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Sequence-Specific Unusual (1→2)-Type Helical Turns in α/β-Hybrid Peptides

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Abstract: This article describes novel conformationally ordered α/β-hybrid peptides consisting of repeating L-proline-anthranilic acid building blocks. These oligomers adopt a compact, right-handed helical architecture determined by the intrinsic conformational preferences of the individual amino acid residues. The striking feature of these oligomers is their ability to display an unusual periodic *pseudo* β-turn network of nine-membered hydrogen-bonded rings formed in the forward direction of the sequence by 1→2 amino acid interactions both in solid-state and in solution. Conformational investigations of several of these oligomers by single-crystal X-ray diffraction, solution-state NMR, and *ab initio* MO theory suggest that the characteristic steric and dihedral angle restraints exerted by proline are essential for stabilizing the unusual *pseudo* β-turn network found in these oligomers. Replacing proline by the conformationally flexible analogue alanine (Ala) or by the conformationally more constrained α-amino isobutyric acid (Aib) had an adverse effect on the stabilization of this structural architecture. These findings increase the potential to design novel secondary structure elements profiting from the steric and dihedral angle constraints of the amino acid constituents and help to augment the conformational space available for synthetic oligomer design with diverse backbone structures.

Introduction

In the course of mimicking nature's mystifying events, the bottom-up approach involving the design and synthesis of non-natural oligomers with well-defined conformations has attained an extensive impact in diverse fields of research.[1,2] These unnatural oligomers are able to mimic many secondary structural elements of native peptides and proteins, such as helices,[3] sheets,[4] turns,[5] etc., by secondary interactions, especially hydrogen bonding.[6] Recently, attempts to mimic more complex tertiary and quaternary structures of the biomachineries with non-natural analogues have yielded fruitful results, albeit to a limited extent, which would increase our understanding about the intrinsic relationship between sequence, conformation, and functions of biomacromolecules.[7]

The role of steric and conformational bias of individual amino acids in dictating the structural architecture of biomacromolecules has long been a subject of intense research interest.[8] The pioneer research of G. N. Ramachandran highlighted the importance of dihedral angle constraints of the α-amino acids [N−C^{α} (ϕ) and C^{α}−CO (ψ)] in correlating their secondary structural preferences in polypeptides and proteins.[9] The distribution of the ϕ, ψ angles of α-amino acids, as given by the Ramachandran plot, provides a rational basis for describing all stereochemically possible structures of polypeptides.[9,10] Whereas the sterically least demanding α-amino acid glycine

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is a welcome candidate in all four quadrants of the Ramachandran plot and occurs in various secondary structure elements, as suggested from its flexible dihedral angle tolerance,[11] the distinctive cyclic structure imparts proline an exceptional conformational rigidity with a restricted N−C$^{\alpha}$ torsion angle ϕ of about −60° allowing it to seek spaces in the "turn" regions of proteins.[12] Although it is possible to predict the conformation of polypeptides to a reasonable degree of accuracy[13] based on the well-documented conformational bias of the α-amino acids using advanced computational tools, the lack of adequate knowledge about the conformational propensities of the vast pool of unnatural amino acids makes comparable procedures troublesome.[14] This is particularly true in the case of hybrid oligomers containing natural and unnatural amino acid residues, wherein the overall conformation is often unpredictable.[4g]

In this article, we describe synthetic hybrid oligomers containing sequentially repeating L-proline (Pro) and anthranilic acid (Ant) residues, which belong to the general category of α/β-hybrid peptides.[15−18] The investigation of α/β-,[15a,b] α/γ-, and β/γ-[15c] hybrid peptides and hetero γ- peptides[15d] led to the discovery of various ordered secondary structures, the latter class showing interesting nine-helical structure as confirmed by extensive NMR studies. Some of the α/β-hybrid peptides have been shown to possess biological activity.[18] Influenced by the rigid aromatic backbone, homo oligomers of anthranilic acid form extended sheet-like structures, as disclosed by Hamilton's group.[19] Now, it might be interesting to study the mutual influence of the conformational constraints of two different amino acid constituents, L-proline and anthranilic acid, on the secondary structure formation in repeating dimer units, which could generate completely novel secondary structure elements. This might augment the conformational space available for synthetic oligomer design by taking profit from the steric and dihedral angle constraints of the individual amino acid residues. Furthermore, the enormous possibility of incorporating functional side chains on the Ant and Pro residues[20] would render their structural modification easy which may enable modulating

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their structural features for possible applications involving protein recognition.[21]

Results and Discussion

Synthesis. The Pro-Ant oligomers **2**, **3** were assembled starting from the BOC-Pro-Ant-OMe building block **1a**. Efforts to synthesize the reverse sequence [(Ant-Pro)$_n$] employing the "reversed building blocks" as the starting point did not succeed since Ant-Pro esters were highly vulnerable to cyclization yielding cycloanthranilylproline,[22] apparently due to the closer positioning of the amino and ester termini of the building block. However, introduction of Ant at the N-terminal could be undertaken at a later stage as demonstrated in the case of **2e**. Thus, starting from the dipeptide Boc-Pro-Ant-OMe **1a**, we obtained the higher oligomers **2** and **3** following a "segment doubling strategy" (Scheme 1).[23] Coupling of dipeptide acid **1b** and the corresponding amine hydrochloride **1c**, using TBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate) as a coupling agent and DIEA (*N*,*N*-diisopropylethylamine) as the base, afforded the tetrapeptide **2a** in 78% yield after chromatographic purification. Similarly, the octapeptide **3a** was prepared by coupling the tetrapeptide acid **2b** with the corresponding amine hydrochloride **2c**. To have an oligomer with Ant at the N-terminal, amine hydrochloride **2c** was coupled with Boc-Ant-OH to furnish the pentapeptide **2e**. The pivaloyl substituted analogues were made by reacting the corresponding free amines with excess pivaloyl chloride in the presence of triethyl amine as a base.

Crystal Structure Investigations. Extensive efforts to crystallize the oligomers resulted in crystals of **2b**. Analysis of the X-ray diffraction data revealed the presence of an unusual nine-membered hydrogen-bonded ring connecting the amide NH (N2) of the Ant2 residue and the carbonyl (O5) of the Pro3 residue (Figure 1). Despite formal similarity, there are striking differences between this pseudo β-turn and the β-turns found in native peptides. Whereas β-turns consisting of 10-membered hydrogen-bonded rings are formed in the backward direction of the sequence between the amino acids ($i + 3$) and i (4→1 interaction),[24,25] the C9 turn in **2b** is closed by hydrogen bonding in the forward direction within the dipeptide unit (1→2 interaction). Reverse β-turns realized *via* four amino acids are characterized by the ϕ, ψ dihedral angles of the two central amino acid residues in the turn region ($i + 1$) and ($i + 2$).[24,25] The two amino acids Ant and L-Pro forming the C9 turn in **2b** have the following dihedral angles: $\phi_{Ant2} = -166°$, $\theta = 3°$, $\psi_{Ant2} = -102°$ and $\phi_{Pro3} = -64°$, $\psi_{Pro3} = +170°$, respectively, wherein the Pro dihedral angles are typical of the poly proline II semiextended conformation.[24,25]

Surprisingly, the formation of a C9 turn in **2b** was at the cost of the S(6)-type[27] six-membered ring H-bonding (N2...O4,

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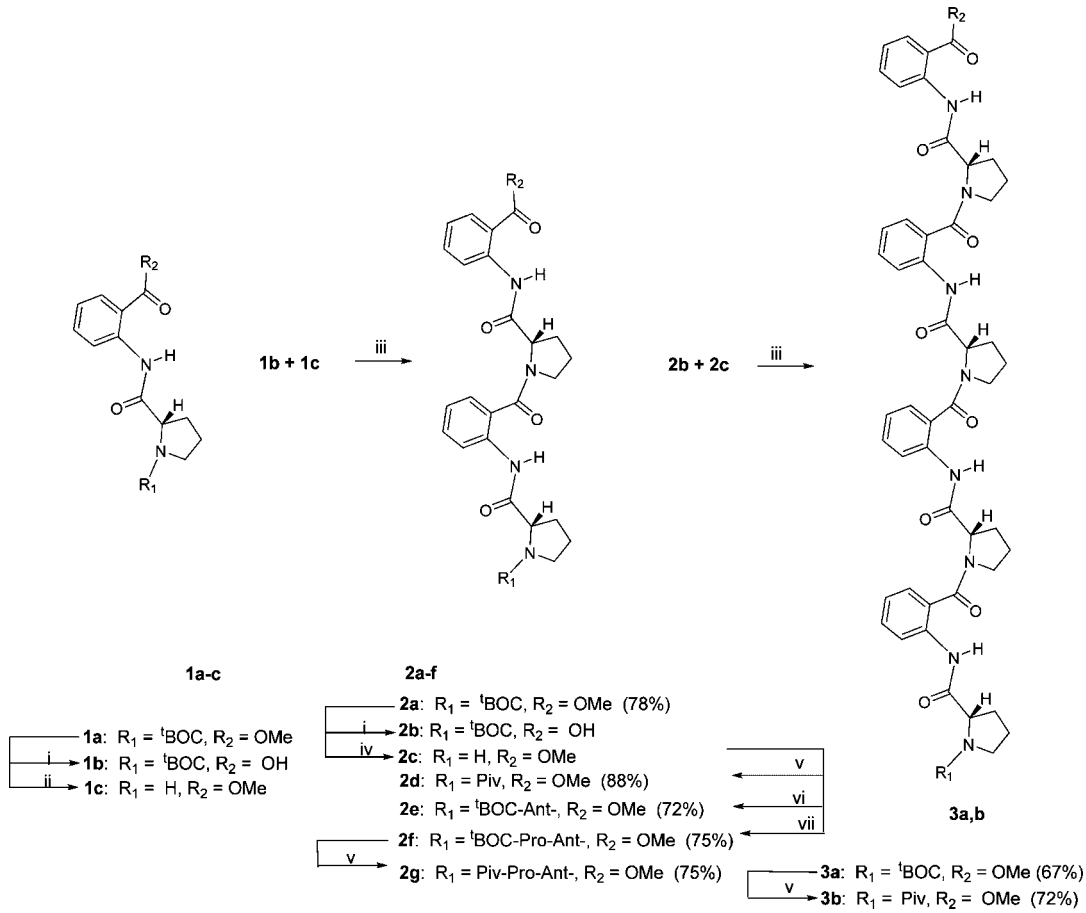
Scheme 1. Synthesis of Oligomers **2** and **3**[a]



[a] Reagents and conditions: (i) LiOH, MeOH, rt, 12 h; (ii) dry HCl (gas), ether, 0°C, 10 min; (iii) TBTU, DIEA, MeCN, rt, 12 h. (iv) TFA, DCM, rt, 1 h. (v) Piv-Cl, Et_3N, dry DCM, rt, 4 h. (vi) Boc-Ant-OH, TBTU, DIEA, MeCN, rt, 12 h. (vii) **1b**, HBTU, DIEA, MeCN, rt, 12 h.

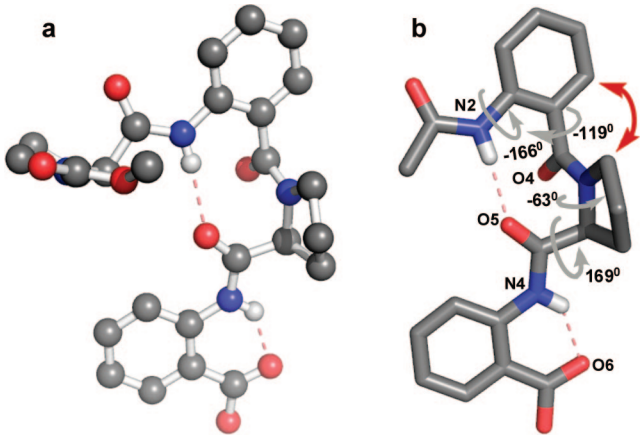


Figure 1. Crystal structure of **2b**.[26] (a) Crystal structure in ball and stick representation (without *tert*-butyl group). (b) Annotated structure (selected region) in tube representation. Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity.

Figure 1b) which is, otherwise, almost always observed in *N*-acyl anthranilamides.[19,28] Apparently, the steric clash between the Ant2 aryl ring and the five-membered proline ring (Pro3),[29]marked by a double headed arrow in Figure 1b, enforces the H-bonding sites (Ant2 amide NH and Pro3 amide CO) to come closer together resulting in the formation of a stronger hydrogen bond in the C9 turn. The H-bond geometry of the C9 turn is characterized by the bond distances d(N...O) = 2.9 Å and d(H...O) = 2.1 Å, the bond angle (N—H...O) = 168°, and the torsion angle (N—H...O═C) = −150°. A consequence of this interaction is the weakening of the S(6)-type[27] hydrogen bonding in Ant2[19,28] (N2...O4) as documented by the H-bond distances d(N...O) = 3.1 Å and d(H...O) = 2.7 Å, the bond angle (N—H...O) = 112°, and the torsion angle (N—H...O═C) = 84°. In contrast, the C-terminal Ant4 residue in **2b** exhibits the perfect geometry for S(6)-type[27] hydrogen bonding with the bond distances d(N...O) = 2.6 Å and d(H...O) = 1.9 Å, the bond angle (N—H...O) = 138°, and the hydrogen bond torsion angle (N—H...O═C) = 5° usually found in N-acylated anthranilamides.[19,28] It is noteworthy that the close proximity of the Ant2 amide NH and the Pro3 carbonyl groups, documented by a distance of d(N2...O5) = 2.9 Å in the crystal structure of **2b**, explains why Ant-Pro-esters (free amines) show

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(26) Crystal structure was solved by direct method and refined by full matrix least squares on F^2 for all data using SHELXTL software (Ver. 6.14, Bruker-AXS, 2003). The hydrogen atom of hydroxy group was located on the difference map and refined isotropically. Other hydrogen atoms were refined in the riding mode. CIF files are available in the Supporting Information.

(27) For the application of graph notation to hydrogen bonding motifs, see: Etter, M. C. *Acc. Chem. Res.* **1990**, *23*, 120–126.

a high tendency to undergo cyclization yielding cycloanthranilylproline.[22]

Conformational Investigation of Oligomers 2 and 3 in Solution. To investigate the solution-state conformation, we performed a detailed NMR study of the oligomers in solution ($CDCl_3/CD_2Cl_2$, 500 MHz). The oligomers **2** and **3** were readily soluble in nonpolar organic solvents (≫100 mM in $CDCl_3$) at ambient temperature suggesting that the polar hydrogen-bonding groups are strongly solvent-shielded, preventing the formation of polymeric aggregates.[30] Inspection of the ^{1}H spectra of the N-BOC oligomers **2a,f** and **3a** (Supporting Information (SI), pp S20, S23, and S26, respectively) revealed *cis*−*trans* isomerization at the N-terminal carbamate carbonyls, a well-known feature in polypeptides with an N-terminal Pro residue,[31] presumably caused by the free rotation of the amide bond connecting the pyrrolidine ring and the ^{t}BOC group. However, the *cis*−*trans* isomerism could be effectively arrested by capping their N-terminal Pro residues with a pivaloyl group (Piv),[8e] as demonstrated in the case of the tetrapeptide **2d**, hexapeptide **2g**, and the octapeptide **3b**. The pivaloyl N-capped oligomers show a single set of sharp signals in their ^{1}H spectra (SI, pp S21, S23, and S26, respectively). The *cis*−*trans* isomerism could also be effectively arrested by the introduction of an Ant residue at the N-terminal, as demonstrated in the case of **2e**, which gives rise to a well-dispersed single set of sharp signals in its NMR spectrum (SI, p S21). The (1→2)-type nine-membered-ring pseudo β-turn, as observed in the solid-state structure of **2b**, was unambiguously confirmed in the solution state as well by the observed characteristic dipolar couplings (NOEs) from the 2D NOESY NMR spectrum of **2e** (Figure 2).

Analysis of the crystal structure of **2b** suggested that the most characteristic NOE to support the (1→2)-type nine-membered hydrogen-bonded turn conformation would be a diagnostic long-range inter-residual dipolar coupling between αH of Pro1 and the aromatic protons of Ant4 (Pro1-αH vs Ant4-C24H, see Figure S26 on page, S81 in the SI). Although these residues are separated by two amino acids, the peculiar nine-membered ring pseudo β-turn conformation requires the peptide backbone to fold back significantly resulting in the short distance, as evident from the crystal structure of **2b** [distance d(α1H−C24H) = 2.47 Å, see Figure S26 on p S81 in the SI]. The analysis of the 2D NOESY data of **2e** indeed revealed the existence of an inter-residual NOE between αH of Pro2 residue and C31 Ar−H of the Ant5 residue (Figure 2b) suggesting the prevalence of a pseudo β-turn structure involving Ant3 and Pro4 residues. The αH of Pro2 is found to have NOE with the amide NH of Ant1 (Figure 2b). Long-range inter-residual NOEs were also observed between the β-protons of Pro2 and C31 Ar−H of the Ant5 residue (Figure 2d). Further expansion of the Figure 2d in various modes and comparison with its 2D COSY clearly confirm that it is the β'1H (of the Pro2 residue) that gives long-range dipolar coupling with the C31 Ar−H (2D excerpts in the SI, p S77, Figure S20). It is noteworthy that the observed strong NOEs between Pro α-H and the amide NH of the subsequent Ant residues (α1H vs NH2, α2H vs NH3, Figure 2a) are clearly indicating their proximity, which was again revealed in the crystal structure of **2b**. Other selected inter-residual NOEs are between the Pro β-protons and the amide NHs of the subsequent Ant residues (β1H vs NH2, β2H vs NH3, Figure 2c) and between the Pro δ-protons and the amide NHs of the preceding Ant residues (δ1H vs NH1, δ2H vs NH2, Figure 2e). The Pro δ-protons also showed dipolar coupling to their respective preceding aromatic protons (δ1H vs C4H, δ2H vs C16H, Figure 2f), since the folded conformation required their closer positioning, as indicated by the crystal structure of **2b**. Since **2e** lacks a preceding Pro residue at the N-terminal, the diagnostic long-range NOE required for confirming the C9 turn at the N-terminal (Pro α-H vs Ar−H; both separated by two residues) could not be observed, and therefore, the conformation of **2e** at the N-terminal involving Ant1 and Pro2 could not be ascertained. Analogous dipolar coupling patterns were observed in the oligomers **2a** and **3a** wherein the N-terminal was capped with the ^{t}BOC group (Figures S21 and S23, pp S78 and S79 in the SI) suggesting similar conformational features for these oligomers in the solution state, although rotamer formation was observed in these oligomers because of the *N*-terminal "free" Pro residues, as discussed above.[31] It is noteworthy that the pivaloyl N-capped oligomers, the tetrapeptide **2d**, and the hexapeptide **2g** displayed sharp signals in their ^{1}H spectra, and the diagnostic inter-residual long-range NOE essential for supporting the folded conformation, as previously discussed (Pro−α-H vs Ar−H separated by two residues), was clearly evident in their 2D NOESY spectra (SI, p S80, Figures S24, S25, respectively). Thus, this study also supports the utility of the pivaloyl group[8e] in arresting the *cis*−*trans* isomerization of N-terminal Pro residues containing carbamate protecting groups.

Solvent titration and dilution experiments are particularly useful for differentiating the nature of hydrogen bonding interactions (inter vs intra) in the solution state, wherein intermolecular hydrogen bonding interactions (solvent exposed NHs) are relatively more vulnerable to environmental effects.[32] Intramolecular hydrogen bonding interactions, on the contrary, are usually little affected by the environmental effects such as solvent dilution and solvent polarity, since the protons involved in such interactions are solvent shielded.[32] Further experimental support for the prevalence of intramolecular hydrogen bonding interactions in oligomers **2a**, **2e**, and **3a** came from DMSO titration and $CDCl_3$ dilution studies of the oligomers (Figure 3).

Notably, all the amide NHs of the oligomers showed negligible shifts (Δδ NH: <0.15 ppm) when solutions of **2a**,

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(29) (a) It is known that *trans*-Xaa-Pro peptide bonds cause steric interactions between the pyrrolidine ring δ-position and the preceding residue. For a relevant reference, see: Mikhailov, D.; Daragan, V. A.; Mayo, K. H. *Biophys. J.* **1995**, *68*, 1540–1550.

(30) (a) Compounds having solvent-exposed acidic protons usually show poor solubility in organic solvents due to the aggregation phenomenon. For instance, see: Mathias, J. P.; Simanek, E. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1994**, *116*, 4326–4340. (b) Damodaran, K.; Sanjayan, G. J.; Rajamohanan, P. R.; Ganapathy, S.; Ganesh, K. N. *Org. Lett.* **2001**, *3*, 1921–1924.

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(32) (a) For leading references, see: Gellman, S. H.; Dado, G.; Liang, G. B.; Adams, B. *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173. (b) Dado, G.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228–4245. (c) Sharma, G. V. M.; Manohar, V.; Dutta, S. K.; Subash, V.; Kunwar, A. C. *J. Org. Chem.* **2008**, *73*, 3689–3698.

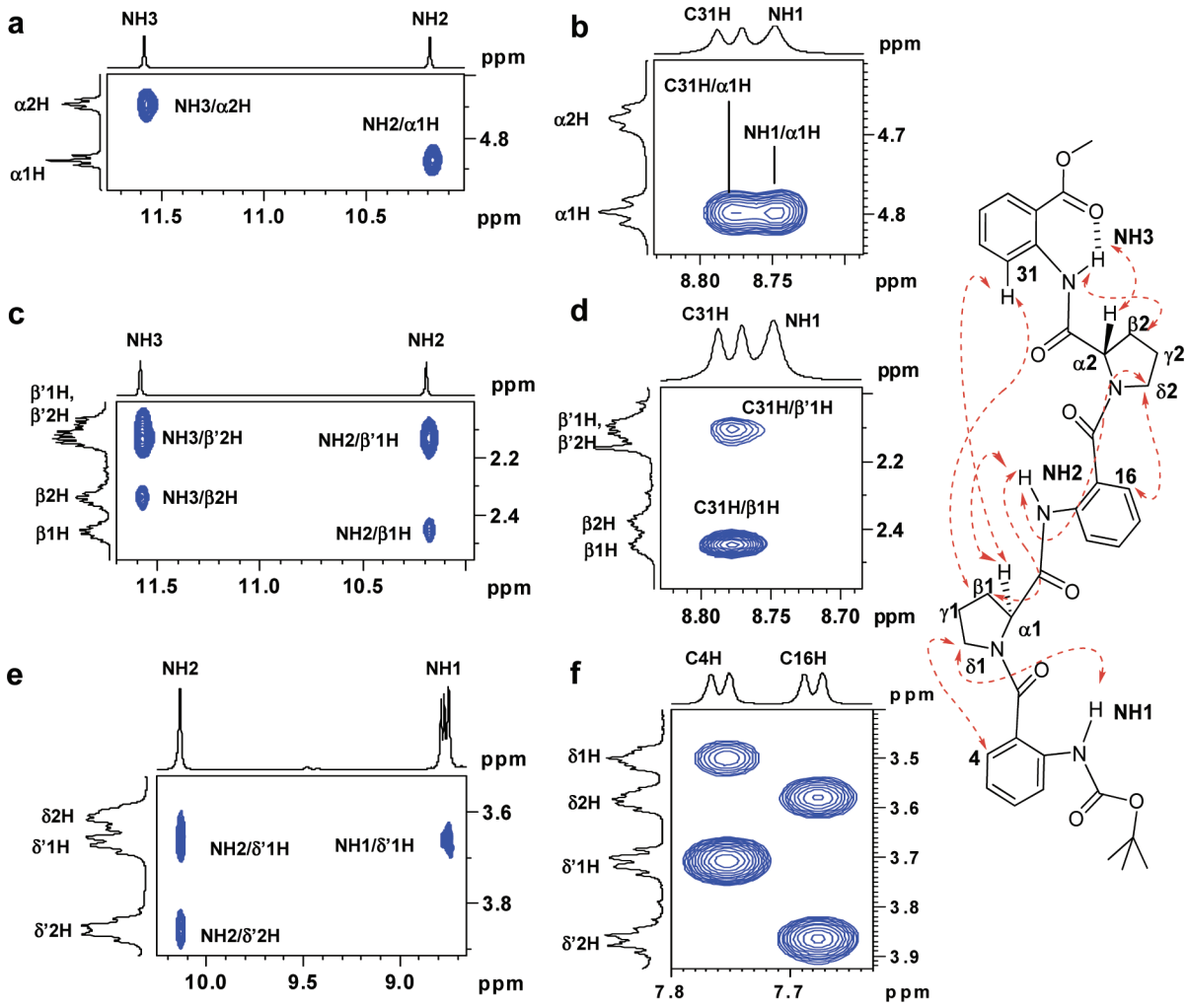

Figure 2. Selected NOE extracts from the 2D NOESY data of **2e** ($CDCl_3/CD_2Cl_2$, 500 MHz). (a) αH vs NH region ($CDCl_3$); (b) αH vs Ar−H region (CD_2Cl_2); (c) βH vs NH region ($CDCl_3$); (d) βH vs Ar−H region (CD_2Cl_2); (e) δH vs N−H region (CD_2Cl_2); (f) δH vs Ar−H region ($CDCl_3$). *Note*: For improved spectral dispersion in the aryl region, NMR studies were performed in CD_2Cl_2.

2e, and **3a** in $CDCl_3$ were titrated gradually with DMSO-d_6 (5 μL on each addition). The same trend was also observed in a solvent dilution study, as demonstrated in the case of the pentapeptide **2e** showing a marginal shift of $\Delta\delta$ (NH) < 0.1 ppm. Thus, the results from the solvent titration and dilution studies further support the intramolecular hydrogen bonding interactions in the oligomers **2e** and **3a**. An analogous trend was also observed in the dilution (SI, pp S44, S48) and titration (SI, pp S45, S49) studies on the Piv-substituted oligomers **2d** and **2g**. Further proof of involvement of the Ant amide NHs in intramolecular hydrogen bonding interactions came from variable temperature studies (SI, pp S55, S56) and H/D exchange experiments (SI, pp S57). Whereas the amide NH chemical shifts of the Piv-tetrapeptide **2d** showed negligible chemical shift difference upon varying the temperature from 268 − 323 K ($\Delta\delta/\Delta T = -4$ ppb/ deg K), nearly complete H/D exchange could be observed for **2d** in methanol-d_4 in 720 min (12 h), further confirming that the amide NHs are clearly involved in intramolecular hydrogen bonding interactions.

Circular dichroism (CD) spectra provide characteristic signature for the conformational features of ordered chiral oligomers. The Pro-Ant oligomers **2a**, **2f**, and **3a** featuring a Pro residue at the N-terminal displayed maxima at around 200 nm, zero crossing at 206 nm and minima at 219 nm. A strong Cotton effect was also observed (second minima) around 262 nm owing to the backbone aromatic groups/aromatic electronic transitions in the oligomers. The pentapeptide **2e** featuring an Ant residue at the N-terminal (in contrast to other oligomers featuring Pro at the N-terminal) showed a maxima at 205 nm and minima at 224 nm, slightly different from others in the series.

Theoretical Studies. The difficulty in crystallizing the oligomers prompted us to examine their conformation by *ab initio* MO theory. Computational investigations employing *ab initio* MO theory have considerably contributed to predict and to understand the folding patterns in a wide variety of foldamer structures.[14b−d,f,g,33] The (1→2)-type nine-membered hydrogen-bonded pseudo β-turn conformation found in the crystal structure of **2b** was supported by *ab initio* MO theory as well at the Hartree−Fock (HF) and B3LYP approximation levels employing the 6−31G* basis set (Table S19 in SI, pp S82), which justifies the application of these methods for the study of the larger oligomer systems (structural details in Tables S20−S22 of the SI, pp S82, S83). The hexapeptide **2g** displays two nine-membered-ring pseudo β-turns assuming an overall right-handed helical architecture, according to the theoretical studies (Figure 5, left). As seen in the crystal structure of **2b**, the Ant residues involved in the C9-turn formation are deprived of effective six-membered ring H-bonding interaction because of the misalignment of their H-bonding sites, although the C-terminal Ant

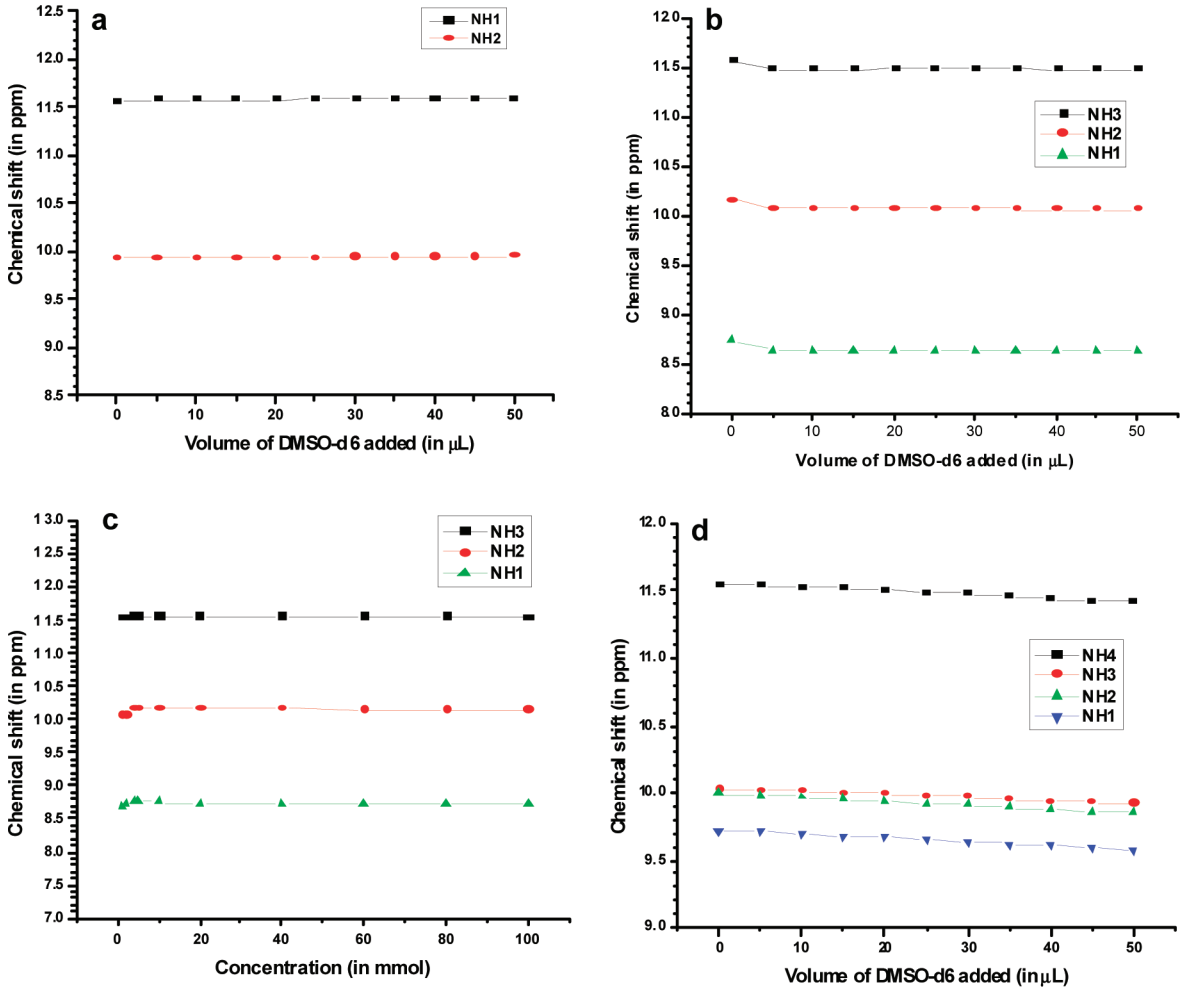

Figure 3. DMSO-d_6 NMR titration/$CDCl_3$ dilution plots of the oligomers. (a) DMSO-d_6 titration plot of tetrapeptide **2a**; (b) DMSO-d_6 titration plot of pentapeptide **2e**; (c) $CDCl_3$ dilution plot of pentapeptide **2e**; (d) DMSO-d_6 titration plot of octapeptide **3a**. *Note*: The assignments of the amide NHs should be from the N-terminal of the peptides, according to the molecular structures given in Scheme 1. DMSO-d_6 (5 μL) was used for each addition in all titration experiments. The initial concentration of the sample in $CDCl_3$ was 10 mM, and the total amount of DMSO-d_6 used was 8.3% of the total volume.

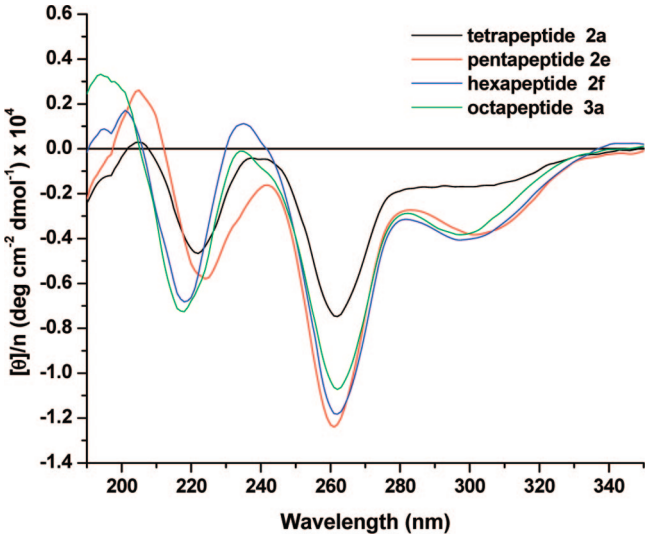

Figure 4. Representative CD spectra of Pro-Ant oligomers: tetrapeptide **2a**, pentapeptide **2e**, hexapeptide **2f**, and octapeptide **3a**, in acetonitrile. All spectra were recorded at 298 K with a concentration of 0.2 mM.

residue clearly retains such an interaction owing to the absence of a successive Pro residue.

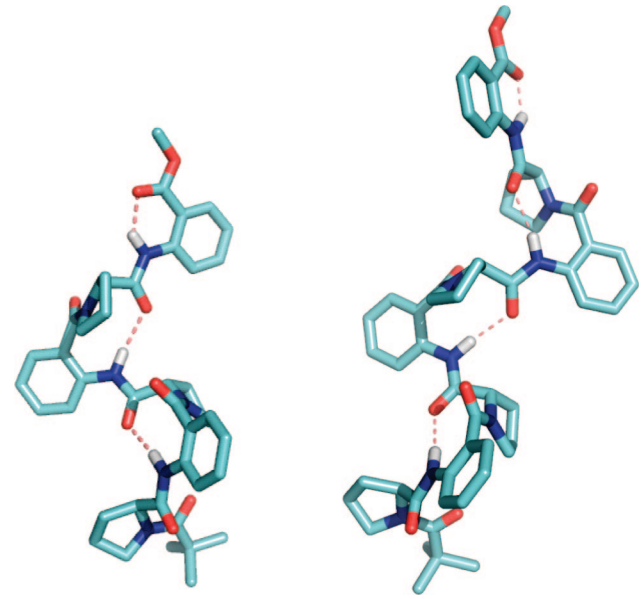

Figure 5. Structural architecture of oligomers **2g** (left) and **3b** (right) displaying C9 helical turns at the HF/6-31G* level of *ab initio* MO theory. Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity.

The conformational analysis on the octapeptide **3a** containing a N-terminal ^{t}BOC-Pro residue at the same approximation levels provided two energetically nearly equivalent conformers, which differ only by a *cis* or *trans* orientation of the N-terminal carbamate carbonyls (Figure S29, SI p S84). In the gas phase, the *trans* conformation is slightly favored, in an aqueous environment the *cis* conformation (Tables S21 and S22 in the SI p S83). The possibility of these conformational alternatives was clearly shown in the NMR studies by the multiple signals mainly arising from the N-terminal Pro residues. As already mentioned, such conformational flipping could be effectively circumvented by capping the N-terminal with a pivaloyl group[8e] as seen in **3b** (Figure 5, right). Interestingly, the pentapeptide **2e** containing a N-terminal BOC-Ant residue also displayed two structural alternatives according to *ab initio* studies (Figure S28, SI p 84).[34] The octapeptide **3b**, having diagonally placed similar residues in the right-handed helical framework, clearly replicates the turn structures observed in the crystal structure of its shorter analogue **2b**. The averaged hydrogen bonding geometry of the C9 pseudo β-turns in **3b** is characterized by the bond distances d(N...O) = 3.2 Å and d(H...O) = 2.2 Å, and the bond angle (N−H...O) = 169°. These data are in fair agreement with the crystal structure parameters of **2b** given above and confirm the reliability of the theoretical predictions.

A helix type with only nine-membered hydrogen-bonded repeating rings arising from nearest-neighbor (1→2) interactions was not found in α/β-hybrid peptides until now. Nine-membered hydrogen-bonded rings formed by 1→2 amino acid interaction occur in mixed or β-helices of α/β-hybrid peptides, where they alternate with 11-membered hydrogen-bonded pseudocycles resulting from 4→1 backward interactions.[16c,d] Mixed helices are excluded in the present case due to the absence of the amide hydrogen in the proline residue. Thus, only one hydrogen-bonded ring type is possible. The alternative with only 11-membered rings,[16d] is obviously prevented by the rigid planar structure of the Ant residues. Hints for a turn with a 9-membered ring in an α/β-hybrid peptide sequence were obtained in the Seebach group.[17e]

Role of Pro in the C9 Turn Formation. To investigate the role of the Pro residue in the C9 turn formation, we synthesized the oligomers **4** and **5** featuring Ala and Aib residues as substitutes of Pro (experimental details in SI). Whereas Ala is a conformationally flexible analogue of Pro, Aib is considerably constrained with a dihedral angle preference different from proline. [35] Interestingly, the crystal structure studies indicated no C9 turn formation in the oligomers **4** and **5** containing the Pro substitutes (Figure 6a,b). They revealed that the amide NHs

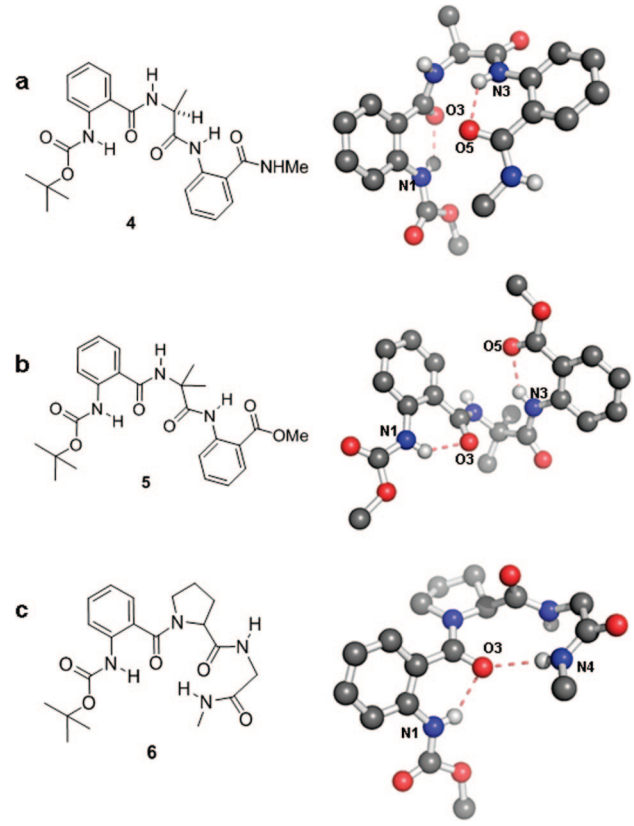

Figure 6. Crystal structures of the shorter analogues **4** (a), **5** (b), and **6** (c) (right) along with their molecular structures (left). Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity in the crystal structures.

of the Ant1 residue are clearly involved in strong intramolecular six-membered-ring hydrogen bonds with H-bond geometries for Ant1 in **4** corresponding to bond distances d(N...O) = 2.7 Å and d(H...O) = 2.0 Å and a bond angle (N−H...O) = 137°). For Ant1 in **5**, the bond distances are d(N...O) = 2.69 Å and d(H...O) = 2.0 Å, and the bond angle is (N−H...O) = 135°. It may be recalled that the S(6)-type interaction was weaker in **2b** owing to the C9 turn, as evident from the H-bond geometry with the distances d(N...O) = 3.1 Å and d(H...O) = 2.7 Å and the bond angle (N−H...O) = 112°. The C-terminal Ant3 residues in **4** and **5** keep also the geometries for S(6)-type intramolecular H-bonding interactions and exhibit the bond distances d(N...O) = 2.66 Å and d(H...O) = 2.02 Å and the bond angle (N−H...O) = 130° for **4** and d(N...O) = 2.65 Å and d(H...O) = 1.93 Å and the bond angle (N−H...O) = 139° in **5** in fair agreement with the values in **2b** given above. It becomes clear from the crystal structures that the NH and CO groups of the central amino acids in both **4** and **5** are pointing away from the backbone. This facilitates their participation in intermolecular hydrogen bonding (Figure S27, p S81 of SI). Comparison of

(33) (a) Wu, Y.-D.; Wang, D.-P. *J. Am. Chem. Soc.* **1998**, *120*, 13485–13493. (b) Wu, Y.-D.; Wang, D.-P. *J. Am. Chem. Soc.* **1999**, *121*, 9352. (c) Zanuy, D.; Alemán, C.; Muñoz-Guerra, S. *Int. J. Biol. Macromolecules* **1998**, *23*, 175. (d) Möhle, K.; Hofmann, H.-J. *J. Pept. Res.* **1998**, *51*, 19. (e) Möhle, K.; Günther, R.; Thormann, M.; Sewald, N.; Hofmann, H.-J. *Biopolymers* **1999**, *50*, 167–184. (f) Günther, R.; Hofmann, H.-J. *Helv. Chim. Acta* **2002**, *85*, 2149–2168. (g) Baldauf, C.; Günther, R.; Hofmann, H.-J. *Helv. Chim. Acta* **2003**, *86*, 2573–2588. (h) Beke, T.; Csizmadia, I. G.; Perczel, A. *J. Comput. Chem.* **2004**, *25*, 285. (i) Baldauf, C.; Günther, R.; Hofmann, H.-J. *Biopolymers* **2005**, *80*, 675–687. (j) Baldauf, C.; Günther, R.; Hofmann, H.-J. *Phys. Biol.* **2006**, *3*, S1−S9. (k) Buffeteau, T.; Ducasse, L.; Poniman, L.; Delsuc., N.; Huc, I. *Chem. Commun.* **2006**, 2714–2716. (l) Kendhale, A. M.; Gonnade, R.; Rajamohanan, P. R.; Hofmann, H.-J.; Sanjayan, G. J. *Chem. Comm.* **2008**, 2541–2543.

(34) An alternate closely related conformation with comparable energy is possible for **2e**, wherein the N-terminal Ant-Pro segment is devoid of a C9 turn conformation but retains C9 turn in the middle region (pp S84 in the Supporting Information).

(35) (a) α-Amino isobutyric acid (Aib) is considerably conformationally restricted, with an allowed conformation range largely lying in the region $\phi = \pm 60°$ and $\psi = \pm 30°$; see: Prasad, B. V. V.; Sasisekharan, V. *Macromolecules* **1979**, *12*, 1107–1110. (b) Prasad, B. V.V.; Balaram, P. *CRC Crit. Rev. Biochem.* **1984**, *16*, 307–348. (c) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756. (d) Balaram, P. *Curr. Opin. Struct. Biol.* **1992**, *2*, 845–851. (e) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers* **2002**, *60*, 396–419. (f) Kaul, R.; Balaram, P. *Bioorg. Med. Chem.* **1999**, *7*, 105–117. (g) Srinivas, D.; Gonnade, R.; Ravindranathan, S.; Sanjayan, G. J. *J. Org. Chem.* **2007**, *72*, 7022–7025.

the ϕ, ψ dihedral angles of the α-amino acid residues in **4**, **5**, and **2b** gives a realistic picture of the requirements for appropriate dihedral angles of α-amino acid residues in the formation of the C9 turn. The crystal structure data for the ϕ, ψ dihedral angles of the Pro residue involved in the C9 turn formation of **2b** are $\phi_{Pro3} = -63°$ and $\psi_{Pro3} = +169°$. These values differ considerably from the dihedral angles of the Ala and Aib residues in **4** and **5**, which are $\phi_{Ala} = -103°$, $\psi_{Ala} = -3°$ and $\phi_{Aib} = -52°$, $\psi_{Aib} = -39°$, respectively. The ϕ, ψ values for the Aib residue correspond to those in βIII-turns or 3_{10}-helices, which are preferred by this constrained α-amino acid.[35] These findings suggest that the dihedral angles ϕ and ψ of the α-amino acids play a crucial role in the conformational bias for the stabilization of the (1→2)-type pseudo β-turn conformation with nine-membered rings.

Interestingly, the model peptide **6** having an Ant residue preceding a Pro-Gly dipeptide, which is well-known for its ability to induce βII-turns in peptides and proteins,[25,36] did not show any folded conformation, which suggests that issues other than steric and dihedral angles might also be involved in the stabilization of the unusual (1→2)-type nine-membered-ring pseudo β-turn conformation described herein.[37] As in **4** and **5**, Ant1 in **6** was found to be clearly involved in six-membered H-bonding interactions. The corresponding bond distances are d(N...O) = 2.79 Å and d(H...O) = 2.13 Å and the bond angle is (N−H...O) = 133°). Furthermore, the carbonyl of Ant1 in **6** also participates in βII-turn formation (Figure 6c).[36a]

Summary. In conclusion, α/β-hybrid peptides consisting of L-Pro-Ant motifs adopt an unusual right-handed helical structural architecture, displaying a pseudo β-turn conformations with nine-membered hydrogen-bonded rings. The hydrogen bonds are formed in the forward direction of the sequence by 1→2 amino acid interactions. This structure is in stark contrast to the extended sheet structure reported for oligo-anthranilamides.[19] The intrinsic conformational bias of the individual amino acids Pro and Ant helps the oligomer backbone to adopt the folded conformation. This becomes evident by a comparison between the structure of the Pro-Ant oligomers and some peptide analogues. The requirement of Pro as a determining amino acid for the formation of such a structural architecture suggests that steric and dihedral angle constraints play a key role in the stabilization of the folded conformation.[38] These findings help to design novel conformationally restricted structures taking profit from the steric and dihedral angle constraints of the selected amino acids. This increases the conformational space available for synthetic oligomer design with diverse backbone structures. The ease of synthesis and the simplicity of the backbone are attractive features of these hybrid oligomers whose structural architecture could be modulated by altering the chirality[15,39] of the proline residues, which is a subject of current investigations in these laboratories.

Experimental Section

Single Crystal X-ray Crystallographic Studies. Crystal Data. Data for the compounds **4**−**6** were collected at $T = 293$ K, on SMART APEX CCD Single Crystal X-ray diffractometer using Mo Kα radiation ($\lambda = 0.7107$ Å), crystal data of **2b** were collected at 120 K on a Bruker SMART CCD 6000 diffractometer using the same radiation. The structures were solved by direct methods using SHELXTL. All the data were corrected for Lorentz polarization and absorption (except **2b**) effects. SHELX-97 (ShelxTL) was used for structure solution and full matrix least-squares refinement on F^2. Hydrogen atoms were included in the refinement in the riding mode. The refinements were carried out using SHELXL-97.

Crystal Data for 2b. Prismatic crystals of **2b** were obtained from a mixture of methanol with PEG-200. A small piece (0.24 × 0.17 × 0.07 mm^3) of a large prism has been used for the data collection. $C_{29}H_{34}N_4O_7$, 0.5 H_2O, $M = 559.61$, monoclinic, space group $P2_1$, $a = 9.6923$ (5) Å, $b = 9.4608$ (4) Å, $c = 15.6706$ (7) Å, $\beta = 93.158(2)°$, $V = 1434.76$ (12) Å^3, $F(000) = 594$, $Z = 2$, $D_c = 1.295$ mg m^{-3}, $\mu = 0.094$ mm^{-1} (Mo Kα, $\lambda = 0.710\,73$ Å), $T = 120(2)$ K. 17 934 reflections ($1.30 \le \theta \le 29.0°$) were collected ($\omega$-scan, 0.3°/frame) yielding 4027 unique data ($R_{merg} = 0.1064$). Final $wR_2(F^2) = 0.0949$ for all data (375 refined parameters), conventional $R(F) = 0.0411$ for 5539 reflections with $I \ge 2\sigma$, GOF = 0.960.

Crystal Data for 4. Single crystals of **4** were grown by slow evaporation of the mixture of ethyl acetate and pet. ether. A colorless needle of approximate size 0.41 × 0.28 × 0.08 mm^3 was used for data collection. Multiscan acquisition. Total scans = 3, total frames = 1271, Oscillation/frame −0.3°, exposure/frame = 15.0 s/frame, θ range = 2.51° to 25.00°, completeness to θ of 25.0° is 99.9%. SADABS correction applied, $C_{23}H_{28}N_4O_5$, $M = 440.49$. Crystals belong to monoclinic, space group $P2_1/n$, $a = 15.219$ (1) Å, $b = 9.4069(6)$ Å, $c = 16.268(1)$ Å, $\beta = 101.440(1)°$. $V = 2282.6$ (3) Å^3, $Z = 4$, $D_c = 1.282$ mg m^{-3}, μ(Mo K_α) = 0.092 mm^{-1}, 11 233 reflections measured, 4016 unique [$I > 2\sigma(I)$], R value 0.0433, $wR_2 = 0.1039$.

Crystal Data for 5. Colorless plate crystals of the compound were grown by slow evaporation of methanol. A crystal size of 0.52 × 0.20 × 0.17 mm^3 was used for data collection. Multiscan data acquisition. Total scans = 4, total frames = 2199, Oscillation/frame −0.3°, exposure/frame = 15.0 s/frame, θ range = 2.39° to 25.00°, completeness to θ of 25.0° is 100.0%. SADABS correction applied, $C_{24}H_{29}N_3O6$, $M = 455.50$. Crystals belong to orthorhombic, space group $Pna2_1$, $a = 10.1637(6)$ Å, $b = 15.6505(8)$ Å, $c = 15.0116(9)$ Å, $V = 2387.9(2)$ Å^3, $Z = 4$, $D_c = 1.267$ mg m^{-3}, μ(Mo K_α) = 0.092 mm^{-1}, 19 877 reflections measured, 4192 unique [$I > 2\sigma(I)$], R value 0.0354, $wR_2 = 0.0821$.

Crystal Data for 6. Single crystals of the compound were grown by slow evaporation of the solution mixture of methanol and DCM. Colorless plate of approximate size 0.22 × 0.18 × 0.09 mm^3 was used for data collection. Multiscan data acquisition. Total scans = 3, total frames = 1271, Oscillation/frame −0.3°, exposure/frame = 15.0 s/frame, θ range = 2.40° to 25.00°, completeness to θ of 25.0° is 100.0%. SADABS correction applied, $C_{20}H_{28}N_4O_5$, $M = 404.46$. Crystals belong to the orthorhombic, space group $Pbca$, $a = 10.867$ (1) Å, $b = 16.478$ (3) Å, $c = 24.053$ (2) Å, $V = 4306.9$ (10) Å^3, $Z = 8$, $D = 1.248$ g cm^{-3}, $\mu = 0.091$ mm^{-1}, $F(000) = 1728$, 20 702 reflections measured, 3796 unique [$I > 2\sigma(I)$], R = 0.0768. $Rw = 0.1355$.

CD Spectra. The CD spectra were obtained with a Jasco J-815 spectropolarimeter. Rectangular fused quartz cells of 0.1 cm path length were used, with the sample as a 200 μM solution in acetonitrile. The values are expressed in mean residue ellipticity [θ]/n (deg cm^{-2} dmol^{-1}).

Quantum Chemical Calculations. Geometry optimizations of various conformational alternatives of **2b,e,g** and **3a,b** were

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(37) Interestingly, a crystal structure of a small dipeptide Boc-Ant-D-Pro-OMe shows only S(6)-type H-bond interaction; see: ref 22a.

(38) (a) Woolfson, D. N.; Williams, D. H. *FEBS Lett.* **1990**, *277*, 185–188. (b) Chang, D.-K.; Cheng, S.-F.; Trivedi, V. D.; Lin, K.-L. *J. Struct. Biol.* **1999**, *128*, 270–279. (c) Cordes, F. S.; Bright, J. N.; Sansom, M. S. P. *J. Mol. Biol.* **2002**, *323*, 951–960.

(39) (a) Sharma, G. V. M.; Ravinder Reddy, K.; Radha Krishna, P.; Ravi Sankar, A.; Narsimulu, K.; Kiran Kumar, S.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 13670–13671. (b) Sharma, G. V. M.; Ravinder Reddy, K.; Radha Krishna, P.; Ravi Sankar, A.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3961–3965.

performed at the HF/6−31G*, DFT/B3LYP/6−31G*, and PCM//HF/6−31G* levels of *ab initio* MO theory employing the Gaussian03 software package.[41]The backbone torsion angles and total energies of the preferred conformations are given in the Supporting Information.

Experimental Procedures. *tert*-Butyl 2-(2-(methoxycarbonyl)phenylcarbamoyl)pyrrolidine-1-carboxylate 1a. A solution containing Boc-proline (4 g, 18.6 mmol, 1 equiv) and Ant-OMe (2.8 g, 18.6 mmol, 1 equiv) in dry acetonitrile (30 mL) was cooled to 0 °C. TBTU (7.16 g, 22.4 mmol, 1.2 equiv) was added followed by DIEA (4.8 mL, 27.8 mmol, and 1.5 equiv). The reaction mixture was stirred at 0 °C for 10 min and at room temperature for 12 h. The reaction mixture was stripped off the solvent under reduced pressure, the residue was taken in dichloromethane and the organic layer was washed sequentially with potassium hydrogen sulfate solution, saturated sodium bicarbonate, and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which on purification by column chromatography (80:20 pet. ether/ethyl acetate, R_f: 0.3) afforded **1a** as a waxy liquid (5.8 g, 90%). $[\alpha]^{25}_D$: −127.3° (*c* 0.11, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3269, 2984, 2941, 2908, 2878, 1738, 1703, 1587, 1529, 1450, 1371, 1240, 1047; ^1H (200 MHz, $CDCl_3$) δ: $11.57_{rotamer}$ (0.45H), $11.47_{rotamer}$ (0.55H), 8.77−8.73 (d, J = 8.3 Hz, 1H), 8.03−8.0 (d, J = 7.58 Hz, 1H), 7.57−7.50 (t, J = 7.35 Hz, 1H), 7.12−7.05 (t, J = 7.54 Hz, 1H), $4.44_{rotamer}$ (0.45H), $4.31_{rotamer}$ (0.55H), 3.89 (s, 3H), 3.68 (m, 2H), 2.18 (m, 2H), 1.91 (m, 2H), $1.49_{rotamer}$ (4H), $1.33_{rotamer}$ (5H); ^{13}C (100 MHz, $CDCl_3$) δ: 172.4, 168.2, 154.3, 141.2, 140.9, 134.6, 131.0, 130.7, 122.7, 120.3, 120.1, 115.3, 80.3, 62.7, 62.1, 52.4, 52.1, 47.2, 46.8, 31.5, 30.5, 28.4, 28.2, 24.4, 23.8; ESI Mass: 349.09 $(M + H)^+$, 371.07 $(M + Na)^+$, 387.05 $(M + K)^+$. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.20; H, 7.02; N, 7.92.

2-(1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carboxamido)benzoic Acid 1b. To a solution of **1a** (2 g, 5.7 mmol, 1 equiv) in methanol (15 mL) $LiOH\cdot H_2O$ (0.49 g, 11.5 mmol, 2 equiv) in water (6 mL) was added, and the reaction mixture was stirred for 12 h. After complete consumption of the starting material, the solvent was evaporated under reduced pressure, and the free acid was liberated by treating with aq. potassium hydrogen sulfate solution followed by extraction with dichloromethane (2 × 25 mL). The residue obtained after evaporation of the solvent under reduced pressure was carried forward for the next reaction, without further purification.

Methyl 2-(Pyrrolidine-2-carboxamido)benzoate, Hydrochloride Salt 1c. To an ice cold solution of **1a** (2.2 g, 6.3 mmol) in dry ether (10 mL) dry HCl gas was passed for 10 min. The solvent was stripped off under reduced pressure, and the residue was dried in KOH desiccator overnight. The crude product was used for the next step without further purification.

***tert*-Butyl 2-(2-(2-(2-(methoxycarbonyl)phenylcarbamoyl)-pyrrolidine-1-carbonyl) phenyl carbamoyl)pyrrolidine-1-carboxylate 2a.** The acid **1b** (1.6 g, 4.8 mmol, 1 equiv) was coupled with amine **1c** (1.36 g, 4.8 mmol, 1 equiv) using TBTU (1.84 g, 5.7 mmol, 1.2 equiv) and DIEA (2 mL, 12 mmol, 2.5 equiv). Workup, as described in the case of **1a**, followed by column chromatographic purification (eluent: pet. ether/ethyl acetate: 40:60, R_f 0.6) yielded **2a** (2.1 g, 78%); fluffy solid, mp: 68−70 °C, $[\alpha]^{26}_D$: −130° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3271, 3016, 2980, 1693, 1682, 1589, 1526, 1452, 1393, 1267, 1215, 1164, 1089; ^1H (400 MHz, $CDCl_3$) δ: 11.57 (s, 1H), 9.93 (s, 1H), 8.75 (bs, 1H), $8.49_{rotamer}$ (0.5H), $8.41_{rotamer}$ (0.5H), 8.03−8.01 (d, J = 6.91 Hz, 1H), 7.74 (d, J = 7.75 Hz, 1H), 7.55 (m, 1H), 7.43 (bs, 1H), 7.13−7.07 (m, 2H), 4.86 (m, 1H), $4.43_{rotamer}$ (0.5H), $4.26_{rotamer}$ (0.5H), 3.92 (s, 3H), 3.83−3.79 (m, 1H), 3.63−3.33 (m, 3H), 2.46−2.39 (m, 2H), 2.21−2.13 (m, 2H), 2.10−2.02 (m, 2H), 1.91−1.87 (m, 2H), $1.48_{rotamer}$ (4H), $1.35_{rotamer}$ (5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.6, 170.7, 169.7, 168.7, 154.0, 141.2, 136.7, 134.6, 134.3, 131.4, 130.8, 128.0, 122.7, 121.7, 120.4, 115.2, 79.9, 62.2, 61.5, 52.4, 50.5, 47.16 46.65, 31.39 30.22, 30.01, 28.3, 25.4, 24.3, 23.8; ESI-MS *m*/*z*: 565.47, $(M+H)^+$, 587.46 $(M + Na)^+$, 603.44 $(M + K)^+$; Anal. Calcd for $C_{30}H_{36}N_4O_7$: C, 63.82.; H, 6.43.; N, 9.92. Found: C, 63.98.; H, 6.46.; N, 9.82.

2-(1-(2-(1-(*tert*-Butoxycarbonyl)pyrrolidine-2-carboxamido)-benzoyl)pyrrolidine-2-carboxamido)benzoic Acid 2b. Compound **2a** (0.8 g, 1.4 mmol, 1 equiv) was subjected to ester hydrolysis with 2 N LiOH solution in MeOH. The progress of the ester hydrolysis was monitored by TLC. After the complete consumption of ester (12 h), the solvent was evaporated under vacuum, and the free acid was liberated by treating with aq. potassium hydrogen sulfate solution followed by extraction with dichloromethane (2 × 25 mL). The residue obtained after evaporation of the solvent under vacuum was carried forward for the next reaction. Crystals, suitable for single crystal analysis, could be obtained by crystallizing a small amount of the residue from methanol containing a few drops of PEG 200. Mp: 180−181 °C; IR ($CHCl_3$) ν (cm^{-1}) 3211, 3015, 2980, 1686, 1589, 1526, 1452, 1398, 1296, 1217, 1163, 1088, 1045; ESI Mass: 551.05 $(M + H)^+$, 573.03 $(M + Na)^+$, 589.0 $(M + K)^+$.

Methyl 2-(1-(2-(Pyrrolidine-2-carboxamido)benzoyl)pyrrolidine-2-carboxamido)benzoate 2c. The tetrapeptide **2a** (1.1 g, 1.9 mmol) was subjected to N-BOC deprotection using DCM/TFA (50%, 10 mL). After completion of the reaction (1 h), the reaction mixture was stripped off the solvent, neutralized with saturated sodium bicarbonate solution, and diluted with dichloromethane (10 mL), and the product was repeatedly extracted into dichloromethane (2 × 15 mL). The crude product, obtained after evaporating the solvent under reduced pressure, was used for the next step without further purification.

Methyl 2-(1-(2-(1-Pivaloylpyrrolidine-2-carboxamido)benzoyl)pyrrolidine-2-carboxamido) Benzoate 2d. A solution containing the tetrapeptide **2a** (0.5 g, 0.8 mmol) in dichloromethane (5 mL) was subjected to Boc deprotection using DCM/TFA (50%, 2 mL). After completion of the reaction (1 h), the reaction mixture was stripped off the solvent, neutralized with saturated sodium bicarbonate solution, and diluted with dichloromethane (10 mL), and the product was extracted into an organic layer (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated, and dried, and the residue obtained was taken in dry dichloromethane (10 mL). The reaction mixture was cooled to 0 °C, pivaloyl chloride (0.13 mL, 1 mmol, 1.2 equiv) was added followed by Et_3N (0.25 mL, 1.8 mmol, 2 equiv), and the reaction mixture was allowed to stir at room temperature for 4 h. The reaction mixture was diluted with more dichloromethane (10 mL) and washed sequentially with potassium hydrogen sulfate solution, sat. sodium bicarbonate solution, and water. The organic layer was dried over anhydrous sodium sulfate, and the crude product obtained on removal of solvent under reduced pressure was subjected to column purification (eluent 70% AcOEt/pet. ether, R_f 0.3) yielding **2d** (0.43 g, 88%), mp: 83−85 °C; $[\alpha]^{25}_D$: −76.36 ° (*c* 0.55, $CHCl_3$); IR (ν) $CHCl_3$ (cm^{-1}) 3275, 3016, 1697, 1686, 1624, 1589, 1526, 1508, 1452, 1406, 1298, 1265, 1215, 1164, 1143, 1091; ^1H (400 MHz, $CDCl_3$) δ: 11.57 (s, 1H), 9.74 (s, 1H), 8.75−8.73 (d, J = 8.51 Hz, 1H), 8.44−8.42 (d, J = 8.51 Hz, 1H), 8.06−8.03 (dd, J = 1.24 Hz, 8.11 Hz, 1H), 7.67−7.65 (d, J = 7.70 Hz, 1H), 7.56−7.52 (t, J = 7.84 Hz, 1H), 7.42−7.38 (t, J = 7.70 Hz, 1H), 7.12−7.05 (m, 2H), 4.82−4.79 (m, 1H), 4.69−4.66 (m, 1H), 3.92 (s, 3H), 3.86−3.80 (m, 1H), 3.78−3.73 (m, 1H), 3.69−3.63 (m, 1H), 3.61−3.56 (m, 1H), 2.48−2.40 (m, 1H), 2.23−1.82 (m, 8H), 1.29 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.5, 171.5, 170.9, 169.5, 168.7, 141.2, 136.8, 134.5, 131.0, 130.8, 127.7, 124.1, 122.8, 122.7, 121.6, 120.3, 151.2, 63.5, 62.3, 52.4, 50.4, 48.4, 40.0, 30.0, 27.4, 25.4; ESI MS: 549.36 $(M + H)^+$, 571.35 $(M + Na)^+$, 587.32 $(M + K)^+$. Elemental analyses calculated for $C_{30}H_{36}N_4O_6$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.90; H, 6.49; N, 10.05.

Methyl 2-(1-(2-(1-(2-(*tert*-Butoxycarbonylamino)benzoyl)pyrrolidine-2-carboxamido)benzoyl) pyrrolidine-2-carboxamido)-benzoate 2e. Boc-Ant-OH **8** (0.24 g, 1 mmol, 1 equiv) was coupled with **2c** (0.5 g, 1 mmol, 1 equiv) using TBTU (0.385 g, 1.2 mmol, 1.2 equiv) and DIEA (0.4 mL, 2.5 mmol, 2.5 equiv). Workup, as described in the case of **1a**, followed by column chromatographic purification (eluent: pet. ether/ethyl acetate: 40:60, R_f 0.6) yielded the pentapeptide **2e** (0.49 g, 72%); as a fluffy solid, mp: 116−118 °C; $[\alpha]^{26}_D$: −108° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3273, 3018, 2995, 1724, 1686, 1624, 1589, 1522, 1452, 1412, 1393, 1298, 1267, 1248, 1219, 1092, 1047; ^1H (400 MHz, $CDCl_3$) δ: 11.55 (s, 1H), 10.15 (s, 1H), 8.75−8.73 (m, 2H), 8.52−8.49 (d, J = 8.04 Hz, 1H), 8.17−8.15 (d, J = 8.30 Hz, 1H), 8.04−8.02 (d, J = 6.96 Hz, 1H), 7.73−7.71 (d, J = 7.23 Hz, 1H), 7.66−7.64 (d, J = 7.50 Hz, 1H), 7.56−7.52 (m, 1H), 7.43−7.36 (m, 2H), 7.14−7.08 (m, 3H), 4.85−4.83 (m, 1H), 4.69−4.66 (m, 1H), 3.91 (s, 3H), 3.87−3.80 (m, 1H), 3.71−3.65 (m, 1H), 3.58−3.53 (m, 1H), 3.50−3.45 (m, 1H), 2.47−2.39 (m,1H), 2.37−2.30 (m,1H), 2.14−1.76 (m, 6H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.8, 170.6, 170.1, 169.6, 168.7, 153.1, 141.1, 137.6, 136.8, 134.6, 131.2, 130.9, 128.3, 127.7, 123.96, 123.1, 122.9, 122.8, 121.7, 121.4, 120.3, 120.1, 115.2, 80.1, 62.3, 61.9, 52.4, 50.7, 50.4, 30.0, 29.98, 28.3, 25.5, 25.4; ESI Mass: 684.16 $(M + H)^+$, 706.11 $(M + Na)^+$, 722.07 $(M + K)^+$. Anal. Calcd for $C_{37}H_{41}N_5O_8$: C, 64.99; H, 6.04; N, 10.24. Found: C, 65.10; H, 6.18; N, 10.16.

***tert*-Butyl 2-(2-(2-(2-(2-(2-(methoxycarbonyl)phenylcarbamoyl)pyrrolidine-1-carbonyl)phenylcarbamoyl)pyrrolidine-1-carbonyl)phenylcarbamoyl)pyrrolidine-1-carboxylate 2f.** A solution containing the tetrapeptide **2a** (1.2 g, 2.1 mmol) in dichloromethane (8 mL) was subjected to Boc deprotection using DCM/TFA (50%, 2 mL). After completion of the reaction (1 h), the reaction mixture was stripped of the solvent, neutralized with saturated sodium bicarbonate solution, and diluted with dichloromethane (10 mL), and the product was extracted into the organic layer (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated, and dried, and the residue obtained was taken in dry acetonitrile (10 mL). The reaction mixture was cooled to 0 °C. To this stirring solution, the acid **1b** was added followed by HBTU (1.2 g, 3.2 mmol, 1.5 equiv) and DIEA (0.76 mL, 4.2 mmol, 2 equiv). The reaction mixture was allowed to stir at room temperature for 12 h. Workup as described in the case of tetrapeptide **2a** and purification of the crude product by column chromatographic purification (eluent: 80% AcOEt/pet. ether, R_f 0.3) yielded **2f** (1.24 g, 75%) as a fluffy compound; mp: 119−121 °C; $[\alpha]^{27}_D$: −85.71° (*c* 1.4, $CHCl_3$); IR (ν) nujol (cm^{-1}): 3264, 1687, 1625, 1586, 1520, 1456; ^1H (400 MHz, $CDCl_3$) δ: 11.54 (s, 1H), $10.07-10.02_{rotamer}$ (1H), $9.90_{rotamer}$ (0.6H), $9.84_{rotamer}$ (0.4H), 8.75−8.73 (d, J = 8.51 Hz, 1H), 8.47−8.26 (m, 2H), 8.06−8.04 (d, J = 7.96 Hz, 1H), 7.75−7.53 (m, 3H), 7.41 (b, 2H), 7.20−7.10 (m, 3H), $4.94_{rotamer}$ (0.55H), $4.87_{rotamer}$ (0.45H), 4.76 (b, 1H), $4.43_{rotamer}$ (0.45H), $4.27_{rotamer}$ (0.55 H), 3.93 (s, 3H), 3.87 (b, 1H), 3.69 (b, 1H), 3.55 (b, 3H), 3.34 (b, 1H), 2.45−2.36 (m, 2H), 2.25−1.8 (m, 10H), $1.46_{rotamer}$ (3H), $1.32_{rotamer}$(6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.0, 171.9, 171.0, 170.8, 169.9, 169.6, 169.3, 169.0, 168.7, 155.0, 154.0, 141.1, 136.8, 136.4, 136.1, 135.9, 135.8, 134.5, 131.2, 130.9, 130.8, 127.95, 127.6, 127.5, 125.7, 124.95, 123.7, 123.6, 123.3, 122.9, 122.3, 122.1, 121.1, 120.8, 120.3, 115.2, 79.7, 62.1, 61.5, 61.4, 61.1, 52.4, 50.6, 50.0, 47.1, 46.7, 31.4, 30.1, 29.9, 28.4, 28.2, 25.4, 25.3, 24.3, 23.8; ESI MS 781.84 $(M + H)^+$, 803.84 $(M + Na)^+$, 819.82 $(M + K)^+$. Elemental analyses calculated for $C_{42}H_{48}N_6O_9$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.25; H, 6.37; N, 11.02.

Methyl 2-(1-(2-(1-(2-(1-Pivaloylpyrrolidine-2-carboxamido)-benzoyl)pyrrolidine-2-carboxamido)benzoyl)pyrrolidine-2-carboxamido)benzoate 2g. A solution containing the hexapeptide **2f** (0.5 g, 0.6 mmol) in dichloromethane (5 mL) was subjected to Boc deprotection using DCM/TFA (50%, 2 mL). After completion of the reaction (1 h), the reaction mixture was stripped of the solvent, neutralized with saturated sodium bicarbonate solution, diluted with dichloromethane (10 mL), and the product was extracted into organic layer (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated, and dried, and the residue obtained was taken in dry dichloromethane (10 mL). The reaction mixture was cooled to 0 °C, pivaloyl chloride (0.1 mL, 0.8 mmol, 1.3 equiv) was added followed by Et_3N (0.17 mL, 1 mmol, 2 equiv), and the reaction mixture was allowed to stir at room temperature for 4 h. The reaction mixture was diluted with more dichloromethane (10 mL) and washed sequentially with potassium hydrogen sulfate solution, sat. sodium bicarbonate solution, and water. The organic layer was dried over anhydrous sodium sulfate, and the crude product obtained on the removal of solvent under reduced pressure was subjected to column purification (eluent: 90% AcOEt/pet. ether, R_f 0.3) yielded **2g** (0.37 g, 75%) mp: 110−112 °C; $[\alpha]^{25}_D$: −60.47° (*c* 0.43, $CHCl_3$); IR (ν) $CHCl_3$ (cm^{-1}): 3286, 3018, 2880, 1695, 1686, 1636, 1623, 1618, 1589, 1541, 1531, 1522, 1518, 1508, 1473, 1452, 1437, 1416, 1296, 1267, 1215, 1164, 1143, 1091, 1047; ^1H (400 MHz, $CDCl_3$) δ: 11.52 (s, 1H), 10.01 (s, 1H), 9.66 (s, 1H), 8.73−8.71 (d, J = 8.40 Hz, 1H), 8.27−8.22 (t, J = 8.67 Hz, 1H), 8.04−8.02 (d, J = 7.42 Hz, 1H), 7.66−7.65 (d, J = 7.42 Hz, 1H), 7.57−7.52 (m, 2H), 7.42−7.34 (m, 2H), 7.18−7.08 (m, 3H), 4.86−4.83 (t, J = 7.26 Hz, 1H), 4.79−4.76 (t, J = 6.39 Hz, 1H), 4.65−4.62 (m, 1H), 3.91 (s, 3H), 3.81−3.65 (m, 4H), 3.60−3.54 (m, 1H), 3.36−3.31 (m, 1H), 2.45−2.36 (m, 2H), 2.22−1.77 (m, 10 H), 1.25 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.96, 171.8, 171.2, 171.9, 169.4, 169.2, 168.7, 141.1, 135.97, 135.7, 134.5, 130.8, 130.7, 130.5, 127.4, 126.1, 123.7, 123.6, 122.8, 122.6, 122.4, 120.3, 115.1, 63.0, 62.1, 61.6, 52.3, 50.1, 49.9, 48.5, 38.8, 30.1, 30.0, 27.3, 25.3; ESI MS 765.43 $(M + H)^+$, 782.47 $(M + Na)^+$, 737.39 $(M + K)^+$. Elemental analyses calculated for $C_{42}H_{48}N_6O_8$: C, 65.95; H, 6.33; N, 10.99. Found: C, 66.24; H, 6.15; N, 10.78.

***tert*-Butyl 2-(2-(2-(2-(2-(2-(2-(2-(Methoxycarbonyl)phenylcarbamoyl)pyrrolidine-1-carbonyl) phenylcarbamoyl)pyrrolidine-1-carbonyl)phenylcarbamoyl)pyrrolidine-1-carbonyl)phenylcarbamoyl)pyrrolidine-1-carboxylate 3a.** The acid **2b** (0.6 g, 1.1 mmol, 1 equiv) was coupled with amine **2c** (0.55 g, 1.1 mmol, 1 equiv) using TBTU (0.42 g, 1.3 mmol, 1.2 equiv) and DIEA (0.48 mL, 2.7 mmol, 2.5 equiv), and the reaction was allowed to proceed for 12 h, at room temperature. Workup, as described in the case of **1a**, followed by column chromatographic purification (eluent: ethyl acetate/MeOH: 98:2, R_f 0.3), afforded **3a** (0.73 g, 67%) as a fluffy solid; mp: 145−146 °C; $[\alpha]^{24}_D$: −60° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3271, 3124, 3016, 2980, 1693, 1682, 1626, 1589, 1528, 1454, 1416, 1296, 1276, 1215, 1165, 1092; ^1H (400 MHz, $CDCl_3$) δ: 11.56 (s, 1H), 10.12 (s, 1H), 10.03 (s, 1H), $9.81_{rotamer}$ (0.8H), $9.73_{rotamer}$ (0.2H), 8.74 (d, J = 8.31 Hz, 1H), 8.51−8.35 (m, 3H), 8.07 (d, J = 7.71 Hz, 1H), 7.70−7.47 (m, 4H), 7.46−7.32 (m, 3H), 7.25−7.10 (m, 4H), 5.05−4.7 (m, 3H), $4.48_{rotamer}$ (0.2H), $4.30_{rotamer}$(0.8H), 3.94 (s, 3H), 3.9−3.2 (m, 8H), 2.5−1.6 (m, 16H), $1.46_{rotamer}$ (3H), $1.35_{rotamer}$ (6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.13, 171.5, 168.85, 168.8, 168.7, 154.1, 141.1, 136.7, 135.9, 134.7, 130.96, 130.92, 130.6, 127.2, 127.16, 127.1, 123.98, 123.9, 122.98, 122.9, 121.7, 121.6, 121.1, 120.9, 115.3, 62. 0, 61.97, 61.37, 61.1, 50.31, 49.9, 47.2, 46.7, 31.3, 30.19, 30.01, 29.7, 24.9, 25.43, 25.2, 23.9; ESI Mass: 998.04 $(M + H)^+$, 1019.99 $(M + Na)^+$, 1035.95 $(M + K)^+$. Anal. Calcd for $C_{54}H_{60}N_8O_{11}$: C, 65.05.; H, 6.07.; N, 11.24. Found: C, 65.28.; H, 6.20.; N, 11.10.

Methyl 2-(1-(2-(1-(2-(1-(2-(1-Pivaloylpyrrolidine-2-carboxamido)benzoyl) pyrrolidine-2-carboxamido)benzoyl)pyrrolidine-2-carboxamido)benzoyl) pyrrolidine-2-carboxamido)benzoate 3b. A solution containing the octapeptide **3a** (0.3 g, 0.3 mmol) in dichloromethane (5 mL) was subjected to Boc deprotection using DCM/TFA (50%, 2 mL). After completion of the reaction (1 h), the reaction mixture was stripped of the solvent, neutralized with saturated sodium bicarbonate solution, and diluted with dichloromethane (10 mL), and the product was extracted into the organic layer (2 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated, and dried, and the residue obtained was

taken in dry dichloromethane (10 mL). The reaction mixture was cooled to 0 °C, pivaloyl chloride (0.05 mL, 0.4 mmol, 1.3 equiv) was added followed by Et_3N (0.08 mL, 0.6 mmol, 2 equiv), and the reaction mixture was allowed to stir at room temperature for 4 h. The reaction mixture was diluted with more dichloromethane (10 mL) and washed sequentially with potassium hydrogen sulfate solution, sat. sodium bicarbonate solution, and water. The organic layer was dried over anhydrous sodium sulfate, and the crude product obtained on the removal of solvent under reduced pressure was subjected to column purification (eluent: AcOEt, R_f 0.3) to yield **3b** (0.21 g, 72%) as a fluffy solid; mp: 147.9−149.6 °C; $[\alpha]^{25}_D$: −61.90° (*c* 0.42, $CHCl_3$); IR (ν) $CHCl_3$ (cm^{-1}): 3020, 1684, 1619, 1528, 1407; ^1H (400 MHz, $CDCl_3$) δ: 11.58 (s, 1H), 10.07 (s, 1 h), 9.98 (s, 1H), 9.63 (s, 1H), 9.75−9.73 (d, J = 8.07 Hz, 1H), 8.39−8.34 (m, 2H), 8.25−8.23 (d, J = 8.08 Hz, 1H), 8.07−8.05 (d, J = 8.08 Hz, 1H), 7.65−7.63 (d, J = 7.41 Hz, 1H), 7.60−7.54 (m, 2H), 7.45−7.34 (m, 2H), 7.45−7.34 (m, 4H), 7.22−7.11 (m, 4H), 4.92−4.83 (m, 2H), 4.77−4.70 (m, 2H), 3.94 (s, 3H), 3.84−3.67 (m, 5H), 3.58−3.53 (m, 1H), 3.42−3.32 (m, 2H), 2.46−2.36 (m, 3H), 2.19−1.7 (m, 13H), 1.27 (9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.8, 171.9, 171.5, 171.2, 170.9, 169.2, 169.2, 168.9, 168.8, 141.1, 136.1, 135.7, 135.4, 134.7, 130.9, 130.85, 130.45, 130.4, 127.5, 127.4, 127.0, 126.9, 126.1, 125.2, 124.2, 123.5, 123.5, 123.0, 122.5, 122.1, 121.7, 120.3, 115.2, 63.0, 62.2, 61.6, 61.2, 52.5, 50.1, 49.8, 49.7, 48.6, 38.8, 30.2, 30.12, 30.1, 29.7, 27.4, 25.4, 25.3; ESI MS 981.85 $(M + H)^+$, 1003.86 $(M + Na)^+$, 1019.89 $(M + K)^+$. Elemental analyses calculated for $C_{42}H_{48}N_6O_8$: C, 66.11; H, 6.16; N, 11.42. Found: C, 65.88; H, 11.27; N, 6.53.

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Supporting Information Available: Experimental procedures for compounds **4**−**6**; ^1H, ^{13}C, and DEPT-135 NMR spectra; ESI mass spectra and 2D spectra of compounds; details of *ab initio* MO calculations; HF/6−31G* structures of **2e** and **3a**, and crystal data[40] of **2b**, **4**, **5**, and **6** (CIF format); complete ref 41. This material is available free of charge via the Internet at http://pubs.acs.org.

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(40) Crystallographic data of **2b** and **4**−**6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-687326, 679457, 679458, and 679456, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

(41) Frisch, M. J., et al. *Gaussian03*, revision B.04; Gaussian Inc.: Pittsburgh, PA, 2003.