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Secondary Structure of Short β-Peptides as the Chiral Expression of Monomeric Building Units: A Rational and Predictive Model

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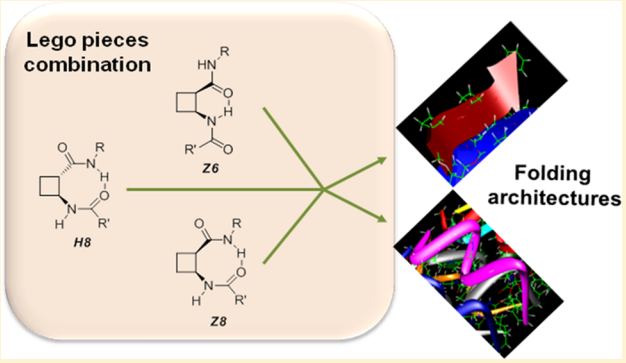
Supporting Information

ABSTRACT: Chirality of the monomeric residues controls and determines the prevalent folding of small oligopeptides (from di- to tetramers) composed of 2-aminocyclobutane-1-carboxylic acid (ACBA) derivatives with the same or different absolute and relative configuration. The *cis*-form of the monomeric ACBA gives rise to two conformers, namely, Z6 and Z8, while the *trans*-form manifests uniquely as an H8 structure. By combining these subunits in oligo- and polypeptides, their local structural preference remains, thus allowing the rational design of new short foldamers. A lego-type molecular architecture evolves; the overall look depends only on the conformational properties of the structural building units. A versatile and efficient method to predict the backbone folds of designed cyclobutane β-peptides is based on QM calculations. Predictions are corroborated by high-resolution NMR studies on selected stereoisomers, most of them being new foldamers that have been synthesized and characterized for the first time. Thus, the chiral expression of monomeric building units results in the defined secondary structures of small oligomers. As a result of this study, a new set of chirality controlled foldamers is provided to probe as biocompatible biopolymers.



INTRODUCTION

Structural mimetics of peptides (β-hairpins, isolated helices, etc.) are studied to understand folding and stability of secondary structural elements of proteins and thus to design standalone foldamers.[1] Several of these *ab initio* designed and/or fold-optimized foldamers are to fulfill biological functionalities related to DNA- and RNA-binding and ATP-, metal-, etc.-accommodation.[2] Unlike peptides composed of α-amino acid residues, even short β-peptides have the ability to fold at high percentage and thus have the potential to adopt the biologically relevant conformer in a time average manner. A β-peptide of appropriate structure can imitate α-peptidic ligands in peptide−protein and protein−protein interactions of increased proteolytic and metabolic stability.[3]

The enhanced ability of β-peptides and β-foldamers to fold has promoted a research field of growing interest in the search for tools by the rational design of foldamers with specific properties.[4] The use of chiral carbocycles as rigid scaffolds has been successfully employed in the synthesis of $\beta^{2,3}$-disubstituted β-peptide foldamers.[5] These compounds present restricted μ torsion in such a way that the dihedral angle θ(N−C^3−C^2−CO) depends on the carbocycle *cis*/*trans* configuration, which governs the *gauche* or *anti* disposition of the N−C^3 and C^2−CO fragments. Therefore, the chirality of the carbocyclic residues incorporated into β-oligomers plays a decisive role in the adoption of determined secondary structural motives such as helices or strand-mimics.[6]

Secondary structures of β-peptides have been the object of several computational studies attempting to rationalize factors controlling the different motifs usually manifested by these oligomers. Perczel et al. described a rational design of β-peptide structures by using a 3D Ramachandran cube and methodologies previously developed.[7a] Structure and stability of single- and double-stranded sheet-like conformers of β-peptides were studied, and never before seen new foldamers were reported based on quantum mechanic (QM) calculations.[7b] The spontaneous formation of nanotubes of different size from parallel or polar strands of β-peptides was first predicted[7c] and subsequently described experimentally.[7d] Energetic and structural features of such β-polypeptides are hoped to stimulate experimental research, as presented herein.

Efforts to rationalize and predict the preferred folding induced by the configuration of the backbone atoms in carbocyclic rings containing β-oligomers have been made. Recently, Martinek and Fülöp described a stereochemical patterning approach for the design of peptidic foldamer helices.[8] Proposed methodology is based on a set of

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geometrical requirements of the periodic secondary structure formation, but the design method cannot handle all the conditions required for folding in solution.

Short β-peptides (two−four residues) are promising compounds as they are more soluble and easy to synthesize than their longer counterparts. Even though their size is usually too small for the formation of 10−14 helices,[9] defined conformational bias in solution has been described for dimers and tetramers of conformationally constrained cyclobutane and norbornene derivatives.[10] For instance, dimeric cyclobutane β-peptides have shown their ability to act as low-molecular-weight gelators (LMWG) affording gels and xerogels as the result of hierarchical folding and self-assembly.[11] Cyclobutane β-dipeptides have been used as a self-assembling component in functional organic fibers for the preparation of conducting materials.[12] Moreover, hybrid dipeptides consisting of cyclobutane β-amino acids and linear residues showed biological activity as metallocarboxypeptidase inhibitors.[13]

In derivatives of *cis*-(*R*,*S*)-2-aminocyclobutane-1-carboxylic acid, the formation of an intraresidual six-membered hydrogen-bonded ring (6-strand) was described for the protected parent compound, as well as for the series of *all-cis* homo-oligomers, from dimer to octamer.[11a,14] In contrast, a H12 fold was recently described for the cyclobutane *all-trans*-hexa- and octamer.[15] Nevertheless, for smaller oligomers, H8 was observed for *trans*,*trans*-(*S*,*S*,*S*,*S*)- and *trans*,*cis*-(*S*,*S*,*S*,*R*)-dimers, as the result of inter-residual hydrogen bonds between N*H*(*i*) and C*O*(*i* + 1), (*i*) and (*i* + 1) being two consecutive residues in the polypeptide backbone.[16] However, data for *all-trans*-tetramer and other tetramers consisting of cyclobutane residues with different chirality (heterochiral β-tetrapeptides) have not previously been reported.

Thus, a total of 256 (4^4) stereoisomers could be formed from the combination of the four stereoisomeric cyclobutane monomers. The chirality of the four residues implied with their sequential position should be known to answer the question of how to design a tetrameric cyclobutane β-peptide of a given fold. In this paper, we propose a simple approach based on QM calculations to demonstrate that the chirality of the monomers is responsible for and controls the dominant conformation of short cyclobutane β-peptides containing 2−4 residues in total. The predictive power of this model has been satisfactorily verified by comparison of the theoretical predictions with their experimental counterparts.

By combining the optimized structures of the monomeric building units (Figure 1), 16 dimeric alternative structures were obtained and refined by QM calculations. Furthermore, mixing these dimeric structures, 64 different tetramers, among the total number of 256 possible stereoisomers, were designed. QM calculations completed on some of the above foldamers confirmed that the conformations of the different dimers and tetramers can easily be predicted by wisely summing the energies of the building units.

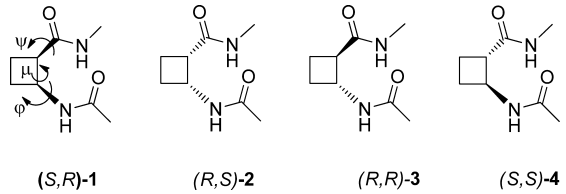

Figure 1. Structures of all four stereoisomers of the monomeric building units considered in this work. φ, μ, and ψ torsion angles are shown.

Computational predictions have been satisfactorily verified by NMR-based structure elucidation, which was performed on selected cyclobutane β-peptides. These compounds were chosen as instances of representative combinations of monomers of different chirality within the sequential backbone. The results of this work provide a rationale for the design of a broad variety of conformationally controlled homo- or heterochiral β-foldamers.

RESULTS AND DISCUSSION

With the aim to validate the model presented herein, *N*-Boc/*N*-Ac- and CO-NHMe-protected monomeric building units (Figure 1), as well as the selected oligomer derivatives depicted in Figures 2 and 3, were synthesized and studied by high resolution ^{1}H NMR techniques. From the experimental data obtained, NOE restraints and coupling constants, the prevalent conformers of these compounds were deduced, and the resultant structures were compared with those predicted by QM calculations. In these computations, for simplicity, *N*-Ac derivatives were considered only. First, the synthesis and ^{1}H NMR experiments are presented. Second, the results of QM calculations and their comparison with experimental data are discussed. Finally, the generalization of such QM-based fold prediction is outlined.

Synthesis of Mono- and Oligomeric β-Peptides. The syntheses of the monomeric building units (*S*,*R*)-**1** and (*S*,*S*)-**4** were achieved by using the corresponding *N*-Boc-ACBA diastereomers,[16] respectively (synthesis of (*S*,*R*)-**1** is depicted in Scheme 1). The carboxylic acid precursor was coupled with methylamine in the presence of pentafluorophenyl diphenylphosphinate (FDPP) as coupling agent and *N*,*N*-diisopropylamine (DIPEA) in dichloromethane. Subsequent removal of the *N*-Boc protecting group with TFA and $EtSi_3H$, followed by acetylation of the resultant primary amine with acetic anhydride in the presence of dimethylaminopyridine (DMAP), afforded (*S*,*R*)-**1** in 81% overall yield for the three steps. (The diastereomer (*S*,*S*)-**4** was obtained in 79% yield).

Dimers and tetramers (Figures 2 and 3, respectively) were selected as representative diastereoisomers containing backbone residues of the same, alternate, or "random" chirality. These compounds were synthesized to study their secondary structure type and to emphasize the role of the absolute and relative stereochemistry of the monomer with respect to the overall fold. Synthesis was conducted as summarized in Scheme 2.

Free carboxylic acids were prepared by mild saponification of the corresponding methyl esters with 0.25 M NaOH in 1:10 THF−water at 0 °C. Epimerization of the stereogenic centers was avoided under these conditions. Amines were deprotected by reaction with TFA for *N*-Boc derivatives and hydrogenolysis in the presence of 10% Pd/C as catalyst for *N*-CBz compounds. Coupling from the suitable stereoisomeric precursors was achieved by using FDPP and DIPEA in anhydrous DMF. For instance, compound **5**[14a] (Figure 2) was prepared in this way (see Supporting Information). Saponification of the methyl ester as described above followed by coupling with methylamine afforded dimers **6**−**12** (Figure 2), which are new products that were fully characterized (see the Supporting Information). The same strategy was applied for the synthesis of the tetramers. Compounds **14**[15] and **15**[14b] were described

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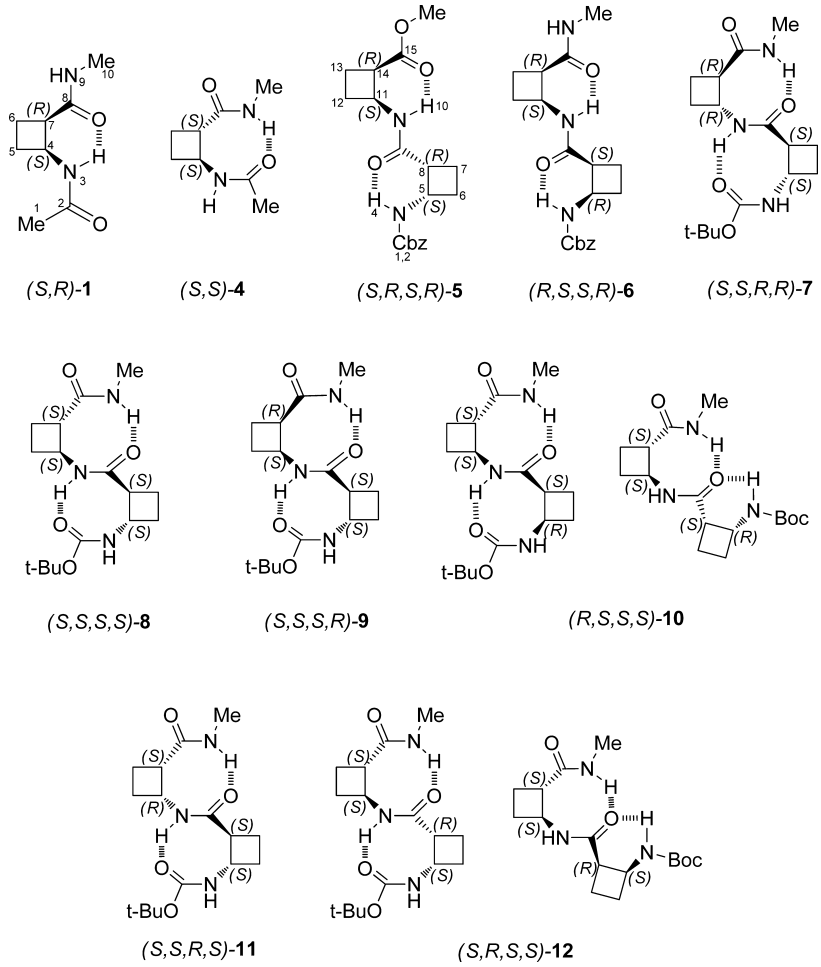

Figure 2. Prevalent molecular structures of synthesized monomers and dimers predicted by QM calculations and verified by ^{1}H NMR. (Dashed lines are for H-bonds).

earlier, while additional tetrapeptides (Figure 3) are new products and thus are fully characterized here.

Structure in Solution Elucidated by ^{1}H NMR Spectroscopy. Experiments were carried out in $CDCl_3$, a solvent of low dielectric constant (<5), suitable for conformational analysis comparable with QM results of oligomers and related cyclobutane β-peptides.[14,16] Furthermore, self-association does not occur in this solvent at the concentrations used (ca. 5−10 mM).[11b,16] These compounds are not soluble in protic solvents such as water or methanol. Moreover, protic solvents could alter the molecular fold as they would compete in the formation of hydrogen bonds. First, standard 1D and 2D high resolution correlation NMR spectra allowed the assignment of all protons and carbon atoms. In this study, NMR not only served as analytical tool to ensure that the desired products were obtaine; in addition, ^{1}H−^{1}H NOESY experiments made it possible to establish intra- and inter-residue connectivities. Both NOE contacts and HH scalar coupling constants, $^3J_{HH}$, confirm the *trans* stereochemistry of all amide bonds within the major conformer of each peptide (see the Supporting Information). Solution state NMR-derived 3D structures are in good agreement with conformers obtained by QM calculations (*vide infra*).

As an instance of the usual procedure followed, experiments and results for dimer (*S*,*R*,*S*,*S*)-**12** are described and discussed. By means of 1D selective TOCSY experiments, the isolated selection of NH_4, NH_{10}, and NH_{16} protons and posterior magnetization transfer to the whole spin system permits editing the ^{1}H NMR spectrum of each residue into separated subspectra (Figure 4). The spatial disposition between residues was disclosed by inspection of 1D selective NOE experiments on NH_4, NH_{10}, and NH_{16} protons (Figure 5). NOE enhancements were used to restrain internuclear distances while experimental ^3J(NHCH) couplings were related to dihedral angles *via* the Karplus-type curve proposed by Ludvingsen for (H−N−C_α−H_α) torsion angle in peptides[17] (see Tables S1 and S2 in the Supporting Information). Based on the above NMR-restraints completed in an iterative conformational search, two possible conformers of **12** are identified in solution, namely, that of Z6H8 and Z8H8 (Figure 6), with the slight predominance of Z6H8 structure, analogously to those encountered by DFT calculations (see the Supporting Information for details and next section for Z*n*H*n* definition). A comparison of the NMR derived and QM obtained structural information is provided in Tables 3, 4, and 6 (*vide infra* in QM calculations).

The comprehensive chemical shift analysis of the experimental and QM calculated (GIAO, B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d)) NH_4, NH_{10}, and NH_{16} resonances shows a convincing similarity. Even though amide N*H*s are extremely sensitive to secondary structure, solvent, temperature, etc., the semiquantitative similarity of these resonances, as calculated

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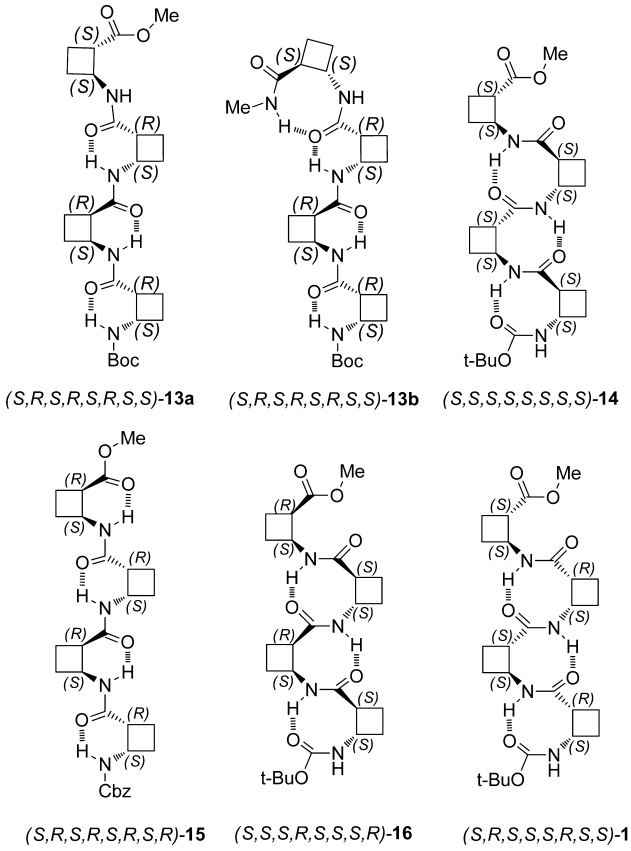


Figure 3. Prevalent molecular structures of synthesized tetramers predicted by QM calculations and verified by ^{1}H NMR. (Dashed lines are for H-bonds).

Scheme 1

and determined by ^{1}H NMR, strongly supports the exclusive presence of the above determined Z6H8 and Z8H8 conformational mixture (Table 1).

Thus, experimental evidence suggests that NOE, *J* couplings, and chemical shift values are all in excellent agreement and suggests a conformational mixture of Z6H8 and Z8H8 of similar population, exhibiting a fast exchange on the NMR time scale of motion (Figure 6).

Similar studies were carried out for all synthesized dipeptides (triamides). For instance, Figure 7 shows ^{1}H NMR comparative results of dimers (*R,S,S,R*)-**6**, (*S,S,R,R*)-**7**, and (*R,S,S,S*)-**10**, all

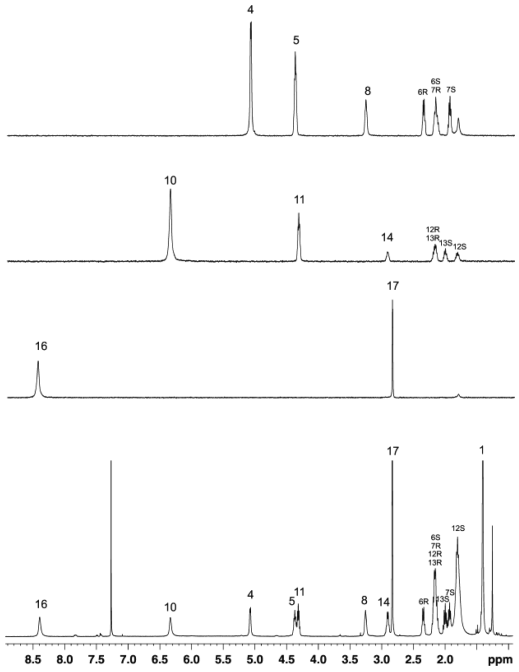


Figure 4. 1D selective TOCSY NMR experiments on N*H*s of (*S,R,S,S*)-**12** used for characterization purposes. (TOCSY mixing time was 60 ms at 298 K in $CDCl_3$ (600 MHz); ^{1}H NMR at the bottom for visual comparison).

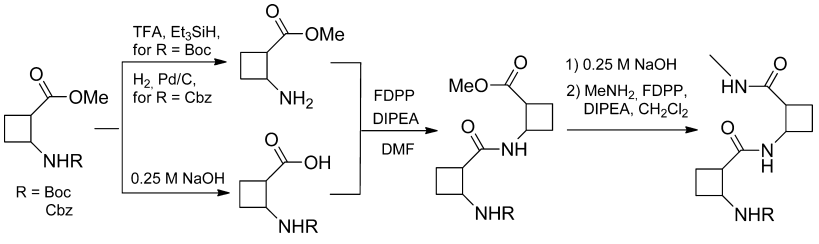
composed of heterochiral residues. As long as **6** adopts exclusively the Z6Z6 backbone fold, **7** has an H8H8 prevalent conformation. Furthermore, as seen for **12**, dipeptide **10** exhibits also the conformational ensemble of Z8H8 and Z6H8 in rapid equilibrium. It is noteworthy that N*H* chemical shifts have marked differences as they depend strongly on the type of secondary structure element(s) formed as also seen for *α*-peptides.[18]

As MeNH−CO-tetramers are insoluble in $CDCl_3$, **13b** was characterized in DMSO-d_6. Therefore, the conformational study of tetramers was completed on MeO−CO derivatives (**13a-17**) depicted in Figure 3. Detailed NMR data for all studied molecules is presented in the Supporting Information except for tetramer **15**, which had been described previously.[14b]

QM Calculations. The zigzag (Z) and helix (H) are two motifs frequently recognized in *β*-peptides and stabilized by internal H-bonds (Figure 8). Thus symbols Z*n* and H*n* mean zigzag and helical conformers, respectively, where *n* is the number of atoms involved in the intramolecular H-bonded pseudo-ring. P indicates plus (or clockwise), and M stands for minus (or counter clockwise) twist or helicity.[7a]

Monomeric building units (Figure 1) were constructed and optimized at the RHF/3-21G level of theory. Subsequently, a

Scheme 2



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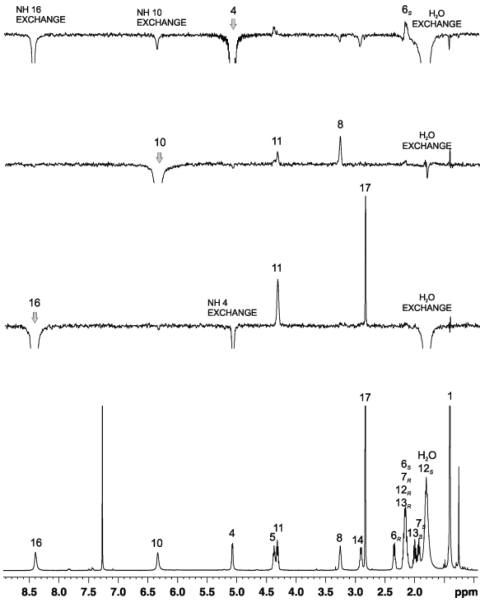
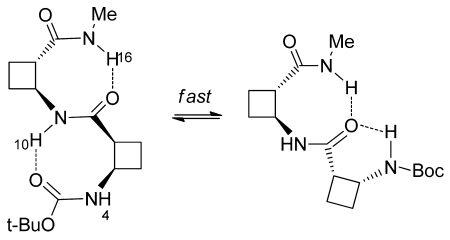


Figure 5. 1D selective NOESY NMR experiments on N*H*s of (*S*,*R*,*S*,*S*)-**12** used for product characterization and conformational studies. (NOESY mixing time was 500 ms at 298 K in $CDCl_3$ (600 MHz); ^{1}H NMR at the bottom for visual comparison).



(*S*,*R*,*S*,*S*)-**12**

Figure 6. Fast equilibrium between Z8H8 and Z6H6 conformation for dimer (*S*,*R*,*S*,*S*)-**12**.

Table 1. Comparison of GIAO Calculated[a] and Experimental ^{1}H NMR Chemical Shifts for (*S*,*R*,*S*,*S*)-12

	GIAO[a] (ppm)	exptl δ (ppm)	$\Delta(\delta_{GIAO}-\delta_{exp})$
Z8H8 conformer			
NH_{16}	8.81	8.39	0.42
NH_{10}	6.73	6.33	0.40
NH_4	4.27	5.07	−0.80
Z6H8 conformer			
NH_{16}	8.22	8.39	−0.17
NH_{10}	5.48	6.33	−0.85
NH_4	5.21	5.07	0.14

[a]B3LYP/6-311+G(2d,p). TMS was used as reference.

full 3D scan was completed taking as variables backbone torsion angle φ and ψ, whereas μ is constrained by the ring itself. The potential energy surface (Ramachandran cube) shows at each value-pair of φ and ψ its energy, and thus its relative stability can easily be derived (Figure 9).

The relative stabilities of all the energy minima considered from the potential surfaces were calculated for each compound at the B3LYP/6-31G(d) level of theory in the gas phase

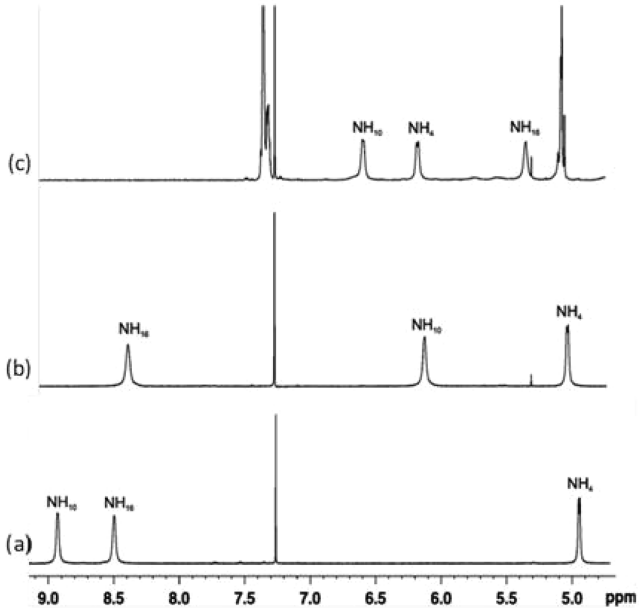
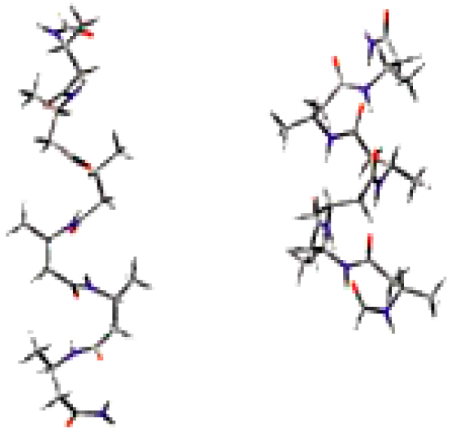


Figure 7. N*H* pattern in the 600 MHz ^{1}H NMR spectrum ($CDCl_3$, 298 K) of dimers (a) (*S*,*S*,*R*,*R*)-**7**, corresponding to H8H8 structure; (b) (*R*,*S*,*S*,*S*)-**10**, corresponding to Z8H8−Z6H6 equilibrium, and (c) (*R*,*S*,*S*,*R*)-**6**, corresponding to Z6H6.



zigzag *helical*

Figure 8. Molecular pictogram of the zigzag (Z) and the helical (H) backbone folds of β-peptides (from ref 7a).

(vacuum) and in $CHCl_3$. The results as well as the Boltzmann distribution are listed in Table 2.

An acyclic monomeric building unit is rather flexible and thus resulting in up to 3^3 possible backbone folds (see the appropriate 3D Ramachandran cube).[7a] However, the cyclobutane ring constrains torsion angle μ leading to the reduction of the number of possible conformers to $3 \times 3 = 9$ or less backbone structures.[19] The geometry scans show that the conformational prevalence of the building unit (lego element) is narrowed down and fully controlled by local chirality (Figure 10).

Geometry scans reveal for the monomeric building unit that the *trans* forms, both the (*S*,*S*)- and (*R*,*R*)-stereoisomers, have more constrained structures, resulting in 2 minima only, namely, that of H8 of greater and Z6 of lower stability. Those of *cis* configuration have a total of 4 minima, out of which 2

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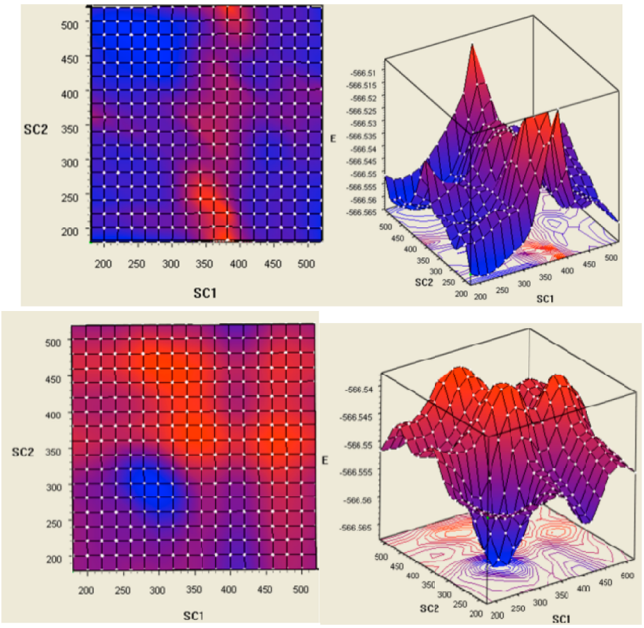

Figure 9. Ramachandran-type surface of (*S*,*R*)-**1** (top) and (*S*,*S*)-**4** (bottom) at the RHF/3-21G level of theory. Their enantiomeric structures, (*R*,*S*)-**2** and (*R*,*R*)-**3**, respectively, have potential energy surfaces that are non-superimposable mirror images.

have greater stability, namely, those of Z6 and Z8 ($\Delta G_{Z8} - \Delta G_{Z6}$ = 0.8 kcal mol^{-1} in vacuum and 0.7 kcal mol^{-1} in chloroform, respectively). H12 conformers have the highest relative energy, and thus their population is the lowest (<1%).

The most stable structures as computed are in perfect accordance with the NMR data (Table 3). Both methods show that Z6 is the exclusive conformer of the *cis*-, and H8 is that of the *trans*-configuration (Figure 2 and Table 3). Thus, the *trans* lego element (*S*,*S*) results in $H8_P$, while its enantiomeric structure, (*R*,*R*), adopts solely (>96%) the $H8_M$ conformer. In addition, the *cis*-(*S*,*R* and *R*,*S*)-diastereomers have also $Z6_P$ (77%) as the prevalent conformer, accompanied by $Z8_P$ as the minor conformer (23%) of the ensemble.

The configuration driven conformer selection of foldamers is even more pronounced as the length of the polypeptide chain increases. With the combination of the above 4 lego elements 16 stereoisomers in total could be obtained for dimers. Due to their inherent symmetry only the 8 different diastereomers have to be synthesized and studied. For each foldamer composed of two ACBA lego units described above, a total of 81 (9 × 9) conformers could have been expected. However, configuration-induced structure selection is so extent for dimers, that for compounds **5**, **6**, **7**, **8**, **9**, and **11** a single conformer dominates the solution state in line with our QM calculations. Only for compounds **10** and **12** is more than a single conformer present. Once again, the NMR restrains provided and the *ab initio* determined structures are very similar, namely, $Z6_MH8_P$ plus $Z8_MH8_P$ for **10**, and $Z6_MH8_P$ plus $Z8_PH8_P$ for **12** (Table 4).

Thus, for selected dimers, when *trans* and *cis* building blocks are both combined, the appearance of novel foldamers is predicted (Table 4 and Figure 2). A Z6 to H8 "transition"-like fold of nearly equal stability as that of Z8H8 conformer is observed for *cis*,*trans*-(*S*,*R*,*S*,*S*)-**12**. Its stability arises from a bifurcated hydrogen bond involving the C-terminal carbonyl group of the first residue. On the contrary, *trans*,*cis*-(*S*,*S*,*S*,*R*)-**9** forms solely the Z8H8 structure, as here the carbonyl group is not accessible and the amino group cannot form the aforementioned bifurcated H-bond. This QM-predicted structure is fully confirmed by NMR spectroscopy: a conformational equilibrium was present between the Z6H8 and Z8H8 structures of *cis*,*trans*-(*S*,*R*,*S*,*S*)-**12** of similar population for both conformers. Even though *N*-Cbz- and *N*-Boc-protected model peptides were used for NMR measurements and computations were carried out on acetyl-protected moieties, experimental and theoretical results correlate very well. Thus, for longer polypeptides the N- and C-terminal protecting groups seems to have negligible effect on the overall backbone fold.

The relative stabilities of the dipeptides were first estimated from the stabilities of their parent monomers and, subsequently, QM fully optimized. A correlation of high significance is obtained between fragment-based and fully optimized stabilities (Table 5), pointing out that folding of longer chain(s) is strongly predictable. This methodology could be of value in designing even longer foldamers.

For trimers and tetramers a total of 64 (4^3) and 256 (4^4) compounds, respectively, could have been synthesized to study the complete set of foldamers. Thus, for a single stereoisomer of a tetramer of $(ACBA)_4$, a total of 9^4 = 6561 conformers could have been present. However the structure selection of ACBA is so robust, that the 3D structure of even longer systems is fully predictable, simply based on the structural properties of their lego elements. For a few chosen compounds, **13**, **14**, **15**, **16**, and **17**, the QM-predicted single and dominant conformer out of the 6561 possible ones was confirmed by NMR structure elucidation (Figure 3 and Table 6). In a similar manner, any tetramer fold can be rationally designed and synthesized to obtain the requested 3D foldamer.

Table 2. Conformer Relative Stabilities of the *cis*-(*S*,*R* or *R*,*S*)- and *trans*-(*S*,*S* or *R*,*R*)-Monomeric Building Units Derived from 2-Aminocyclobutane-1-carboxylic Acid (Figure 1) and Their Relative Abundances (in Parentheses); Most Stable Conformers Are in Bold

		ΔG^a (kcal mol^{-1}) (% population)^b				ΔG^a (kcal mol^{-1}) (% population)^b	
compound	conformation	vacuum	$CHCl_3$	compound	conformation	vacuum	$CHCl_3$
(*S*,*R*)-**1**	$Z8_P$	8.3^c	8.1^c	(*R*,*S*)-**2**	$Z8_M$	8.3^c	8.1^c
	$Z6_P$	1.6 (79)	1.7 (77)		**$Z6_M$**	1.6 (79)	1.7 (77)
	$Z8_P$	2.4 (21)	2.4 (23)		**$Z8_M$**	2.4 (21)	2.4 (23)
	$H12_M$	10.2^c	8.4^c		$H12_P$	10.2^c	8.4^c
(*S*,*S*)-**3**	$H8_P$	0.0	0.0 (96)	(*R*,*R*)-**4**	$H8_M$	0.0	0.0 (96)
	$Z6_M$	3.2^c	1.9 (4)		$Z6_P$	3.2^c	1.9 (4%)

^{a}As obtained at the B3LYP level of theory. ^{b}Relative abundances from Boltzmann distribution at T = 298.15 K. ^{c}Conformers with less than 1% relative abundance.

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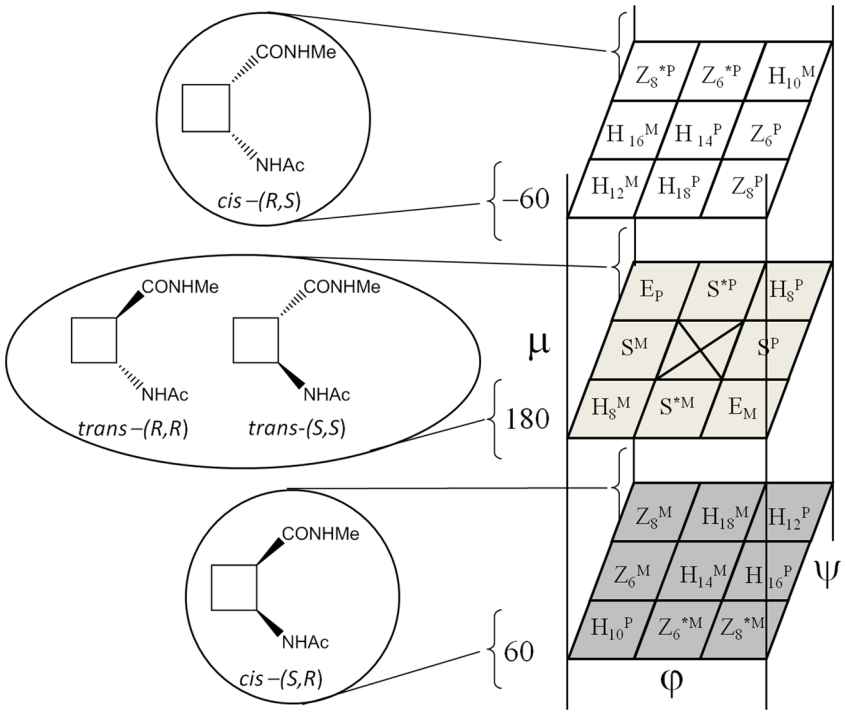

Figure 10. Selected cross sections of the Ramachandran-type cubes of the *cis*-(*S*,*R* or *R*,*S*)- and *trans*-(*S*,*S* or *R*,*R*)-monomeric building units derived from 2-aminocyclobutane-1-carboxylic acid (Figure 1). S (spiral) and E (elongated) refer to additional possible conformations according to ref 7a.

Table 3. Selected Computed and NMR Determined Structural Parameters of the Most Stable *cis*-(*S*,*R* or *R*,*S*)- and *trans*-(*S*,*S* or *R*,*R*)-Conformers of the Monomeric Building Units of 2-Aminocyclobutane-1-carboxylic Acid (Figures 1 and 2)

	computed torsional angles[a,b] (deg)				exptl torsional angles[b,c] (deg)		
configuration/structure	φ	μ	ψ	ΔG (kcal mol^{-1}) vacuum (*CHCl*$_3$)	φ	μ	ψ
(*S*,*R*)/Z6$_P$	−124	−27	149	1.6 (*1.7*)	−119	−20	157
(*S*,*R*)/Z8$_P$	−69	−24	90	2.4 (*2.4*)			
(*R*,*S*)/Z6$_M$	124	27	−149	1.6 (*1.7*)	119	20	−157
(*R*,*S*)/Z8$_M$	69	24	90	2.4 (*2.4*)			
(*S*,*S*)/H8$_P$	−87	104	−35	0.0 (*0.0*)	−85	102	−18
(*R*,*R*)/H8$_M$	87	−104	35	0.0 (*0.0*)	85	−102	18

[a]B3LYP level of theory. [b]All amide torsion angles are ∼180°. [c]Values obtained by NMR in $CDCl_3$.

CONCLUSION

In hunting for a larger arsenal of foldamers, new skeletons are required. Polyamide systems are of great interest due to their favorable biological properties and powerful applicability. The functionalized cyclobutane derivatives, oligomers of 2-aminocyclobutane-1-carboxylic acid (ACBA), are promising candidates along this line. The *cis*-form of ACBA gives rise to two backbone folds only, Z6 and Z8, whereas the *trans*-form manifests exclusively as an H8 structure. By combing these subunits their structural preference remains and this feature of ACBA allows the rational design of a new series of foldamers, by simply combing the appropriate lego building units (Figure 11).

Based on the structural properties of a given protein fragment, epitope, or subunit to be mimicked by foldamers, the right combination of the appropriate ACBA stereoisomer subunits makes feasible the unambiguous design and subsequent synthesis of new cyclobutane β-peptides presenting solely the desired secondary structural form. Biologically active foldamers of almost any kind can now be issued by simply condensing the correct sequence of lego elements.

EXPERIMENTAL SECTION

NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 for ^{1}H at 500 or 600 MHz and for ^{13}C at 125 or 150 MHz. $CDCl_3$ (δ 7.26/77.16 ppm) and DMSO-d_6 (δ 3.33/39.53 ppm) served as internal standards for ^{1}H/^{13}C NMR. Melting points were determined on a hot stage and are uncorrected. TLC was performed using silica on aluminum plates (0.20 mm thickness). Visualization was accomplished with UV light, vanillin, and/or anisaldehyde. Column chromatography was performed on silica gel (mean pore: 60 Å; particle size: 0.04−0.06 mm, 230−400 mesh).

General Procedure for the Synthesis of the Building Units (*S*,*R*)-1 and (*S*,*S*)-4. The free acid of the *N*-Boc-protected cyclobutane derivative is coupled to methylamine using 1.2 equiv of pentafluorophenyl diphenyl phosphinate (FDPP), 3.0 equiv of *N*,*N*-diisopropylethylamine (DIPEA), dichloromethane, and 2.5 equiv of a 2 M solution in THF of the methylamine. The mixture is stirred overnight, and then the solvent is evaporated under vacuum to dryness. The crude can be purified by column chromatography.

The obtained *N*-Boc derivative is then hydrolyzed by addition of trifluoroacetic acid (10 equiv) in the presence of Et_3SiH (3.0 equiv) in dry dichloromethane. After 2 h, the solvent is evaporated, and the excess of TFA is lyophilized. The obtained crude is used in the next step without further purification. A solution in EtOAc of the amine obtained, DMAP (0.2 equiv), Et_3N (2.5 equiv), and acetic anhydride

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Table 4. Selected Computed and NMR Determined Structural Parameters of the Most Stable Conformers of Dimers of 2-Aminocyclobutane-1-carboxylic Derivatives (Figure 2)

compound/ conformation	computed torsional angles[a,b] (deg)						ΔG (kcal mol^{-1}) computed	exptl torsional angles[b,c] (deg)						diff[d]
	φ	μ	ψ	φ	μ	ψ		φ	μ	ψ	φ	μ	ψ	
(*S*,*R*,*S*,*R*)-**5**/ $Z6_PZ6_P$	−129	−27	153	−125	−27	152	4.4	−147	−21	171	−144	−20	170	38
(*R*,*S*,*S*,*R*)-**6**/ $Z6_MZ6_P$	127	27	−151	−129	−27	151	4.2	121	19	−146	−163	−20	−177	49
(*S*,*S*,*R*,*R*)-**7**/ $H8_PH8_M$	−86	103	−32	86	−104	35	0.4	−85	99	−19	76	−110	48	22
(*S*,*S*,*S*,*S*)-**8**/ $H8_PH8_P$	−87	103	−37	−88	104	−34	0.0	−83	102	−34	−86	100	−15	20
(*S*,*S*,*S*,*R*)-**9**/ $H8_PZ8_P$	−87	103	−33	−70	−23	87	3.9	−61	106	−56	−66	−25	96	36
(*R*,*S*,*S*,*S*)-**10**/ $Z6_MH8_P$	121	26	−145	−87	104	−34	3.0	153	21	−164	−69	110	−43	43
(*R*,*S*,*S*,*S*)-**10**/ $Z8_MH8_P$	69	24	−87	−90	103	−33	3.4	62	33	−93	−88	108	−37	15
(*S*,*S*,*R*,*S*)-**11**/ $H8_PZ8_M$	−88	101	−31	69	22	−87	3.0	−71	107	−50	66	22	−90	27
(*S*,*R*,*S*,*S*)-**12**/ $Z6_PH8_P$	−122	−27	146	−87	104	−35	3.4	−152	−21	164	−80	99	−24	38
(*S*,*R*,*S*,*S*)-**12**/ $Z8_PH8_P$	−70	−23	83	−90	104	−35	3.5	−62	−33	79	−96	93	−19	24

[a]B3LYP level of theory. [b]All amide torsion angles are ∼180°. [c]Values obtained by NMR in $CDCl_3$. [d]Diff is a term that defines the difference between computed and experimental values according to the Hunt-McIlroy algorithm (Hunt, J. W.; McIlroy, M. *Computing Science Technical Report*, Bell Laboratories, **1976**, vol. *41*).

Table 5. Conformer Relative Stabilities of β-Dipeptides Composed of 2-Aminocyclobutane-1-carboxylic acid (Figure 2) and Their Relative Abundances; Most Stable Conformers Are in Bold

compound	conform	ΔG (kcal mol^{-1}) computed (predicted[a]) % population[b]	compound	conform	ΔG (kcal mol^{-1}) computed (predicted[a]) % population[b]	compound	conform	ΔG (kcal mol^{-1}) computed (predicted[a]) % population[b]
(*S*,*R*,*S*,*R*)-**5**	**$Z6_PZ6_P$**	**4.4 (3.2) 88%**	(*R*,*S*,*S*,*R*)-**6**	**$Z6_MZ6_P$**	**4.2 (3.2) 93%**	(*S*,*S*,*S*,*R*)-**9**	**$H8_PZ8_P$**	**3.9 (2.4) 96%**
	$Z6_PZ8_P$	5.7 (4.0) 10%		$Z6_MZ8_P$	6.7 (4.0) 1%		$Z6_MZ6_P$	5.9 (4.8) 3%
	$Z8_PZ8_P$	6.6 (4.8) 2%		$Z8_MZ8_P$	5.9 (4.8) 5%		$Z6_MZ8_P$	7.3 (5.6)
	$Z8_PZ8_M$	9.5 (10.7)		$Z6_MZ8_M$	11.9 (9.9)		$H8_PZ8_M$	8.9 (8.3)
	$Z6_MZ6_P$	9.7 (−)		$Z6_MH12_M$	13.1 (11.8)		$H8_PH12_M$	9.7 (10.2)
	$Z8_PZ8_P$	10.7 (10.7)		$Z8_MZ8_M$	11.8 (10.7)		$Z6_MZ8_M$	12.7 (11.5)
	$H12_MZ6_P$	12.5 (11.8)		$Z8_MH12_M$	12.9 (12.6)		$Z6_MH12_M$	13.6 (13.4)
	$Z6_PZ8^*_M$	12.7 (9.9)		$Z6_MZ6_M$	11.3 (--)			
	$H12_MZ8_P$	12.8 (12.6)		$Z8_MZ8_P$	12.1 (10.7)	(*S*,*S*,*R*,*S*)-**11**	**$H8_PZ8_M$**	**3.0 (2.4) 99%**
	$Z6_PH12_M$	13.1 (11.8)		$H12_PZ6_P$	13.6 (11.8)		$Z6_MZ6_M$	6.3 (4.8)
	$Z8_PH12_M$	14.0 (12.6)		$H12_PZ8_P$	16.9 (12.6)		$Z6_MZ8_M$	6.3 (5.6)
	$Z8_PZ8_P$	17.4 (16.6)		$Z8_MZ8_P$	14.6 (16.6)		$H8_PZ8_P$	8.9 (8.3)
	$Z8_PH12_M$	18.0 (18.5)		$Z8_MH12_M$	18.5 (12.6)		$H8_PH12_P$	10.5 (10.2)
	$H10_PZ8_P$	18.0 (19.0)		$H12_PZ8_P$	18.5 (12.6)		$Z6_MH12_P$	13.8 (13.4)
	$H12_MH12_M$	18.6 (20.4)		$H12_PH12_M$	20.5 (20.4)		$Z6_MZ8_P$	12.2 (11.5)
(*S*,*R*,*S*,*S*)-**12**	**$Z6_PH8_P$**	**3.4 (1.6) 54%**	(*R*,*S*,*S*,*S*)-**10**	**$Z6_MH8_P$**	**3.0 (1.6) 66%**	(*S*,*S*,*S*,*S*)-**8**	**$H8_PH8_P$**	**0.0 (0.0) 100%**
	$Z8_PH8_P$	**3.5 (2.4) 45%**		**$Z8_MH8_P$**	**3.4 (2.4) 34%**		$Z6_MH8_P$	4.0 (3.2)
	$Z6_PZ6_M$	6.1 (4.8)		$Z6_MZ6_M$	6.2 (4.8)		$H8_PZ6_M$	4.5 (3.2)
	$Z8_PZ6_M$	6.7 (5.6)		$Z8_MZ6_M$	7.2 (5.6)		$Z6_MZ6_M$	6.9 (6.4)
	$Z8_MH8_P$	8.3 (8.3)		$Z8_PH8_M$	9.0 (8.3)	(*S*,*S*,*R*,*R*)-7	**$H8_PH8_M$**	**0.4 (0.0) 100%**
	$H12_MH8_P$	10.5 (10.2)		$H12_PH8_P$	10.1 (10.2)		$H8_PZ6_M$	4.6 (3.2)
	$H12_MZ6_M$	12.0 (13.5)		$H12_PZ6_M$	13.6 (13.4)		$Z6_PH8_P$	4.2 (3.2)
	$Z8_MZ6_M$	12.6 (11.5)		Z_PZ6_M	11.9 (11.5)		$Z6_MZ6_P$	7.2 (6.4)

[a]All possible conformers were computed at B3LYP level of theory as well as fragment-based predicted from the parent monomeric units (values in parentheses). [b]Relative abundances from Boltzmann distribution at T = 298.15 K.

(1.0 equiv) is stirred for 18 h at room temperature. Afterward, the mixture is washed with sodium bicarbonate, and the organic phase is dried over magnesium sulfate. Solvent is removed, and the residue is chromatographed on silica gel obtaining the desired product.

Data for Building Unit 1. Purified by column chromatography (19:1 DCM/MeOH). Isolated yield 130 mg (81%); colorless crystals (EtOAc); mp 129 °C (polymorphism change), 164−165 °C; $[\alpha]^{25}_D$ −231 (*c* 0.3 in MeOH); IR (ATR) 3318, 3297, 2943, 1738, 1645

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Table 6. Selected Computed[a,b] and NMR Determined[c] Structural Parameters of the Most Stable Conformers of Selected Tetramers of 2-aminocyclobutane-1-carboxylic Acid (Figure 3)

		calculated and experimental torsional angles (deg)												ΔG (kcal mol^{-1})		
														predicted		
compound/conformation		φ	μ	ψ	φ	μ	ψ	φ	μ	ψ	φ	μ	ψ	from monomers	from dimers	calcd
(*SRSRSRSS*)-**13b**/$Z6_pZ6_pZ6_pZ8_p$	calc	−127	−27	152	−127	−27	153	−120	−26	147	−87	104	−35	4.80	7.80	9.38
	expt	−144	−21	174	−136	−22	170	−157	−20	176	−86	101	−44			
(SSSSSSSS)-**14**/$H8_pH8_pH8_pH8_p$	calc	−87	103	−37	−88	102	−36	−90	102	−35	−88	104	−35	0.00	0.00	0.00
	expt	−79	107	−46	−87	102	−23	−78	107	−45	−76	101	−42			
(*SRSRSRSR*)-**15**/$Z6_pZ6_pZ6_pZ6_p$	calc	−127	−27	151	−130	−27	155	−128	−27	157	−125	−27	153	6.40	8.80	9.31
	expt	−158	−22	180	−167	−20	−173	−175	−20	−168	−172	−21	−164			
(SSSRSSSR)-**16**/$Z8_pZ8_pZ8_pZ8_p$	calc	−87	103	−33	−69	−23	89	−94	103	−33	−70	−23	86	4.80	7.80	7.47
	expt	−89	101	−23	−63	−23	99	−82	100	−23	−63	30	71			
(SRSSSRSS)-**17**/$Z8_pZ8_pZ8_pZ8_p$	calc	−70	−23	83	−90	104	−55	−67	−23	83	−90	104	−35	4.80	7.00	7.03
	expt	−67	−18	99	−81	102	−27	−99	30	51	−67	103	−42			

[a]B3LYP level of theory. [b]All amide torsion angles are ~180°. [c]Values obtained by NMR in $CDCl_3$.

Figure 11. The rational design of any short new β-peptide with a defined folding becomes possible by simply combing the appropriate lego conformational building units.

cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.68 (br. s, 1H), 5.77 (br. s, 1H), 4.57 (m, 1H), 3.23 (m, 1H), 2.69 (s, 3H), 2.25 (c. a, 2H), 2.03 (m, 1H), 2.01 (m, 1H), 1.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.8, 170.0, 45.7, 45.1, 28.5, 26.1, 23.1, 18.7; HRMS (ESI-TOF) *m*/*z* calcd for $C_8H_{14}N_2O_2Na$ (M + Na)$^+$ 193.0947, found 193.0949.

Data for Building Unit 4. Purified by column chromatography (19:1 DCM/MeOH). Isolated yield 260 mg (79%); colorless crystals (EtOAc); mp change of polymorphism at 134 °C, 169−171 °C; $[\alpha]^{25}_D$ +18 (*c* 0.3 in MeOH); IR (ATR) 3349, 2962, 1745, 1687 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.50 (br. s, 1H), 7.04 (br. s, 1H), 4.27 (m, 1H), 2.87 (m, 1H), 2.73 (d, *J* = 4.6 Hz, 3H), 2.11 (c. a, 2H), 1.97 (s, 3H), 1.94−1.82 (c. a., 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.0, 171.8, 49.7, 48.2, 26.2, 24.1, 22.8, 19.1; HRMS (ESI-TOF) *m*/*z* calcd for $C_8H_{14}N_2O_2Na$ (M + Na)$^+$ 193.0947, found 193.0949.

General Procedure for the Synthesis of the β-Peptides. The MeO-protected amino acid or precursor peptide is dissolved in 1:10 THF−water, and 0.25 M NaOH is added. The mixture is stirred at 0 °C for 2 h. The mixture is washed with CH_2Cl_2 before being acidified to pH 2 with 2 M HCl. The aqueous layer is extracted with EtOAc, and the organic layer is dried over $MgSO_4$, filtered, and evaporated under high vacuum to produce the corresponding carboxylic acid. This compound is used directly in next step without further purification.

The *N*-Boc-protected amino acid or precursor peptide is dissolved in dry CH_2Cl_2 and Et_3SiH and TFA are added. The mixture is stirred at room temperature for 2 h. The reaction mixture is concentrated under reduced pressure obtaining the free amine, which is used in next step without further purification. After this, a solution containing the free carboxylic acid, the free amine, DIPEA (3 equiv), and FDPP (1.2 equiv) in anhydrous DMF is stirred at room temperature overnight. Then, ethyl acetate is added, and the combined organic layers are washed with saturated aqueous $NaHCO_3$. The organic layer is dried over $MgSO_4$, and solvents are removed under reduced pressure. The residue is purified by column chromatography to afford the desired peptide.

In the case of MeNH-protected peptides, first the MeO-protected peptide is submitted to saponification following the procedure described above. Then the free acid obtained is coupled with methylamine (2.5 equiv of a 2 M solution in THF) by adding FDPP (1.2 equiv) and DIPEA (3.0 equiv) in dichloromethane. The mixture is stirred overnight, and then the solvent is evaporated under vacuum to dryness. The crude can be purified by column chromatography to produce the desired peptide.

Data for Dipeptide 6. Purified by column chromatography (EtOAc). Isolated yield 130 mg (99%); colorless crystals (EtOAc/pentane); mp 184−186 °C; $[\alpha]^{25}_D$ −73 (*c* 0.6 in CH_2Cl_2); IR (ATR) 3419, 3314, 2970, 1726, 1643 cm^{-1}; IR ($CDCl_3$) 3460, 3425, 2953,

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1714, 1661 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.35 (c. a, 5H), 6.15 (br.s, 1H), 6.58 (d, J = 7.3 Hz, 1H), 5.34 (br. s, 1H), 5.07 (c. a, 2H), 4.66 (m, 1H), 4.47 (m, 1H), 3.10 (c. a, 2H), 2.68 (d, J = 4.2 Hz, 3H), 2.39−2.21 (c. a, 4H), 1.94 (c. a, 4H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.8 (2C), 173.1, 155.6, 136.8, 128.7, 128.2, 128.1, 66.6, 46.3 (4C), 45.9, 44.8, 28.7, 26.7, 30.6 (4C), 29.3, 19.6, 19.3; HRMS (ESI-TOF) m/z calcd for $C_{19}H_{25}N_3O_4Na$ $(M + Na)^+$ 382.1737, found 382.1736.

Data for Dipeptide 7a (Methyl Ester Precursor of 7). Purified by column chromatography (EtOAc). Isolated yield 130 mg (71%); colorless crystals (CH_2Cl_2/pentane); mp 153 °C (polymorphism change), 174−178 °C; $[\alpha]^{25}_D$ −34 (c 0.9 in CH_2Cl_2); IR (ATR) 3352, 3311, 2981, 1724, 1681, 1648 cm^{-1}; IR ($CDCl_3$) 3448, 3288, 2985, 1731, 1694, 1656 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.74 (d, J = 5.4 Hz, 1H), 4.89 (d, J = 6.2 Hz, 1H), 4.49 (m, 1H), 4.10 (m, 1H), 3.66 (s, 3H), 3.00 (dd, J = 8.7, J = 18.2 Hz, 1H), 2.88 (dd, J = 9.4, J = 18.3 Hz, 1H), 2.30 (m, 1H), 2.17 (m, 1H), 2.00−1.85 (c. a, 4H), 1.71 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.6, 172.3, 156.5, 80.7, 51.7, 50.3, 48.7, 47.4, 46.4, 28.3, 27.1, 24.6, 18.8, 18.4; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{26}N_2O_5Na$ $(M + Na)^+$ 349.1735, found 349.1734.

Data for Dipeptide 7. Purified by column chromatography (EtOAc). Isolated yield 30.7 mg (49%); colorless crystals (EtOAc/pentane); mp 72−75 °C; $[\alpha]^{25}_D$ −58 (c 0.3 in MeOH); IR (ATR) 3334, 3286, 2923, 1689, 1660, 1646 cm^{-1}; IR ($CDCl_3$) 3448, 3275, 2984, 1728, 1692, 1651 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.92 (br. s, 1H), 8.49 (br.s, 1H), 4.94 (d, J = 6.4 Hz, 1H), 4.26 (m, 1H), 4.10 (m, 1H), 2.91 (dd, J = 9.4 Hz, J = 18.4 Hz, 1H), 2.85 (dd, J = 9.2 Hz, J = 17.9 Hz, 1H), 2.78 (d, J = 4.7 Hz, 3H), 2.23−2.14 (c. a, 4H), 1.97 (c. a, 2H), 1.88 (m, 1H), 1.75 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.4 (2C), 174.2, 157.8, 81.11, 50.2 (4C), 49.9, 48.8, 48.2, 28.5, 26.2, 24.6 (4C), 23.7, 19.2, 18.7; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{27}N_3O_4Na$ $(M + Na)^+$ 348.1894, found 348.1897.

Data for Dipeptide 8. Purified by column chromatography (EtOAc). Isolated yield 160 mg (76%); colorless crystals (EtOAc/pentane); mp 224−227 °C; $[\alpha]^{25}_D$ 24 (c 1.2 in MeOH); IR (ATR) 3305, 2970, 1738, 1681, 1650 cm^{-1}; IR ($CDCl_3$) 3694, 3608, 3440, 3302, 2960, 1703, 1651 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.72 (d, J = 4.4 Hz, 1H), 8.53 (d, J = 2.3 Hz, 1H), 5.06 (br. s, 1H), 4.23 (m, 1H), 4.13 (qt, J = 8.3 Hz, 1H), 3.31 (dd, J = 9.3 Hz, J = 18.1 Hz, 1H), 2.89 (dd, J = 9.6 Hz, J = 18.4 Hz, 1H), 2,79 (d, J = 4.7 Hz, 3H), 2.24−2.15 (c. a, 2H), 2.14−2.06 (c. a, 2H), 2.01−1.88 (c. a, 3H), 1.77 (qt, J = 9.9 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.2 (2C), 156.6, 80.9, 50.1 (4C), 49.7, 48.7, 48.1, 28.3, 26.1, 24.6 (4C), 23.3, 18.9, 18.3; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{28}N_3O_4$ $(M + H)^+$ 326.2079, found 326.2087.

Data for Dipeptide 9. Purified by column chromatography (EtOAC). Isolated yield 180 mg (87%); colorless crystals (EtOAc/pentane); $[\alpha]^{25}_D$ −71 (c 0.9 in MeOH); IR (ATR) 3328, 2942, 1738, 1680, 1645 cm^{-1}; IR ($CDCl_3$) 3450, 3271, 2965, 1696, 1663, 1651 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.29 (br. s, 1H), 5.86 (br. s, 1H), 4.89 (d, J = 6.5 Hz, 1H), 4.52 (m, 1H), 4.09 (m, 1H), 3.31 (m, 1H), 2.85 (dd, J = 8.9, J = 17.4 Hz, 1H), 2.78 (d, J = 4.8 Hz, 3H), 2.43−1.90 (c. a, 4H), 1.75 (c. a, 4H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9, 172.8, 156.0, 80.4, 49.7 (4C), 48.8, 46.4, 46.0, 28.5, 26.6 (4C), 25.4, 19.1, 18.8; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{27}N_3O_4Na$ $[M + Na]^+$ 348.1899, found 348.1897.

Data for Dipeptide 10a (Methyl Ester Precursor of 10). Purified by column chromatography (3:2 EtOAc/hexane). Isolated yield 0.41 g (52%); colorless crystals (EtOAc/pentane); $[\alpha]^{25}_D$ +121 (c 0.9 in CH_2Cl_2); IR (ATR) 3352, 3324, 2982, 1731, 1681, 1651; IR ($CDCl_3$) 3439, 2984, 1731, 1705, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.74 (br. s, 1H), 5.35 (br. s, 1H), 4.48 (m, 1H), 4.40 (m, 1H), 3.71 (s, 3H), 3.13 (m, 1H), 3.02 (dd, J = 8.9, J = 18.0 Hz, 1H), 2.33−2.19 (c. a, 3H), 2.06 (m, 1H), 2.02−1.97 (c. a, 2H), 1.93−1.82 (c. a, 2H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.4, 172.3, 155.4, 79.4, 51.6, 47.6, 47.0, 46.4, 46.3, 29.3, 28.4, 26.6, 18.5, 17.9; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{26}N_2O_5Na$ $(M + Na)^+$ 349.1734, found 349.1737.

Data for Dipeptide 10. Purified by column chromatography (19:1 DCM/MeOH). Isolated yield 160 mg (77%); colorless crystals (CH_2Cl_2/pentane); mp 163−166 °C; $[\alpha]^{25}_D$ 91 (c 1.2 in CH_2Cl_2); IR (ATR) 3311, 2978, 1682, 1649 cm^{-1}; IR ($CDCl_3$) 3442, 3303, 2984, 1706, 1652 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.38 (br. s, 1H), 6.11 (br.s, 1H), 5.02 (d, J = 6.7 Hz, 1H), 4.38 (m, 1H), 4.32 (m, 1H), 3.24 (m, 1H), 2.84 (d, J = 4.6 Hz, 3H), 2.35 (m, 1H), 2.22−2.10 (c. a, 4H), 2.01 (m, 1H), 1.92 (m, 1H), 1.76 (m, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.8 (2C), 155.6, 80.3, 50.0 (4C), 48.3, 47.0, 46.1, 28.5, 28.2 (4C), 26.4, 24.4, 19.0, 17.9; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{27}N_3O_4Na$ $(M + Na)^+$ 348.1894, found 348.1899.

Data for Dipeptide 11a (Methyl Ester Precursor of 11). Purified by column chromatography (3:2 EtOAc/hexane). Isolated yield 210 mg (39%); colorless crystals (EtOAc/pentane); mp 129−131 °C; IR (ATR) 3341, 2970, 1738, 1683, 1650 cm^{-1}; IR ($CDCl_3$) 3448, 3279, 2984, 1726, 1699, 1655 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.87 (br. s, 1H), 4.85 (s, 1H), 4.71 (m, 1H), 4.09 (m, 1H), 3.64 (s, 3H), 3.40 (m, 1H), 2,83 (m, 1H), 2.39−2.27 (c. a, 2H), 2.20−1.87 (c. a, 5H), 1.75 (qt, J = 9.8 Hz, 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.9, 172.5, 155.9, 80.5, 51.6, 49.7, 49.0, 45.4, 45.1, 28.5, 28.1, 25.3, 19.0, 18.3; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{26}N_2O_5Na$ $(M + Na)^+$ 349.1734, found 349.1732.

Data for Dipeptide 11. Purified by column chromatography (EtOAc). Isolated yield 35.3 mg (38%); colorless crystals (EtOAc/pentane); mp 160 °C (polymorphism change), 207−210 °C; $[\alpha]^{25}_D$ 74 (c 0.5 in MeOH); IR (ATR) 3317, 2975, 1684, 1645 cm^{-1}; IR ($CDCl_3$) 3695, 3608, 3450, 3279, 2960, 1690−1650 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.82 (br. s, 1H), 5.68 (br.s, 1H), 4.86 (br. s, 1H), 4.54 (m, 1H), 4.11 (m, 1H), 3.26 (m, 1H), 2.80 (m, 1H), 2.72 (d, J = 4.7 Hz, 3H), 2.34−2.05 (c. a, 4H), 2.01 (c. a, 1H), 1.92 (m, 1H), 1.86 (m, 1H), 1.76 (m, 1H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ173.5, 172.8, 155.9, 80.6, 49.7 (4C), 48.9, 46.4, 46.0, 28.5, 27.1 (4C), 26.2, 25.5, 18.7, 18.3; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{27}N_3O_4Na$ $(M + Na)^+$ 348.1894, found 348.1901.

Data for Dipeptide 12. Purified by column chromatography (EtOAc). Isolated yield 100 mg (82%); colorless crystals (EtOAc/pentane); mp 193 °C (polymorphism change), 235−236 °C; $[\alpha]^{25}_D$ −93 (c 0.7 in CH_2Cl_2); IR (ATR) 3456, 3306, 2970, 1738, 1685, 1645 cm^{-1}; IR ($CDCl_3$) 3694, 3609, 3448, 3282, 2965, 1692, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.39 (br. s, 1H), 6.15 (br.s, 1H), 5.08 (d, J = 7.0 Hz, 1H), 4.45−4.29 (c. a, 2H), 3.26 (m, 1H), 2.91 (m, 1H), 2,86 (d, J = 4.5 Hz, 3H), 2.36 (c. a, 1H), 2.26−2.09 (c. a, 4H), 2.00 (c. a, 2H), 1.81 (c. a, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ174.2 (2C), 156.0, 80.2, 50.0 (4C), 48.6, 47.2, 46.5, 28.7, 26.6, 28.6 (4C), 24.7, 19.3, 18.4; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{27}N_3O_4Na$ $(M + Na)^+$ 348.1899, found 348.1896.

Data for Tetrapeptide 13a. Purified by crystallization (EtOAc). Isolated yield 140 mg (44%); colorless crystals (EtOAc); mp 245 °C (polymorphism change), 264−266 °C; $[\alpha]^{25}_D$ −206 (c 0.1 in MeOH); IR (ATR) 3318, 2947, 1732, 1683, 1651 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 9.19 (d, J = 7.1 Hz, 1H), 8.97 (d, J = 5.7 Hz, 1H), 5.76 (d, J = 5.7 Hz, 1H), 5.46 (d, J = 6.2 Hz, 1H), 4.68 (m, 1H), 4.60 (c. a, 1H), 4.48 (m, 1H), 4.38 (m, 1H), 3.69 (s, 3H), 3.16 (m, 2H), 3.09 (m, 1H), 2.95 (m, 1H), 2.34−2.11 (c. a, 7H), 2.02−1.84 (c. a, 8H), 1.77 (m, 1H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ173.3, 173.0, 172.9, 172.0, 155.2, 79.4, 52.1, 47.4, 46.8, 46.5, 46.0 (2C), 45.9, 45.0, 44.7, 29.8, 29.1, 28.5, 28.4, 27.3, 19.1−18.7 (4C); HRMS (ESI-TOF) m/z calcd for $C_{26}H_{40}N_4O_7Na$ $(M + Na)^+$ 543.2789, found 543.2797.

Data for Tetrapeptide 13b. Purified by crystallization (EtOAc/MeOH). Isolated yield 50 mg (66%); mp 254 °C (dec); $[\alpha]^{25}_D$ +276 (c 0.1 in MeOH); IR (ATR) 3325, 3313, 2945, 1683, 1648 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) 2 possible conformers (see NMR studies in Supporting Information) δ 8.10 (d, $1H_{conformer1}$, J = 7.6 Hz), 8.01 (d, $1H_{conformer\ 2}$, J = 6.7 Hz), 7.94 (d, $1H_{conformer\ 1}$, J = 4.9 Hz), 7.55 (d, 1H, J = 7.0 Hz), 7.44 (d, 1H, $^3J_{H-H}$= 6.5 Hz), 5.60 (d, $^3J_{H-H}$= 8.0 Hz), 4.39−4.49 (c.a., 2H), 4.24 (m, $1H_{conformer1}$), 4.08−4.18 (c.a., $1H_{both\ conformers}$, $1H_{conformer2}$), 3.05−3.16 (c.a., 3H), 2.91 (m, $1H_{conformer1}$), 2.83 (m, $1H_{conformer2}$), 2.52 (d, 3H, J = 4.6 Hz), 1.99−2.11 (c.a., 6H), 1.63−1.98 (c.a., 10H, $6H_{both\ conformers}$, $4H_{conformer1}$, $4H_{conformer2}$), 1.32 (s, 9H); ^{13}C NMR (DMSO-d_6, 125 MHz) 2 possible conformers (see NMR studies in Supporting Information) δ 174.19, 172.33, 171.66, 171.32, 171.16, 170.94, 154.33, 77.82, 47.13,

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46.54 (2C), 45.95, 45.82, 44.76−44.64 (3C), 44.42 (2C), 28.16, 27.85 (3C), 27.68, 27.37, 26.17, 25.73, 25.47, 24.01, 18.32−17.69 (5C); HRMS (ESI-TOF) m/z calcd for $C_{26}H_{41}N_5O_6Na$ $(M + Na)^+$ 542.2949, found 542.2953.

Data for Tetrapeptide 14. Purified by crystallization (EtOAC). Isolated yield 140 mg (65%); colorless crystals; mp 194 °C (polymorphism change), 246−248 °C; $[\alpha]^{25}_D$ 71 (c 0.5 in MeOH); IR (ATR) 3294, 2949, 1732, 1683, 1650 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 9.19 (d, J = 5.8 Hz, 1H), 8.97 (d, J = 7.8 Hz, 1H), 8.67 (d, J = 5.7 Hz, 1H), 5.00 (d, J = 6.4 Hz, 1H), 4.55 (m, 1H), 4.27 (c. a, 2H), 4.14 (m, 1H), 3.68 (s, 3H), 3.14 (m, 1H), 2.99−2.86 (c. a, 3H), 2.31−2.20 (c. a, 2H), 2.20−2.08 (c. a, 4H), 2.05−1.90 (c. a, 9H), 1.78 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.4, 174.3, 173.8, 172.7, 156.5, 80.9, 51.7, 50.1, 49.8, 49.6, 48.8, 48.2, 48.0, 47.2, 46.7, 29.7, 28.3, 26.9, 24.6, 23.4, 18.6 (2C), 18.4, 18.2; HRMS (ESI-TOF) m/z calcd for $C_{26}H_{40}N_4O_7Na$ $(M + Na)^+$ 543.2789, found 543.2797.

Data for Tetrapeptide 16. Purified by column chromatography (9:1 DCM/MeOH). Isolated yield 87 mg (58%); colorless crystals (MeOH); mp 271 °C (dec); IR (ATR) 3308, 2944, 1722, 1684, 1649, 1532 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.98 (d, J = 8.0 Hz, 1H), 8.89 (d, J = 5.9 Hz, 1H), 6.65 (d, J = 5.2 Hz, 1H), 4.93 (d, J = 6.5 Hz, 1H), 4.66 (m, 1H), 4.39 (m, 1H), 4.22 (m, 1H), 3.99 (m, 1H), 3.61 (s, 3H), 3.39 (m, 1H), 3.30 (m, 1H), 2.82 (m, 2H), 2.42−2.31 (c. a, 2H), 2.26 (c. a, 2H), 2.13 (c. a, 3H), 2.06 (c. a, 3H), 1.99−1.88 (c. a, 2H), 1.81 (c. a, 2H), 1.71 (qt, J = 10.1 Hz, 1H), 1.61 (qt, J = 9.4 Hz, 1H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.5, 173.8, 173.0, 172.7, 156.4, 80.7, 51.9, 49.8−49.5 (2C), 48.8, 48.2, 46.9, 45.7, 45.6, 45.4, 28.4, 28.1, 26.0, 25.0, 24.6, 19.0 (4C); HRMS (ESI-TOF) m/z calcd for $C_{26}H_{40}N_4O_7Na$ $(M + Na)^+$ 543.2789, found 543.2789.

Data for Tetrapeptide 17. Purified by column chromatography (EtOAC). Isolated yield 120 mg (50%); colorless crystals (EtOAc); mp 259 °C (dec); $[\alpha]^{25}_D$ = −38 (c 0.4 in MeOH); IR (ATR) 3306, 2970, 1737, 1681, 1648 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.80 (br. s, 1H), 6.24 (d, J = 6.6 Hz, 1H), 6.02 (br. s, 1H), 5.28 (d, J = 7.0 Hz, 1H), 4.55−4.47 (c. a, 2H), 4.35 (m, 1H), 4.27 (m, 1H), 3.69 (s, 3H), 3.29 (dd, J = 7.4 Hz, J = 13.9 Hz, 1H), 2.99 (dd, J = 8.9 Hz, J = 17.9 Hz, 1H), 2.87 (dd, J = 9.2 Hz, J = 17.9 Hz, 1H), 2.43 (m, 1H), 2.29 (c. a, 2H), 2.23 (c. a, 2H), 2.15 (c. a, 4h), 1.98 (c. a, 4H), 1.88 (c. a, 2H), 1.78 (qt, J = 10.2 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ174.1−171.5 (4C), 156.7, 79.8, 52.0, 49.4, 48.1, 47.6, 47.0, 46.9, 46.6, 46.2, 46.0, 28.5, 28.3, 26.9, 26.4, 24.8, 19.1 (4C), 19.0, 18.9, 18.0; HRMS (ESI-TOF) m/z calcd for $C_{26}H_{41}N_4O_7$ $(M + H)^+$ 521.2970, found 521.2986.

Computational Details and Considerations. For the conformational search based on NMR data, NOE enhancements and J coupling constants were used as to define distance and torsion angle constraints for use within a conformational search by using the MMFF force field[20] implemented in the Macromodel 7.0 program.[21] The $CHCl_3$ solvent effect was included through the GB/SA method.[22] NOE enhancements were integrated with respect to the saturated signal (−100% integral) and were categorized as very strong, strong, medium, medium-weak, and weak signals (see the Supporting Information for an example).

For structure prediction, all computations were carried out using the Gaussian 09 software package.[23] All four stereoisomers of 2-aminocyclobutanecarboxylic acid derivatives were subjected to full geometry optimization at the HF/3-21G level of theory and each of them to a subsequent grid scan ($0° < \gamma < 360°$, $0° < \psi < 360°$, as μ fixed by the ring); a total of 324 (18 × 18) grid points (20° intervals) were obtained. All minima of PES at the B3LYP/6-31G(d) level of calculation[24] were fully characterized through the calculation of harmonic vibrational frequencies. The results of frequency calculations were used to compute thermodynamic parameters H, G, and S. The energies of all of these structures were recalculated using the 6-311++G(d,p) basis set to minimize the Basis Set Superposition Error (BSSE). This appproach was found to be adequate to characterize peptide systems of similar size.[7a] The energies were also recalculated in chloroform solution using the B3LYP/6-31G(d) basis set and PCM solvation model.[25]

Finally, NMR chemical shift calculations were completed at the B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) level of theory using the GIAO method.[26]

By using the optimized monomeric structures, all 8 types of dipeptides were generated and submitted to geometry optimization and frequency calculations at the B3LYP/6-31G(d) level of theory. Subsequently, computations on selected tetrapeptides were carried out using the procedures outlined above.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Selected CD spectra, high-resolution NMR studies, NMR-based conformational studies, list of computed energies, full ref 23. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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