii) BF_3OEt_2, Et_3SiH; (c) O_3, Ph_3P; (d) (i) PDC, DMF; (ii) MeI, $NaHCO_3$, DMF; (e) (i) TFA; (ii) pivaloyl chloride, Et_3N; (iii) H_2, Pd−C; (f) (i) first, **18**, TFA; (ii) then, RCO_2H, Et_3N, DIC, HOBt, DMAP, CH_2Cl_2; (g) LiOH; (h) H_2, Pd−C.

10. The 2,4-*cis*-substituted analogues **11** and **12** were synthesized from **3** in an analogous fashion with the exception that the PTAA was protected as the benzyl ester and deprotection of this ester was carried out through hydrogenolysis.

The synthesis of the water-soluble PPII mimics was accomplished through elaboration of another synthon derived from pyroglutamic acid (Scheme 2).[9] Enolization (LHMDS) and alkylation of the protected pyroglutamate[10] **13** with 4-bromo-2-methyl-2-butene afforded a 5:1 mixture of diastereomers **14** (63% yield) and **15** (Scheme 2). Lactam **14** was reduced to the pyrrolidine by the two-step sequence involving superhydride reduction of the lactam to the hemiaminal and subsequent reduction of the hemiaminal with Et_3SiH/BF_3OEt_2 in 82% yield for two steps as described by Ruano.[11] Ozonolysis of the alkene **16** afforded the aldehyde **17** in 90% yield. Subsequent oxidation of the aldehyde to the acid with PDC in DMF and methylation of the resultant acid with $MeI/NaHCO_3$ in DMF gave the diester **18** in 82% yield. Again, to avoid diketopiperazine formation, the synthesis of these oligomers was performed in an N-terminal to C-terminal sense. Therefore, **18** was Boc-deprotected and reprotected as the pivalamide with $PivCl/Et_3N$. Hydrogenolysis of the benzyl ester allowed selective α-carboxy deprotection to give the PTAA **19**. The fully protected dimer was obtained by Boc deprotection of **18** (HCl/HOAc) and DIC coupling with **19**, which furnished the dimer−triester **20** in 84% yield. Finally, hydrolysis of **20** with LiOH gave the dimer triacid **21** in 91% yield. The tetracid **24** was obtained in a similar manner from **20** through the hydrogenolysis, coupling, and hydrolysis sequence used to make **21**.

Conformational Studies. While circular dichroism is most commonly used to assign the PPII secondary structure, this method can be less reliable for short peptides. We instead decided to use the nuclear Overhauser effect to investigate the conformational properties of these PPII mimics. It was expected that, in the PPII conformation, a positive NOE would be evident between αH(*i*) and δH(*i*+1) since the minimization of pseudo-A(1,3) strain would place αH(*i*) in close proximity to δH(*i*+1). Furthermore, this NOE would be evident in the amide *trans* conformation ($\omega \sim 180°$) as required for the PPII secondary structure (Figure 4). Of note, all peptides utilized in this study exhibited the predominance of a single conformation by ^1H and ^{13}C NMR spectroscopy. Thus, these oligomers do not exist as a random mixture of *cis*/*trans* proline amide bond rotamers, but exist in a *single conformation* (within our detection limits). Figure 4 summarizes the results of our NOE experiments with the dimeric and trimeric PPII mimics. The NOESY spectrum of the 2,4-*trans*-substituted dimer **9** in $CDCl_3$ exhibits the desired NOEs between the α-hydrogen of the N-terminal PTAA and the δ-hydrogens of the C-terminal PTAA, in agreement with results expected for PTAA oligomers in the PPII conformation. In addition, the trimer **10** also exhibited analogous NOEs between αH(*i*) and δH(*i*+1) and between αH(*i*+1) and δH(*i*+2). Although these NOEs are not considered diagnostic for the PPII conformation, a qualitative statement can be made regarding the conformational properties of these peptides from a comparison of the intensity of these NOEs with additional inter-residue NOEs.

For these molecules for which rotation about ψ is possible, the NOE intensity between δH(*i*+1) and *x*H(*i*) (where *x*H(*i*) denotes additional (*i*)-residue protons) will be a function of the average distance between these protons as influenced by the population of the ψ-rotamers. Since different ψ-rotamers will exhibit δH(*i*+1)−*x*H(*i*) distances equal or greater than the δH(*i*+1)−αH(*i*) distance at $\psi \sim 145°$, a comparison of NOE intensities between δH(*i*+1) and *x*H(*i*) can provide a qualitative measure of the most populated ψ-rotamer. This is best observed by examination of the NOESY rows at the shift corresponding to the δ-protons. Figure 5 shows the NOESY rows of the trimer **10** at the shifts corresponding to δtH(*i*), δtH(*i*+1), δtH(*i*+2), δcH(*i*)/δcH(*i*+2), and δcH(*i*+1) (*cis*/*trans* (c/t) is defined relative to αH). The row corresponding to the shift of δtH(*i*) shows a strong geminal NOE with δcH(*i*) and a medium vicinal NOE with γtH(*i*) and, not surprisingly, no measurable NOEs with *x*H(*i*+1). The analogous intra-residue NOEs can also be observed in the row corresponding to the shift of δtH(*i*+1). However, δtH(*i*+1) row also exhibits three inter-residue NOEs: a medium NOE with αH(*i*) and two weak NOEs with βtH(*i*) and βcH(*i*). The row corresponding to the shift of δcH(*i*+1) also shows a medium NOE with αH(*i*) and no additional

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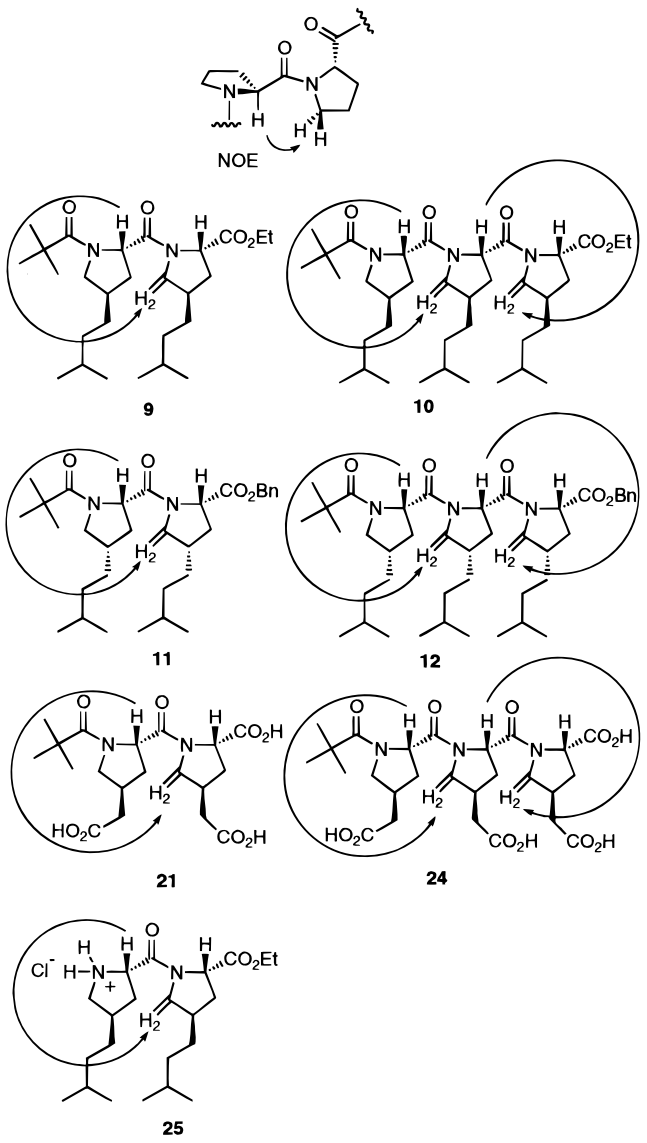


Figure 4. αH(*i*)−δH(*i*+1) and αH(*i*+1)−δH(*i*+2) NOEs for dimeric and trimeric PPII mimics. NOESY spectra for **9**−**12** were obtained in $CDCl_3$ and for **21**, **24**, and **25** in D_2O.

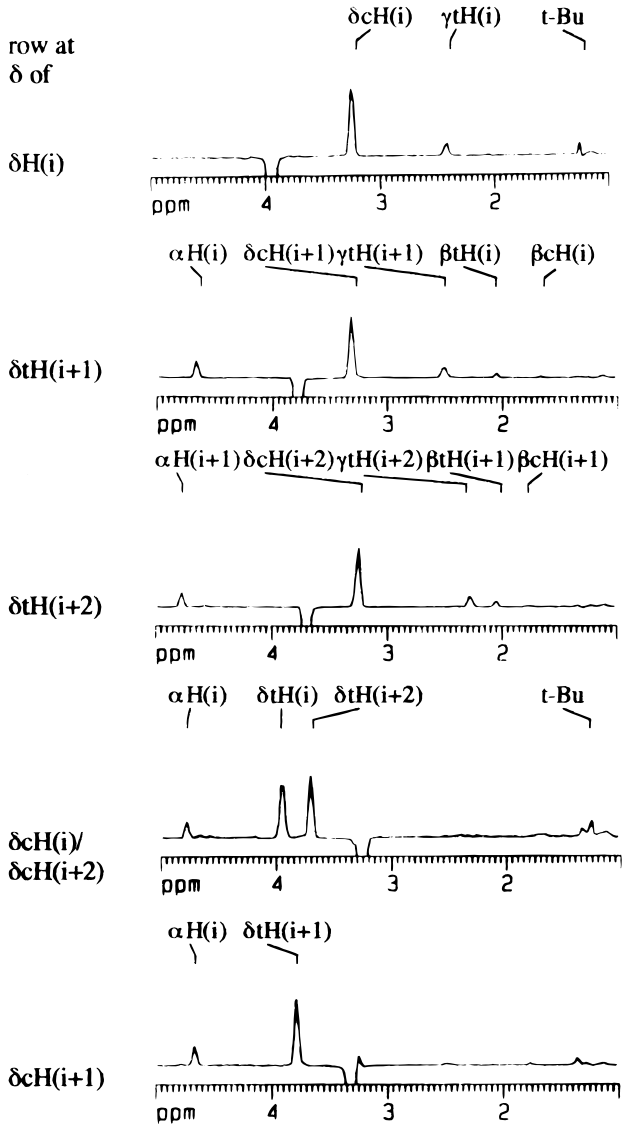
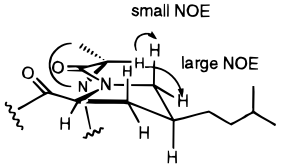


Figure 5. NOESY rows of **10** at the δ corresponding to δtH(*i*), δtH(*i*+1), δtH(*i*+2), δcH(*i*)/δcH(*i*+2), and δcH(*i*+1). *Cis* and *trans* (c/t) protons are defined relative to αH.

inter-residue NOEs of equal magnitude. From this data it is apparent that the strongest δH(*i*+1)−*x*H(*i*) NOEs are between δH(*i*+1) and αH(*i*), consistent with a significantly populated conformation in which δH(*i*+1) and αH(*i*) are in close proximity (i.e., when $\psi \sim 145°$) as expected for the minimization of pseudo-A(1,3) strain. Analysis of the rows corresponding to the shifts of δtH(*i*+2) and δcH(*i*+2) also reveals that the strongest inter-residue NOEs involve δH(*i*+2)−αH(*i*+1), again consistent with a populated conformation in which $\psi \sim 145°$. The cumulative NOE data presented for the trimer **10** demonstrates that this PPII mimic, composed of 2,4-*trans*-substituted PTAAs, is conformationally constrained by the minimization of pseudo-A(1,3) strain and preferentially populates the PPII conformation. Analogous results are also observed for the dimer **9**, e.g. the NOESY spectrum of **9** also exhibits the strongest inter-residue NOEs between δH(*i*+1) and αH(*i*) (data not shown). These data also illustrate that the minimization of pseudo-A(1,3) strain can conformationally bias a unit as small as a dimer.

Analysis of the NOESY spectra of the additional PPII mimics synthesized for this study reveals similar trends. For example, the NOESY spectra of the 2,4-*cis*-substituted dimer **11** and trimer **12** also exhibit the desired NOE intensity profile in $CDCl_3$.[12] Thus, peptides composed of 2,4-*cis*-substituted PTAAs also preferentially populate the PPII conformation in solution. Since peptides can exhibit different conformational behavior in different solvents, it is necessary to determine if

(12) For the PPII mimics **11** and **12**, the NOEs between the N-terminal α-Hs and the *cis* and *trans* δ-Hs (*cis* and *trans* are assigned relative to the proline carbonyl) are of unequal intensity with the NOE between the *trans* δ-H and the α-H being more pronounced. Furthermore, the NOE between the *tert*-butyl group and the N-terminal *trans* δ-H is also greater than between the *tert*-butyl group and the N-terminal *cis* δ-H. Although pyrrolidine ring conformations are difficult to assign due to the small energy differences between conformers and the low energy of activation for five-member ring conformational interconversion, these observed NOEs can be tentatively assigned to a populated C*γ exo* pyrrolidine ring conformation. The C*γ exo* pucker thus places the *trans* δ-H into closer spacial relationship to the α-H of an N-terminally positioned PTAA (or to the *tert*-butyl group in the N-terminal residue).



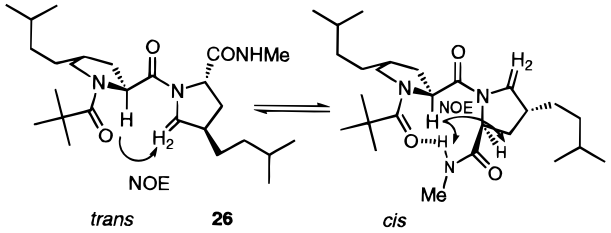


Figure 6. NOEs of *cis* and *trans* amide bond rotamers of dimer **26**.

the PTAA approach will provide agents which preferentially populate the PPII conformation in aqueous medium. To this end, 2D NOE studies of the PPII mimics **21** and **24** possessing hydrophilic side chains were conducted in D_2O. The NOESY spectra of **21** and **24** in D_2O also exhibited the diagnostic NOE intensity profile, thus confirming the PPII conformation for these peptides in water. Finally, to ascertain the resistance of PTAA oligomers possessing hydrocarbon chains to hydrophobic collapse in aqueous medium, the HCl salt **25** was also investigated in D_2O (the trimer was not studied due to difficulties with solubility in D_2O). This PPII dimer also showed the desired NOEs indicative of the PPII conformation. Thus, the conformational control elements which stabilize an all-proline helix still function in aqueous medium with a PTAA dimer which possess hydrophobic side chains. For all peptides used in this study, correlation between the α-H and *δ*-H resonances of individual residues was determined through COSY or TOCSY spectroscopy. Sequence assignments were made by first identifying *δ*H(*i*) from the NOE with the pivaloyl *tert*-butyl group and then performing the (*i*), (*i*+1), and (*i*+2) sequence assignments through the αH(*i*)−*δ*H(*i*+1) NOEs.

To establish the validity of the positive NOEs in assigning the amide *trans* conformation, we sought to study a system which possessed a *cis* amide bond rotamer.[13] Previously Naider[13] and co-workers had suggested that the Pro−Pro amide bond in the trimer Boc-Pro-Pro-Gly-OMe existed as a mixture of *cis*−*trans* rotamers and that the *cis* conformation was stabilized by hydrogen bonding of the Gly amide to the Boc carbonyl oxygen. Analogously, it was found that the dimer **26** (Figure 6) exists as a ∼1:1 mixture of conformations in $CDCl_3$. Subsequent 2D NOE experiments indicated that one rotamer exhibits an NOE between αH(*i*) and *δ*H(*i*+1), while the other rotamer (*cis*) does not, thus establishing that the αH(*i*) and *δ*H(*i*+1) NOEs are valid in assigning the *trans* conformation. Furthermore, the *cis* conformer exhibited an NOE between αH(*i*) and αH(*i*+1) as expected. In addition, an NOE was also evident between the N-terminal α-hydrogen and the hydrogen-bonded NH (Figure 6), further confirming the conformational assignment. It should be noted that stabilization of the *cis* conformation by intramolecular hydrogen bonding is more predominant in $CDCl_3$ than in more polar solvents. In a hydrogen-bonding solvent such as CD_3OD for example, the population of the *cis* conformation diminishes to afford an 85:15 *trans*/*cis* mixture of rotamers. It is thus expected that, in aqueous medium, a natural amino acid incorporated on the C-terminal end of a PPII mimic will not significantly contribute to this intramolecular hydrogen bonding.

The PTAA approach has thus been successfully used to synthesize peptide mimics of the PPII secondary structure which can incorporate nonprolyl side chain functionality. The feasibility of this approach is highlighted by the fact that a unit as small as a dimer is conformationally constrained. Furthermore, the potential application of this approach to the design of bioactive agents is especially attractive since these agents preferentially populate the PPII conformation in aqueous medium. Further work is now ongoing to expand the number of PTAAs which serve to form PPII mimics. Additional work is ongoing to synthesize PPII mimics which target specific signaling pathways in which the PPII secondary structure has been implicated.

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Experimental Section

General Procedure. Unless otherwise specified, all reagents were obtained from commercial sources and were used without further purification. THF was distilled from sodium benzophenone ketyl, and CH_2Cl_2 was distilled from CaH_2. All reactions were carried out under an N_2 atmosphere unless otherwise specified. 1D and 2D spectra were collected on a Bruker ARX 500-MHz NMR spectrometer. 2D NMR spectra were collected using standard pulse sequences offered by Bruker. All TOCSY spectra were obtained using a 60-ms mlevtp spin lock and 2-s repetition delay except the TOCSY spectrum of **11** which was obtained with a 100-ms mlevtp spin lock. NOESY spectra were obtained using a 2-s repetition delay and a 400-ms mixing time. All 2D spectra were obtained in $CDCl_3$ except the 2D spectra of **21**, **24**, and **25**, which were collected in D_2O.

Alkylation of *O,N*-Acetal 1. A solution of **1** (5.98 g, 29.4 mmol) in THF (75 mL) was cooled to −78 °C, and LDA (14.7 mL, 29.4 mmol) was added dropwise as a 2.0 M solution in heptane/THF/benzene. After 30 min, 2-methyl-4-bromobutene (3.7 mL, 32 mmol) was added dropwise. The reaction was maintained at −78 °C for 15 min and then quenched by the addition of water (15 mL). The resulting suspension was allowed to warm to room temperature (rt) and diluted with EtOAc (50 mL). The layers were separated, and the organic layer was washed with water (20 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated. Flash chromatography (25% EtOAc/hexanes) afforded 4.56 g (57%) of **2** and 1.64 g (21%) of **3** as colorless oils. **2**: ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.43−7.41 (m, 2H), 7.37−7.27 (m, 3H), 6.30 (s, 1H), 5.14 (m, 1H), 4.17 (dd, app t, J = 6.9 Hz, 2H), 4.01 (m, 1H), 3.37 (dd, app t, J = 8.4 Hz, 1H), 2.70 (m, 1H), 2.46 (m, 1H), 2.34 (m, 1H), 2.03 (dd, app t, J = 6.6 Hz, 1H), 1.69 (s, 3H), 1.62 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 180.8, 139.6, 135.1, 128.9, 128.8, 126.4, 120.8, 87.7, 71.9, 57.9, 45.8, 31.0, 28.2, 26.4, 18.4 ppm; IR (film) 1705, 1377, 1350, 1253, 1221, 1027 cm^{-1}; MS (CI) *m*/*z* 272 (MH), 203, 165, 97, 69; $[\alpha]^{25}_D$ = +196.2° (c = 0.13, EtOAc). Anal. Calcd for $C_{17}H_{21}NO_2$: C, 75.25; H, 7.80; N, 5.16. Found: C, 75.07; H, 7.77; N, 5.08. **3**: ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.51−7.29 (m, 5H), 6.33 (s, 1H), 5.10 (m, 1H), 4.23 (dd, app t, J = 6.4 Hz, 1H), 4.06 (m, 1H), 3.48 (dd, app t, J = 7.8 Hz, 1H), 2.92 (m, 1H), 2.58−2.48 (m, 2H), 2.17 (m, 1H), 1.71 (s, 3H), 1.64 (s, 3H), 1.58 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 178.9, 139.5, 134.7, 129.1, 129.0, 126.6, 121.5, 87.4, 73.0, 57.3, 46.1, 32.0, 29.6, 26.4, 18.6 ppm; IR (film) 2915, 1700, 1576, 1260; MS (CI) *m*/*z* 272 (MH), 203, 194; $[\alpha]^{25}_D$ = +198.4° (c = 0.19, EtOAc). Anal. Calcd for $C_{17}H_{21}NO_2$: C, 75.25; H, 7.80; N, 5.16. Found: C, 75.06; H, 7.88; N, 5.10.

***trans*-4-(3-Methylbut-2-enyl)-*N*-benzyl-L-prolinol.** A suspension of LAH (0.69 g, 18 mmol) in THF (20 mL) was brought to a gentle reflux, and **2** (3.28 g, 12.1 mmol) was added dropwise as a solution in THF (10 mL). The suspension was maintained at reflux for 1 h and then cooled to 0 °C. Saturated aqueous Na_2SO_4 was added slowly until a white granular precipitate formed. The mixture was diluted with EtOAc (30 mL), filtered through Celite, and concentrated to afford 3.26 g of a colorless oil. Flash chromatography (5% MeOH/20% EtOAc hexanes) afforded 2.75 g (88%) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 7.33−7.23 (m, 5H), 5.05 (m, 1H), 3.93 (d, J = 13.0 Hz, 1H), 3.61 (dd, J = 10.7, 3.4 Hz, 1H), 3.97−3.35 (m, 2H), 3.05 (dd, J = 8.7, 6.2 Hz, 1H), 2.81 (m, 1H), 2.13−1.92 (m, 5H), 1.65 (s, 3H), 1.62−1.57 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) 139.9, 132.7, 129.3, 128.9, 127.7, 123.4, 64.8, 62.9, 61.3, 59.4, 38.6, 35.3, 33.0, 26.3, 18.4 ppm; IR (film) 3400, 2924, 1653, 1454, 1375, 1139 cm^{-1}; MS (CI) *m*/*z* 260 (MH), 228, 91; $[\alpha]^{25}_D$ = −90.0° (c = 0.15, EtOAc). Anal. Calcd for $C_{17}H_{25}NO$: C, 78.72; H, 9.71; N, 5.40. Found: C, 78.83; H, 9.82; N, 5.48.

***trans*-4-(3-Methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-prolinol (4).** The *N*-benzylprolinol (2.28 g, 8.79 mmol) was dissolved in 2:1 acetic acid/EtOAc (45 mL), and 10% Pd−C (0.47 g) was added. The mixture was shaken under H_2 (60 PSI) for 3 h and then filtered through Celite. The filtrate was concentrated and made basic to pH 13 with aqueous 30% KOH. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated to afford 1.40 g of a pale yellow oil which was used without further purification: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.51 (dd, J = 10.2, 3.8 Hz, 1H), 3.37−3.29 (m, 2H), 3.07 (dd, J = 14.8, 7.4 Hz, 1H), 2.54 (dd, J = 10.1, 7.9 Hz, 1H), 2.02 (m, 1H), 1.65 (m, 1H), 1.52−1.41 (m, 2H), 1.33−1.28 (m, 2H), 1.19−1.16 (m, 2H), 8.6 (d, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 65.5, 59.6, 53.2, 39.9, 38.3, 34.8, 32.6, 28.7, 23.2, 23.1 ppm; IR (film) 3273, 2953, 1640, 1366, 1048 cm^{-1}; MS (CI) *m*/*z* 172 (MH), 154, 140. The deprotected amine (1.11 g, 6.48 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. Di-*tert*-butyl pyrocarbonate (1.40 g, 6.46 mmol) in CH_2Cl_2 (10 mL) was added dropwise, and the resulting solution was stirred at 0 °C for 20 min. The solution was concentrated under reduced pressure, and the resulting oil was subjected to flash chromatography (5% MeOH/20% EtOAc/hexanes) to afford 1.38 g of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.31 (sb, 1H), 4.04 (sb, 1H), 3.60 (m, 2H), 3.48 (m, 1H), 2.96 (t, J = 8.5 Hz, 1H), 2.10 (m, 1H), 1.67 (m, 1H), 1.58−1.46 (m, 11H), 1.34−1.29 (m, 2H), 1.21−1.13 (m, 2H), 0.86 (d, J = 6.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) 157.9, 80.9, 68.7, 60.3, 53.7, 38.1, 38.0, 35.3, 31.8, 29.2, 28.8, 23.3 ppm; IR (film) 3422, 1699, 1674, 1398, 1169 cm^{-1}; MS (CI) *m*/*z* 272 (MH), 240, 216, 184; $[\alpha]^{25}_D$ = −23.7° (c = 0.13, EtOAc). Anal. Calcd for $C_{15}H_{29}NO_3$: C, 66.38; H, 10.77; N, 5.16. Found: C, 66.13; H, 10.71; N, 5.07.

***trans*-4-(3-Methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-proline (5).** The alcohol **4** (1.15 g, 4.24 mmol) in acetone (3 mL) was added dropwise over 3 h to a solution of Jones reagent (3.1 mL, prepared by dissolving 27 g of CrO_3, in 23 mL of H_2SO_4, and diluting to 100 mL with H_2O) in acetone (3 mL) at 0 °C. After the addition was complete, the mixture was maintained at 0 °C for 1 h. Isopropyl alcohol (0.5 mL) was added, and after 30 min, the solvent was decanted and concentrated to afford a green oil. Purification by flash chromatography (10% MeOH/20% EtOAc in hexanes) afforded 0.766 g of product as a colorless oil (63%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.35 (d, J = 8.5 Hz, 0.5H), 4.24 (d, J = 8.2 Hz, 0.5H), 3.71 (dd, app t, J = 9.1 Hz, 0.5H), 3.57 (dd, app t, J = 8.4 Hz, 0.5H), 2.95 (dd, app t, J = 9.3 Hz, 0.5H), 2.87 (dd, app t, J = 9.8 Hz, 0.5H), 2.30−2.13 (m, 2H), 1.84 (m, 0.5H), 1.68 (m, 0.5H), 1.53−1.10 (m, 15H), 0.84 (d, J = 6.3 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 179.3, 176.6, 156.5, 154.5, 81.6, 80.9, 59.8, 59.6, 53.0, 52.4, 38.4, 37.9, 37.4, 37.3, 35.6, 31.4, 29.0, 28.9, 28.7, 23.0 ppm; IR (film) 3212, 2956, 1701, 1653 cm^{-1}; MS (CI) *m*/*z* 286 (MH), 230, 214, 186, 184, 140; $[\alpha]^{25}_D$ = −25.0° (c = 0.1, EtOAc). Anal. Calcd for $C_{21}H_{40}NO_4$ (cyclohexylamine salt; mp 166−171 °C): C, 65.56; H, 10.48; N, 7.28. Found: C, 65.36; H, 10.51; N, 7.28.

***trans*-4-(3-Methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-proline Ethyl Ester (6).** The acid **5** (196 mg, 0.687 mmol) and DMAP (42 mg, 0.34 mmol) were dissolved in anhydrous ethanol (2 mL), and diisopropylcarbodiimide (DIC) (0.13 mL, 0.82 mmol) was added dropwise. The mixture was maintained at rt overnight and was then concentrated and purified by flash chromatography (15% EtOAc/hexanes) to afford 185 mg (94%) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.31 (d, J = 8.5 Hz, 0.4H), 4.21 (dd, J = 9.0, 1.8 Hz, 0.6H), 4.18−4.09 (m, 2H), 3.71 (dd, J = 10.2, 7.8 Hz, 0.6H), 3.64 (dd, J = 9.7, 8.2 Hz, 0.4H), 2.95 (dd, app t, J = 9.1 Hz, 0.6H), 2.89 (dd, app t, J = 9.5 Hz, 0.4H), 2.19 (m, 1H), 2.04 (m, 1H), 1.76 (m, 1H), 1.56−1.21 (m, 17H), 0.85−0.83 (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 173.9, 173.6, 155.0, 154.5, 80.4, 61.51, 61.46, 59.9, 59.6, 52.8, 52.5, 38.3, 38.0, 37.43, 37.40, 36.6, 31.6, 31.5, 29.1, 29.0, 28.8, 28.7, 23.2, 23.1, 15.0, 14.9; IR (film) 1749, 1705, 1395, 1188 cm^{-1}; MS (CI) *m*/*z* 314 (MH), 258, 240, 214, 184, 140; $[\alpha]^{25}_D$ = −23.7° (c = 0.12, $CHCl_3$). Anal. Calcd for $C_{17}H_{31}NO_4$: C, 65.14; H, 9.97; N, 4.47. Found: C, 65.22; H, 10.05; N, 4.47.

Synthesis of *trans*-4-(3-Methylbutyl)-Boc-dimer 8. A solution of **6** (0.327 g, 1.05 mmol) in 1 M HCl/HOAc (4 mL) was maintained at rt for 1 h. The solution was concentrated. Toluene was added and removed under reduced pressure (2 × 15 mL) to remove residual HOAc and H_2O. This oil was dissolved in CH_2Cl_2 (3 mL) and added to a separate flask containing **5** (0.296 g, 1.04 mmol), DIC (0.33 mL, 2.1 mmol), 1-hydroxybenzotriazole (HOBt) (0.16 g, 1.0 mmol), DMAP (0.128 g, 1.05 mmol), and Et_3N (0.43 mL, 3.1 mmol) in CH_2Cl_2 (3 mL). The mixture was maintained at rt overnight and concentrated. Purification by flash chromatography (30% EtOAc/hexanes afforded 0.437 g (86%) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.61 (d, J = 8.8 Hz, 0.6H), 4.58 (d, J = 8.7 Hz, 0.4H), 4.49 (d, J = 8.7 Hz, 0.6H), 4.39 (d, J = 8.0 Hz, 0.4H), 4.19−4.11 (m, 2H), 3.82−3.70 (m, 2H), 3.29 (dd, app t, J = 9.4 Hz, 0.6H), 3.15 (dd, app t, J = 8.9 Hz, 0.4H), 2.97 (dd, app t, J = 9.3 Hz, 0.4H), 2.92 (dd, app t, J = 9.6 Hz, 0.6H), 2.44−2.30 (m, 2H), 2.13−2.05 (m, 2H), 1.82−1.74 (m, 2H), 1.55−1.13 (m, 22H), 0.92−0.85 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) 172.0, 171.8, 171.0, 170.7, 154.3, 153.5, 79.1, 79.0, 60.8, 60.7, 58.6, 58.5, 57.6, 52.3, 52.0, 51.9, 51.7, 38.24, 38.19, 37.2, 37.1, 37.0, 36.2, 35.7, 35.1, 34.6, 34.5, 31.1, 31.0, 30.7, 30.5, 30.3, 28.3, 28.2, 27.90, 27.88, 27.8, 22.30, 22.28, 22.2, 13.9 ppm; IR (film) 2955, 1743, 1700, 1668, 1397, 1184 cm^{-1}; MS (CI) *m*/*z* 481 (MH), 381, 240, 184, 140; $[\alpha]^{25}_D$ = −34.0° (c = 0.1, EtOAc).

Synthesis of *trans*-4-(3-Methylbutyl)-Piv-dimer 9. A solution of **8** (154 mg, 0.330 mmol) in 1 M HCl/HOAc (2 mL) was maintained at rt for 1 h. The solvent was removed under reduced pressure. Toluene was added and removed under reduced pressure (2 × 15 mL) to azeotropically remove residual HOAc and H_2O. The resultant oil was dissolved in CH_2Cl_2 (1 mL). To this solution was added pivaloyl chloride (0.81 mL, 0.66 mmol) followed by Et_3N (0.14 mL, 0.99 mmol). After 15 min, the mixture was diluted with 20% EtOAc/hexanes (20 mL) and washed with H_2O (2 mL), dried (Na_2SO_4), and concentrated. Flash chromatography (20% hexanes/EtOAc) afforded 133 mg (87%) of product as a thick colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.62 (dd, J = 8.9, 2.7 Hz, 1H), 4.57 (d, J = 8.9 Hz, 1H), 4.07 (m, 2H), 3.91 (dd, J = 9.4, 7.3 Hz, 1H), 3.68 (dd, app t, J = 8.7 Hz, 1H), 3.27 (dd, app t, J = 9.5 Hz, 1H), 3.21 (dd, app t, J = 9.3 Hz, 1H), 2.42 (m, 1H), 2.25 (m, 1H), 2.01−1.95 (m, 2H), 1.72 (ddd, J = 12.3, 9.3, 9.3 Hz, 1H), 1.64 (ddd, J = 12.2, 9.1, 9.1 Hz, 1H), 1.48−1.05 (m, 22H), 0.81−0.79 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) 177.2, 172.9, 171.6, 61.4, 60.2, 59.2, 54.6, 52.5, 39.4, 39.2, 38.9, 37.9, 37.8, 35.4, 33.5, 31.6, 31.3, 27.7, 27.9, 13.0, 13.7 ppm; IR (film) 2870, 1744, 1662, 1623 cm^{-1}; MS (CI) *m*/*z* 465 (MH), 379, 268, 252, 224, 140; $[\alpha]^{25}_D$ = −41.9° (c = 0.09, $CHCl_3$). Anal. Calcd for $C_{27}H_{48}N_2O_4$: C, 69.79; H, 10.41; N, 6.03. Found: C, 69.70; H, 10.50; N, 6.00.

Ester Hydrolysis of 9. A solution of the ester **9** (0.123 g, 0.272 mmol) and LiOH (42 mg, 1.0 mmol) in 2:1 dioxane/H_2O (3 mL) was maintained at rt for 6 h. The solution was cooled to 0 °C, made acidic to pH 2 with aqueous 10% HCl, and extracted with EtOAc (3 × 5 mL). The combined organic extracts were dried and concentrated to afford 0.141 g of a pale yellow oil. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded 78 mg (67%) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.68 (d, J = 8.8 Hz, 1H), 4.65 (dd, J = 9.0, 3.0 Hz, 1H), 3.98 (dd, J = 9.5, 7.2 Hz, 1H), 3.65 (dd, app t, J = 8.3 Hz, 1H), 3.35−3.28 (m, 2H), 2.51 (m, 1H), 2.41−2.34 (m, 2H), 1.96 (m, 1H), 1.74−1.66 (m, 2H), 1.56−1.14 (m, 19H), 0.88−0.85 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) 177.7, 174.5, 173.7, 60.5, 60.2, 54.7, 53.3, 39.6, 39.4, 39.2, 38.0, 37.9, 34.0, 33.7, 31.5, 31.0, 28.8, 28.7, 27.9, 27.8, 23.2, 23.1 ppm; IR (film) 3136, 2968, 1734, 1653, 1456 cm^{-1}; MS (CI) *m*/*z* 437 (MH), 252, 224; $[\alpha]^{25}_D$ = −72.3° (c = 0.4, CH_2Cl_2).

Synthesis of *trans*-4-(3-Methylbutyl)-Piv-trimer 10. Boc deprotection of **6** (73.1 mg, 234 mmol) was accomplished as described for the synthesis of **8**. The hydrochloride salt was dissolved in CH_2Cl_2 (2 mL) and added to a separate reaction flask containing DIC (0.40 mL, 0.26 mmol), Et_3N (0.65 mL, 0.47 mL), and the dimer−acid (0.102 g, 0.233 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was allowed to warm to rt and then maintained at rt overnight. The mixture was concentrated and purified by flash chromatography (25% EtOAc/hexanes) to afford 0.102 g (74%) of **10** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.74 (d, J = 8.7 Hz, 1H), 4.64 (d, J = 8.8 Hz, 1H), 4.54 (d, J = 8.9 Hz, 1H), 4.15−4.05 (m, 2H), 3.92 (dd, apparent t, J = 7.5 Hz, 1H), 3.75 (dd, app t, J = 8.3 Hz, 1H), 3.67 (dd, app t, J = 8.2 Hz, 1H), 3.29 (dd, app t, J = 9.0 Hz, 1H), 3.24−3.21 (m, 2H),

2.48 (m, 1H), 2.40 (m, 1H), 2.26 (m, 1H), 2.05−1.99 (m, 3H), 1.77−1.63 (m, 3H), 1.49−1.10 (m, 27H), 0.83−0.81 (m, 18H); ^{13}C NMR ($CDCl_3$, 125 MHz) 177.2, 172.8, 171.4, 171.1, 61.5, 60.4, 59.4, 58.4, 54.7, 53.1, 52.6, 39.4, 39.2, 38.9, 38.5, 38.0, 37.9, 37.8, 35.5, 34.8, 33.6, 31.7, 31.3, 28.74, 28.69, 28.0, 23.1, 23.0, 14.7 ppm; IR (film) 2955, 1744, 1653, 1625, 1185 cm^{-1}; MS (CI) *m*/*z* 632 (MH), 252, 143, 101, 87; $[\alpha]^{25}_D = -41.3°$ ($c = 0.16$, $CHCl_3$). Anal. Calcd for $C_{37}H_{65}N_3O_5$: C, 70.32; H, 10.37; N, 6.65. Found: C, 70.17; H, 10.38; N, 6.63.

Synthesis of *trans*-4-(3-Methylbutyl)-Piv-dimer 26. A solution of 2,4-*trans*-PTAA-PTAA-OH (51.9 mg, 0.122 mmol) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (28.5 mg, 0.145 mmol) in CH_2Cl_2 (1 mL) was maintained at 0 °C for 20 min, and methylamine (46 μL, 0.37 mmol) was added as an 8 M solution in EtOH and allowed to warm to rt. After 6 h, the suspension was diluted with EtOAc (10 mL) and washed with 10% aqueous HCl (2 × 1 mL) and saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded 18 mg (33%) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.21 (qb, $J = 4.5$ Hz, 0.5H), 7.02 (sb, 0.5H), 4.67 (dd, $J = 8.7$, 3.3 Hz, 0.5H), 4.63 (d, $J = 8.2$ Hz, 0.5H), 4.35 (dd, $J = 8.9$, 2.7 Hz, 0.5H), 4.27 (d, $J = 8.1$ Hz, 0.5H), 4.02 (dd, $J = 9.6$, 7.1 Hz, 0.5H), 3.98 (dd, $J = 9.8$, 7.1 Hz, 0.5H), 3.68 (dd, $J = 11.7$, 7.8 Hz, 0.5H), 3.61 (dd, app t, $J = 8.4$ Hz, 0.5H), 3.36−3.28 (m, 1H), 3.23 (dd, app t, $J = 9.0$ Hz, 0.5H), 3.08 (dd, app t, $J = 11.0$ Hz, 0.5H), 2.82 (d, $J = 4.7$ Hz, 1.5H), 2.74 (d, $J = 4.8$ Hz, 1.5H), 2.65 (dd, $J = 12.2$, 5.7 Hz, 0.5H), 2.52−2.42 (m, 2H), 2.10 (m, 0.5H), 1.89−1.81 (m, 1H), 1.76−1.12 (m, 21H), 0.92−0.85 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) 177.6, 177.4, 173.7, 172.8, 172.0, 171.8, 62.1, 60.9, 60.5, 60.3, 54.9, 54.7, 53.5, 53.0, 39.8, 39.7, 39.3, 38.3, 38.1, 38.0, 37.9, 36.3, 34.0, 33.7, 33.6, 31.5, 31.41, 31.38, 28.78, 28.76, 28.73, 28.70, 28.0, 27.9, 27.2, 26.7, 23.21, 23.19, 23.17, 23.10 ppm; IR (CH_2Cl_2) 3455, 3324, 1654, 1609 cm^{-1}; MS (CI) *m*/*z* 450 (MH), 252, 224; $[\alpha]^{25}_D = -65.6°$ ($c = 0.16$, $CHCl_3$).

***cis*-4-(3-Methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-prolinol.** A suspension of LAH (0.85 g, 22 mmol) in THF (30 mL) was brought to a gentle reflux, and **3** (4.05 g, 14.9 mmol) was added dropwise as a solution in THF (10 mL). The suspension was maintained at reflux for 1 h and then cooled to 0 °C. Saturated aqueous Na_2SO_4 was added slowly until a white granular precipitate formed. The mixture was diluted with EtOAc (40 mL), filtered through Celite, and concentrated to afford 3.86 g of a colorless oil. This oil was dissolved in 2:1 acetic acid/EtOAc (60 mL), and 10% Pd−C (0.79 g) was added. The mixture was shaken under H_2 (60 PSI) for 3 h and then filtered through Celite. The filtrate was concentrated and made basic to pH 13 with 30% KOH. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated to afford 2.49 g of a pale yellow oil which was used without further purification. This oil was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. Di-*tert*-butyl pyrocarbonate (3.30 g, 15.1 mmol) in CH_2Cl_2 (20 mL) was added dropwise, and the resulting solution was stirred at 0 °C for 20 min. The solution was concentrated under reduced pressure, and the resulting oil was subjected to flash chromatography (5% MeOH/20% EtOAc/hexanes to afford 3.13 g (77% from **3**) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.25 (d, $J = 8.4$ Hz, 1H), 3.90 (m, 1H), 3.65 (m, 2H), 3.55 (m, 1H), 2.77 (dd, apparent t, $J = 10.3$ Hz, 1H), 2.13 (m, 1H), 1.96 (m, 1H), 1.54−1.40 (m, 11H), 1.33−1.30 (m, 2H), 1.20−1.27 (m, 2H), 0.86 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 157.7, 81.0, 68.6, 62.0, 54.0, 38.4, 38.1, 36.3, 31.4, 29.2, 28.8, 23.2; IR (film) 3412, 2956, 1699, 1669, 1418 cm^{-1}; MS (CI) *m*/*z* 272 (MH) 240, 216, 200, 184, 172, 140, 58; $[\alpha]^{25}_D = -32.4°$ ($c = 0.14$, $CHCl_3$). Anal. Calcd for $C_{15}H_{29}NO_3$: C, 66.38; H, 10.77; N, 5.16. Found: C, 66.13; H, 10.66; N, 5.07.

***cis*-4-(3-Methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-proline.** Oxidation of the 2,4-*cis*-4-(3-methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-prolinol (1.26 g, 4.64 mmol) was accomplished as described for the synthesis of **5**. NMR of the crude reaction mixture revealed the presence of ∼10% *cis*-4-(3-methylbut-2-enyl)-*N*-(*tert*-butoxycarbonyl)-L-proline as a byproduct which was inseparable by chromatography. The crude reaction mixture was hydrogenated (0.49 g of 10% Pd−C, H_2, 1 atm) and purified by flash chromatography (5% MeOH/CH_2Cl_2) to afford 0.84 g (64%) of product as a colorless solid: mp 69−72 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.31−4.16 (m, 1H), 3.77−3.71 (m, 1H), 2.98−2.92 (m, 1H), 2.45−2.33 (m, 1H), 2.07−1.95 (m, 1H), 1.60−1.40 (m, 12H), 1.25−1.12 (m, 3H), 0.88 (d, $J = 6.5$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 179.5, 177.4, 156.1, 154.4, 81.4, 81.0, 60.0, 59.8, 53.3, 52.7, 39.4, 39.0, 38.0, 37.9, 36.2, 31.1, 30.3, 29.0, 28.9, 28.6, 23.0 ppm; IR (film) 2927, 1734, 1702, 1635, 1435 cm^{-1}; MS (CI) *m*/*z* 286 (MH) 230, 214, 186, 140; $[\alpha]^{25}_D = -56.7°$ ($c = 0.07$, EtOAc). Anal. Calcd for $C_{15}H_{27}NO_4$: C, 63.13; H, 9.53; N, 4.91. Found: C, 63.35; H, 9.26; N, 4.90.

***cis*-4-(3-Methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-proline Benzyl Ester.** A solution of *cis*-4-(3-methylbutyl)-*N*-(*tert*-butoxycarbonyl)-L-proline (0.447 g, 1.56 mmol), diisopropylcarbodiimide (0.49 mL, 3.1 mmol), benzyl alcohol (0.24 mL, 2.4 mmol), and DMAP (95 mg, 0.78 mmol) in CH_2Cl_2 (3 mL) was stirred at rt overnight. The suspension was filtered and concentrated. Flash chromatography (10% EtOAc/hexanes) afforded 0.512 g (87%) of product as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36−7.30 (m, 5H), 5.27 (d, $J = 12.5$ Hz, 0.4H), 5.15 (s, 1.2H), 5.07 (d, $J = 12.5$ Hz, 0.4H), 4.31 (dd, app t, $J = 8.9$ Hz, 0.4H), 4.23 (dd, app t, $J = 8.1$ Hz, 0.6H), 3.78 (dd, $J = 10.3$, 7.5 Hz, 0.6H), 3.66 (dd, $J = 10.0$, 7.6 Hz, 0.4H), 3.01−2.95 (m, 1H), 2.43−2.38 (m, 1H), 2.09−2.04 (m, 1H), 1.55−1.18 (m, 13H), 1.15−1.12 (m, 2H), 0.85 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 173.8, 173.5, 155.1, 154.3, 136.7, 136.3, 129.3, 129.1, 129.0, 128.7, 80.6, 80.5, 67.2, 60.2, 59.9, 53.2, 52.8, 39.6, 38.9, 38.0, 37.9, 36.9, 31.3, 31.2, 29.1, 28.9, 28.7, 28.6, 23.1 ppm; IR (film) 2955, 1750, 1704, 1401, 1160 cm^{-1}; MS (CI) *m*/*z* 376 (MH), 348, 320, 276, 240, 184, 140, 119, 91, 57; $[\alpha]^{25}_D = -68.3°$ ($c = 0.1$, $CHCl_3$). Anal. Calcd for $C_{22}H_{33}NO_4$: C, 70.37; H, 8.86; N, 3.73. Found: C, 70.46; H, 8.93; N, 3.76.

Synthesis of *cis*-4-(3-Methylbutyl)-Boc-dimer. Synthesized as described for **8** (82% yield): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.27 (m, 5H), 5.23 (d, $J = 12.3$ Hz, 0.5H), 5.22 (d, $J = 12.3$ Hz, 0.5H), 5.04 (d, $J = 12.3$ Hz, 0.5H), 5.02 (d, $J = 12.3$ Hz, 0.5H), 4.54 (dd, app t, $J = 8.2$ Hz, 0.5H), 4.47−4.43 (m, 1H), 4.36 (dd, $J = 9.2$, 7.6 Hz, 0.5H), 4.02 (dd, app t, $J = 7.9$ Hz, 0.5H), 3.89 (dd, app t, $J = 7.9$ Hz, 0.5H), 3.75 (dd, $J = 10.1$, 7.4 Hz, 0.5H), 3.66 (dd, $J = 10.2$, 7.4 Hz, 0.5H), 3.08−2.96 (m, 2H), 2.42−1.98 (m, 5H), 1.55−1.08 (m, 20H), 0.87−0.84 (m, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz) 172.2, 171.8, 171.4, 171.0, 154.3, 153.4, 135.8, 135.7, 128.42, 128.39, 128.2, 128.1, 128.07, 128.04, 79.4, 79.3, 66.7, 66.6, 59.4, 59.2, 58.0, 57.7, 52.7, 52.5, 52.2, 39.7, 39.4, 38.5, 37.4, 37.25, 37.21, 36.5, 35.4, 35.2, 30.5, 30.3, 30.2, 28.5, 28.3, 28.1, 27.9, 22.5, 22.42, 22.40, 22.37 ppm; IR (film) 2955, 1749, 1700, 1664, 1404, 1169 cm^{-1}; MS (CI) *m*/*z* 543 (MH), 471, 443, 240, 184, 140, 91, 57; $[\alpha]^{25}_D = -89.9°$ ($c = 0.15$, $CHCl_3$). Anal. Calcd for $C_{32}H_{50}N_2O_5$: C, 70.81; H, 9.23; N, 5.16. Found: C, 70.65; H, 9.20; N, 5.15.

Synthesis of *cis*-4-(3-Methylbutyl)-Piv-dimer 11. The synthesis of **11** was accomplished as described for **9** (87%) from the 2,4-*cis*-Boc-PTAA-PTAA-OBn-dimer: mp 85−86 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.29 (m, 5H), 5.25 (d, $J = 12.4$ Hz, 1H), 5.02 (d, $J = 12.4$ Hz, 1H), 4.64 (dd, app t, $J = 9.0$ Hz, 1H), 4.53 (dd, $J = 9.1$, 7.8 Hz, 1H), 4.14 (dd, app t, $J = 8.3$ Hz, 1H), 3.99 (dd, $J = 9.5$, 6.9 Hz, 1H), 3.22 (dd, app t, $J = 10.5$ Hz, 1H), 3.09 (dd, app t, $J = 9.5$ Hz, 1H), 2.40 (m, 1H), 2.35−2.23 (m, 2H), 2.06 (m, 1H), 1.57−1.08 (m, 21H), 0.88 (d, $J = 6.6$ Hz, 6H), 0.85 (d, $J = 5.9$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) 177.0, 172.9, 172.1, 136.6, 129.1, 128.8, 128.7, 67.3, 60.5, 59.9, 55.1, 53.4, 41.4, 40.1, 39.1, 38.1, 38.0, 36.1, 34.2, 31.3, 30.1, 28.9, 28.7, 28.0, 23.19, 23.16, 23.0 ppm; IR (film) 2954, 1748, 1662, 1623, 1410, 1171 cm^{-1}; MS (CI) *m*/*z* 527 (MH), 252, 224; $[\alpha]^{25}_D = -80.0°$ ($c = 0.1$, $CHCl_3$). Anal. Calcd for $C_{32}H_{50}N_2O_4$: C, 72.96; H, 9.57; N, 5.32. Found: C, 72.93; H, 9.54; N, 5.25.

Synthesis of *cis*-4-(3-Methylbutyl)-Piv-trimer 12. A suspension of Pd−C (29 mg) and **11** (0.130 g, 0.253 mmol) in EtOAc (2 mL) was stirred under a hydrogen balloon for 6 h. The suspension was filtered through Celite and concentrated to afford 107 mg of the acid as a colorless oil which was homogenous by TLC. The trimer **12** was obtained as described for **10** from the 2,4-*cis*-Piv-PTAA-PTAA-OH-dimer. Purification by flash chromatography (30% EtOAc/hexanes) afforded 153.6 mg (89% from **11**) of product as a colorless glassy solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33−7.29 (m, 5H), 5.24 (d, $J =$

12.4 Hz, 1H), 5.02 (d, J = 12.4 Hz, 1H), 4.69 (dd, app t, J = 8.7 Hz, 1H), 4.64 (dd, app t, J = 8.9 Hz, 1H), 4.53 (dd, app t, J = 8.5 Hz, 1H), 4.11 (dd, app t, J = 8.0 Hz, 1H), 4.07 (dd, app t, J = 8.3 Hz, 1H), 3.98 (dd, J = 10.6, 6.9 Hz, 1H), 3.20 (dd, app t, J = 10.6 Hz, 1H,) 3.12 (dd, app t, J = 9.8 Hz, 1H), 3.05 (dd, app t, J = 9.6 Hz, 1H), 2.41−2.22 (m, 5H), 2.04 (m, 1H), 1.54−1.07 (m, 27H), 0.87−0.84 (m, 18H); ^{13}C NMR ($CDCl_3$, 125 MHz) 176.9, 172.8, 171.5, 171.3, 136.5, 129.1, 128.8, 67.2, 60.7, 59.7, 58.7, 55.1, 53.5, 53.2, 41.3, 40.3, 40.0, 39.1, 38.04, 38.00, 37.9, 35.9, 35.2, 34.2, 31.2, 31.0, 30.6, 28.8, 28.7, 28.6, 28.0, 23.1, 23.07, 23.0 ppm; IR (film) 2955, 1749, 1652, 1623, 1456, 1170 cm^{-1}; MS (CI) *m*/*z* 694 (MH), 101, 87, 69; $[\alpha]^{25}_D$ = −75.4° (c = 0.5, CH_2Cl_2). Anal. Calcd for $C_{42}H_{67}N_3O_5$: C, 72.69; H, 9.73; N, 6.05. Found: C, 72.46; H, 9.80; N, 6.02.

Benzyl (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2-methylbut-2-enyl)-pyroglutamate (14). The pyroglutamic acid derivative **13** (6.36 g, 19.9 mmol) in THF (40 mL) was added dropwise to a 1.0 M solution of LHMDS in THF (19.9 mL, 19.9 mmol) at −78 °C. After 30 min, a cold solution (0 °C) of 4-bromo-2-methyl-2-butene (2.55 mL, 19.9 mmol) in THF (10 mL) was added dropwise, and the resulting solution was maintained at −78 °C for 2 h. Saturated aqueous $NaHCO_3$ (50 mL) was then added, and the resulting suspension was allowed to warm to room temperature. The layers were separated, and the aqueous layer was extracted with 1:1 hexanes−ethyl acetate (3 × 50 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated. ^{1}H NMR of the crude product indicated the ratio of 5:1 of *trans*/*cis* product. Flash chromatography of the residue on silica gel (3:1 hexanes−ethyl acetate) afforded **14** (4.86 g, 63%) as colorless oil: $[\alpha]^{25}_D$ = −35.3° (c = 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35 (m, 5H), 5.21 (d, J = 12.2 Hz, 1H), 5.18 (d, J = 12.2 Hz, 1H), 5.04 (m, 1H), 4.58 (dd, J = 1.5, 9.7 Hz, 1H), 2.65 (m, 1H), 2.51 (m, 1H), 2.17 (m, 2H), 1.98 (m, 1H), 1.69 (s, 3H), 1.60 (s, 3H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) 174.5, 171.2, 149.4, 135.1, 134.7, 128.6, 128.5, 128.4, 119.7, 83.4, 67.2, 57.1, 41.9, 28.4, 27.8, 27.7, 25.7, 17.8 ppm; IR (film) 2978, 2931, 1793, 1752, 1718, 1457, 1393, 1368, 1317, 1251, 1186, 1155, 965, 853, 776, 751, 699, 668 cm^{-1}; MS (CI) *m*/*z* 332, 316, 288, 219, 196, 152, 119, 91, 57. Anal. Calcd for $C_{22}H_{29}O_5N$: C, 68.20; H, 7.54; N, 3.62. Found: C, 68.32; H, 7.58; N, 3.62.

***trans*-1-(*tert*-Butoxycarbonyl)-4-(2-methyl-2-butenyl)-L-proline Benzyl Ester (16).** A 1.0 M solution of lithium triethylborohydride in THF (13.0 mL, 13.0 mmol) was added to a solution of **14** (4.16 g, 10.7 mmol) in THF (10 mL) at −78 °C. After 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (40 mL) and warmed to 0 °C. Then 30% H_2O_2 (0.5 mL) was added, and the resulting mixture was stirred at 0 °C for 20 min. The organic solvent was removed under reduced pressure, and the aqueous layer was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated. The crude product was used without further purification. A solution of the crude product and triethylsilane (3.47 mL, 21.5 mmol) in CH_2Cl_2 (40 mL) was cooled to −78 °C, and boron trifluoride etherate (2.92 mL, 23.6 mmol) was then added dropwise. After 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (40 mL) and warmed to room temperature, the layers were separated, and the aqueous layer was then extracted with CH_2Cl_2 (3 × 40 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated. Flash chromatography of the residue on silica gel (5:1 hexanes−ethyl acetate) yielded **16** (3.28 g, 82%) as a colorless oil: $[\alpha]^{25}_D$ = −33.0° (c = 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34 (m, 5H), 5.15 (m, 2H), 5.10 (m, 0.4H), 5.07 (m, 0.6H), 4.32 (dd, J = 2.1, 8.8 Hz, 0.4 H), 4.30 (J = 2.6, 8.8 Hz, 0.6H), 3.69 (dd, J = 7.6, 10.3 Hz, 0.6H), 3.62 (dd, J = 7.7, 10.1 Hz, 0.4 H), 3.05 (dd, J = 8.3, 10.2 Hz, 0.6H), 2.99 (dd, J = 8.5, 10.2 Hz, 0.4H), 2.28 (m, 1H), 2.03 (m, 3H), 1.90 (m, 1H), 1.69 (s, 1.2H), 1.68 (s, 1.8H), 1.58 (m, 3H), 1.46 (s, 3.6H), 1.34 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 125 MHz) 172.9, 172.7, 154.3, 153.7, 135.8, 135.6, 133.2, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 121.6, 121.5, 79.7, 79.6, 66.5, 59.1, 58.8, 51.5, 51.2, 37.8, 36.9, 36.2, 35.3, 31.1, 31.0, 28.4, 28.1, 25.6, 17.7 ppm; IR (film) 2974, 2930, 1749, 1702, 1478, 1456, 1398, 1366, 1258, 1178, 1124, 970, 892, 772, 751, 698, 668 cm^{-1}; MS (CI) *m*/*z* 374 (MH), 318, 302, 274, 238, 210, 182, 138, 121, 119, 91, 68, 59, 58, 57. Anal. Calcd for $C_{22}H_{31}O_4N$: C, 70.75; H, 8.37; N, 3.75. Found: C, 70.68; H, 8.43; N, 3.71.

***trans*-1-(*tert*-Butoxycarbonyl)-4-(2-oxoethyl)-L-proline Benzyl Ester (17).** The alkene **16** (2.06 g, 5.52 mmol) in CH_2Cl_2 (20 mL) was cooled to −78 °C, and ozone was bubbled through the solution until a blue color persisted. Nitrogen was then bubbled through the solution for 15 min to drive off excess ozone. Triphenylphosphine (2.17 g, 8.28 mmol) was added, the resulting mixture was warmed to room temperature over 2 h, and the solvent was removed under reduced pressure. Flash chromatography on silica gel (2:1 hexanes−ethyl acetate) gave **17** (1.73 g, 90%) as pale yellow oil: $[\alpha]^{25}_D$ = −36.3° (c = 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.71 (s, 1H), 7.35 (m, 5H), 5.15 (m, 2H), 4.42 (dd, J = 2.9, 8.8 Hz, 0.4H), 4.31 (dd, J = 2.9, 8.9 Hz, 0.6H), 3.83 (dd, J = 7.8, 10.4 Hz, 0.6H), 3.79 (dd, J = 7.9, 10.1 Hz, 0.4H), 3.04 (dd, J = 8.3, 10.2 Hz, 0.6H), 2.98 (dd, J = 8.3, 10.2 Hz, 0.4H), 2.75 (m, 1H), 2.52 (m, 2H), 2.20 (m, 1H), 1.88 (m, 1H), 1.45 (s, 3.6H), 1.33 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 125 MHz) 199.8, 199.7, 172.3, 172.1, 153.9, 153.3, 135.5, 135.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 79.8, 66.5, 58.6, 58.4, 51.3, 51.0, 46.7, 46.6, 36.0, 35.1, 31.2, 30.4, 28.2, 28.0 ppm; IR (film) 2975, 1746, 1724, 1698, 1455, 1398, 1367, 1258, 1181, 1159, 1129, 740, 699 cm^{-1}; MS (CI) *m*/*z* 348 (MH), 308, 292, 276, 264, 248, 228, 212, 202, 184, 172, 156, 129, 119, 112, 91, 68, 59, 58, 57. Anal. Calcd for $C_{19}H_{25}O_5N$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.44; H, 7.26; N, 4.09.

***trans*-1-(*tert*-Butoxycarbonyl)-4-((methoxycarbonyl)methyl)-L-proline Benzyl Ester (18).** PDC (3.62 g, 9.62 mmol) was added to a solution of **17** (1.67 g, 4.81 mmol) in DMF (15 mL), the resulting suspension was stirred at rt for 9 h. H_2O (150 mL) was added, and the aqueous layer was extracted with Et_2O (6 × 120 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated. The crude acid was used without further purification. $NaHCO_3$ (0.81 g, 9.62 mmol) and iodomethane (2.99 mL, 48.1 mmol) were added to the crude acid in DMF (15 mL), and the resulting suspension was stirred at room temperature for 20 h. Et_2O (100 mL) and H_2O (100 mL) were then added, and the organic layer was washed with H_2O (3 × 100 mL), dried (Na_2SO_4), and concentrated. Flash chromatography of the residue on silica gel (4:1 hexanes−ethyl acetate) gave **18** (1.48 g, 82%) as yellowish oil: $[\alpha]^{25}_D$ = −30.8° (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35 (m, 5H), 5.15 (m, 2H), 4.43 (dd, J = 2.6, 8.8 Hz, 0.4H), 4.31 (dd, J = 2.6, 8.8 Hz, 0.6H), 3.81 (dd, J = 8.0, 10.4 Hz, 0.6H), 3.77 (dd, J = 8.0, 10.4 Hz, 0.4H), 3.66 (s, 3H), 3.10 (dd, J = 8.1, 10.4 Hz, 0.6H), 3.03 (dd, J = 8.1, 10.4 Hz, 0.4H), 2.71 (m, 1H), 2.38 (m, 2H), 2.18 (m, 1H), 1.93 (m, 1H), 1.45 (s, 3.6H), 1.33 (s, 5.4H); ^{13}C NMR ($CDCl_3$, 125 MHz) 172.5, 172.2, 172.0, 171.9, 154.1, 153.5, 135.7, 135.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 80.0, 79.9, 66.6, 58.8, 58.6, 51.6, 51.5, 51.2, 37.1, 36.1, 35.3, 33.7, 32.9, 28.3, 28.0 ppm; IR (film) 2976, 2878, 1741, 1701, 1479, 1456, 1438, 1400, 1367, 1258, 1173, 1127, 1004, 891, 773, 699 cm^{-1}; MS (CI) *m*/*z* 378 (MH), 322, 306, 278, 242, 214, 186, 142, 119, 91, 68, 59, 58, 57, 52. Anal. Calcd for $C_{20}H_{27}O_6N$: C, 63.65; H, 7.21; N, 3.71. Found: C, 63.41; H, 7.16; N, 3.73.

***trans*-1-(2,2-Dimethylpropanoyl)-4-((methoxycarbonyl)methyl)-L-proline Benzyl Ester. 18** (600 mg, 1.59 mmol) was treated with TFA (0.5 mL), and the resulting mixture was stirred at room temperature for 30 min. TFA was evaporated, and the residue was dried *in vacuo* overnight and then dissolved in CH_2Cl_2 (3.0 mL). Triethylamine (0.55 mL, 4.0 mmol) and trimethylacetyl chloride (0.22 mL, 1.8 mmol) were successively added, and the resulting mixture was stirred at room temperature for 1 h. After dilution with CH_2Cl_2 (10 mL), the reaction mixture was washed with 0.25 N HCl (10 mL), 5% $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes−ethyl acetate) afforded benzyl ester **19** (546 mg, 95%) as a yellowish oil: $[\alpha]^{25}_D$ = −51.8° (c = 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34 (m, 5H), 5.23 (d, J = 12.4 Hz, 1H), 5.07 (d, J = 12.4 Hz, 1H), 4.60 (br d, J = 5.8, 1H), 4.02 (dd, J = 7.8, 8.7 Hz, 1H), 3.68 (s, 3H), 3.41 (dd, J = 7.9, 9.5 Hz, 1H), 2.79 (m, 1H), 2.40 (dd, J = 6.5, 15.9 Hz, 1H), 2.36 (dd, J = 8.1, 15.9 Hz, 1H), 2.04 (m, 1H), 1.85 (td, J = 9.0, 13.0 Hz, 1H), 1.24 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) 176.8, 172.2, 171.9, 135.7, 128.4, 128.1, 128.0, 66.6, 60.3, 53.0, 51.7, 38.6, 36.7, 35.2, 33.1, 27.1 ppm; IR (film) 2957, 1740, 1627, 1478, 1457, 1437, 1406, 1361, 1172, 1082, 1003, 756, 699, 668 cm^{-1}; MS (CI) *m*/*z* 362 (MH), 254, 226, 142, 119, 91, 87,

85, 68, 59, 58, 57, 52. Anal. Calcd for $C_{20}H_{27}O_5N$: C, 66.46; H, 7.53; N, 3.88. Found: C, 66.46; H, 7.63; N, 3.82.

***trans*-1-(2,2-Dimethylpropanoyl)-4-((methoxycarbonyl)methyl)-L-proline (19).** Benzyl ester **19** (450 mg, 1.25 mmol) in MeOH (2 mL) was treated with 10% Pd−C (45 mg), and the resulting mixture was stirred under hydrogen balloon overnight and then filtered through Celite. The filtrate was concentrated, and crystallization of the residue in 1:2 hexanes−ethyl acetate gave **19** (308 mg, 91%) as colorless needles: mp 150−151 °C; $[\alpha]^{25}_D = -112.8°$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.36 (br s, 1H), 4.60 (br d, $J = 7.3$ Hz, 1H), 4.03 (dd, $J = 8.1$, 8.8 Hz, 1H), 3.70 (s, 3H), 3.41 (dd, $J = 8.8$, 9.4 Hz, 1H), 2.82 (m, 1H), 2.49 (dd, $J = 5.8$, 15.9 Hz, 1H), 2.37 (dd, $J = 8.6$, 15.9 Hz, 1H), 2.29 (m, 1H), 1.80 (dt, $J = 9.2$, 12.7 Hz, 1H), 1.27 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) 178.8, 174.8, 172.0, 60.9, 53.3, 51.8, 39.0, 36.7, 35.2, 32.5, 27.1 ppm; IR (film) 3100 (br), 2967, 1736, 1734, 1718, 1653, 1635, 1559, 1540, 1507, 1457, 1419, 1172, 668 cm^{-1}; MS (CI) *m*/*z* 272 (MH), 254, 227, 226, 216, 202, 188, 185, 142, 85, 68, 59, 58, 57. Anal. Calcd for $C_{13}H_{21}O_5N$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.55; H, 7.79; N, 5.13.

***trans*-4-((Methoxycarbonyl)methyl)-dimer 20.** The PTAA **18** (340 mg, 0.90 mmol) was treated with TFA (0.5 mL), and the resulting mixture was stirred at room temperature for 30 min and concentrated under reduced pressure. The residue was dried *in vacuo* overnight. In a separate flask, 1,3-diisopropylcarbodiimide (0.29 mL, 1.80 mmol) was added to a solution of **19** (245 mg, 0.90 mmol), DMAP (110 mg, 0.90 mmol), and HOBt (138 mg, 0.90 mmol) in CH_2Cl_2 (2 mL) at 0 °C, and the resulting mixture was stirred at 0 °C for 0.5 h. A solution of the Boc-deprotected PTAA and triethylamine (0.16 mL, 1.10 mmol) in CH_2Cl_2 (1 mL) was added, and the reaction mixture was warmed to rt and stirred for 15 h. CH_2Cl_2 (8 mL) was added, and the reaction mixture was washed with aqueous 0.25 N HCl (10 mL), aqueous 5% $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL). The organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography of the residue on silica gel (1:1.5 hexanes−ethyl acetate) gave **20** (401 mg, 84%) as colorless oil: $[\alpha]^{25}_D = -64.5°$ ($c = 0.3$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.33 (m, 5H), 5.22 (d, $J = 12.3$ Hz, 1H), 5.04 (d, $J = 12.3$ Hz, 1H), 4.72 (dd, $J = 1.8$, 9.2 Hz, 1H), 4.66 (dd, $J = 3.7$, 8.8 Hz, 1H), 4.06 (dd, $J = 6.9$, 10.0 Hz, 1H), 3.89 (dd, $J = 8.0$, 9.3 Hz, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 3.45 (m, 2H), 2.88 (m, 1H), 2.77 (m, 1H), 2.46 (dd, $J = 6.4$, 16.3 Hz, 1H), 2.29 (dd, $J = 9.0$, 15.6 Hz, 1H), 2.40 (dd, $J = 8.2$, 16.3 Hz, 1H), 2.39 (dd, $J = 5.6$, 15.6 Hz, 1H), 2.16 (ddd, $J = 1.8$, 6.5, 13.0 Hz, 1H), 1.98 (ddd, $J = 3.9$, 6.4, 12.5 Hz, 1H), 1.90 (ddd, $J = 9.3$, 11.2, 13.0 Hz, 1H), 1.76 (td, $J = 8.6$, 12.6 Hz, 1H), 1.26 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) 176.8, 172.1, 172.0, 171.7, 170.9, 135.6, 128.5, 128.2, 128.1, 66.9, 58.9, 58.3, 53.3, 51.7, 51.6, 51.5, 38.6, 37.2, 36.9, 35.1, 34.3, 32.4, 27.2 ppm; IR (film) 2955, 1736, 1662, 1622, 1436, 1408, 1361, 1172, 1001, 756, 700, 668 cm^{-1}; MS (CI) *m*/*z* 531 (MH), 254, 226, 91, 85, 58. Anal. Calcd for $C_{28}H_{38}O_8N_2$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.11; H, 7.29; N, 5.18.

***trans*-4-(Carboxymethyl)-dimer 21.** **20** (53.1 mg, 0.10 mmol) was treated with a 1 M solution of LiOH in 3:1 H_2O−MeOH (1.0 mL), and the resulting mixture was stirred at rt for 3 h. H_2O (2 mL) was then added, and the organic solvent was evaporated *in vacuo*. The resulting solution was applied to a column of Dowex-50W HCR-W2 resin (H_2O) and eluted with H_2O affording **21** (37 mg, 91%) as white solid: mp 118−121 °C; $[\alpha]^{25}_D = -43.0$ ($c = 0.1$, EtOH); ^{1}H NMR (D_2O, 500 MHz) δ 4.59 (m, 1H), 4.39 (dd, $J = 3.0$, 9.1 Hz, 1H), 3.90 (dd, $J = 6.6$, 10.7 Hz, 1H), 3.82 (dd, $J = 7.4$, 10.1 Hz, 1H), 3.52 (dd, $J = 6.2$, 10.8 Hz, 1H), 3.34 (dd, $J = 8.7$, 10.0 Hz, 1H), 2.68 (m, 2H), 2.45 (d, $J = 7.3$ Hz, 2H), 2.38 (d, $J = 7.4$ Hz, 2H), 2.14 (ddd, $J = 3.0$, 6.3, 13.1 Hz, 1H), 1.95 (m, 3H), 1.11 (s, 9H); ^{13}C NMR (CD_3OD, 125 MHz) δ 178.9, 175.7, 175.6, 175.3, 173.0, 60.8, 60.1, 54.8, 52.9, 39.7, 37.9, 37.7, 36.7, 35.7, 35.4, 33.2, 27.7 ppm; IR (film) 3431 (br), 2967, 1731, 1623, 1587, 1425, 1366, 1232, 1175, 869 cm^{-1}; MS (CI) *m*/*z* 413 (MH), 311, 258, 240, 213, 212, 188, 171, 156, 143, 128, 103, 87, 85, 73, 69, 59, 58, 57.

Dimer 22. **20** (250.0 mg, 0.47 mmol) in MeOH (2 mL) was treated with 10% Pd−C (25 mg), and the resulting mixture was stirred under hydrogen balloon overnight, then filtered through Celite, and washed with MeOH (15 mL). The filtrate was concentrated and dried *in vacuo*, giving **22** as a white solid (206.5 mg, 100%): mp 57−59 °C; $[\alpha]^{25}_D = -69.8°$ ($c = 0.3$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.68 (br d, $J = 8.7$ Hz, 1H), 4.65 (dd, $J = 4.0$, 8.7 Hz, 1H), 4.07 (dd, $J = 6.8$, 10.0 Hz, 1H), 3.86 (t, $J = 8.9$ Hz, 1H), 3.70 (s, 3H), 3.68 (s, 3H), 3.47 (m, 2H), 2.97 (m, 1H), 2.79 (m, 1H), 2.57 (dd, $J = 5.4$, 16.3 Hz, 1H), 2.40 (m, 3H), 2.06 (m, 1H), 1.82 (m, 2H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) 177.2, 173.6, 172.4, 172.1, 172.0, 59.3, 58.9, 53.3, 52.2, 51.8, 38.6, 37.0, 36.7, 35.3, 34.6, 33.0, 32.5, 27.2 ppm; IR (film) 3158 (br), 2957, 2873, 1736, 1661, 1622, 1587, 1436, 1368, 1203, 1172, 1082, 999, 858, 734 cm^{-1}.

***trans*-4-((Methoxycarbonyl)methyl)-trimer 23.** The synthesis of **23** was accomplished from **18** (151 mg, 0.40 mmol) and **22** (175.8 mg, 0.40 mmol) as described for the synthesis of **20**. Flash chromatography of the residue on silica gel (ethyl acetate) gave **23** (240 mg, 86%) as white solid: mp 134−135 °C; $[\alpha]^{25}_D = -54.7°$ ($c = 0.3$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32 (m, 5H), 5.22 (d, $J = 12.3$ Hz, 1H), 5.04 (d, $J = 12.3$ Hz, 1H), 4.75 (brd, $J = 8.6$ Hz, 1H), 4.66 (brd, $J = 8.5$ Hz, 2H), 4.04 (dd, $J = 6.9$, 9.9 Hz, 1H), 3.93 (t, $J = 8.6$ Hz, 1H), 3.83 (t, $J = 8.5$ Hz, 1H), 3.68 (s, 6H), 3.67 (s, 3H), 3.45 (t, $J = 7.7$ Hz, 1H), 3.44 (t, $J = 9.3$ Hz, 1H), 3.36 (t, $J = 9.4$ Hz, 1H), 2.87 (m, 2H), 2.76 (m, 1H), 2.42 (m, 4H), 2.30 (m, 2H), 2.17 (ddd, $J = 1.4$, 6.5, 12.9 Hz, 1H), 2.10 (ddd, $J = 3.8$, 6.4, 12.8 Hz, 1H), 2.02 (ddd, $J = 1.6$, 6.8, 12.6 Hz, 1H), 1.84 (m, 3H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) 176.7, 172.1, 172.0, 171.9, 171.5, 170.6, 170.2, 135.5, 128.5, 128.2, 128.1, 66.9, 59.0, 58.4, 57.2, 53.4, 51.9, 51.7, 51.6, 51.4, 38.6, 37.3, 37.2, 36.9, 35.1, 34.3, 34.2, 33.9, 33.5, 32.4, 27.2 ppm; IR (film) 2955, 2876, 1737, 1651, 1621, 1435, 1172, 1089, 1004, 748, 705 cm^{-1}; MS (CI) *m*/*z* 700 (MH), 475, 447, 423, 254, 226, 143, 115, 107, 101, 92, 91, 87, 85, 69, 60, 59, 52. Anal. Calcd for $C_{36}H_{49}O_{11}N_3$: C, 61.79; H, 7.06; N, 6.00. Found: C, 61.86; H, 7.12; N, 5.93.

***trans*-4-(Carboxymethyl)-trimer 24.** As described for the synthesis of **21**. **24** was obtained (66 mg, 90%) from **23** (88.0 mg, 0.13 mmol) as a white solid, which was crystallized from MeOH−EtOAc, yielding colorless cubic crystals (56 mg, 76%): mp 159−161 °C; $[\alpha]^{25}_D = -71.5°$ ($c = 0.2$, EtOH); ^{1}H NMR (D_2O, 500 MHz) δ 4.64 (m, 2H), 4.40 (dd, $J = 1.3$, 8.5 Hz, 1H), 3.88 (dd, $J = 6.6$, 10.5 Hz, 1H), 3.80 (t, $J = 8.7$ Hz, 2H), 3.52 (dd, $J = 6.0$, 10.5 Hz, 1H), 3.38 (t, $J = 7.8$ Hz, 1H), 3.37 (t, $J = 8.8$ Hz, 1H), 2.68 (m, 3H), 2.46 (d, $J = 7.0$ Hz, 2H), 2.45 (d, $J = 7.0$ Hz, 2H), 2.38 (m, 2H), 2.14 (m, 1H), 2.08 (m, 1H), 1.92 (m, 4H), 1.12 (s, 9H); ^{13}C NMR (CD_3SOCD_3, 125 MHz) 174.7, 173.4, 173.2, 173.1, 173.0, 170.0, 169.7, 58.7, 58.1, 56.9, 51.5, 51.0, 37.9, 37.0, 36.9, 36.4, 34.7, 34.0, 33.8, 33.1, 31.9, 27.1 ppm; IR (film) 3452 (br), 3089 (br), 2972, 2883, 2612 (br), 1729, 1617, 1431, 1365, 1172, 876. Anal. Calcd for $C_{26}H_{37}O_{11}N_3$: C, 55.02; H, 6.57; N, 7.40. Found: C, 55.02; H, 6.61; N, 7.37.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for **2**−**6**, **8**−**12**, **14**, **16**−**24**, and **26** and TOCSY and NOESY spectra for **9**−**12**, **21**, **24**, and **26** (60 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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