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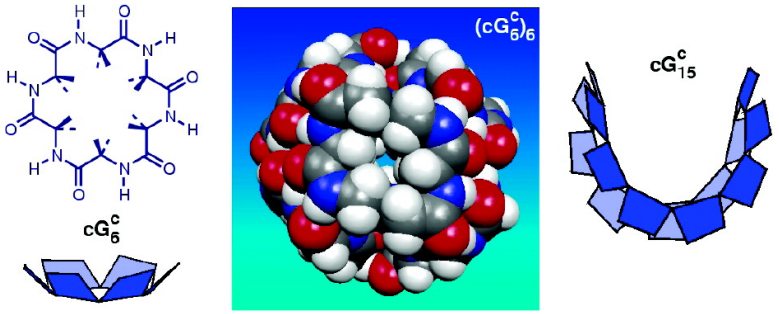

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All-*cis* Cyclic Peptides

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Abstract: Amide bonds −NH−CO− preferentially exist in *trans* conformations, the *cis* conformation being thermodynamically unfavored with respect to the *trans* by about 2 kcal/mol. Yet, the main reason most proteins or peptides cannot be made from *cis*-peptide plaques only lies in that connecting them into open chains appears to be sterically impracticable. It is possible, however, to build all-*cis* cyclic peptides in which all *cis*-plaques are efficiently locked. The present work examines, through quantum calculations, the structural and energetic issues associated with these peculiar arrangements. Systematic exploration at DFT-B3LYP level of the potential-energy surfaces for all-*cis* cyclopolyglycines cG^c_n ($n = 2-10,15$), and to a lesser extent, for all-*cis* cyclopolyalanines and all-*cis* cyclopolyphenylalanines confirms that all these structures are true minima. Optimal ring size occurs around eight peptide units, resulting in planar cG^c_7, cG^c_8, and cG^c_9. In smaller systems, the ring strain is relieved through nonplanar cup-like distortions, particularly in cG^c_6. From 10 peptide units and beyond, the ring framework distorts into a saddle-edge shape. These molecules disclose some molecular flexibility, as combinatorial tilting of the plaques may give sets of minima close in energy. Indexes based on isodesmic reactions are used to estimate the energy for joining all-*cis* or all-*trans* plaques into cyclic peptides. One of them, the mean plaque-junction energy (MPJE) suggests that within sensible sizes from six peptide units and beyond, all-*cis* plaque association is almost equally favorable as all-*trans* one. The frame of radiating *cis*-amide bonds can be considered as defining a new kind of peptidic material, endowed with specific self-assembling properties.

Introduction

The peptide bond −NH−CO− is most generally found in a *trans* conformation (or configuration), either in naturally occurring proteins and peptides or in synthetic peptidic matter. The occasional occurrence of *cis*-type arrangements mainly involves proline amino acids in -X-Pro- sequences.[1−5] Non-proline *cis* peptide bonds are extremely rare in proteins (<0.03%, usually involved in β turns), and equally scarce in peptides.[5−8] The reason invoked for accounting for the large primacy of *trans* peptide links is their intrinsic thermodynamic preference with respect to the *cis* arrangement, usually (but somewhat oversimplistically) attributed to steric hindrance between the C_α carbons.[9] Outside the peptide world, the secondary amide group −NH−CO− in *cis* geometry can be encountered in lactams, although this organic function brings back to proteins, owing to lactam bridges[10] and pyroglutamic acid.

The parent representative of the peptide bond is *N*-methylacetamide (NMA) $CH_3-CO-NH-CH_3$,[11] which has been extensively studied either theoretically,[12−22] or experimentally,[23−26] regarding in particular its (O)C−N rotational

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(11) In many theoretical works, formamide H_2N-CHO is taken as a proper model for a peptide bond or a peptide plaque. The peculiar organization of the presently addressed systems requires, for obvious reasons, that methyl groups must be explicited at both amino and carbonyl ends. This is why reasonable models for peptide plaques and plaque junctions are here chosen as *N*-methylacetamide and *N*-acetylmethylaminoglycine, respectively.
(12) A query of the *Quantum Chemistry Literature Database QCLDB II* provided 34 occurrences for *N*-methylacetamide. Only some recent contributions are given in refs 13−22.
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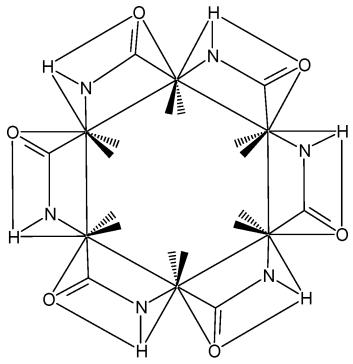
coordinate. Both approaches point to a preferred *trans* conformation, with a barrier of about 16 kcal/mol to overcome to reach the *cis* form, lying at 2.3 kcal/mol higher in energy. These values also apply to secondary amide bonds in dipeptides,[5,27] and presumably in polypeptides in general.

When one tries to draw a sequence of *cis* peptidic plaques,[28] it is virtually impossible to envisage any linear or kinked chain. The only viable architecture that comes to mind is a cyclic picture such as a the cyclohexamer **1**.



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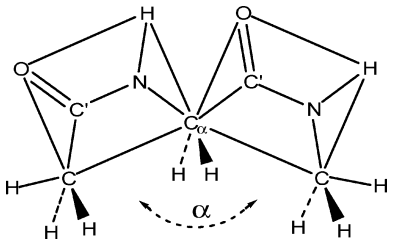
Because of the 16 kcal/mol local barriers to overcome at each peptidic-link unit, one can reasonably hope that once trapped in such layouts, these systems should remain efficiently locked in them.

The present work proposes a theoretical preliminary exploratory work to test the viability of such potential molecular arrangements. To this end, standard quantum-chemistry calculations, essentially performed at the DFT (B3LYP/6-31G(d)) level, will be used to analyze their structural features and to try to estimate their kinetic and thermodynamic stabilities, in particular relative to all-*trans* classical alternatives. We will focus on *non-proline cis* peptide bonds linking α-amino acids, mostly limited to polyglycine cases. This work is organized into two parts. The first three sections deal with the results, analyses, and comments on monomeric cyclic units. They are followed by two sections examining and discussing their potential properties and applications, regarding in particular their intermolecular organization. All methodological details are given in the Computational Section.

Structural Range. A helpful prerequisite for the following analyses is the knowledge of the shape of the Ramachandran conformational map for the simplest and most reliable model of two adjacent *cis* plaques, namely the all-*cis* dipeptide model $CH_3CONH-CH_2-CONHCH_3$. All of the results for this ϕ/ψ exhaustive exploration will be detailed elsewhere, but we must mention at this point that three minima are found. As expected, the preferred conformation corresponds to the coplanar arrangement of the peptide plaques (Scheme 1), reminiscent of the β region in a classical Ramachandran plot, while the two other

Scheme 1



minima essentially correspond to the two possible quasi-orthogonal arrangements of the plaques.[29]

In this preferred "extended" conformation, an interesting geometrical parameter is the angle α between the three C_α carbon atoms $H_3C\cdots CH_2\cdots CH_3$. This is a *virtual* or geometrical angle in the sense that it is neither a real valence angle nor a real dihedral angle, but it embodies the *valence* angle between the plaques at their junction. This angle is calculated, in the all-*cis* dipeptide model, at 135°. In a context of *cyclic planar* arrangement, it already suggests which size will be favored, since in this case, the valence angle between the plaques is nothing else than the interior angle of the corresponding regular polygon. By definition, it writes $\alpha = 180(n-2)/n$, with n being the number of sides—in our case the number of elementary *cis* plaques. Inversion of the previous relation yields $n = 360/(180-\alpha)$, and it comes up that a size around eight units meets quite well the condition on α (i.e., $\alpha = 135°$), suggesting that cyclooctapeptides and close companions in size should adopt such a regular planar arrangement of the plaques. Actually, the geometry with C_{nh} planar symmetry is found to be the preferred form for both cycloheptaglycine cG_7^c, cyclooctaglycine cG_8^c, and cyclononaglycine cG_9^c (**7**, **8**, and **9** respectively).[30]

Cyclohexaglycine. For cG_6^c, the system preferably adopts slightly curved cupel-like (or vase-like or vessel-like or bowl-like) forms, the C_{6h}-constrained planar-symmetrical form (**6**) lying about 10 kcal/mol above in energy as a saddle-point of index 5 (see Table 1). In these various vase forms, close in energy, the constraint on α has been broken up by allowing skewed arrangements of the plaques (see Figure 1). In the absolute minimum of C_2 symmetry (**6a**)—a shape reminiscent of an open book or a butterfly—the two opposite junctions lying on the hinge axis carry an orientation of the plaques at about 60°, the four remaining junctions keeping close to the coplanar arrangement. In the nearly-degenerate C_3 form (**6b**), the picture is slightly different in that there are two alternate sets of junctions: one, again, aims at the coplanar orientation of the peptidic planes, while the other one undergoes a 50°-skewness of the plane. The global shape is that of a nearly regular C_6 cupel, slightly distorted to alleviate the interaction between the hydrogens of the CH_2 groups, in particular those CH bonds that are located inside the concavity. The C_3 distortion splits these

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(28) The peptide plaque corresponds to the mean plane associated with the six atoms $C_\alpha C'ONHC_\alpha$ involved in the amide bond. In most cases, these atoms are roughly coplanar.
(29) This is in agreement with previous results found on the simplified model $HCONH-CH_2-CONH_2$ (*cis*): Baldoni, H. A.; Zamarbide, G. N.; Enriz, R. D.; Jauregui, E. A.; Farkas, Ö.; Perczel, A.; Salpietro, S. J.; Csizmadia, I. G. *J. Mol. Struct. (THEOCHEM)* **2000**, *500*, 97.
(30) From now on, a bold integer label will refer to the number of peptide units forming the labeled all-*cis* cyclopeptide (e.g., **6** designates all-*cis* cyclohexaglycine). Conventionally, a naked integer will refer to the C_{nh} regular planar-symmetry form, while adjunction of a letter (e.g., **6a**) will designate a conformer with no longer regular planar-symmetry geometry. More generally, using the single-letter code for α-amino acids, we will indicate the *trans* or *cis* geometry of the peptide bond by adding a t or c exponent to the residue letter. As an exemple, cG_6^c stands for all-*cis* cyclohexaglycine, while cG_6^t stands for normal all-*trans* cyclohexaglycine.

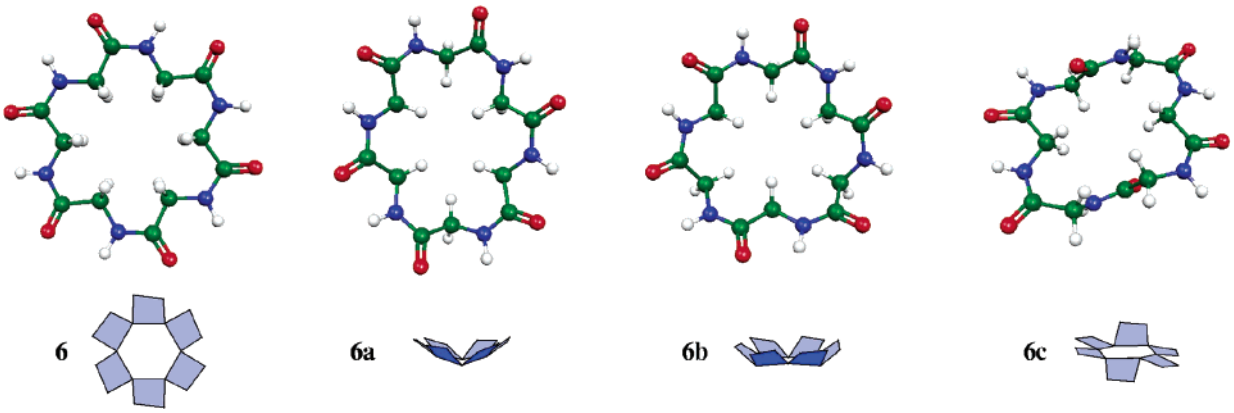

Figure 1. Optimal structures of the four minima for all-*cis* cyclohexaglycine cG^c_6.

Table 1. Summary of the Energetics Calculated for Some All-*cis* Cyclopolyglycines[a]

				relative energies			MPJE		
			i	ΔE	ΔE_{ZPE}	$\Delta G°$	ΔE	ΔE_{ZPE}	$\Delta G°$
cG^c_2	**2**	C_{2h}	1	0.1	0.1	0.7	−1.3	−2.8	−6.2
	2a	C_2	0	0.	0.	0.	−1.4	−2.8	−6.6
cG^c_3	**3**	C_{3h}	6	65.3	63.9.	66.6	22.9	21.6	21.1
	3b	C_1	0	2.7	2.3	1.9	2.1	1.1	−0.4
	3a	C_3	0	0.	0.	0.	1.2	0.3	−1.1
cG^c_4	**4**	C_{4h}	3	102.1	100.7	102.0	26.2	24.9	24.9
	4b	C_2	0	0.0	0.3	0.	0.7	−0.2	−0.7
	4a	C_i	0	0.	0.	0.4	0.6	−0.3	−0.5
cG^c_5	**5**	C_{5h}	4	40.3	39.1	42.3	7.5	6.3	7.1
	5c	C_1	0	1.6	1.6	1.8	−0.2	−1.2	−1.0
	5b	C_1	0	0.1	0.	0.8	−0.6	−1.5	−1.2
	5a	C_1	0	0.	0.1	0.	−0.6	−1.5	−1.4
cG^c_6	**6**	C_{6h}	5	10.3	8.9	14.0	−0.0	−1.2	0.1
	6c	C_i	0	2.0	2.1	2.7	−1.4	−2.4	−1.8
	6b	C_3	0	0.5	0.8	1.3	−1.6	−2.6	−2.0
	6a	C_2	0	0.	0.	0.	−1.7	−2.7	−2.2
cG^c_7	**7**	C_{7h}	0				−2.8	−4.0	−3.2
cG^c_8	**8b**	C_i	0	14.5	15.4	15.0	−1.8	−2.8	−1.9
	8a	C_1	0	13.3	16.0	17.1	−2.0	−2.8	−1.6
	8	C_{8h}	0	0.	0.	0.	−3.6	−4.8	−3.7
cG^c_8	**9**	C_{9h}	0				−3.5	−4.6	−3.6
cG^c_{10}	**10**	C_{10h}	2	0.8	0.6	2.7	−3.0	−4.2	−2.8
	10a	C_2	0	0.	0.	0.	−3.1	−4.2	−3.1
cG^c_{15}	**15**	C_{15h}	8	45.1			0.4		
	15a	C_1	0	0.			−2.6		

[a] In kcal/mol; i: number of imaginary frequencies; ΔE_{ZPE}: energy difference after zero-point energy vibrational corrections; $\Delta G°$ at 298 K; the MPJE (mean plaque junction energy) is defined from the formal equation: *N*-methylacetamide → CH_4 + 1/*n* cG^c_n, see text.

six hydrogens into two sets, coplanar three-by-three, with the two corresponding planes sufficiently apart to diminish the interactions. A closer look at the baseline formed by the six plaques reveals they form a boatlike arrangement in **6a** and a chairlike one in **6b**. A third minimum, lying 2 kcal/mol higher in energy, realizes a different compromise by adopting a chair shape derived from the former arrangements by flipping over two opposite plaques, above and below the plane defined by the four other plaques now nearly coplanar (**6c**). To do so, two opposite junctions aim at coplanarity, while the four other junctions aim at orthogonality, close to the orthogonal minima found in the dipeptide model. Not surprisingly, the boat-type counterpart to this chair-type arrangement is lying just 0.6 kcal/mol above in energy (this form is not shown in Figure 1, nor discussed in Table 1). Almost degenerate in energy, a variant of this boat form is obtained by bringing closer the two facing plaques, leading in this case to a real transanular interaction. Because severe ring strain largely counterbalances the two weak hydrogen bonds, this arrangement is overall not favored (2.5 kcal/mol above **6a**). The sequences of the corresponding interplaque dihedral angles θ and "valence" angles α are tentatively illustrated in Figure 2. These results further suggest there are probably other local minima for cG^c_6, corresponding to various combinations of ϕ/ψ sets associated to the fundamental minima or low-energy regions on the conformational map of the all-*cis* dipeptide model, and accordingly described by various plaque-flipping schemes.

Large Sizes. Beyond the cyclononamer cG^c_9, a new picture is selected for nonplanar deformation (optimized structures of all-*cis* cyclopeptides are given in Figure 3, using the peptide plaque representation, thus helping to understand the geometries). In the cyclodecamer cG^c_{10} and its following larger analogues, the constraint is now spread out all along the chain: any two consecutive plaques are slightly staggered by about the same amount, corresponding, in **10a**, to a dihedral angle θ between two adjacent plaques of only 10−15°, thus reducing α from 144° (i.e. 180(n − 2)/n, with n = 10) to 137° (Figure 2). This confers to the backbone a shape of nonplanar regular wave, reminiscent of the exterior edge (or rim) of a saddle or of a potato chip (in the following, such geometries will be labeled as chip-shaped, or saddle-rim-shaped. Due to the existence of the backbone direction, there remains little symmetry, strictly speaking, in such nonplanar arrangements. With an even number of units as in cG^c_{10} (**10a**), we have a C_2 symmetry, whereas for an odd number of units such as in cG^c_{15} (**15a**), we have no symmetry at all. A consequence of this state of things is that, unlike C_{nh} planar forms, such layouts are now chiral, even with glycine as residues.[31]

Although the energy gained by the buckling of C_{10h} **10** into C_2 **10a** is weak (less than one kcal/mol), for longer rings, deeper distortions are expected, resulting in larger energy differences. Indeed, for cG^c_{15} the energy difference between the C_{15h}-constrained planar-symmetry form **15** and the strongly buckled saddle-shaped C_1 form **15a** is as high as 45 kcal/mol. Associated with several imaginary frequencies, none of these planar forms,

(31) Note that without the directionality of the backbone, for even sizes such as cG^c_{10}, the symmetry would be D_{2d}, as in tub-like cyclooctatetraene—clearly, there is in cG^c_{10} a pseudosymmetry higher than C_2.

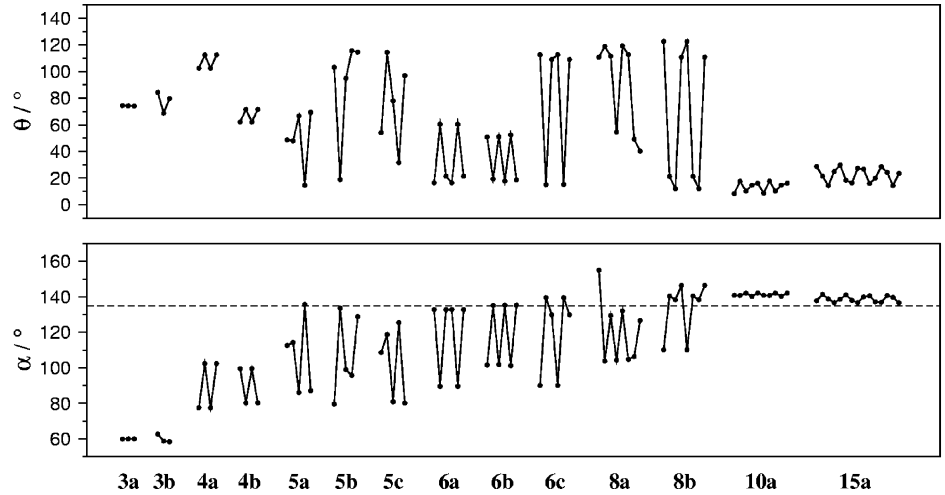

Figure 2. Interplaque geometrical angles α, and dihedral angles θ, plotted along the ring in nonplanar minima. α is defined as ($C_\alpha C_\alpha C_\alpha$); θ is defined as the average of (OC′N,OC′N) and (C′NC_α,C_αC′N) between two consecutive plaques. The dashed line corresponds to the optimal angle α = 135° (see text).

however, correspond to a barrier to planarity, as was found either for the cyclohexamer and its lower analogues except cG_2^c (see Table 1). The chip-shaped arrangement therefore seems to be the preferred form adopted from cG_{10}^c (slight such distortion) to cG_{15}^c (large such distortion), and quite presumably for higher sizes, since these forms are further associated with quite favorable energetics, as we will see below. Again, as illustrated in Figure 2, the buckling leads to an α value close to the 135° optimal angle. In other words, once such kind of minimum is caught for cG_{10}^c, it should be kept as the chosen geometrical solution for the following 10 or so higher all-*cis* cyclopeptides.

Small Sizes. In all-*cis* cyclopeptides with sizes smaller than six peptide units, due to higher cyclic constraints, the regular cupel-like arrangements are now in competition with less regular forms, involving quasi-orthogonal junctions between consecutive plaques or, in other words, allowing one or several plaques to be oddly flipped. Such is the case of the cyclopentamer cG_5^c. Its preferred form **5a** is still the regular vase form, with large concavity (strictly of C_1 symmetry, but nearly C_5 insofar as all peptide plaques are almost equivalent), but it is nearly degenerate in energy with a more irregular arrangement allowing several skewed junctions (**5b**). Still more uneven, another combination (**5c**) is lying less than 2 kcal/mol above in energy. Other such minima probably exist between **5a** and the C_{5h}-constrained planar-symmetry form (**5**), which admits four imaginary frequencies, and is lying 40 kcal/mol higher in energy.

In the cyclotetramer cG_4^c, the more stable conformation (**4a**) is again nearly degenerate in energy with the regular cup-form arrangement (**4b**). It has a global chairlike shape of C_i symmetry, with two opposite plaques coplanar with the C_α mean plane, and the two other plaques flipped over below and above this plane (see Figure 3). Here again, the boatlike variant of this form, also found from molecular-mechanics calculations,[32] lies only 0.5 kcal/mol above in energy. The C_{4h}-constrained planar-symmetry arrangement (**4**), which admits three imaginary frequencies, is now lying at more than 100 kcal/mol above the preferred form—a result not unexpected from the above-discussed condition on the α angle.

In all-*cis* cyclotriglycine cG_3^c, the preferred form (**3a**) is a C_3 fully symmetrical form, a regular vase-like form with significant tilting of the plaques on a same side of the plane defined by the C_α triangle. Lying 2.7 kcal/mol above in energy, a second minimum (**3b**) can be constructed by keeping one plaque parallel to the C_α plane and flipping out the two other plaques above and below this mean plane, respectively, at about 90°. As expected, the C_{3h}-constrained planar-symmetry form for cG_3^c (**3**) is unfavored in energy, lying 65 kcal/mol above **3a**. This is not worse than in planar cG_4^c, but now it is a saddle point of index six, instead of three.

The cyclodimer cG_2^c is nothing else than diketopiperazine, the structure of which is largely documented either from experimental and theoretical approaches.[33,34] The $C_{2h} \rightarrow C_2$ nonplanarity distortion here is very weak, corresponding at our level of description at only 0.1 kcal/mol, but nevertheless surviving the zero-point vibrational-energy corrections. Unlike in the above-discussed cG_n^c systems, the CH bonds borne by the two opposite C_α atoms are here oriented outside the ring, raising the question, at which size does the outward → inward "transition" occur. Actually, it does so between three and four plaque units, as long as a planar-symmetry C_{nh} constraint is maintained. However, given the puckered real geometries for the various minima of cG_3^c and cG_4^c, this geometrical feature is here little relevant.

Complementary Considerations. Although the absolute minima are the regular planar crowns for cG_7^c, cG_8^c, and cG_9^c, it is likely that several other arrangements using noncoplanar junctions of the plaques also exist as local minima on the corresponding potential surfaces. Some of these alternatives have been sought after for the cyclooctamer cG_8^c. Two were found, lying at around 15 kcal/mol above the C_{8h} minimum: (i) a conformation of chair-type (**8b**), derived from the planar crown by flipping two opposite plaques upside and downside the main planar frame, resulting in a C_i arrangement as in **4a** and **6c** and (ii) an odd conformation (**8a**), totally unsymmetrical, in which two opposite plaques are partly oriented inward, affording a

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(33) Bettens, F. L.; Bettens, R. P. A.; Brown, R. D.; Godfrey, P. D. *J. Am. Chem. Soc.* **2000**, *122*, 5856.

(34) For a review on the syntheses, conformations, and applications of diketopiperazines, see: Fischer, P. M. *J. Peptide Sci.* **2003**, *9*, 9.

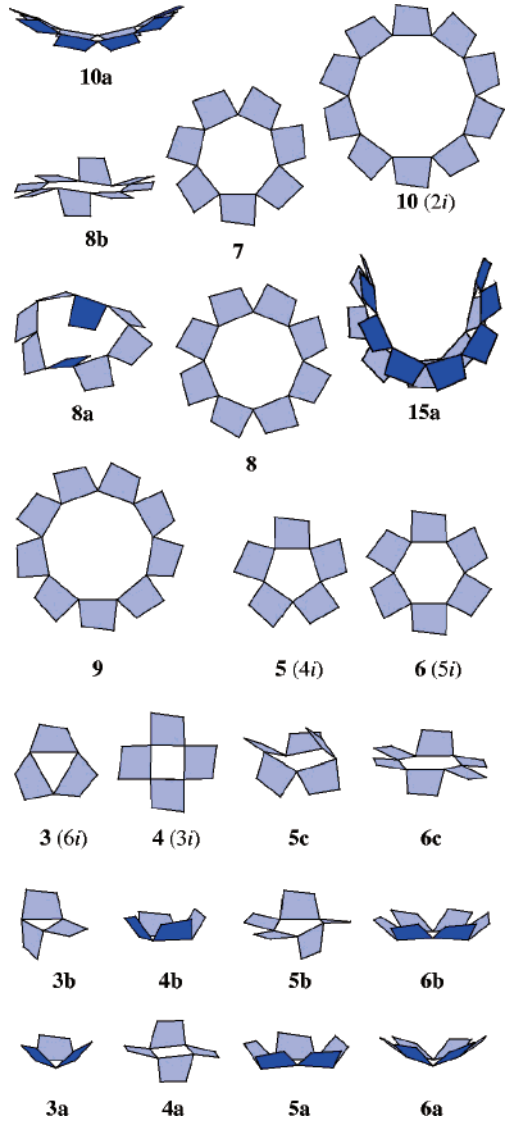


Figure 3. Optimal arrangements of peptide plaques in various all-*cis* cyclic peptides. The number of imaginary frequencies are given in parentheses for structures which are not minima. Each individual peptide plaque is drawn ideally planar.

transanular interplaque weak hydrogen bond. This interaction no doubt stabilizes this "improbable" form, which is found to be more stable than **8b** by 1 kcal/mol, this ordering being reversed, however, after taking into account the ZPE energies. At this point, let us emphasize how such a high-complexity layout, echoing the *trans* peptide world, contrasts with the geometries of all the other forms discussed above. Still, we tend to regard all-*cis* cyclic peptides as low-conformational entropy systems.

If the plaque picture helps explain the geometries by reducing the information, especially for large systems, it must not be forgotten that the real chemical bonds—the true topological link—is the backbone. The small systems, particularly, should be better viewed as nonplanar and irregular nine-membered rings for cG_3^c and twelve-membered rings for cG_4^c all the more that significant deviations to planarity occur here in some *cis*-peptide plaques.[35] Such nonzero values of the peptide angles ω ($C_\alpha-C'-N-C_a$) are calculated at up to 18° in **4a**, 22° in **4b**, and 23° in **3b**, emphasizing how the cyclicity constraints, normally distributed among interplaque parameters, may also mobilize

Table 2. Mean Geometrical Parameters (Å and deg) for the *cis* Plaques in All True Minima; *cis*-*N*-Methylacetamide (NMA) Is Given for Comparison

		cG_n^c	
	cis-NMA	mean	SD
$C_\alpha-C'$	1.522	1.542	0.003
$C'=O$	1.224	1.224	0.002
$C'-N$	1.372	1.361	0.007
$N-H$	1.011	1.017	0.003
$N-C_\alpha$	1.452	1.449	0.006
$C_\alpha C'N$	116.1	117.3	1.5
$C'NC_\alpha$	127.3	128.4	1.5
$C_\alpha C'O$	122.7	120.0	1.0
$OC'N$	121.3	122.7	0.9
$C'NH$	113.7	115.3	2.3
HNC_α	118.9	114.6	1.6
$\omega(C_\alpha C'NC_\alpha)$	0.	2.4	11.2
(NH,C′O)	55.0	58.0	3.2

intraplaque contributions as well. Incidentally, compiling, sorting in decreasing order, and plotting against rank all ω's (in absolute value) in all the presently calculated minima reveal a rather uniform distribution of $|\omega|$ between 0° and 23° (exceptional higher values of 25° and 28° are calculated in **8a**). Consequently, any value of ω within these limits can be mobilized to help relieve high geometrical constraints, if necessary, by use of peptidic plaque nonplanarity.

In the regular cup-like forms, the pitch of the plaques above the mean C_α baseline is a critical parameter for plaque-to-plaque dimerization into a regular capsule. The larger the bending, the better the C=O···H−N intermolecular alignment, and the stronger the binding energy. This is actually observed when going from the six-plaque cyclohexamer to the smaller analogues with five, four, and three plaques. But this effect is counterbalanced by the number of hydrogen bonds involved, which decreases by two-counts at each size reduction, from twelve in cG_6^c to six in cG_3^c. The balance of these two effects will need to be taken into account when addressing the kinetics and thermodynamics of the encapsulation process.

Finally, the extent to which the *cis* peptide plaque reflects an autonomous structural entity in all-*cis* cyclic peptides can be illustrated by the standard deviation of their mean geometrical parameters calculated from the set of 125 plaques forming the various cyclic true minima explored throughout this work.[36] As can be seen in Table 2, these values happen to be weakly scattered. Not unexpectedly, the more dispersed parameter is the dihedral angle ω that, as mentioned, oscillates around the value for planarity with a standard deviation up to 11°. The valence angles have standard deviations of 1−2°, with a maximum value of 2.3° for C′NH, in accordance with possible nitrogen pyramidalization. Note that the mean angle between C=O and N−H, 58°, is very close to $\pi/3$. As a consequence, the association of three such plaques into a cyclic trimer should exhibit a nearly perfect C=O···H−N alignment for the three hydrogen bonds, resulting in a favorable intermolecular alternative. The main difference between *cis*-NMA and these mean values concerns the $C_\alpha-C'$ distance, which is 0.02-Å shorter

(35) For the nonplanarity of peptide bonds, see: (a) Zimmerman, S. S.; Scheraga, H. A. *Macromolecules* **1976**, *9*, 408. (b) MacArthur, M. W.; Thornton, J. M. *J. Mol. Biol.* **1996**, *264*, 1180. (c) Rick, S. W.; Cachau, R. E. *J. Chem. Phys.* **2000**, *112*, 5230.

(36) For better uniformity, diketopiperazine is not included in this statistics. Taking it into account changes very little the values of Table 2.

in NMA, while $C_\alpha-N$ keeps about the same value of 1.45 Å in both. The same effect is observed in the *trans* cyclic peptides cG_6^t and cG_8^t with respect to *trans*-NMA. In summary, our mean *cis* peptide plaque can be seen as an uneven quadrilateral with side lengths of 2.40, 2.99, 2.09, and 2.46 Å along the $O-C_\alpha-C_\alpha-H-O$ path, and interior angles of 88, 84, 82, and 106° on the O, C_α, C_α, H vertexes, respectively.

Energy Assessment. Wondering about the stability of all-*cis* cyclopeptides entails the question of a reference state: stable with respect to what? As mentioned previously, all-*cis* cyclopeptides are unfavored with respect to all-*trans* cyclopeptides by an irreducible destabilization of about 2.3 kcal/mol (the present computational level gives 2.5 kcal/mol, see the Computational Section). This increment being essentially local and additive, one therefore expects for a cyclopeptide of size *n*, a *cis*−*trans* energy difference around 2.3*n* kcal/mol. Each plaque being efficiently locked, this increment is not of much interest, here, and one must search for more informative indicators, such as, for instance, those able to measure the *cis* vs *trans* plaque-coupling stability. To do so, we turned toward a concept familiar in applied quantum chemistry, the bond separation energies (BSE). This index allows measuring the effects of the immediate environment on the stability of a chemical bond. The following isodesmic reaction, for instance

$$H_2C{=}CH_2 + H_3C{-}CH_3 + H_2C{=}CH_2 \rightarrow H_2C{=}CH{-}CH{=}CH_2 + 2\ CH_4$$

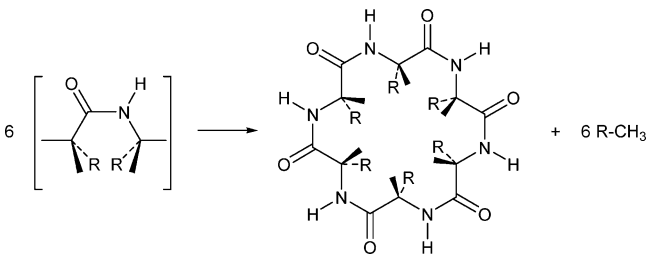
can be used to estimate the stabilizing effect due to conjugative vicinity of two double bonds. The BSE is given straightaway by the calculated exothermicity of the reaction, in this case about 10 kcal/mol. In this spirit, one can write, for the dipeptide model, the following reaction

$$H_3C{-}CONH{-}CH_3 + H_3C{-}CONH{-}CH_3 \rightarrow H_3C{-}CONH{-}CH_2{-}CONH{-}CH_3 + CH_4$$

which, in fact, reflects a plaque coupling more than a bond separation.

Plaque Coupling. The energy of this isodesmic reaction measures the energetic cost or gain brought about by the fusion or junction of two plaques at the C_α atom. Inside the β region of the Ramachandran map, some extra conjugation between the two π-conjugated CO−NH amide groups is brought by hyperconjugation through the $C_\alpha H_2$ (or $C_\alpha HR$ in natural peptides), so that the above process is expected to be exothermic. Actually, with *trans* peptide bonds, the energy of the above reaction (2 *trans*-NMA → all-*trans*-dipeptide model + CH_4) is calculated at −4.7 kcal/mol (−5.7 kcal/mol after ZPE). In the following, we shall call *plaque junction energy* (PJE) this amount of reaction energy. By changing its sign, this number would refer of course to the plaque separation energy. Note that in both definitions, these indexes can be negative or positive. In the case of the coupling of two *cis*-plaques into the extended C_s form of the dipeptide model (same β region, see Scheme 1), one gets a value of only −3.1 kcal/mol (−4.4 kcal/mol after ZPE). The difference originates in possibly less conjugation when *cis* amides are involved, and a significant steric interference between the two end methyl groups and the central $C_\alpha H_2$ group. Since the peptide backbone is oriented, combining both types of peptide bonds would need to consider two types of

Scheme 2



coupling reactions:

$$\textit{trans}\text{-NMA} + \textit{cis}\text{-NMA} \rightarrow \textit{trans,cis}\text{-dipeptide model} + CH_4$$

$$\textit{trans}\text{-NMA} + \textit{cis}\text{-NMA} \rightarrow \textit{cis,trans}\text{-dipeptide model} + CH_4$$

Actually, the two PJE are calculated at −5.3 and −2.6 kcal/mol for these two reactions, respectively, which happen to be rather different. For an open polyglycine chain, the total PJE refers to the reaction

$$n\ H_3C{-}CONH{-}CH_3 \rightarrow CH_3{-}(CONH{-}CH_2)_{n-1}{-}CONH{-}CH_3 + (n-1)\ CH_4$$

Since it depends on the size of the system, it is more informative and relevant to reduce it to the PJE per plaque, by dividing it, in this case, by $n-1$. One gets this way a *mean* plaque junction energy (MPJE). In an extended β strand of all-*trans* polyglycine, such MPJE should be close to the PJE calculated in the corresponding extended dipeptide model. However, due to the C_α hyperconjugation, one expects an increasing of the MPJE, as occurs in coupling double bonds in linear polyenes. For cyclopolypeptides, the definition now refers to the reaction:

$$n\ H_3C{-}CONH{-}CH_3 \rightarrow (CONH{-}CH_2)_n + n\ CH_4$$

or, using the proper acronyms:

$$n\ \text{NMA} \rightarrow cG_n + n\ CH_4$$

Scheme 2 (R = H) illustrates the all-*cis* cyclohexamer case. The mean PJE is obtained, here, by dividing the total PJE by *n*, so that finally the MPJE for cyclopolyglycines is given by the calculated energy associated to the formal equation:

$$\text{NMA} \rightarrow (1/n)\ cG_n + CH_4$$

decomposing into two cases

$$\textit{trans}\text{-NMA} \rightarrow (1/n)\ cG_n^t + CH_4$$

$$\textit{cis}\text{-NMA} \rightarrow (1/n)\ cG_n^c + CH_4$$

These calculated indexes are given in Tables 1 and 3. Let us illustrate their use, first, in analyzing the constraint carried in normal *trans* cyclopolyglycines. In regular S_6 cG_6^t, the MPJE is calculated at −2.9 kcal/mol, which is less than the PJE calculated in the unconstrained dipeptide model (−4.7 kcal/mol). It is a direct measure of the constraint that cyclicity imposes to the plaque junctions in such an arrangement. It is a global index in that it also accounts for possible plaque deformations, but since the plaques are coarsely the unchanging

Table 3. Comparative Energetics for Some All-*cis* and All-*trans* Peptides[a]

			relative energies			plaque junction energies		
			ΔE	ΔE_{ZPE}	$\Delta G°$	ΔE	ΔE_{ZPE}	$\Delta G°$
N-methylacetamide	*cis*	C_s	2.5	2.5	3.0			
	trans	C_s	0.	0.	0.			
dipeptide model	*cis*	C_s	6.5	6.5	7.5	−3.1	−4.4	−2.2
	trans	C_s	0.	0.	0.	−4.7	−5.7	−4.2
cyclohexaglycine cG_6	*cis*	C_2	21.7	20.1	20.1	−1.7	−2.7	−2.2
	trans	S_6	0.	0.	0.	−2.9	−3.6	−2.6
cyclooctaglycine cG_8	*cis*	C_{8h}	21.5	19.1	19.5	−3.6	−4.8	−3.7
	trans	S_8	0.	0.	0.	−3.8	−4.6	−3.2

[a] In kcal/mol; ΔE_{ZPE}: energy differences after zero-point vibrational-energy corrections; $\Delta G°$ at 298 K; for the dipeptide model $CH_3-CONH-CH_2-CONH-CH_3$, the plaque junction energy (PJE) is the energy of the reaction 2 *N*-methylacetamide → dipeptide model + CH_4; for cyclic systems, it is defined from the formal reaction *N*-methylacetamide → $1/n$ cG_n + CH_4; for cG_6^c (C_2), this is a *mean* PJE.

and transferable structural entities, MPJE describes, basically, the junction.[37] As the ring extends, the junction constraint is reduced. For S_8 cG_8^t, MPJE returns to −3.8 kcal, a value closer to that in the dipeptide model, but still not as large.

In the case of all-*cis* cyclohexaglycine in its C_2-preferred puckered form, **6a**, the MPJE is calculated at −1.7 kcal/mol, which is 1.4 kcal/mol less than in the dipeptide model, in line with a nonplanar framework. The total energy difference between the *cis* and *trans* cyclohexamers can be retrieved from these elemental increments, giving a total energy difference of $6\{(-1.7 - (-2.9)) + 2.5\} = 22$ kcal/mol. In all-*cis* cyclooctaglycine cG_8^c, of perfect planar symmetry, the MPJE is calculated at −3.6 kcal/mol, which is now *better* than in the dipeptide model. Here, the planar arrangement of the plaques ensures full conjugation at all C_α, providing strong stabilization as predicted from cyclic tight-binding arguments. This illustrates a fundamental difference between the two kinds of cyclic peptides. In the normal all-*trans* case, the peptide plaques are laid along a ribbon perpendicular to the ring plane, inevitably breaking conjugation. In all-*cis* cyclopeptides with planar optimal size around eight units, all plaques are lying coplanar along a planar disk, where nothing impedes the conjugation. As a consequence, both cyclooctapeptides finally have close MPJE, reflecting a similar amount of plaque-junction stabilization in both cases.

The mean plaque junction energies for the planar C_{nh} cyclic peptides are listed in Table 1 and drawn in Figure 4. For the favorable cases where these regular forms are the absolute minima on the potential surface (cG_7^c, cG_8^c, cG_9^c), as well as for C_{10h} cG_{10}^c (a saddle-point of index 2 lying only 0.8 kcal/mol above the bent C_2 absolute minimum), MPJE is calculated at −2.8 to −3.6 kcal/mol, which is close to, and even better than, the value obtained for the dipeptide model, −3.1 kcal/mol. Below and beyond these sizes, MPJE becomes less favorable, as expected from ring strain. While in cG_6^c and cG_{10}^c to cG_{15}^c it corresponds almost to thermoneutrality; for smaller sizes, MPJE becomes positive, reaching 26 kcal/mol for cG_4^c and 23 kcal/mol for cG_3^c. Note that the constraint has vanished in diketopiperazine, which exhibits an MPJE of −1.4 kcal, a value

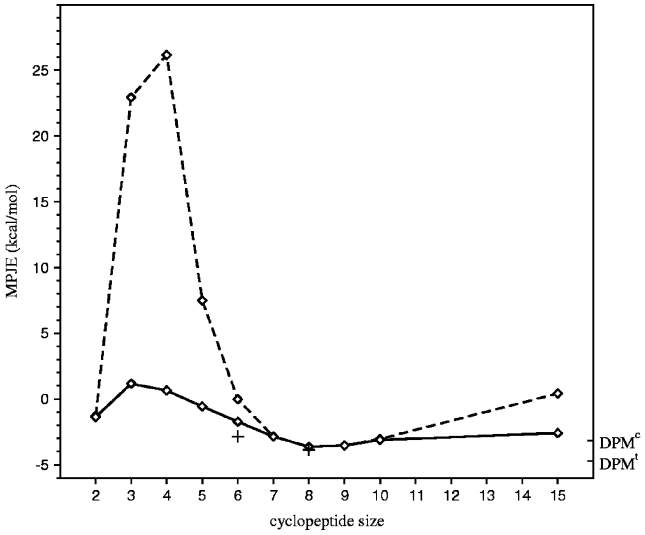


Figure 4. Mean plaque-junction energies (MPJE) calculated in all-*cis* cyclic peptides. Dashed curve: planar-symmetry constrained forms; plain curve: absolute minima; (+): all-*trans* cG_6^t and cG_8^t. (DPM):dipeptide model.

weaker than that calculated in the dipeptide model, but still reflecting a profitable coupling.

None of these strained small rings, however, are the preferred conformations, as mentioned earlier. In the distorted bent forms corresponding to the absolute minima, the MPJE are calculated to more reasonable values: −1.7, −0.6, 0.6, and 1.2 kcal/mol in **6a**, **5a**, **4a**, and **3a**, respectively (lower curve in Figure 4). Interestingly, for the buckled minimum of cG_{15}^c, MPJE is calculated at −2.6 kcal/mol. As disclosed in Figure 4, this would suggests that within the range of 7−20 units, building an all-*cis* cyclic peptide from elemental *cis* plaques should be as favorable a process as the coupling of two such plaques into a β-extended dipeptide model. In both cases, the energy benefit per junction is roughly that of a weak hydrogen bond. It is interesting to note in Table 1 that the comparison of stabilities in terms of Gibbs free energies ($\Delta G°$) does not yield different conclusions.

Other Coupling Scheme. To keep close to the more intuitive idea of building polypeptides from polycondensation of amino acids, one can also search for a coupling scheme in which the building blocks would be representative of amino acid residues rather than peptidic plaques. The following isodesmic reaction has been proposed by Perczel et al. for the building of a β chain of polyalanine from peptide subunits:[38]

$$n\ HCO-NHCH(CH_3)CO-NH_2 \rightarrow HCO-(HNCH(CH_3)CO)_n-NH_2 + (n-1)\ HCO-NH_2$$

At RHF-level, the calculations give, for this process, very low energies, so that this reaction is described as thermoneutral.[39] This reaction, however, hardly any better reflects the process taking place in protein biosynthesis or peptide synthesis but rather translates the coupling of unconstrained junctions into a more or less constrained final polypeptide. In the present case, using this reaction to construct cyclic peptides instead of

(37) Actually, the ω dihedral angles deviate here by 13° with respect to the ideal *trans* value of 180°, indicating significant nonplanarity in cG_6^t. In S_8 cG_8^t, this deviation of ω is 10°.

(38) Perczel, A.; Hudaki, P.; Füzéry, A. K.; Csizmadia, I. G. *J. Comput. Chem.* **2004**, *25*, 1084.

(39) In an ideal extended β strand, some π conjugation occurs through the π-type orbitals at C_α. This delocalization effect should induce a size dependence in the corresponding isodesmic reaction energy. Actually the RHF results of ref 38 disclose this trend, although to a weak extent.

Scheme 3

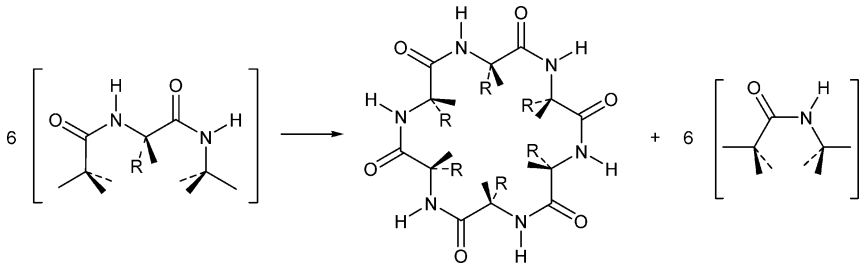


Table 4. Mean Junction-Coupling Energies (MJCE) for Cyclohexaglycine and Cyclooctaglycine[a]

		ΔE		ΔE_{ZPE}	
		cG_6	cG_8	cG_6	cG_8
R = CH_3	*trans*	+1.9	+0.9	+2.2	+1.1
	cis	+1.4	−0.5	+1.5	−0.5
R = H	*trans*	+1.9	+0.9	+1.8	+0.8
	cis	+1.7	−0.2	+1.7	−0.3

[a] Defined as the energy (kcal/mol) of the reaction R−CONH−CH_2−CONH−R → 1/*n* cG_n + R−CONH−R. ZPE refers to the taking into account of zero-point vibrational-energy corrections.

constraint-free open chains should result in substantial positive energies, and may provide an interesting comparative tool. Applied to the building of cyclopolyglycines, the above equation is written:

$$n\ \mathrm{HCO{-}NHCH_2CO{-}NH2} \rightarrow \mathrm{cG}_n + n\ \mathrm{HCO{-}NH_2}$$

or, with our methylated models:

$$n\ \mathrm{CH_3CO{-}NHCH_2CO{-}NHCH_3} \rightarrow \mathrm{cG}_n + n\ \mathrm{CH_3CO{-}NHCH_3}$$

Scheme 3 illustrates the cases of all-*cis* cyclohexamers. Again, scaling the energetics per residue or junction unit leads to

$$\mathrm{CH_3CO{-}NHCH_2CO{-}NHCH_3} \rightarrow (1/n)\ \mathrm{cG}_n + \mathrm{CH_3CO{-}NHCH_3}$$

Further abridging it by using the proper acronyms for the dipeptide model (DPM) and *N*-methylacetamide (NMA)

$$\mathrm{DPM} \rightarrow (1/n)\ \mathrm{cG}_n + \mathrm{NMA}$$

enables the parallel with the former plaque-coupling equation:

$$\mathrm{NMA} \rightarrow (1/n)\ \mathrm{cG}_n + \mathrm{CH_4}$$

Still comparing the *cis* vs *trans* forms of cyclohexaglycine cG_6 and cyclooctaglycine cG_8, we therefore calculated the energy for the following set of reactions:

$$\textit{trans,trans}\text{-DPM} \rightarrow (1/n)\ \mathrm{cG}_n^t + \textit{trans}\text{-NMA}$$

$$\textit{cis,cis}\text{-DPM} \rightarrow (1/n)\ \mathrm{cG}_n^c + \textit{cis}\text{-NMA}$$

For $n = 6$ and $n = 8$, these reaction energies, reported in Table 4, suggest that, according to this criterion, and in the favorable size range, the building of an all-*cis* cyclopeptide is less difficult than the building of an all-*trans* one. Even more encouraging is the negative sign obtained for cG_8^c, indicating an exothermic process where the *trans* case remains endothermic. As mentioned earlier, this probably originates in some extent of conjugation occurring along the planar backbone of *cis*-cyclooctaglycine, while *trans*-cyclopeptides necessarily have nonplanar backbones. Since one can synthesize all-*trans* cyclopeptides, this result is a promising sign for cG_8^c as an achievable synthetic target. This stability index (that we could define to parallel MPJE as a *mean junction-coupling energy* (MJCE)) exhibits some robustness since (1) the above values are little modified when including the zero-point vibrational energy (ZPE) corrections and (2) using a dipeptide model with hydrogens instead of end methyl groups (in this case, *N*-formyl aminoglycine stands for DPM in the left-hand side of the equation, while formamide stands for NMA in the right-hand side) also leads to similar energies, either with or without including ZPE corrections (see Table 4).

In fact, it is easy to show that this index is nothing more than the MPJE *minus* the PJE calculated for the dipeptide models. Therefore, all these indexes can be deduced from the values listed in Tables 1 and 3 augmented by 3.1 kcal/mol for the *cis* rings and 4.7 kcal/mol for the *trans* rings. In other words, to know these mean junction-coupling energies along our cG_n^c series, one just has to push upwards by 3.1 kcal/mol the lower curve in Figure 4. The point to notice, here, is that according to the results for cG_6^t and cG_8^t, the *trans* curve is just below the *cis* curve, whereas it is the opposite for the above-discussed junction-coupling indexes, the *trans* values being above the *cis* values.

As soon as non-glycine α-amino acids are considered, all of the above-discussed energetics should of course depend on the relative configurations at each C_α. Typically, as we shall see below, alternating L- and D-residues along the backbone is expected to be more fortunate than the uniform juxtaposition of L- or D-building blocks.

The preceding energy results can be recapitulated as follows. The making of a *cis* peptide bond cannot avoid a penalty of 2.3−2.5 kcal/mol with respect to a *trans* peptide bond. However, for certain sizes beyond 6 units, the juxtaposition of *cis* plaques into all-*cis* cyclopeptides can be as favorable as, or better than, the juxtaposition of normal *trans* plaques into existing all-*trans* cyclopeptides. On the other hand, by their proper topology and low conformational complexity, all-*cis* cyclopeptides could further lead to advantageous intermolecular arrangements, as we shall see later on.

Influence of Lateral Chains. Due to the polarity of the peptide backbone, most *cis*-cyclopeptides are chiral, either because of their nonplanar geometries, or because a substituent R—the lateral chain—is borne at one or several alpha carbons. Only those parent *cis*-cyclopolyglycines that have perfect planar-symmetry, cG_7^c, cG_8^c, and cG_9^c, are achiral. For non-glycine peptides of planar symmetry built from homogeneous sets of L

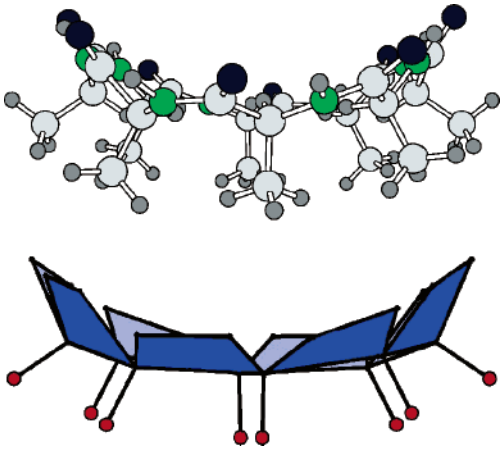


Figure 5. Optimized structure of all-*cis* L-cyclooctaalanine (cA_8^c).

or D series, there are, for each set, two possible enantiomers of equivalent energy. When R = CH_3 for instance, the C_{nh}-constrained planar symmetry all-*cis* cyclopolyalanine cA_n^c would admit four stereoisomers of equal energy. In most cases, however, any substitution at C_α induces a concavity, as illustrated in Figure 5 for cA_8^c. Depending on the position of the lateral chain with respect to this concavity (convex side or concave side), the two resulting isomers are now of unequal energy. From obvious steric argument, the preferred location of R is expected at the outside of the cup, the convex side. Starting from a planar cA_n^c, as the ring is kinked, the four energy-equivalent isomers are split into two unequivalent sets of two enantiomers, but here comes some unavoidable restrictions due to steric hindrance. Simple model building of cup-like all-*cis* cyclohexaalanine for instance, rules out the possibility for two adjacent C_α methyl groups within the *concave* side of the cup. Searching such an arrangement for c*L*-A_6^c failed, indeed, and the minimum found corresponds to a regular cupel like **6b**, with all lateral methyl groups pointing toward the convex side of the cupel.

Even concave-side substitution at every other C_α in $cG^cA^cG^cA^cG^cA^c$ appears unachievable. Starting an optimization process from such an arrangement led, through inversion of the ring curvature, to a rearrangement into the more stable isomer with the three methyl groups pointing toward the convex side. On the other hand, alternating *L*- and *R*-series along the ring, i.e., inverting the absolute configuration from one C_α to another as in c(*L*-A^c-*R*-A^c)$_4$, should restore the planarity of the cyclic backbone, although reducing the global symmetry, in this case, from C_{8h} to C_{4h}.

Methylation is expected to bring extra curvature to cupel-like forms such as cG_6^c. Comparing cG_6^c (**6b**) with cA_6^c, both of C_3 symmetry, actually disclose this trend since in the latter, the plaques have been tilted with respect to the mean basal plane by an extra 10° in average (55° vs 45°). This trend is not observed for C_3 c(G^cA^c)$_3$, in which the methyl groups are more distant. While C_{8h} cG_8^c (**8**) is planar, one can see in Figure 5 that in C_2 cA_8^c there are two sets of opposite plaques: one set is tilted by about 35° in average, with respect to the mean basal plane, and the second set is tilted by about 65°.

The calculation of MPJE in non-glycine systems requires the use of *N*-alkyl alkylamide instead of *N*-methylacetamide as building blocks in the relevant isodesmic reaction. A problem we face here is the choice of this elemental plaque, since several possibilities seem equivalent:

$$n\ cis\ RCH_2{-}NHCO{-}CH_3 \rightarrow \text{all-}cis\ (CO{-}NH{-}CHR)_n + n\ CH_4$$

$$n\ cis\ CH_3{-}NHCO{-}CH_2R \rightarrow \text{all-}cis\ (CO{-}NH{-}CHR)_n + n\ CH_4$$

$$n\ cis\ RCH_2{-}NHCO{-}CH_2R \rightarrow \text{all-}cis\ (CO{-}NH{-}CHR)_n + n\ RCH_3$$

Reduced to single junction, for alanine these reactions write:

$$Me{-}CH_2NHCOCH_3 \rightarrow 1/n\ cA_n^c + CH_4$$

$$CH_3NHCOCH_2{-}Me \rightarrow 1/n\ cA_n^c + CH_4$$

$$Me{-}CH_2NHCOCH_2{-}Me \rightarrow 1/n\ cA_n^c + CH_3{-}Me$$

The last one was previously given in Scheme 2 (with R = CH_3). By definition, in an isodesmic reaction each member must be taken in its lowest-energy conformation. In the present case this would imply an *anti* orientation of both end methyl groups in the model plaque. However, this conformation does not reflect the environment of the lateral methyl groups in all-*cis* cyclopolyalanine, so that this reaction energy can be overestimated. Setting the methyl ends in *syn* in the building blocks better accounts for their positions in the cyclic product and might seem more reasonable instead (Scheme 2). So, where there was a single nonambiguous definition for the MPJE of glycine, there are as many as six possibilities in the case of alanine.

This also holds for the estimation of the PJE in the dipeptide model of alanine, which may use two possible methylated plaque, in two possible conformations:

$$CH_3CONH{-}CH_2CH_3 + CH_3{-}CONHCH_3 \rightarrow CH_3CONH{-}CH(CH_3){-}CONHCH_3 + CH_4$$

$$CH_3CONH{-}CH_3 + CH_3CH_2{-}CONHCH_3 \rightarrow CH_3CONH{-}CH(CH_3){-}CONHCH_3 + CH_4$$

$$CH_3CONH{-}CH_2CH_3 + CH_3CH_2{-}CONHCH_3 \rightarrow CH_3CONH{-}CH(CH_3){-}CONHCH_3 + CH_3{-}CH_3$$

On the other hand, the calculations of the mean junction coupling energies (MJCE) use the following reaction

$$CH_3CONH{-}CH(CH_3){-}CONHCH_3 \rightarrow 1/n\ cA_n^c + CH_3{-}CONH{-}CH_3$$

which is, here, devoid of any ambiguity (depicted in Scheme 3, with R = CH_3). These MJCE energies were calculated at +0.3 kcal/mol for cA_6^c, and +1.6 kcal/mol for cA_8^c. For the mixed compound c(G^cA^c)$_3$ where an average is needed for the defining dipeptide ($^1/_2${$CH_3CONH{-}CH_2{-}CONHCH_3 + CH_3{-}CONH{-}CH(CH_3){-}CONHCH_3$}), an expected intermediate value of +0.4 kcal/mol is obtained. From these numbers, there is some consistency to use, among the above-discussed definitions, those leading to a PJE of −1.7 kcal/mol for the alanine dipeptide model (this is about half that obtained for the glycine dipeptide model), and an MPJE of −1.4, −0.1, and −2.0 kcal/mol for cA_6^c, cA_8^c, and c(G^cA^c)$_3$ respectively.

Table 5. Comparative Energies (kcal/mol) for Selected All-*cis* and All-*trans* Cyclopeptides

	cG_6^c	cA_6^c	$c(G^cA^c)_3$	cG_8^c	cA_8^c
mean plaque-junction energy MPJE[a]	−1.7	−1.4	−2.0	−3.6	−0.1
mean junction-coupling energy MCJE[a]	+1.4	+0.3	+0.4	−0.5	+1.6
lateral methyl substitution[a]		−2.3	−3.3		+0.9

[a] See text for definitions.

Comparing these numbers with the MPJE of cyclohexaglycine and cyclooctaglycine (Table 5) indicates that according to this criterion, methyl substitution is slightly unfavored in the cyclohexamer and strongly unfavored in the cyclooctamer. On the other hand, comparing the above MJCE with those of cyclohexaglycine and cyclooctaglycine suggest that, according to that criterion, methyl substitution *favors* the cyclohexamer and, again, significantly unfavors the cyclooctamer. The question should be settled through the lateral methyl substitution reaction of cyclopolyglycines into cyclopolyalanines.

Normalized to one peptide unit, the corresponding isodesmic reaction writes

$$1/n\ cG_n^c + CH_3{-}CH_3 \rightarrow 1/n\ cA_n^c + CH_4$$

The energy for this reaction is calculated at −2.3 kcal/mol for $n = 6$ and +0.9 kcal/mol for $n = 8$.[40] As summarized in Table 5, this suggests that MJCE is here a better indicator than MPJE for assessing the effect of substitution on the stability of all-*cis* cyclic peptides. This is not unexpected as substitution alters more the C_α junction rather than the plaque itself. The reason the cyclooctamer is unfavored should certainly be traced to the above-discussed nonplanar distortion of the octapeptide ring induced by the methylation.

Turning back to the mixed compound $c(G^cA^c)_3$, the above isodesmic reaction (now normalized with $n = 1/3$) yields −3.3 kcal/mol. This slight stabilization with respect to cA_6^c should be related to less steric hindrance and consequently a better hyperconjugation between peptide plaques. To more firmly ground this new class of coumpounds, we have addressed the case of $CH_2{-}Ph$ as lateral chains, in other words, the case of phenylalanine residues. Preliminary results are encouraging, since the substitution reaction, which now is written:

$$1/3\ cG_n^c + CH_3{-}CH_2{-}Ph \rightarrow 1/3\ c(G^cF^c)_3 + CH_4$$

yields an energy of −4.9 kcal/mol. Further substitution into cup-like all-*cis* hexaphenylalanine cF_6^c provides a stabilization energy of −0.4 kcal/mol, despite the obvious steric hindrance of proximal phenyl groups.

In summary, the stabilizing effect of lateral chain substitution obtained for the hexamer, as clearly appearing from methyl and phenylmethyl substitution reactions, augurs well for the possibility of making all-*cis* cyclic peptides from naturally occurring amino acids, as well as for designing variously substituted and functionalized all-*cis* cyclopeptides.

Self-Assembling Properties. From the combination of proper substitutions at C_α with the hydrogen-bond capabilities of radiating *cis* amide groups, all-*cis* cyclic peptides could exhibit original properties.

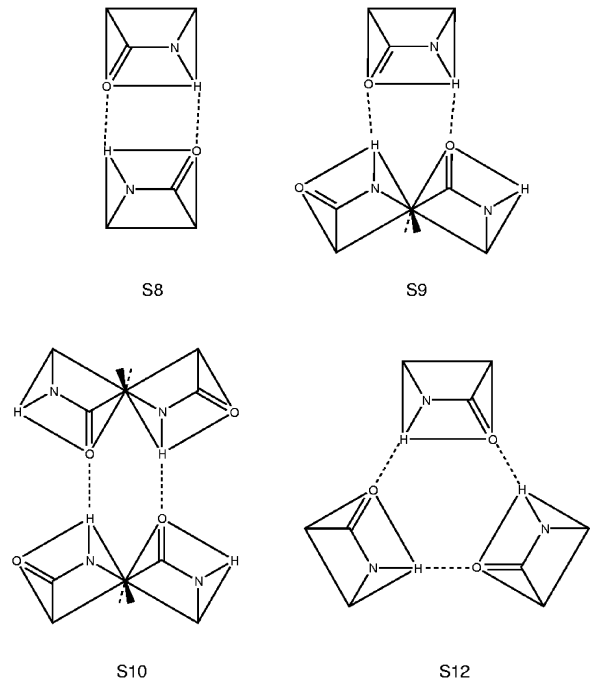


Figure 6. Basic intermolecular arrangement of *cis* plaques.

As a preliminary, let us note that in host−guest chemistry, a behavior of amphi-ionophore as that proposed for normal all-*trans* cyclic peptides[41] is not possible here *by construction*. Consequently, any host−guest complexation will necessarily make use of functionalized lateral chains. These lateral chains can be used for linking transition metal atoms, or organic or inorganic fragments. According to the size of the cycle, polyhapto binding can be envisaged by substituting, for instance, every other alpha carbon in the cyclohexamer or in the cyclooctamer. A consequence of the cup-like form of many of our chelating units is that they can give hemicarcerand cryptants, possibly accommodating large metal entities such as lanthanide or actinide metallic cations.

The very structure of all-*cis* amide rings will bring some restrictions to their association or self-assembling properties. In particular, the well-known vertical stacking of normal all-*trans* cyclic peptides into nanotubes is here no longer possible. Instead, the set of nearly-coplanar, all-side oriented, *cis*-amide plaques will tend to form quasi-two-dimensional (2-D) extended associations, like those proposed for oxopyrrolidine-containing foldamers.[42] Let us inspect, first, the possible hydrogen-bond pairings from our monomers.

The basic options for hydrogen-bond coupling of adjacent *cis* plaques are schematized in Figure 6. The simplest way consists of two plaques facing one another in an antiparallel way, defining a pseudoring of size eight, thus called S8 to keep with the notation of ref 38.[43] The second one consists of coupling a plaque with a junction, thus defining a peudoring S9. Then, by facing two junctions, one gets the S10 arrangement, as in classical antiparallel β sheets. The coplanar coupling of

(40) After ZPE corrections, these numbers become −3.4 and −0.1 kcal/mol, respectively, resulting in ΔG^{298} values of −2.1 and +1.3 kcal/mol, respectively.

(41) Kim, K. S.; Cui, C.; Cho, S. J. *J. Phys. Chem. B* **1998**, *102*, 461.
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(43) In the real geometry, the two plaques are, of course, shifted to maintain N−H···O alignments.

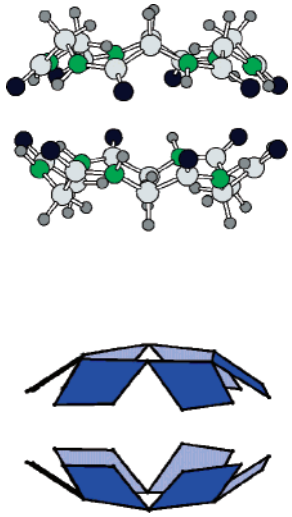

Figure 7. Calculated structure of closed dimer $(cG_6^c)_2$.

three *cis* plaques (S12) should be a priori favored due to the (NH,CO) angle in the *cis* plaque, close to 60° (see above), thus favoring properly aligned three-fold symmetry arrangements. Coarse estimates of the mean energy per hydrogen bond in such arrangements can be obtained from calculations of the corresponding combinations of parent *cis*-NMA and dipeptide model, but these increments (here estimated at 8−9 kcal/mol) can hardly be transferred to more intricate associations, since the whole vicinity of each hydrogen bond can modify its stability.[44]

The self-assembling capabilities of all-*cis* cyclic peptides essentially include capsule dimers, polyhedra, and 2-D extended arrangements. The simplest association of nonplanar cup-shaped cyclopeptides is their dimerization into a capsule, such as that obtained from two units of the cyclohexamer cG_6^c (Figure 7). Actually, the capsule obtained in this case is not tightly bound since all hydrogen bonds are rather out of line due to the weak tilting of the plaques with respect to ring mean planes. While the mean hydrogen bond is estimated here at about 1.2 kcal/mol only, the total binding energy for this assembling, 14 kcal/mol, is however non-negligible. In this example, the cavity is too small to accommodate any chemical group or metal fragment. Increasing the size of the cyclopeptide unfortunately results in even less tilted plaques, so that it appears difficult to form functional capsules from simple dimerizations. One could be tempted to assemble two **15a** elements into a nearly-perfect tennis ball. Again, the essence of the *cis* framework prevents effective hydrogen bonding in such a topology. Strategies for self-assembling the present building blocks into vesicles or designing efficient encapsulation should therefore search toward polyhedron formation.

The formation of polyhedra should be possible by assembling proper combinations of regular or quasi-regular polygons such as most of the present cyclopolymers. Combining octagons (octamers) and squares (tetramers) may be problematical since it should use **4b**, which is symmetrical but highly puckered and therefore badly prepared for hydrogen bonding with the planar ring of **8**. Associating hexagons and pentagons in a rather huge fullerene-like arrangement seems more reasonable, although both building blocks are not planar. If all faces were required to be strictly planar, no doubt this would be too costly for cG_5^c (40 kcal/mol). However, the system should find its way through self-adaptating distribution of all constraints, without full planarization of all faces, the global binding energy overcoming the partial opening of the cupels. Explicit treatment with the present tools of such a cluster being still prohibitive, we studied, as a test case, a smaller globular arrangement made of six units of cyclohexaglycine cG_6^c only, ignoring therefore Eulers's closing rule.[45] Optimizing this complex, at the Hartree−Fock level of calculation and using a smaller basis set (3-21G*), actually led to a rather regular spheroïdal capside of internal volume around 700 Å^3, corresponding to a diameter of 1.4 nm (Figure 8). The capsule is bind by a full set of 36 hydrogen bonds, resulting in a total binding energy of 220 kcal/mol, corresponding to a mean value of 37 kcal/mol per monomer unit, or 6 kcal/mol per hydrogen bond. As checked on the NMA dimer, this procedure overestimates each hydrogen bond by about 2 kcal/mol, and consequently we can extrapolate a mean value of 4 kcal/mol per hydrogen bond. As expected from the constraints applying here on the C═O···H−N alignments, the latter value is less than that calculated in a free NMA dimer (S8) with the same basis set (11.3 kcal/mol per hydrogen bond), even if in the cluster $(cG_6^c)_6$ the plaques have tipped a little with respect to free cG_6^c. Although cycloheptaglycine cG_7^c, cyclooctaglycine cG_8^c, and cyclononaglycine cG_9^c are planar, small plaque bending is presumed to demand little energy. So,

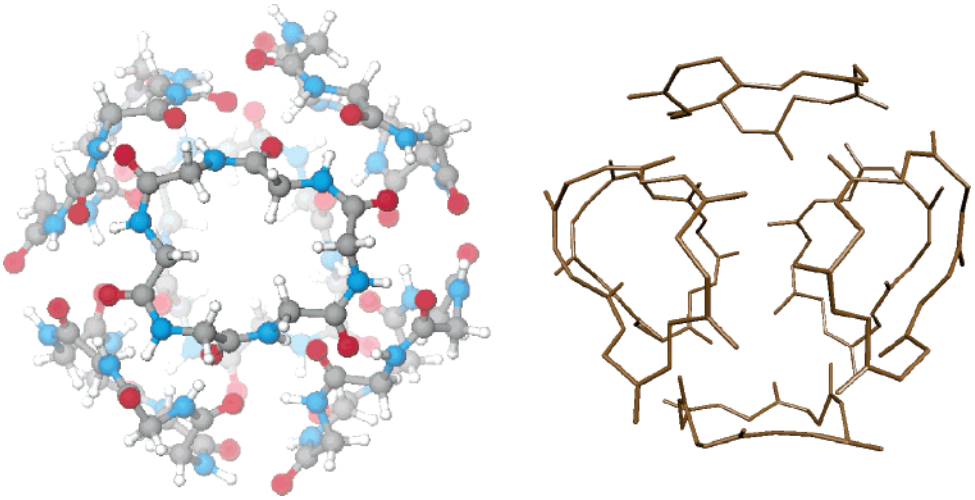

Figure 8. Calculated structure of spheroidal $(cG_6^c)_6$.

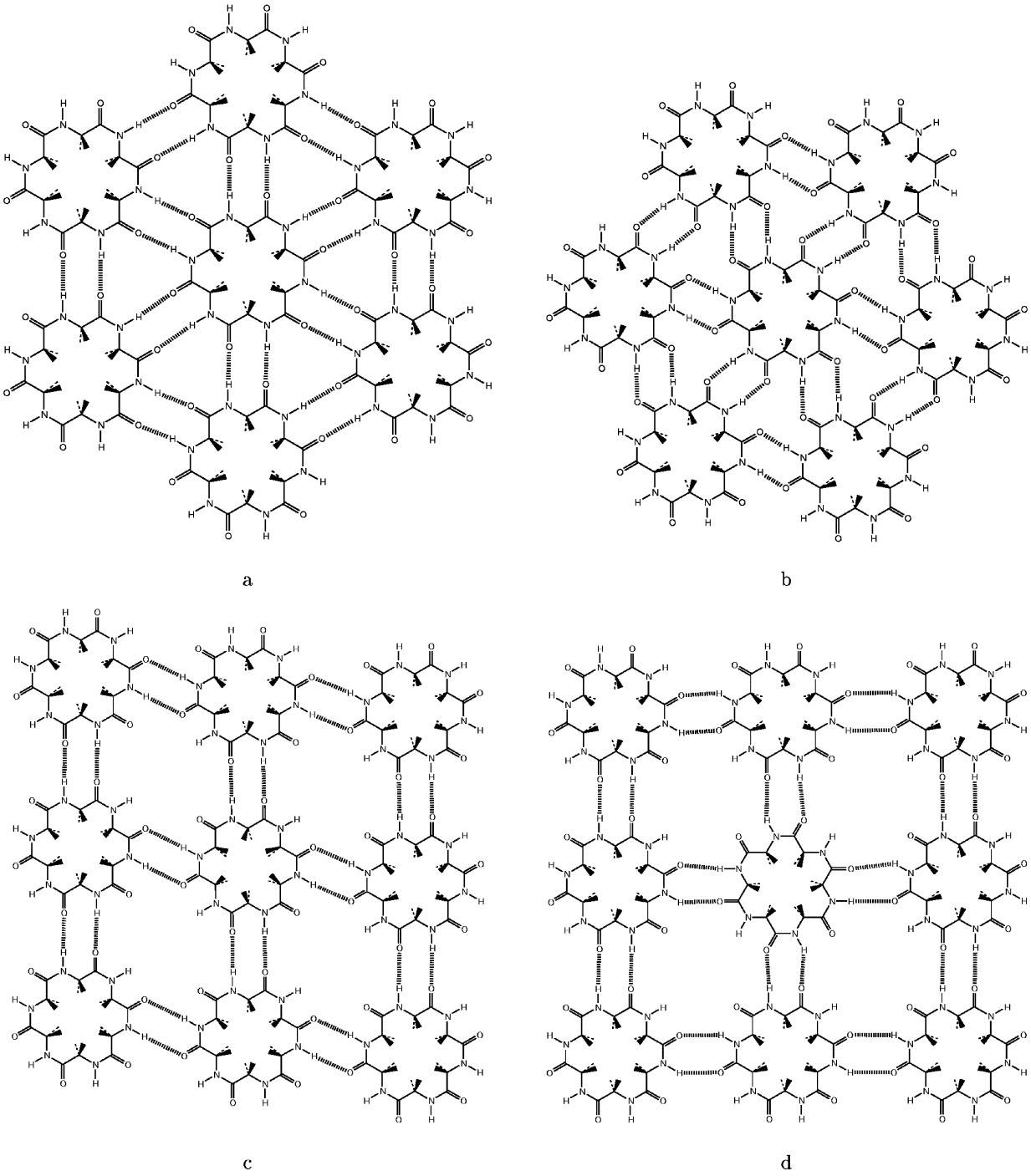

Figure 9. Possible 2-D intermolecular arrangements for cG_6^c.

these nonplanar curved rings could also be combining elements available for polyhedra building, still increasing the range of possibilities.

In contrast with the vertical stacking of all-*trans* cyclic peptides, all-*cis* cyclic peptides could self-assemble into planar or quasi-planar 2-D extended systems. Keeping with the most favorable case of the cG_6^c hexapeptide, once overcome the barrier to planarity, one can conceive compact quasi-hexagonal arrangements fulfilling all the hydrogen-bonding potential with optimal alignments, as illustrated in Figure 9a,b. Assuming total planarity, a cohesive energy of 35−40 kcal/mol per cyclopeptide monomer can be expected ($\sim[((12 \times 8)/2) - 10]$). Again, a balance between less planarity and worse hydrogen bonding could result in layers not fully planar still undergoing decent binding energies. Building regularly undulating layers, however, should meet topological requirements such as a chromatic

(44) As an illustration, at B3LYP/6-31G(d) level, and without taking into account BSSE, the energy per hydrogen bond is calculated at 8.9 kcal/mol in the NMA dimer. In the cyclic trimer of all-*cis* dipeptide models, an arrangement which now includes three such pairs of *cis*-amide plaques facing one another, this value reduces to 6.2 kcal/mol per hydrogen bond, mostly due to the close vicinity of three carbonyl oxygen atoms in the middle of the complex. On another hand, one should trust that the energy per hydrogen bond is larger in S12 than in S8: 9.4 vs 8.9 kcal/mol at B3LYP/6-31G(d) level, and 8.8 kcal/mol vs 8.0 kcal/mol at B3LYP/6-31G(d,p) level.

(45) For the definitions of closing rules and chromatic index, see for instance: Gross, J.; Yellen, J. *Graph Theory and its Applications*; CRC Press: Boca Raton, FL, 1998.

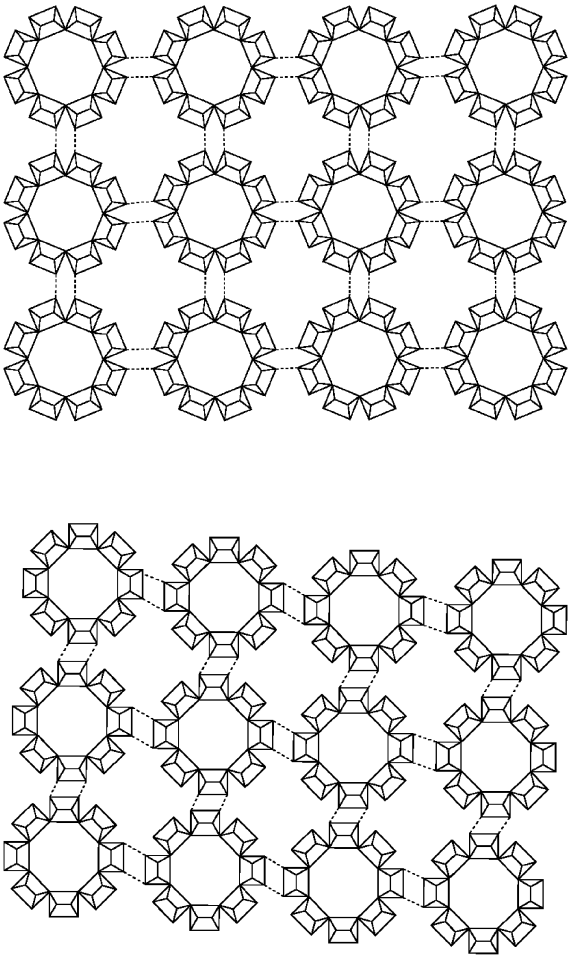

Figure 10. Possible 2-D intermolecular arrangements for cG_8^c.

index of two, which is not the case of the two compact assemblies of Figure 9a,b, both having a chromatic index of three. Assembling planar cyclooctapeptides cG_8^c results in square-planar networks, each monomer contributing to eight hydrogen bonds, as illustrated in Figure 10. Since in this case there is no planarity barrier to overcome, a mean cohesive energy can be anticipated at around 30 kcal/mol per cyclopeptide monomer (∼[(8 × 7)/2]). The large cavity between the octagons has potential for eight hydrogen bonds, and could accommodate any ligand of the right size with proper two- or four-fold symmetry. Although perfectly suited, the tetrapeptide cG_4^c could not be used here since, as mentioned earlier, too much energy is required to bring it close to planarity. On the other hand, smaller amide systems such as diketopiperazine or diazetidinedione $(-CO-NH-)_2$ could properly—albeit partially—fill the gap.

As illustrated in Figure 9c, it is also possible to build a square-planar net from cG_6^c cyclohexapeptides by combining junction pairing along one direction with plaque pairing along the perpendicular direction, or in other words by mixing S8 and S10 couplings. In this case, only four neat hydrogen-bond increments per cyclopeptide unit contribute the cohesive energy. However, ring planarity is no longer needed here since, with a chromatic index of two, this arrangement allows the building of an egg-box-type regular undulating sheet preserving favorable C═O···H−N alignments and resulting in a cohesive energy presumed to be around 25−30 kcal/mol per cyclopeptide

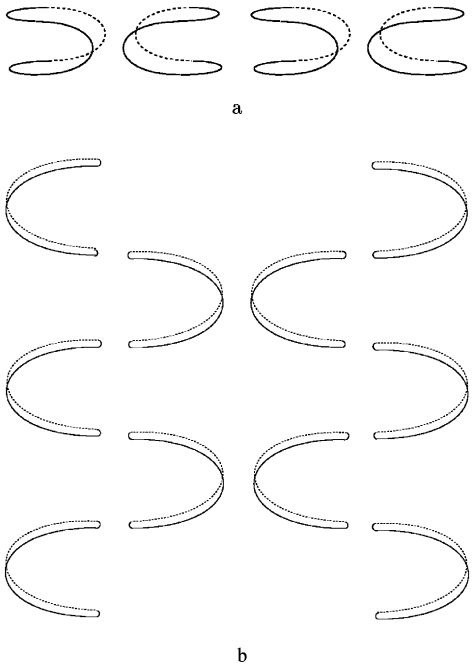

Figure 11. Two coupling schemes for chip-like cG_{15}^c.

monomer (∼[(8 × 6)/2]). Mixing S9, S10, and S12 coupling schemes is also conceivable, as depicted in Figure 9d. The available empty spaces are here smaller than in the preceding cG_8^c grid. With a potential for four hydrogen bonds, they could accommodate quite efficiently ligands such as diketopiperazine. A similar square-grid arrangement can be constructed from **10** and **10a**, leading in this case to a quasi-planar sheet, more supple than sinuous.

Building extended 2-D assemblies from planar or quasi-planar rings is possible not only from even rings, but also from odd rings such as cG_7^c or cG_9^c. In the case of heterogeneous mixing of cyclopeptides, amorphous-like organization should be expected, while quasi-crystal-like arrangements could result from homogeneous odd-unit sets. On the other hand, those chip-like forms such as cG_{15}^c **15a** built from even numbers of plaques share a symmetry allowing efficient head-to-tail coupling into extended 1-D rows, as well as 2-D networks (Figure 11), with expected cohesive energy around 20−25 kcal/mol per molecule (∼[(8 × 6)/2]). Last, other self-assembly is possible, such as the aggregation of the "open box" **4a** into a two-dimensional array with staircase arrangement in one direction.

In all these examples of self-assembly, we must keep in mind that owing to sp^3 α-carbons, the 2-D arrangements are not strictly planar in the sense of graphite or nucleic-acid bases. Through proper substitutions at C_α, various functionalizations can be considered, such as metal ligands in hetero multilayer slabs, stems designed to stand on nanosurfaces, groups favoring encapsulations into dimers or polyhedra, etc. As materials, such molecular plasticity for intermolecular arrangement might be a specific character of the all-*cis* peptide rings that could be put to profit in others contexts, still to find.

Concluding Remarks

This theoretical work has endeavored to explore the idea of a new kind of polycondensation of α-amino acids into all-*cis* cyclic peptides.[46] Exploration of the potential energy surfaces, especially for cyclopolyglycines cG_n^c, with the tools of quan-

tum chemistry led to the following clues. These systems are true minima on the potential surfaces, and should be locked in these arrangements. The optimal size for the thermodynamic stability occurs around eight peptide units and beyond. There, various energy criteria indicate that the juxtaposition of *cis* plaques is as favorable as that of *trans* plaques. The preferred geometries of these species are bowl-like forms (5,6 plaques), planar-symmetry forms (7,8,9 plaques) or more-or-less buckled distorted strings with saddle-edge or chip-edge shape (10 plaques and beyond). Although relative plaque tilting can generate low-lying minima, particularly for cG_4^c, cG_5^c, and cG_6^c, thus conferring some flexibility or plasticity to these rings, the all-*cis* cyclic peptides are basically rigid bodies.

Potential properties for derivatives of these arrangements include metal complexation in host−guest chemistry. Ionophore behavior directly from CO or NH as in normal all-*trans* cyclopeptides is however not possible here, and any binding to metallic fragments should make use of proper substituents at C_α. In this way, efficient kryptant or hemicarcerand activity can be expected, in particular with the bowl structures, evocative of the embedding in calixarenes. With two hydrophobic tails at each C_α, these molecules could be made amphiphilic, resulting in a hydrophobic rod surrounded by a hydrophilic slice in the middle. More attractive applications rest on the self-assembling properties. Where normal all-*trans* cyclic peptides self-assemble into nanotubes or micropore channels, all-*cis* cyclic peptides self-assemble into open or closed 2-D-like extended systems. In particular, organizing such elements into polyhedra or capsules of *desired* size opens the way for encapsulation.[47−52] Tuning size and C_α-substitution of the starting building elements could turn out to be a significant advantage in a context of delivery vectors, transfection vectors, saccharide recognition,[53,54] or heavy-metal trapping.

Although exhibiting some flexibility, the presently proposed cyclic molecules might not be qualified as real foldamers,[55−58] as they remain basically low conformational-entropy systems. Likewise, being made from the same pattern of atoms and covalent-bond as normal peptides, they also contrast with most variants devised in peptidomimetics[59,60] like peptoids,[61] hydrazino peptides,[62] peptidostarands,[63] depsipeptides,[64] azapeptides,[65] azatides, aminoxyacids, carbopeptoides,[55] or other isosteres.[66]

As biomaterials, all-*cis* cyclic peptides can interact noncovalently with normal all-*trans* proteins or peptides, including cyclic ones. Through S10 or S9 multicoupling schemes (Figure 6), they can act as cross linkers, giving more or less entangled networks. Such modes of association will need to be looked at carefully before anticipating to which extent all-*cis* cyclic peptides could interfere with proteins in folding, unfolding, misfolding, aggregation, or desegregation processes. Moreover, for in vivo applications such as delivery vectors, both biocompatibility, sensitivity to proteases and peptidases,[67] and biodegradability in general will need to be appraised before envisaging their possible use in a context of bioavailability. Other more-or-less speculative points about all-*cis* cyclic peptides as bioactive compounds include (i) their possible *cis*→*trans* unlocking provoked in vivo by enzymatic systems,[68] (ii) their ability to pair with nucleic acid bases, (iii) wondering why such structural design seems to have never been selected during the course of evolution (e.g. to store small amounts of energy as in the ATP pathway).

Besides the above structural and energetic preliminary results, this work raises several questions. In addition to the properties of isolated *cis*-amide function, such as trapping of carboxylic acids by diketopiperazine,[69] what kind of specific properties, different from those of lactams, could the proposed systems be endowed with? Can they be described as 2-D glue elements? Can they be covalently coupled in a vertical stack? What properties would have such a stick? Are open-chain helices possible? In what conditions, and to what extent are intraring hydrogen bonds possible? What dynamical properties would their semirigid character confer to cG_{10}^c−cG_{15}^c? What about the chip-type arrangement beyond cG_{15}^c? What about all-*cis* cyclic β peptides? Indeed, considering β-amino acids instead of α-amino acids, nothing prevents all-*cis* β peptides to exist as open chains, and both open and cyclic forms are here expected to be real foldamers, with properties presumably different from those of normal all-*trans* β peptides.[70−72]

To elucidate these different points, a description in terms of molecular mechanics seems now desirable in order to enable efficient treatment of higher-size monomers and intermolecular clusters. While we keep working along these lines, our reflection no doubt would be stimulated by any concrete experimental outcome around the all-*cis* stability proposal. In this respect, classical schemes for amide-bond creation in peptides or lactams should not be operative here,[73−75] any more than thermal or photochemical[76] *cis*−*trans* isomerizations from all-*trans* isomers. The art of synthesis is, however, full of resources, and

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Table 6. Effects of Methods and Basis Sets on Relative (ΔE) and Mean Plaque-Coupling (MPJE) Energies[a]

		ΔE			MPJE		
		B3LYP			B3LYP		
		6-311G(d,p)	6-31G(d)	MP2 6-311G(d,p)	6-311G(d,p)	6-31G(d)	MP2 6-311G(d,p)
cis-NMA	C_1	2.7	2.5	2.2			
trans-NMA	C_1	0.	0.	0.			
cis-dipeptide model	C_s	6.9	6.5	6.1	−2.9	−3.1	−3.7
trans-dipeptide model	C_s	0.	0.	0.	−4.4	−4.7	−5.5
cG_6^c	C_3	24.1	22.2	22.6	−1.4	−1.7	−2.8
cG_6^t	S_6	0.	0.	0.	−2.7	−2.8	−4.4

[a] In kcal/mol; *cis*- and *trans*-NMA correspond to *Esa* and *Zaa* rotamers, respectively, in the definition of ref 17; MP2 values of NMA from ref 17.

we hope there will be synthetic chemists willing to take up the challenge.

Computational Section

All calculations were performed with the *Gaussian 03* quantum chemistry package, at the DFT level of theory, using the B3LYP hybrid functional.[77] Full geometry optimizations were achieved in the gas phase, using the 6-31G(d) basis set. Calculations of vibrational frequencies were systematically done in order to characterize the nature of stationary points. For saddle points of index *N*, geometry deformations associated with each imaginary frequency were carried out in both directions of the corresponding normal mode, to find the stationary points connected by the given saddle point. This procedure is repeated until all the connected minima are found. To assess the quality of our B3LYP/6-31G(d) energies, we have compared them with those obtained from MP2/6-311G(d,p) calculations for parent NMA, methylated dipeptide model, and cyclohexaglycine (Table 6). In the former procedure, the *cis*−*trans* energy differences are increased by only 0.3 kcal/mol for NMA, and 0.4 kcal/mol for the dipeptide model. For cyclohexaglycine, it is decreased by 0.2 kcal/mol. Less agreement is observed for the mean plaque-junction energies, which are systematically lower with MP2. In the dipeptide model, they are decreased by 0.6 and 0.8 kcal/mol for all-*trans* and all-*cis* forms, respectively. In cyclohexaglycine, these underestimations amount to 1.6 and 1.1 kcal/mol for all-*trans* and all-*cis* forms, respectively. Even in this worse case, the *cis*−*trans* differential is however conserved within 0.5 kcal/mol. In other words, according to this criterion, MP2 tends to stress the stability of cyclohexamers with respect to dipeptide models. Nevertheless, since the energy scale is roughly the same, we do not believe that using an alternative method to B3LYP would qualitatively modify the present results. Further checks made at the B3LYP/6-311G(d,p) level still exhibit somewhat less agreement with these MP2/6-311G(d,p) values (see Table 6). Although mainly devoted to monomers, this work also includes some quantitative aspects of intermolecular interactions. For the test case of NMA dimer, both structural parameters and association energies obtained at B3LYP/6-31G(d) and B3LYP/6-311G(d,p) levels compare favorably with those obtained from MP2 calculations using extended basis sets,[14] as recapitulated in Table 7. For the few compounds including intramolecular hydrogen bonds such as **8a**, we have checked that the inclusion of p functions on hydrogen atoms does not alter dramatically the relative energies: at the B3LYP/6-311G(d,p) level, the **8** − **8a** energy difference is calculated at 12.1 kcal/mol, which reflects an expected stabilization of **8a** around 1 kcal/mol with respect to the B3LYP/6-31G(d) value. More generally, preliminary tests done at the B3LYP/6-311G(d,p) level for cG_6^c do not reveal any modification of the isomer ordering. In summary, the presently used B3LYP/6-31G(d) procedure appears as a good cost/accuracy compromise for addressing the structural and energy issues for a wide range of representatives of this new class of molecular compounds.

Table 7. Hydrogen-Bond Parameters (Å and deg) in *cis*-NMA Dimer

		B3LYP	
	MP2[a] Aug-cc-PVXZ	6-31G(d)	6-311G(d,p)
H···O	1.799	1.855	1.858
N···O	2.832	2.884	2.885
N−H···O	177.7	178.9	179.7
H···O═C	118.6	120.4	121.9
ΔE (kcal/mol)	8.6	8.9	8.0

[a] Reference 14; X = D and T for structural parameters and interaction energies, respectively.

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Supporting Information Available: Complete list of authors for ref 77, and total energies and Cartesian coordinates for all stationary points. This material is available free of charge via the Internet at http://pubs.acs.org.

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