Poly-L-proline Type II Peptide Mimics Based on the 3-Azabicyclo[3.1.0]hexane System

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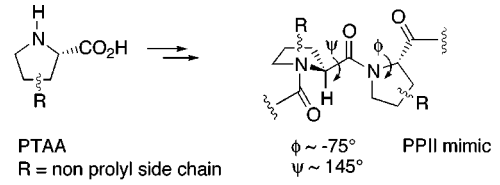
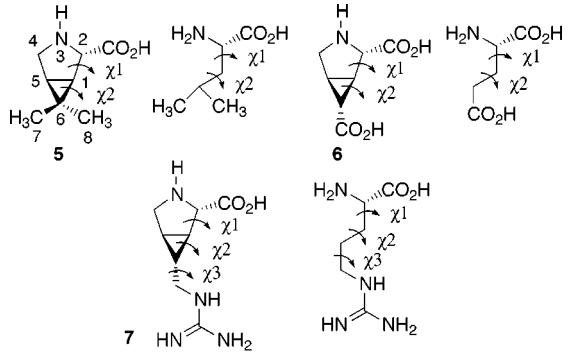
This paper describes conformational studies of proline-templated amino acids (PTAAs) based on the 3-azabicyclo[3.1.0]hexane system as well as conformational studies on short peptides composed of these PTAAs. NOE data, coupling constants, and molecular modeling are consistent with a flattened boat conformation for monomeric and oligomeric residues based on this bicyclic system. NMR studies on dimeric and trimeric oligomers are consistent with a populated poly-L-proline type II conformation in $CDCl_3$ and D_2O. Solution studies and molecular modeling predicts $\phi \sim -70°$, $\psi \sim 131°$, $\chi1 \sim -57°$, and $\chi2 \sim -158°$ for oligomeric residues.

Introduction

Over the past decade, poly-L-proline type II (PPII) secondary structure has been shown to play a critical role in mediating several cellular signaling pathways.[1,2] Due to the potential utility of agents capable of inhibiting these signaling mechanisms, we are developing a program focused on the design and synthesis of mimics of the PPII conformation. PPII helices are characterized by an extended left-handed fold with $\phi \sim -75°$, $\psi \sim 145°$, and $\omega \sim 180°$. Since many PPII helices possess amino acids other than proline, PPII mimics must not only adopt the desired backbone conformation, but also be able to account for the nonprolyl side chains which are often critical for receptor recognition of the PPII helix. Our strategy for mimicking this secondary structure involves first the synthesis of proline-templated amino acids (PTAAs) and then the synthesis of oligopeptides from these PTAAs.[3] OligoPTAAs are thus expected to preferentially populate the PPII conformation in solution since (1) $\phi \sim -75°$ due to the constraints of the pyrrolidine ring, (2) $\psi \sim 145°$ due to pseudo A(1,3)-strain, and (3) $\omega \sim 180°$ because the amide trans conformation is favored. One of the advantages of the PTAA approach lies in that not only the peptide backbone, but also the amino acid side chains can be constrained. Side chain conformation is described by the parameters $\chi1$, $\chi2$, $\chi3$... with $\chi1$ defining



rotation about the αC − βC bond, $\chi2$ defining rotation about the βC − γC bond, etc. Examination of a number



of receptor-bound PPII helices reveals that residues possessing $\chi1 \sim$ gauche(−) and $\chi2 \sim$ trans are common, thus necessitating a strategy to mimic this geometry. With this goal in mind, we envisioned that a PTAA based on the 3-azabicyclo[3.1.0]hexane system would approximate a $\chi1$ angle ~ gauche(−) (compare Newman projections **3** and **4**, Figure 1).[4] Furthermore, due to the greater p-character in the cyclopropane C−C bonds, we expected that $\chi2$ would approximate the desired trans geometry. This paper describes the synthesis and conformational evaluation of PPII mimics based on the 3-azabicyclo-[3.1.0]hexane system in $CHCl_3$ and D_2O.[5−7]

* To whom correspondence should be addressed.

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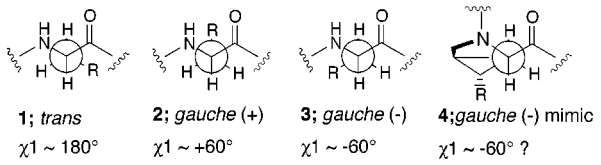
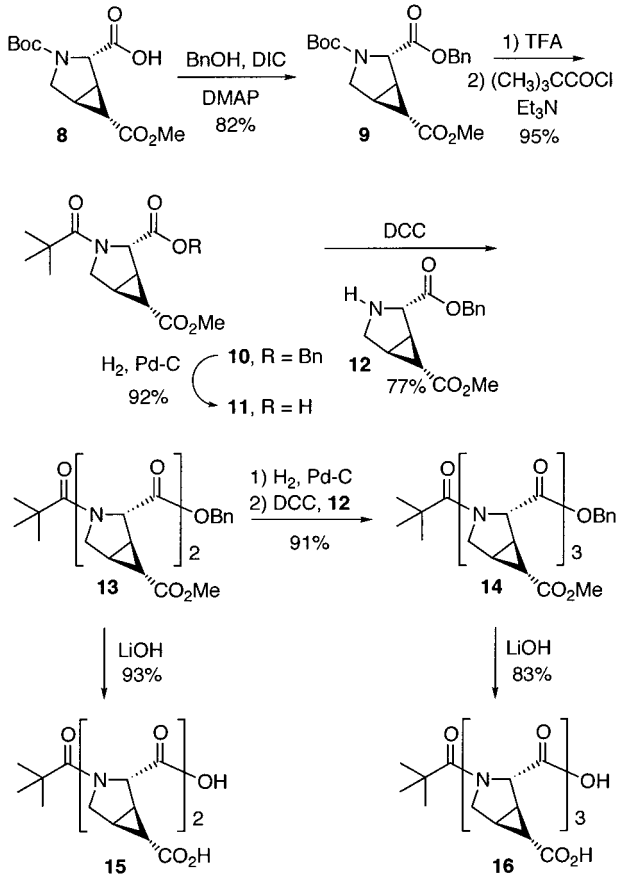


Figure 1. Comparison of side chain orientations.

Scheme 1



Results and Discussion

Synthesis. The synthesis of the oligomeric PTAAs was achieved by a modular assembly described in Scheme 1.[8,9] The synthesis of oligomeric leucine PTAAs have already been reported.[5] The dimeric and trimeric proline-templated glutamic acids (PTE) were synthesized from Boc-PTE(Me)-OH (**8**). Protection of the carboxy terminus with BnOH, diisopropylcarbodiimide (DIC), and DMAP gave the benzyl ester **9** (82%). Boc-deprotection (TFA) and reprotection of the resultant amine afforded the trimethylacetamide **10** in 95% yield. Debenzylation of the ester gave the carboxylic acid **11** which underwent smooth DIC−HOBt-mediated coupling with amino ester **12** to give the dimer **13** in 77% yield. The trimer **14** was obtained by debenzylation of **13** followed by DIC−HOBt coupling with the amine fragment **12** in 91% yield. The ester functionalities were hydrolyzed with LiOH to afford the water soluble dimer **15** (93%) and trimer **16** (83%).

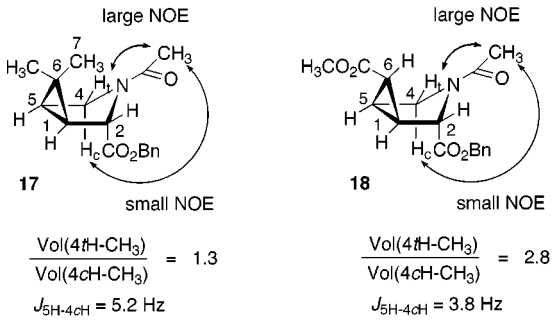


Figure 2. Selected NOEs for **24** and **25**.

Conformation of Monomers. Previous studies have shown that the bicyclo[3.1.0]hexane system populates a flattened boat conformation.[10,11] Although the pucker is slight, the degree of the pucker will influence ϕ, prompting first an investigation of the bicyclic ring system conformation. NOE experiments on the model compounds Ac−PTL-OBn, **17**, and Ac−PTE(Me)-OBn, **18** (Figure 2), were utilized to explore the ring pucker since a populated boat or chair conformation would result in unequal distances from the acyl methyl group to 4*c*H and 4*t*H (*c* and *t* denote a cis and trans relationship relative to the ester carbonyl).[12] NOESY spectra of compounds **17** and **18** reveal that the acyl methyl group exhibits a higher NOE intensity with 4*t*H than 4*c*H as expected for a populated boat conformation. This experiment is dependent on a planar amide bond geometry for which reason, the sterically demanding trimethylacetyl substituted monomers were not utilized. To investigate if the magnitude of the pucker is the same in both PTL **17** and PTE **18**, we determined and compared the volumes of the 4*t*H−CH_3 and 4*c*H−CH_3 NOESY cross-peaks. For PTL **17**, the ratio of the 4*t*H−CH_3/4*c*H−CH_3 cross-peak volumes is 1.3, while the analogous value for PTE **18** is 2.8, consistent with a more pronounced pucker for PTE **18**. A more pronounced pucker for PTE **18** is also supported by coupling constant data (J_{4cH-5H} = 5.2 Hz for **17**, J_{4cH-5H} = 3.8 Hz for **18**).

To gain better insights into the exact geometries of the monomer units, we also investigated Ac-PTL-OMe and Ac-PTE(Me)-OMe by molecular modeling. Geometry optimizations were performed on Ac-PTL-OMe and Ac-PTE-(Me)-OMe using molecular mechanics (MMX force field) and ab initio calculations (HF/6-31g* level). Both molecular mechanics and ab initio methods reproduce the slight pucker (Figure 3). Table 1 compares some torsional angles for the PTL and PTE structures generated by both approaches. A cursory examination of these parameters reveals that in contrast to the NMR data, molecular modeling predicts essentially identical geometries for the PTE and PTL bicyclic framework. This discrepancy can be described by the inherent differences and limitations

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(12) The monomers **17** and **18** exist as 7: 3 mixture of trans/cis amide bond rotamers, respectively.

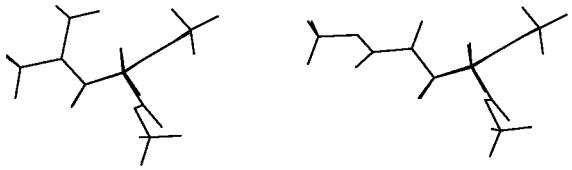

Figure 3. Edge-on view of Ac-PTL-OMe and Ac-PTE(Me)-OMe showing the slight puckering of the flattened boat conformation.

Table 1. Comparison of Some Ac-PTE(Me)-OMe Which Were Geometry Optimized (MMX force field and by ab initio calculations)

	ϕ_{ai}	ϕ_{MMX}	$\chi1_{ai}$	$\chi1_{MMX}$	$\chi2_{ai}$	$\chi2_{MMX}$	$\chi2'_{ai}$	$\chi2'_{MMX}$
PTE[2]	−71.9	−68.7	−55.4	−56.9	−158	−155	–	–
PTL[2]	−71.1	−67.7	−57.0	−59.9	−161	−159	−16.9	−14.9

[a] Torsional angles are in deg.

of NMR and molecular modeling methods. For example, since molecular modeling provides single point minimum energy conformations, it is feasible that the PTL and PTE frameworks have additional local minima which differ in the degree of the pucker. It is thus possible that the geometry optimizations converged on the same conformation and missed the other minima of minimum. However, considering the inherent rigidity of the system, it seems unlikely that there are additional minima that vary in the degree of the pucker. We believe that a more plausible explanation reflects the inherent property that NMR data are time averaged. With this in mind, we are of the opinion that the discrepancy between the NMR and molecular modeling results reflects a different time averaged behavior for PTL and PTE in which the C7-methyl hinders PTL motion toward a more prominent boat conformation. Since the distance between the PTL C7-hydrogen (closest to nitrogen) and N3 is ∼2.5 Å it easy to extrapolate that motion toward a more prominent pucker is less favored for PTL than for PTE and that this different time averaged behavior is reflected in the NMR data. The minima, however, appear to be nearly identical. Further examination of the dihedral angles reveals that the 3-azabicyclo[3.1.0]hexane system serves well to mimic the desired dihedral angles with $\phi \sim -70°$, $\chi1 \sim -57°$ (gauche(−)) and $\chi2 \sim -158°$ (trans). It should be noted that the greater p-character in the cyclopropane bonds do help to approximate the "trans" geometry about the βC−γC bond as evidenced in that a $\chi2$ value $\sim -158°$ comes reasonably close to − 180°. We also explored how the cyclopropane influences the angle $\chi3$ as this would be critical in PTAAs with longer side chains (for example PTR **7**). For PTR **7** there are three minima found at $\chi3$ values of −37°, 85°, and −152° with relative energies 0.88, 0.0, and 0.0 kcal/mol, respectively. The minima are at shallow potential energy wells so that deviations of $\chi3 \pm 20°$ from the minima result in an increase ∼ 0.4 kcal/mol. As such, the three minima are similar to the gauche(−), gauche(+), and trans values expected for the natural amino acid. To evaluate how well our PTAAs mimic the desired geometry, overlays of Leu/PTL, Glu/PTE, and Arg/PTR were generated. The overlays (Figure 4) show that indeed the PTAAs fit remarkably well with the natural amino acids ($RMS_{Leu/PTL} = 0.30$ Å, $RMS_{Glu/PTE} = 0.16$ Å, $RMS_{Arg/PTR} = 0.12$ Å).[9,10]

Conformation of PTL Oligomers in $CDCl_3$. The conformational behavior of the PTL dimer **19** and trimer **20** was next studied in $CHCl_3$. We previously have argued

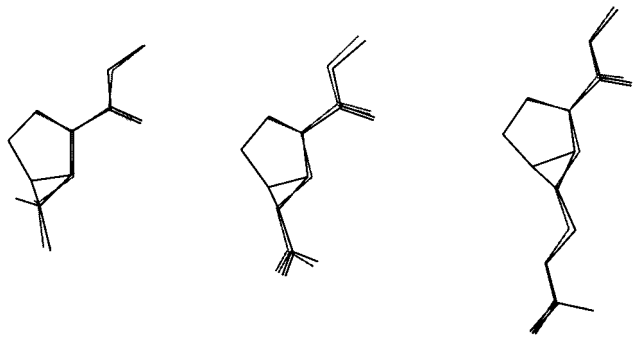

Figure 4. Overlays of Leu/PTL, Glu/PTE, and Arg/PTR. Dark bonds denote the PTAAs.

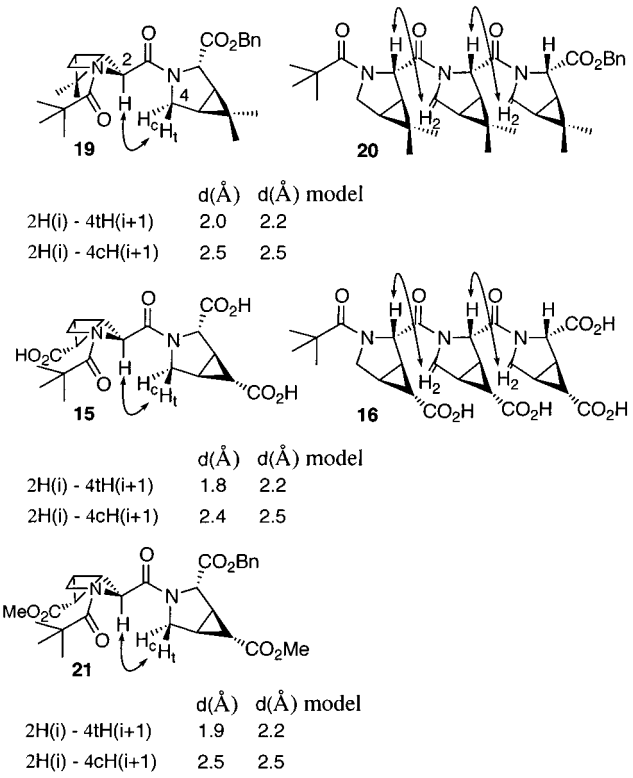

Figure 5. Selected NOEs and theoretical and experimental distances for oligomeric PTAAs.

that the minimization of pseudo A(1,3)-strain between adjacent prolines can define $\psi \sim 145°$ as required in PPII helices. Geometry optimization of dimer **19** (Piv-PTL-PTL-OBn) affords a ψ value = 131°, consistent with the conformational analysis prediction. For Piv-PTL-PTL-OBn the minimization of pseudo A(1,3)-strain should place 2H(*i*) in close spatial relationship to 4*c*H(*i* + 1) and 4*t*H(*i* + 1) when the PTAA−PTAA amide bond is in the trans conformation (Figure 5).[13,14] Indeed molecular modeling provides a 2H(*i*)−4*c*H(*i* + 1) distance = 2.5 Å and a 2H(*i*)−4*t*H(*i* + 1) distance = 2.2 Å. More significantly, for Piv-PTL-PTL-OBn the 2H(*i*)−4*c*H(*i* + 1) distance and the 2H(*i*)−4*t*H(*i* + 1) distance calculated from the NOESY cross-peak volumes are in excellent agreement at 2.5 and 2.0 Å, respectively. These data indicate that a unit as small as a dimer is conformationally constrained to the ϕ/ψ angles of a PPII helix. It

(13) All oligomeric PTAAs (**15**, **16**, **19**, **20**) exhibited only the presence of trans amide bond rotamer.

(14) The designation (*i*) denotes the N-terminal residue.

should be noted that the unequal 2H(i)−4cH(i + 1)/2H(i)−4tH(i + 1) distances are due to the flattened boat conformation and that both residues populate the flattened boat conformation as evidenced by $J_{5H(i)-4cH(i)} = J_{5H(i+1)-4cH(i+1)} = 5.2$ Hz (consistent with $J_{4cH-5H} = 5.2$ Hz for Ac-PTL-OBn). The trimer Piv-PTL-PTL-PTL-OBn is an important compound for our studies since three residues define one turn of a PPII helix. A NOESY spectrum of trimer **20** also reveals the key NOEs between 2H(i)−4H(i + 1) and 2H(i + 1)−4H(i + 2). A statement about the distances and relative NOE intensities between 2H(i)−4cH(i + 1) and 2H(i)−4tH(i + 1) cannot be made in this case because 4cH(i + 1) and 4tH(i + 1) are not resolved. However, since these NOEs are the strongest inter-PTAA NOEs it may be stated that qualitatively trimer **20** preferentially populates a conformation in which $\psi \sim 131°$. It should also be noted that the three residues maintain the flattened boat conformation as evidenced by 5H-4cH coupling constants which are 5.0, 5.2, and 5.2 Hz for the i, i + 1, and i + 2 PTLs, respectively.

Conformation of PTE Oligomers in D_2O. With the establishment that PTL oligomers populate the PPII conformation in solution, we next investigated the conformational behavior of the PTE oligomers in D_2O. Geometry optimization of dimer **15** (Piv-PTE-PTE-OH) also afforded a ψ value = 131°. Furthermore, the optimized structure also exhibited the 2H(i)−4cH(i + 1) and 2H(i)−4tH(i + 1) distances as 2.5 Å and = 2.2 Å, respectively. For Piv-PTE-PTE-OH the 2H(i)−4cH(i + 1) and 2H(i)−4tH(i + 1) distances calculated from the NOESY cross-peak volumes are 2.4 and 1.8 Å respectively. We were somewhat surprised by the shorter 2H(i)−4tH(i + 1) distance. To determine whether this were a function of the PTAA or the solvent (D_2O), we used dimer **21** in $CDCl_3$ as a reference compound which is similar to Piv-PTE-PTE-OH in that it does not possess the C7 methyl as the PTL analogues do. The 2H(i)−4cH(i + 1) and 2H(i)−4tH(i + 1) distances for **21** calculated from the NOESY cross-peak volumes are 2.5 and 1.9 Å, respectively suggesting that the shorter 2H(i)−4tH(i + 1) distance is a function of the PTAA. We suspect that again this reflects the more "pronounced averaged" boat in the PTE framework vs the PTL framework. Coupling constants ($J_{5H(i)-4cH(i)} = J_{5H(i+1)-4cH(i+1)} = 3.9$ Hz) indicate the same conformational behavior for the bicyclic frameworks as is found for the monomer Ac−PTE(Me)-OBn. The PTE trimer also exhibits the 2H(i)−4cH(i + 1)/2H(i)−4tH(i + 1) and the 2H(i + 1)-4cH(i + 2)/2H(i + 1)-4tH(i + 2) NOEs with the 2H(i)−4tH(i + 1) and 2H(i + 1)-4tH(i + 2) NOEs of greater magnitude. Although overlap of the 4cH-4tH NOE cross-peaks does not allow a quantitative analysis of the proton distances, it is possible again to make a qualitative statement that the PTE trimer preferentially populates a conformation in which $\psi \sim 131°$. Furthermore, the bicyclic ring system in trimer **16** also populates a flattened boat conformation as evidenced by $J_{5H(i)-4cH(i)} = 3.8$ Hz $J_{5H(i+1)-4cH(i+1)} = 3.9$ Hz, $J_{5H(i+1)-4cH(i+1)} = 3.8$ Hz. The data provided from these studies provides compelling evidence that even in aqueous medium, dimeric and trimeric water soluble PPII mimics are constrained to the ϕ/ψ angles of a PPII helix.

Conclusions

Our studies show that PTAAs based on the 3-azabicyclo[3.1.0]hexane system possess torsional parameters $\phi \sim -70°$, $\chi 1 \sim -57°$, and $\chi 2 \sim -158°$. Molecular modeling estimates that oligomeric PTAAs based on this ring system exhibit $\psi \sim 131°$. Solution studies of oligoPTLs in $CDCl_3$ and oligoPTEs in D_2O preferentially populate the PPII conformation. These PTAAs should prove useful for the synthesis of conformationally constrained PPII mimics which possess residues with $\chi 1 \sim$ gauche(−) and a $\chi 2 \sim$ trans. Work is ongoing to synthesize and evaluate PPII mimics for a number of pharmacologically relevant receptors.

Experimental Section

General. Unless otherwise specified, all reagents were purchased 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 or Ar atmosphere. Flash chromatography was carried out on Selecto silica gel (230−400 mesh). 1D and 2D NMR spectra were collected in $CDCl_3$ using standard pulse sequences provided by Bruker. NOESY spectra were obtained using a 2 s repetition delay and a 400 ms mixing time with TD1 = 4096 and TD2 = 512. NOESY cross-peak volumes were calculated by the method described by Prestegard.[15] Characterization data as well as ^1H and ^{13}C NMR spectra of the PTL series have been included as Supporting Information in ref 4. To avoid duplicity, these have not been included here.

Molecular Modeling. Structure building was performed with Chem 3D. The structure generated from Chem 3D was then subjected to geometry optimization with Gaussian 98 at the HF/6-31G* level. The Chem 3D structure was also geometry optimized with PCMODEL 7.0 using the MMX force field. The minimum energy value for ψ was obtained using the dihedral driver function of PCMODEL. For the Leu/PTL overlay, the following leucine values were used $\chi 1 = -60°$, $\chi 2 = 180°$, $\chi 2' = -60°$. Cα, Cβ, Cγ, and Cδ as well as the nitrogen and carbonyl carbon of leucine were chosen to be overlaid with the corresponding atoms on PTL. For the Glu/PTE overlay, the following glutamic acid values were used $\chi 1 = -60°$, $\chi 2 = 180°$. Cα, Cβ, Cγ, and Cδ as well as the nitrogen and carbonyl carbon of glutamic acid were chosen to be overlaid with the corresponding atoms on PTE. For the Arg/PTR overlay, the following Arg values were used $\chi 1 = -60°$, $\chi 2 = 180°$, $\chi 3 = 180°$ while for PTR, $\chi 3 = -152°$. Cα, Cβ, Cγ, and Cδ as well as the nitrogen and carbonyl carbon of arginine were chosen to be overlaid with the corresponding atoms on PTR.

Benzyl 1*R*,2*S*,5*S*,6*R*-3-Aza-3-*tert*-butyl(oxycarbonyl)-6-(methoxycarbonyl)bicyclo[3.1.0]hexane-2-carboxylate (9). A solution of diisopropylcarbodiimide (0.56 g, 4.4 mmol), acid **8** (0.84 g, 3.0 mmol), and DMAP (0.36 g, 3.0 mmol) in CH_2Cl_2 (6 mL) was maintained at 0 °C for 30 min. Benzyl alcohol (0.48 g, 4.4 mmol) was added, and the cooling bath was removed. After 12 h, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography (9:1 hexanes−EtOAc) affording the ester **9** as a colorless oil (0.91 g, 82%): $[\alpha]^{25}_D = -54.3°$ (c 0.12, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.23−7.35 (m, 5H), 5.19 (d, J = 12.5 Hz, 0.6H), 5.15 (d, J = 12.5 Hz, 0.6H), 5.10 (d, J = 12.2 Hz, 0.4H), 5.07 (d, J = 12.2 Hz, 0.4H), 4.49 (s, 0.4H), 4.35 (s, 0.6H), 3.67−3.69 (m, 0.4H), 3.62−3.64 (m, 0.6H), 3.60 (s, 3H), 3.59−3.57 (m, 0.6H), 3.54−3.56 (m, 0.4H), 2.15−2.18 (m, 0.6H), 2.12−2.14 (m, 0.4H), 2.05−2.07 (m, 0.6H), 2.02−2.04 (m, 0.4H), 1.58 (dd, app t, J = 3.1 Hz, 0.6H), 1.55 (dd, app t, J = 3.2 Hz, 0.4H), 1.39 (s, 3.6H), 1.27 (s, 5.4H); ^{13}C NMR (125 MHz, $CDCl_3$) 171.9, 170.6, 170.5, 154.3, 153.8, 135.3, 128.5, 128.4, 128.2, 128.0, 80.4, 66.9, 61.1, 60.7, 51.8, 48.0, 47.9, 29.5, 29.1, 28.3, 28.2, 28.0, 25.6, 24.9, 23.8, 23.6 ppm; IR (film) 1744, 1730, 1728, 1708, 1665, 1655 cm^{-1}; MS (CI) m/z 376 (MH). Anal. Calcd for $C_{20}H_{25}O_6N$: C, 63.98; H, 6.71; N, 3.73. Found: C, 63.76; H, 6.72; N, 3.64.

(15) Holak, T. A.; Scarsdale, J. N.; Prestegard, J. H. *J. Magn. Reson.* **1987**, *74*, 546.

Benzyl 1*R*,2*S*,5*S*,6*R*-3-Aza-3-(2,2-dimethylpropanoyl)-6-(methoxycarbonyl)bicyclo[3.1.0]hexane-2-carboxylate (10). The ester **9** (0.50 g, 1.3 mmol) was dissolved in a 1:1 mixture of TFA/CH_2Cl_2 (2 mL). After 30 min at room temperature the volatiles were removed under reduced pressure. The resulting oil was dissolved in CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated to afford the free amine (0.37 g) as a yellow oil which was used without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.26 (m, 5H), 5.14 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 3.81 (s, 1H), 3.60 (s, 3H), 3.19 (dd, J = 3.5, 10.1 Hz, 1H), 3.02 (d, J = 10.1 Hz, 1H), 2.23 (dd, J = 2.9, 6.7 Hz, 1H), 2.02−1.98 (m, 2H), 1.67 (dd, app t, J = 3.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.1, 172.8, 135.5, 128.5, 128.3, 128.1, 66.7, 61.2, 51.6, 47.4, 29.2, 26.4, 21.9 ppm; IR (film) 3405, 1738, 1730 cm^{-1}. The secondary amine from above was dissolved in dry CH_2Cl_2 (4 mL), the solution was cooled to 0 °C under argon, and Et_3N (0.41 g, 4.1 mmol) and $(CH_3)_3$-CCOCl (0.25 g, 2.1 mmol) were successively added. After 1 h, the mixture was washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were dried over Na_2-SO_4 and concentrated. Flash chromatography with 1.5−8.5 hexanes−EtOAc afforded the pivaloyl ester **17** (0.46 g, 95% overall) as a colorless oil: $[\alpha]^{25}_D$ = −47.5° (*c* 0.12, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28−7.23 (m, 5H), 5.20 (d, J = 12.2 Hz, 1H), 5.90 (d, J = 12.2 Hz, 1H), 4.78 (s, 1H), 4.00 (d, J = 10.3 Hz, 1H), 3.82−3.78 (m, 1H), 3.63 (s, 3H), 2.14−2.12 (m, 2H), 1.49−1.46 (m, 1H), 1.17 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) 177.6, 171.1, 170.4, 135.3, 128.5, 128.3, 128.1, 67.0, 62.3, 51.9, 49.1, 38.8, 27.1, 26.3, 23.2 ppm; IR (film) 1737, 1730, 1637 cm^{-1}; MS (CI) *m*/*z* 360 (MH). Anal. Calcd for $C_{20}H_{25}NO_5$: C, 66.83; H, 7.01; N, 3.89. Found: C, 66.65; H, 7.03; N,3.79.

1*R*,2*S*,5*S*,6*R*-3-Aza-3-(2,2-dimethylpropanoyl)-6-(methoxycarbonyl)bicyclo[3.1.0]hexane-2-carboxylic Acid (11). Pd−C (10%) (45 mg) was added to a solution of the amide **10** (0.45 g, 1.3 mmol) in EtOAc (6 mL). This mixture was stirred overnight under a balloon of hydrogen. The catalyst was removed by filtration through a Celite pad. The filtrate was concentrated, and the resulting pale solid was recrystallized from hexanes−EtOAc (4:1) to give the acid **11** (0.31 g, 92%) as a colorless solid: mp 165−167 °C; $[\alpha]^{25}_D$ = −38.9° (*c* 0.35, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45−9.55 (br s, 1H), 4.26 (s, 1H), 3.95 (d, J = 10.6 Hz, 1H), 3.81−3.87 (m, 1H), 3.61 (s, 3H), 2.31 (dd, J = 2.7, 7.1 Hz, 1H), 2.24−2.19 (m, 1H), 1.48 (dd, app t, J = 2.7 Hz, 1H), 1.19 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) 178.8, 173.7, 171.3, 62.4, 51.9, 49.2, 38.9, 27.0, 26.2, 25.8, 23.1 ppm; IR (KBr) 3203, 1737, 1730, 1651 cm^{-1}; MS (CI) *m*/*z* 270 (MH). Anal. Calcd for $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11; N, 5.20. Found: C, 57.74; H, 6.99; N, 5.14.

Synthesis of Dimer 13. The ester **9** (217 mg, 0.58 mmol) was dissolved in a 1:1 mixture TFA/CH_2Cl_2 (1 mL). After 30 min at room temperature, the volatiles were removed under reduced pressure. The resulting oil was dissolved in CH_2Cl_2 (10 mL) and washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 8 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated to afford the free amine (160 mg) as a yellow oil which was used without further purification. This amine was dissolved in dry CH_2Cl_2 (1 mL) and then added to a solution of DCC (120 mg, 0.58 mmol) and the acid **11** (157 mg, 0.58 mmol) in dry CH_2Cl_2 (2 mL). The mixture was stirred for 4 h under argon then washed with 1 N HCl (5 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated. Flash chromatography of the residue on silica gel eluting with 1:3 EtOAc−hexanes afforded the dimeric ester **13** (234 mg, 77%) as a colorless solid: mp 50−52 °C; $[\alpha]^{25}_D$ = −64.1° (*c* 0.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.23 (m, 5H), 5.22 (d, J = 12.2 Hz, 1H), 5.08 (d, J = 12.2 Hz, 1H), 4.82 (s, 1H), 4.69 (s, 1H), 4.11 (d, J = 9.9 Hz, 1H), 3.99−3.96 (m, 2H), 3.87 (dd, J = 3.7, 9.9 Hz, 1H), 3.64 (s, 3H), 3.63 (s, 3H), 2.24−2.15 (m, 3H), 2.09 (dd, J = 3.0, 7.4 Hz, 1H), 1.72 (dd, app t, J = 3.1 Hz, 1H), 1.46 (dd, app t, J = 3.1 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) 177.5, 171.7, 171.5, 170.3, 169.6, 135.0, 128.5, 128.3, 128.1, 67.3, 61.6, 60.5, 51.9, 51.8, 49.7, 48.2, 38.7, 27.4, 27.0, 25.8, 25.5, 23.5, 23.4 ppm; IR (KBr) 1371, 1730, 1673, 1666 cm^{-1}; MS (CI) *m*/*z* 527 (MH). Anal. Calcd for $C_{28}H_{34}N_2O_8$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.65; H, 6.49; N, 5.18.

Synthesis of Trimer 14. Pd−C (10%) (15 mg) was added to a solution of the dimeric ester **13** (134 mg, 0.25 mmol) in EtOAc (3 mL). This mixture was stirred overnight under a balloon of hydrogen. The catalyst was removed by filtration over a Celite pad. The filtrate was concentrated, and the resulting pale solid was crystallized from hexanes−EtOAc (4:1) to give the acid (85 mg, 78%) as a colorless solid: mp 127−129 °C; $[\alpha]^{25}_D$ = −86.4 (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15−7.96 (br s, 1H), 4.78 (s, 1H), 4.71 (s, 1H), 4.13 (d, J = 10.0 Hz, 1H), 4.02−3.96 (m, 2H), 3.88 (dd, J = 4.1, 10.9 Hz, 1H), 3.65 (s, 6H), 2.35 (dd, J = 3.0, 7.1 Hz, 1H), 2.30−2.27 (m, 1H), 2.24−2.21 (m, 1H), 2.11 (dd, J = 2.9, 7.3 Hz, 1H), 1.72 (dd, app t, J = 3.0 Hz, 1H), 1.48 (dd, app t, J = 3.0 Hz, 1H),1.18 (s, 9H); ^{13} C NMR (125 MHz, $CDCl_3$) 177.8, 171.9, 171.6, 170.9, 61.7, 60.7, 51.9, 49.8, 48.3, 47.9, 38.8, 27.3, 27.2, 27.0, 25.8, 25.6, 23.5, 23.4 ppm; IR (KBr) 3174, 1737, 1730, 1673, 1666 cm^{-1}; HRMS (CI) *m*/*z* 437.1923 (437.1927 calcd for $C_{21}H_{29}N_2O_8$, MH). The ester **9** (45 mg, 0.12 mmol) was dissolved in a 1:1 mixture TFA/CH_2Cl_2 (0.5 mL). After 30 min, the volatiles were removed under reduced pressure, and the resulting oil was dissolved in CH_2Cl_2 (5 mL) and then washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated to afford the free amine (33 mg) as a yellow oil which was used without further purification. This amine was dissolved in dry CH_2Cl_2 (0.5 mL) and then added to a solution of DCC (25 mg, 0.12 mmol) and the acid obtained above (52 mg, 0.12 mmol) in dry CH_2Cl_2 (1 mL). The mixture was maintained overnight under argon then washed with HCl (1 N). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic layers were dried over Na_2SO_4 and then concentrated. Flash chromatography of the residue on silica gel eluting with 1:1 EtOAc−hexanes afforded the trimeric ester **14** (75 mg, 91%) as a white solid: mp 114−116 °C; $[\alpha]^{25}_D$ = −75.8 (*c* 0.58, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.29 (m, 5H), 5.23 (d, J = 12.2 Hz, 1H), 5.08 (d, J = 12.2 Hz, 1H), 4.80 (s, 1H), 4.74 (s, 1H), 4.66 (s, 1H), 4.06 (d, J = 9.9 Hz, 1H), 4.01 (d, J = 10.0 Hz, 1H), 3.95−3.92 (m, 3H), 3.85 (dd, J = 3.9, 9.9 Hz, 1H), 3.64 (s, 3H), 3.63 (s, 3H), 3.62 (s, 3H), 2.26−2.23 (m, 1H), 2.21 (dd, J = 3.0, 7.1 Hz, 1H), 2.21−2.16 (m, 2H), 2.14−2.11 (m, 2H), 1.69−1.67 (m, 2H), 1.42 (dd, app t, J = 3.1 Hz, 1H), 1.69 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) 177.4, 171.6, 171.4, 170.1, 169.5, 169.2, 134.9, 128.9, 128.4, 128.2, 67.4, 61.8, 60.7, 60.1, 51.9, 51.8, 49.7, 48.7, 48.1, 38.7, 29.6, 27.3, 27.1, 26.7, 26.1, 25.7, 25.5, 23.7, 23.6, 23.5 ppm; IR (KBr) 1741, 1737, 1732, 1728, 1672, 1655, 1651 cm^{-1}; MS (CI) *m*/*z* 694 (MH). Anal. Calcd for $C_{36}H_{43}N_3O_{11}$: C, 62.33; H, 6.23; N, 6.06. Found: C, 62.47; H, 6.35; N, 5.93.

Synthesis of Dimer 15. The dimeric ester **13** (80 mg, 0.15 mmol) was dissolved in THF (1 mL) and then added to a 1 M solution of lithium hydroxide (2 mL). After 4 h at room temperature, the solution was concentrated to a 1 mL volume under reduced pressure and passed over a DOWEX resin eluting with water to give a colorless solid. This crude compound was crystallized from 1:4 THF−hexanes to give the dimer **15** (58 mg, 93%) as a colorless powder: mp 135−137 °C; $[\alpha]^{25}_D$ = −47.5 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, D_2O) δ 4.73 (s, 1H), 4.61 (s, 1H), 4.10 (d, J = 10.8 Hz, 1H), 4.04 (d, J = 10.8 Hz, 1H), 3.92 (dd, J = 3.8, 10.8 Hz, 1H), 3.88 (dd, J = 4.1, 10.8 Hz, 1H), 2.35 (dd, J = 3.0, 7.1 Hz, 1H), 2.28−2.33 (m, 2H), 2.11 (dd, J = 2.9, 7.3 Hz, 1H), 1.60 (dd, app t, J = 3.0 Hz, 1H), 1.56 (dd, app t, J = 3.0 Hz, 1H), 1.10 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD) 179.6, 174.8, 172.6, 172.3, 63.8, 63.7, 62.1, 51.3, 39.9, 32.7, 28.6, 27.5, 26.7, 24.9, 24.7, 23.6, 23.5 ppm; FTIR (KBr) 3449, 3072, 1735, 1729, 1710, 1670, 1658, 1443, 1437 cm^{-1}; HRMS (CI) *m*/*z* 409.1614 (409.1611 calcd for $C_{19}H_{25}N_2O_8$, MH).

Synthesis of Trimer 16. The ester **14** (61 mg, 0.09 mmol) was dissolved in THF (1 mL) and then added to a 1 M solution of LiOH (1.5 mL). After 4 h at room temperature, the solution was concentrated to a 1 mL volume under reduced pressure and passed over a DOWEX resin eluting with water to give a colorless solid. This crude compound was crystallized from 1:4 THF−hexanes to give the trimer **16** (51 mg, 83%) as a colorless powder: mp 165−167 °C; $[\alpha]^{25}_D = -52.8$ (*c* 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, D_2O) δ 4.78 (s, 1H), 4.71 (s, 1H), 4.62 (s, 1H), 4.08 (d, *J* = 10.1 Hz, 1H), 4.05 (d, *J* = 11.0 Hz, 1H), 4.01 (d, *J* = 11.0 Hz, 1H), 3.91 (dd, *J* = 3.9, 11.0 Hz, 2H), 3.87 (dd, *J* = 4.1, 10.9 Hz, 1H), 2.35−2.32 (m, 3H), 2.30 (dd, *J* = 3.6, 7.7 Hz, 1H), 2.23 (dd, *J* = 3.0, 7.3 Hz, 1H), 2.05 (dd, *J* = 2.9, 7.3 Hz, 1H), 1.65 (dd, app t, *J* = 3.0 Hz, 1H), 1.59 (dd, app t, *J* = 3.0 Hz, 1H), 1.53 (dd, app t, *J* = 3.0 Hz, 1H), 1.04 (s, 9H). ^{13}C NMR (125 MHz, CD_3OD) 179.8, 174.8, 174.7, 172.9, 172.2, 171.2, 63.8, 62.4, 62.1, 62.0, 51.3, 50.2, 49.8, 39.9, 30.7, 28.5, 28.4, 28.0, 27.5, 26.7, 25.2, 25.0, 24.7 ppm; IR (KBr) 3416, 3085, 2858, 1735, 1729, 1722, 1703, 1670, 1665, 1657, 1463, 1430 cm^{-1}; HRMS (CI) *m*/*z* 562.2036 (562.2037 calcd for $C_{26}H_{32}N_3O_{11}$, MH).

Benzyl 1*R*,2*S*,5*S*,6*R*-3-Aza-3-(ethanoyl)-6-(methoxycarbonyl)bicyclo[3.1.0]hexane-2-carboxylate (18). The amine **12** (40 mg, 0.14 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and then cooled to 0 °C. Et_3N (44 mg, 0.44 mmol) and AcCl (23 mg, 0.29 mmol) were added to the mixture. After 30 min at 0 °C, the solution was diluted with CH_2Cl_2 (10 mL) and washed with 1 N HCl (3 mL). The organic layer was dried over Na_2SO_4 and then concentrated. Flash chromatography of the residue on silica gel eluting with 7:3 EtOAc−hexanes afforded the amide **18** (45 mg, 98%) as a colorless oil: $[\alpha]^{25}_D = -54.1$ (*c* 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39−4.33 (m, 5H), 5.22−5.20 (m, 1.3H), 5.16 (d, *J* = 12.3 Hz, 0.7H), 4.77 (s, 0.7H), 4.46 (s, 0.3H), 3.99 (d, *J* = 12.0 Hz, 0.3H), 3.86 (dd, *J* = 3.9, 10.1 Hz, 0.7H), 3.69−3.66 (m, 3.7H), 3.56 (dd, *J* = 4.1, 12.0 Hz, 0.3H), 2.35 (dd, *J* = 3.0, 7.1 Hz, 0.3H), 2.24 (dd, *J* = 3.0, 7.1 Hz, 0.7H), 2.21−2.17 (m, 0.7H), 2.16−2.12 (m, 0.3H), 2.04 (s, 2.1H), 1.91 (s, 0.9H), 1.58 (dd, app t, *J* = 3.1 Hz, 0.7H), 1.54 (dd, app t, *J* = 3.1 Hz, 0.3H); ^{13}C NMR (125 MHz, $CDCl_3$) 171.6, 170.1, 170.0, 169.8, 169.6, 135.2, 134.8, 128.7, 128.5, 128.3, 128.2, 128.0, 67.6, 67.2, 62.0, 60.3, 51.9, 49.2, 47.5, 29.6, 29.2, 27.9, 25.6, 24.2, 23.8, 23.6, 22.2 ppm; IR (film) 1744, 1737, 1666, 1659 cm^{-1}; MS (CI) *m*/*z* 318 (MH). Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.96; H, 6.03; N, 4.41. Found: C, 64.77; H, 6.08; N, 4.25.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, and **18**, and NOESY spectra of **13**, **15**, **16**, and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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