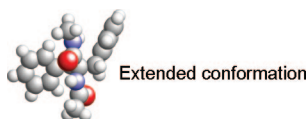# Intrinsic Conformational Preferences of $C^{\alpha,\alpha}$-Dibenzylglycine

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Departament de Química, Escola Politècnica Superior, Universitat de Lleida, c/Jaume II no. 69, Lleida E-25001, Spain, Basic Research Program, SAIC-Frederick, Inc., Center for Cancer Research Nanobiology Program, NCI, Frederick, Maryland 21702, Department of Human Genetics Sackler, Medical School, Tel Aviv University, Tel Aviv 69978, Israel, and Departament d'Enginyeria Química, E. T. S. d'Enginyeria Industrial de Barcelona, Universitat Politècnica de Catalunya, Avda. Diagonal no. 647, Barcelona E-08028, Spain

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Extended conformation

The intrinsic conformational preferences of $C^{\alpha,\alpha}$-dibenzylglycine, a symmetric α,α-dialkylated amino acid bearing two benzyl substituents on the α-carbon atom, have been determined using quantum chemical calculations at the B3LYP/6-31+G(d,p) level. A total of 46 minimum energy conformations were found for the *N*-acetyl-*N'*-methylamide derivative, even though only nine of them showed a relative energy lower than 5.0 kcal/mol. The latter involves C_7, C_5, and α' backbone conformations stabilized by intramolecular hydrogen bonds and/or N—H···π interactions. Calculation of the conformational free energies in different environments (gas-phase, carbon tetrachloride, chloroform, methanol, and water solutions) indicates that four different minima (two C_5 and two C_7) are energetically accessible at room temperature in the gas phase, while in methanol and aqueous solutions one such minimum (C_5) becomes the only significant conformation. Comparison with results recently reported for $C^{\alpha,\alpha}$-diphenylglycine indicates that substitution of phenyl side groups by benzyl enhances the conformational flexibility leading to (i) a reduction of the strain of the peptide backbone and (ii) alleviating the repulsive interactions between the π electron density of the phenyl groups and the lone pairs of the carbonyl oxygen atoms.

Introduction

Conformationally restricted α-amino acids are widely used in the construction of peptide analogues with controlled backbone fold. Among the α-amino acids whose structural rigidity can be exploited in the design of restricted peptides are α,α-dialkylated (also called *quaternary*) amino acids. Tetra-substitution at C^α introduces severe constraints in the backbone dihedral angles, thus stabilizing particular elements of peptide secondary structure.[1,2]

The simplest α,α-dialkylated amino acid is α-aminoisobutyric acid (Aib), that is, C^α-methylalanine. Replacement of the

α-hydrogen in alanine (Ala) by a methyl group results in a drastic reduction of the available conformational space. Theoretical and experimental studies[1–4] have demonstrated the strong tendency of Aib to induce folded structures in the 3_{10}-/α-helical region (φ, $\psi \approx \pm 60°$, $\pm 30°$), while semiextended or fully extended conformations are extremely rare for this residue. In comparison, Ala is easily accommodated in folded or extended structures. For higher homologues of Aib with linear side chains (diethylglycine, dipropylglycine, dibutylglycine, etc.), the stability of helical structures decreases as the side-chain length

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increases and thus these residues have been shown to prefer fully extended conformations.[1,2]

We recently investigated the conformational preferences of the $C^{\alpha,\alpha}$-diphenylglycine residue (Dϕg) using quantum mechanical calculations.[5] Although this residue was crystallized in both the folded and extended conformations depending on the peptide sequence,[6] its intrinsic conformational tendencies were established in our recent work.[5] Furthermore, the influence of the phenyl side groups on the conformational properties of Dϕg were examined by comparing the minimum energy conformations of this amino acid with those of Aib.

Nine minimum energy conformations were characterized at the B3LYP/6-31+G(d,p) level for the *N*-acetyl-*N*′-methylamide derivative of Dϕg within a relative energy range of about 9 kcal/mol. The relative stability of these structures was found to be largely influenced by specific backbone···side chain and side chain···side chain interactions that can be attractive (N—H···π and C—H···π) or repulsive (C=O···π). On the other hand, comparison with the minimum energy conformations calculated for Aib, in which the two phenyl substituents are replaced by methyl groups, revealed that the bulky aromatic rings of Dϕg induce strain in the internal geometry of the peptide.

In this work, we extend our studies on the intrinsic conformational preferences of symmetrically substituted *quaternary* amino acids to the $C^{\alpha,\alpha}$-dibenzylglycine residue (Db$_z$g). It should be noted that the methylene groups connected to the C^{α} are expected to increase significantly the number of minima with respect to Dϕg as well as to decrease the strain induced by the bulky aromatic side groups. Unfortunately, available experimental information about the structural propensities of Dbg is very scarce, a fully extended conformation being detected for some small model peptides.[6b,7] In this study, the potential energy surface of the *N*-acetyl-*N*′-methylamide derivative of Db$_z$g (Ac-Db$_z$g-NHMe, see Figure 1) has been explored using density functional theory (DFT) calculations. The influence of the polarity of the environment on the conformational preferences of this dipeptide has been analyzed using a self-consistent reaction-field (SCRF) method. Finally, the specific role of the benzyl side groups on the strain of the peptide backbone has been determined by comparing the energetics of the minima found for Ac-Db$_z$g-NHMe with those of the corresponding Aib and Dϕg analogues (Figure 1) through controlled isodesmic reactions.

Methods

The conformational properties of Ac-Db$_z$g-NHMe have been investigated using the Gaussian 03 computer program.[8] The conformational search was performed considering that this dipeptide retains the restrictions imposed by side-chain methyl groups on the backbone of Ac-Aib-NHMe. Thus, the five minimum energy conformations characterized for Ac-Aib-NHMe in ref 5 were used to generate the starting structures for Ac-Db$_z$g-NHMe. Although for Ac-Aib-NHMe four of such five minima were 2-fold degenerate
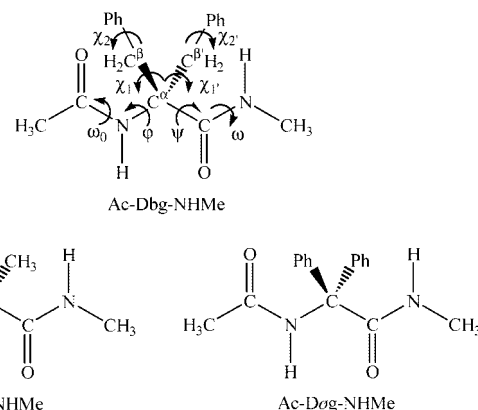
FIGURE 1. Chemical structure of the peptides examined in the present work. The minimum energy conformations of Ac-Db$_z$g-NHMe have been characterized in this study at the B3LYP/6-31+G(d,p) level, while the potential energy surfaces of Ac-Aib-NHMe and Ac-Dϕg-NHMe were reported at the same level of theory in ref 5. The backbone and side-chain dihedral angles are defined for Ac-Db$_z$g-NHMe.

due to the symmetry of the molecule, that is, $\{\varphi,\psi\} = \{-\varphi,-\psi\}$, the achiral nature of the Db$_z$g residue allows maintaining this degeneracy. The arrangement of the benzyl side groups is defined by the flexible dihedral angles $\{\chi_1,\chi_2,\ \chi'_1$ and $\chi'_2\}$, which are expected to exhibit three different minima: *gauche*$^+$ (60°), *trans* (180°) and *gauche*$^-$ (−60°). The dihedral angles that define the rotation of the amide bonds (ω and ω_0) were arranged at 180° in all cases. Consequently, 5 (minima of Ac-Aib-NHMe) × 3^4 (3 minima for each of the four flexible dihedral angles of benzyl side groups) = 405 minima can be anticipated for the potential energy hypersurface (PEH) $E = E(\varphi,\psi,\chi_1,\chi_2,\chi'_1,\chi'_2)$ of Ac-Db$_z$g-NHMe. All these structures were used as starting points for subsequent full geometry optimizations. This systematic conformational analysis strategy has allowed us to explore the PEHs of both small dipeptides[9] and flexible organic molecules.[10]

All geometry optimizations were performed using the Becke's three-parameter hybrid functional (B3)[11] combined with the Lee, Yang and Parr (LYP) expression for the nonlocal correlation[12] and the 6-31+G(d,p) basis set,[13] i.e., B3LYP/6-31+G(d,p) calculations. It should be noted that the PEH of both Ac-Aib-NHMe and Ac-Dϕg-NHMe were studied using the same theoretical level.[5] Frequency analyses were carried out to verify the nature of the

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minimum state of all the stationary points obtained and to calculate the zero-point vibrational energies (ZPVE) and both thermal and entropic corrections. These statistical terms were then used to compute the conformational Gibbs free energies in the gas phase at 298 K (ΔG_{gp}).

To obtain an estimation of the solvation effects on the relative stability of the different minima, single-point calculations were conducted on the optimized structures using a SCRF model. Specifically, the Polarizable Continuum Model (PCM) developed by Tomasi and co-workers[14] was used to describe carbon tetrachloride, chloroform, methanol, and water as solvents. The PCM method represents the polarization of the liquid by a charge density appearing on the surface of the cavity created in the solvent. This cavity is built using a molecular shape algorithm. PCM calculations were performed in the framework of the B3LYP/6-31+G(d,p) level using the standard protocol and considering the dielectric constants of carbon tetrachloride ($\varepsilon = 2.2$), chloroform ($\varepsilon = 4.9$), methanol ($\varepsilon = 32.6$), and water ($\varepsilon = 78.4$) to obtain the free energies of solvation (ΔG_{solv}) of the minimum energy conformations. Within this context, it should be emphasized that previous studies indicated that solute geometry relaxations in solution and single-point calculations on the optimized geometries in gas-phase give almost identical ΔG_{solv} values.[15] The conformational free energies in solution (ΔG_{CCl4}, ΔG_{Chl}, ΔG_{Met} and ΔG_{water}) at the B3LYP/6-31+G(d,p) level were then estimated using the classical thermodynamics scheme, i.e. summing the ΔG_{solv} and ΔG_{gp} values.

Results and Discussion

Conformational Properties. Using as starting points the 405 structures described in the Methods, geometry optimization at the B3LYP/6-31+G(d,p) level led to the characterization of 46 different minimum energy structures for Ac-Db$_z$g-NHMe, which are 2-fold degenerate since the structures with $\{\varphi,\psi,\chi_1,\chi_2,\chi'_1,\chi'_2\}$ and $\{-\varphi,-\psi,-\chi_1,-\chi_2,-\chi'_1,-\chi'_2\}$ are equivalent and isoenergetic. This indicates that the incorporation of one methylene groups into each side chain of Ac-Dϕg-NHMe produces a significant increase in the peptide flexibility, since only 9 different minima were characterized for the latter dipeptide.

Figure 2 represents the backbone φ,ψ angles of the 46 minima found for Ac-Db$_z$g-NHMe. The backbone conformation of the nine minimum energy conformations with relative energy lower than 5.0 kcal/mol are categorized within the Ramachandran map as follows: 5 adopts a C_7 conformation (seven-membered hydrogen bonded ring) with $\varphi,\psi \approx 60°,-60°$ or $\varphi,\psi \approx -60°,60°$, 3 presents a C_5 arrangement (five-membered hydrogen bonded ring) with $\varphi,\psi \approx 180°,180°$, and only 1 shows an α' conformation with $\varphi,\psi \approx 180°, \pm 60°$. The remaining 37 minima, which also include α ($\varphi,\psi \approx \pm 60°, \pm 60°$) and P_{II} ($\varphi,\psi \approx \pm 60°,180°$) backbone conformations, have relative energies ranging from 5.0 to 10.0 kcal/mol (25 minima) or even larger (12 minima).

Table 1 lists the backbone and side chain dihedral angles and relative energies (ΔE) of the more representative minimum energy structures of Ac-Db$_z$g-NHMe in gas-phase, i.e. those with $\Delta E < 5.0$ kcal/mol. In the lowest energy conformation, which has been labeled as C_7-I (Table 1), the backbone dihedral
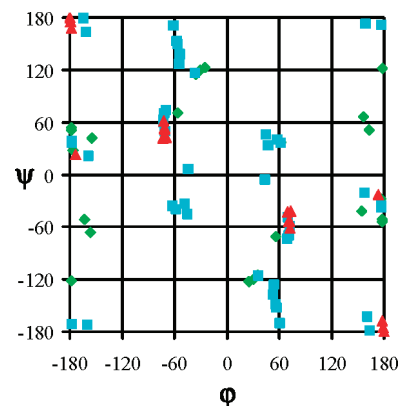
FIGURE 2. Backbone conformational preferences predicted for Ac-Db$_z$g-NHMe at the B3LYP/6-31+G(d,p) level. Minimum energy conformations with relative energies (ΔE) lower than 5.0 kcal/mol are indicated by red triangles. Blue squares and green diamonds refer to conformations with $5.0 < \Delta E < 10$ kcal/mol and $\Delta E \geq 10$ kcal/mol, respectively.

angles ($\varphi,\psi = 69.8°,-42.9°$) lead to the formation of an intramolecular hydrogen bond between the terminal NH and C=O groups [$d(H\cdots O) = 1.880$ Å and $<N-H\cdots O = 154.1°$] defining a seven-membered cycle (C_7 or γ-turn conformation). This structure, which is displayed in Figure 3a, is also stabilized by an $N-H\cdots\pi$ interaction[16] involving the NH group of the Db$_z$g residue and the π electron density of one of the aromatic side groups. The geometric parameters used to characterize this interaction are as follows: the distance between the NH hydrogen atom and the center of the aromatic ring ($d_{H\cdots ring}$) and the angle defined by the N-H bond and the plane of the aromatic ring (θ). These parameters in the C_7-I conformation are $d_{H\cdots ring} = 3.297$ Å and $\theta = 17.4°$. Indeed, $N-H\cdots\pi$ interactions have been frequently cited as stabilizing factors in the structure of peptides and proteins.[17] Furthermore, the intrinsic preferences of different conformationally restricted phenylalanine analogues were found to largely influenced by this specific interaction.[4f,9ef18]

The second minimum, labeled as C_7-II (Figure 3b), is almost equivalent to the C_7-I structure, i.e., backbone and side-chain dihedral angles are very similar but with opposite sign, the only difference being the conformation of one benzyl group (Table 1). Thus, the arrangement of the dihedral angle χ'_1 is *gauche* and *trans* for C_7-I and C_7-II, respectively. However, the two stabilizing interactions found in C_7-I are also detected in C_7-II: the terminal C=O and N-H groups form an intramolecular hydrogen bond [$d(H\cdots O) = 1.871$ Å and $<N-H\cdots O = 153.5°$], while the N-H moiety of the Db$_z$g residue is involved in a $N-H\cdots\pi$ interaction [$d_{H\cdots Ph} = 3.259$ Å and $\theta = 16.4°$]. The rearrangement of one benzyl group produces a destabilization of 1.4 kcal/mol.

In the third minimum, denoted C_5-I (Figure 3c), the backbone dihedral angles ($\varphi,\psi = 179.3°,-175.6°$) define a five-membered intramolecular hydrogen-bonded ring with parameters $d(H\cdots O) = 2.001$ Å and $<N-H\cdots O = 113.4°$. Furthermore, a $N-H\cdots\pi$ interaction involving the N-H of the NHMe blocking group

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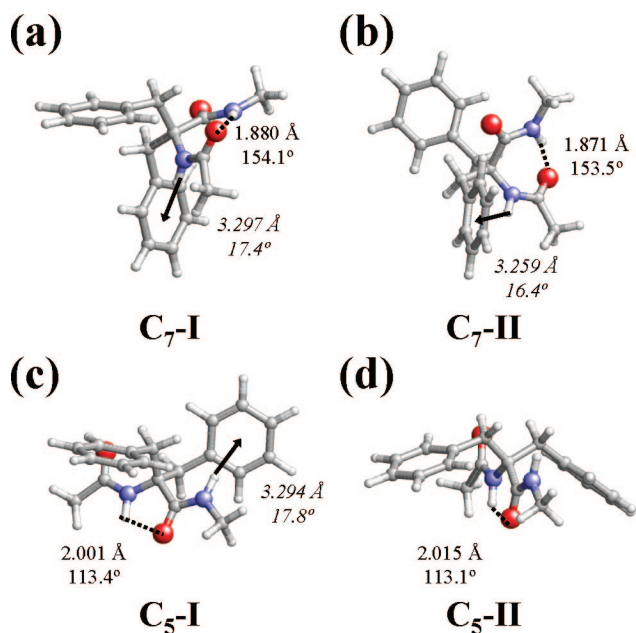
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TABLE 1. Backbone and Side-Chain Dihedral Angles,[a] Selected Bond Angles ($\angle$N$-$C$^\alpha$$-$C and $\angle$C$^\beta$$-C^\alpha$$-C^{\beta'}$)[a], and Relative Energies in the Gas Phase (ΔE)[b] for the More Stable Minimum Energy Conformations[c] of Ac-Db$_z$g-NHMe Characterized at the B3LYP/6-31+G(d,p) Level

no.	ω_0	φ	ψ	ω	χ_1	χ_2	$\chi_{1'}$	$\chi_{2'}$	$\angle$N$-$C$^\alpha$$-$C	$\angle$C$^\beta$$-C^\alpha$$-C^{\beta'}$	ΔE
C$_7$-I	176.1	69.8	$-$42.9	$-$175.5	$-$59.1	$-$93.5/88.6	$-$45.3	102.8/$-$78.1	113.2	109.8	0.0[d]
C$_7$-II	$-$175.4	$-$71.4	52.2	179.7	42.0	$-$102.7/78.3	175.0	83.4/$-$98.5	111.9	109.2	1.4
C$_5$-I	179.2	179.3	$-$175.6	$-$176.1	53.8	87.0/$-$94.5	177.0	99.6/$-$83.5	104.0	111.5	1.7
C$_5$-II	$-$179.3	179.8	178.0	$-$179.6	54.4	86.8/$-$94.4	$-$55.3	92.6/$-$88.6	104.2	107.8	1.8
α'	169.8	173.4	$-$23.2	$-$172.3	60.2	93.0/$-$88.5	$-$49.0	98.5/$-$82.5	108.7	107.7	2.9
C$_5$-III	$-$178.7	178.1	$-$168.1	$-$176.0	52.2	25.0/$-$156.7	179.5	96.7/$-$85.9	104.5	110.8	3.8
C$_7$-III	$-$171.3	$-$71.5	54.4	179.9	$-$41.2	100.0/$-$81.2	$-$171.8	91.9/$-$90.2	111.4	112.1	4.1
C$_7$-IV	$-$171.8	$-$72.7	61.8	$-$178.3	$-$48.7	99.3/$-$82.4	171.0	42.4/$-$141.1	109.5	112.6	4.1
C$_7$-V	$-$176.2	$-$73.2	41.6	173.3	46.2	78.6/$-$102.3	$-$119.2	102.3/$-$80.5	112.8	107.5	4.6

[a] In degrees. See Scheme 1. [b] In kcal/mol. [c] Minima with relative energy lower than 5.0 kcal/mol. [d] $E = -997.338908$ au.

(a) 1.880 Å 154.1° 3.297 Å 17.4° **C$_7$-I**

(b) 1.871 Å 153.5° 3.259 Å 16.4° **C$_7$-II**

(c) 3.294 Å 17.8° 2.001 Å 113.4° **C$_5$-I**

(d) 2.015 Å 113.1° **C$_5$-II**

FIGURE 3. C$_7$-I (a), C$_7$-II (b), C$_5$-I (c), and C$_5$-II (d) minimum energy conformations of Ac-Db$_z$g-NHMe characterized at the B3LYP/6-31+G(d,p) level. Geometric parameters of both intramolecular hydrogen bonds (dashed lines and roman numbers) and N$-$H$\cdots\pi$ interactions (arrows and italic numbers) are indicated.
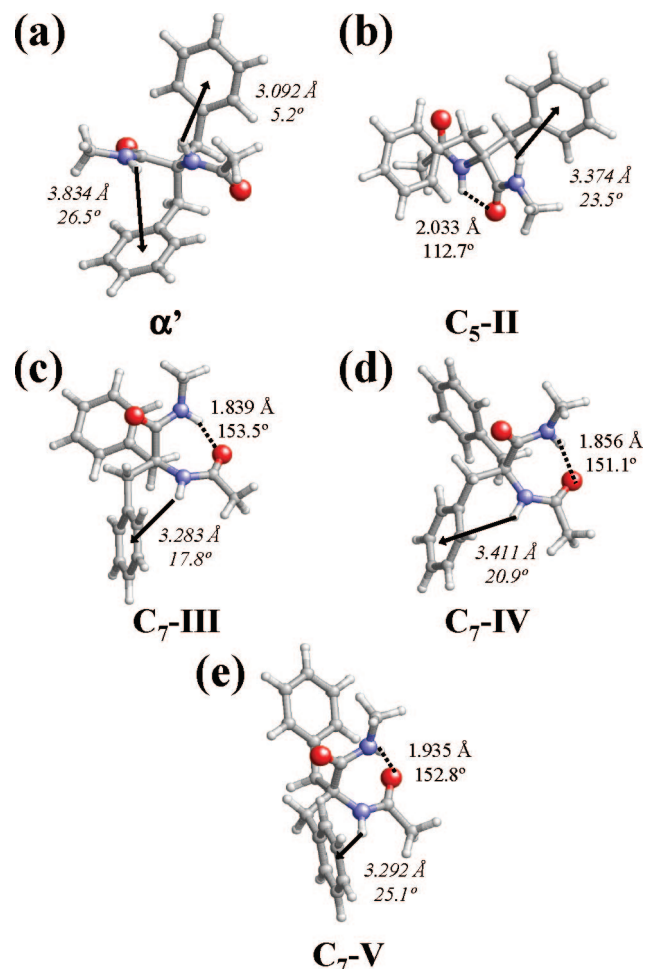
SCHEME 1

Reaction 1
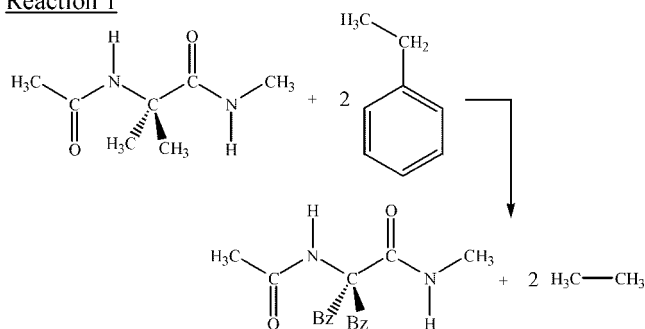
Reaction 2
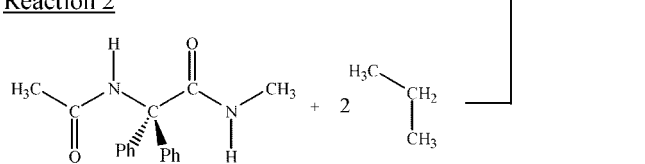
and one side phenyl group ($d_{H\cdots Ph} = 3.294$ Å and $\theta = 17.8°$) is also detected in this conformation. The fourth minimum, labeled as C$_5$-II, also forms a five-membered intramolecular

hydrogen-bonded ring [$d(H\cdots O) = 2.015$ Å and $<$N$-$H$\cdots$O $= 113.1°$]. This minimum only differs from C$_5$-I in the conformation of one side group, this situation being similar to that discussed above for C$_7$-I and C$_7$-II. Interestingly, as can be seen in Figure 3d, the rotation of the dihedral angle χ'_1 precludes the formation of the N$-$H$\cdots\pi$ interaction in C$_5$-II. Conformations C$_5$-I and C$_5$-II are destabilized by 1.7 and 1.8 kcal/mol, respectively.

The relative energies of the other five minima listed in Table 1 range from 2.9 to 4.6 kcal/mol, and therefore, their relative population in gas phase is expected to be negligible. In particular, the fifth minimum energy conformation corresponds to a partially folded α' arrangement, which does not show any stabilizing intramolecular hydrogen bond (Figure 4a). Although the relative energy of this structure is relatively high, i.e., 2.9 kcal/mol, the geometrical disposition of the backbone amide groups and the aromatic rings allows the coexistence of two N$-$H$\cdots\pi$ stabilizing interactions with geometric parameters [$d_{H\cdots Ph} = 3.834$ Å and $\theta = 26.5°$] and [$d_{H\cdots Ph} = 3.092$ Å and $\theta = 5.2°$]. The steric effects produced by the bulky benzyl side groups on the stability of symmetric linear α,α-dialkylated amino acids are more important for the remaining minima of Table 1. These four conformations, which have been denoted C$_5$-III, C$_7$-III, C$_7$-IV, and C$_7$-V, are depicted in Figures 4b$-$e. As can be seen, all these structures present both an intramolecular hydrogen bond and an N$-$H$\cdots\pi$ stabilizing interaction. However, the arrangements adopted by the benzyl side groups lead to strained conformations or involve unfavorable interactions between the lone pairs of the backbone C$=$O groups and the π-clouds of the aromatic rings. These steric and/or electronic effects produce conformations that are destabilized by more than 3.8 kcal/mol with respect to the global minimum.

On the other hand, inspection to the data listed in Table 1 reveals that the $\angle$N$-$C$^\alpha$$-$C bond angle strongly depends on the backbone conformation. Specifically, for the five C$_7$ conformers the values of the $\angle$N$-$C$-$-C angle ranges from 109.5° to 113.2°, while it remains close to 104° in the two C$_5$ minima. This conformational dependency is consequence of the geometrical strain induced by the benzyl side groups.

Thermodynamical Corrections and Solvent effects. Table 2 lists the relative conformational Gibbs free energies in the gas phase at $T = 298.15$ K (ΔG_{gp}) calculated for the nine minima of Ac-Db$_z$g-NHMe analyzed in the previous section. As can be seen, consideration of the ZPVE, thermal, and entropic corrections introduces significant changes to the relative stability of the minima. Specifically, the range that separates the four minima of lower energy, i.e., those with $\Delta E < 2.0$ kcal/mol, is reduced by 0.7 kcal/mol. However, the most remarkable result corresponds to the C$_5$-II minimum, which

FIGURE 4. α′ (a), C$_5$-III (b), C$_7$-III (c), C$_7$-IV (d), and C$_7$-V (e) minimum energy conformations of Ac-Db$_z$g-NHMe obtained at the B3LYP/6-31+G(d,p) level. Geometric parameters of both intramolecular hydrogen bonds (dashed lines and roman numbers) and N−H···π interactions (arrows and italic numbers) are indicated.
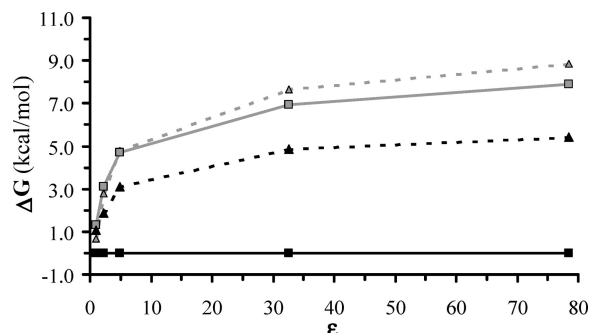

FIGURE 5. Variation of the conformational free energy against the polarity of the environment for the C$_5$-II (solid black line, black squares), C$_7$-I (dashed gray line, gray triangles), C$_5$-I (dashed black line, black triangles), and C$_7$-II (solid gray line, black triangles) minimum energy conformations.

Ac-Db$_z$g-NHMe. For this purpose, the molecular geometry of the C$_5$-II conformation was reoptimized in both chloroform and water solutions. Comparison of the resulting molecular geometries with that obtained in the gas-phase indicated that the variation of bond distances and bond angles was in all cases lower than 0.008 Å and 2.0°, respectively. Moreover, the values of ΔG_{sol} obtained using the geometries optimized in the gas-phase and in solution were almost identical; i.e., the largest variation was 0.2 kcal/mol. Accordingly, the values ΔG_{sol} in carbon tetrachloride, chloroform, methanol and water were calculated for the minimum energy conformations of Ac-Db$_z$g-NHMe using the geometries optimized in the gas-phase.

Table 2 also lists the estimated relative conformational free energies calculated in different solvents. As can be seen, solute−solvent interactions do not alter significantly the relative energy order of the different minima with respect to that obtained in the gas-phase. In all cases the C$_5$-II was the lowest energy conformation. Moreover, the relative stability of this structure increases with the polarity of the solvent. This is clearly reflected in Figure 5, which represents the relative conformational free energies calculated for the more stable structures, i.e. C$_5$-II, C$_7$-I, C$_5$-I, and C$_7$-II, against the polarity of the environment. The solute−solvent interactions associated with the C$_5$-II conformation become so attractive in solution that it is the only significant structure in chloroform, methanol and aqueous solution. According to a Boltzmann distribution, which is usually used to describe the conformational preferences of small peptides, the population of C$_5$-II at room temperature increases from 63% in gas phase to 95%, 99%, 100%, and 100% in carbon tetrachloride, chloroform, methanol, and water solvents, respectively.

The overall of the results obtained for Db$_z$g is in excellent agreement with experimental evidence reported for other α,α-dialkylated amino acids.[1,2,19] Thus, it has been observed that homopeptides consisting of symmetric α,α-dialkylated amino acids with side chains bulkier than a methyl group are able to stabilize a rare secondary structure denoted 2.0$_5$ helix. This helical arrangement is based on the propagation of a fully extended conformation that involves a five-membered hydrogen bonded ring. Furthermore, although very scarce, available

TABLE 2. Relative Conformational Free Energies[a] in the Gas Phase, Carbon Tetrachloride, Chloroform, Methanol, and Aqueous Solutions for the More Stable Minimum Energy Conformations[b] of Ac-Db$_z$g-NHMe

no.	ΔG_{gp}	ΔG_{CCl4}	ΔG_{Chl}	ΔG_{Met}	ΔG_{water}
C$_7$-I	0.7	2.8	4.8	7.7	8.9
C$_7$-II	1.3	3.1	4.7	6.9	7.9
C$_5$-I	1.1	1.9	3.1	4.9	5.4
C$_5$-II	0.0[c]	0.0	0.0	0.0	0.0
α′	2.7	4.0	5.5	6.9	5.9
C$_5$-III	4.9	6.2	7.6	8.8	9.2
C$_7$-III	4.2	5.4	6.7	8.8	9.8
C$_7$-IV	4.0	5.6	7.1	9.5	10.6
C$_7$-V	3.7	5.5	7.4	10.4	11.4

[a] In kcal/mol. [b] Minima with relative energy lower than 5.0 kcal/mol in the gas phase. [c] $E = -997.014910$ au.

showed the largest thermodynamical correction transforming into the most stable conformation. Overall, these results indicate that the C$_5$-II, C$_7$-I, C$_5$-I, and C$_7$-II are the only energetically accessible minima in gas-phase, i.e., the estimated populations are 63%, 20%, 10%, and 7%, respectively, the population expected for the other conformers being negligible.

In spite of a large number of works indicated that the influence of the solvent on the ΔG_{sol} of (bio)organic molecules is negligible, we decided to check that this feature is shared by
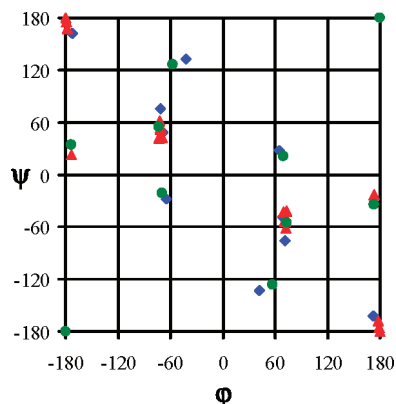
(19) (a) Di Blasio, B.; Pavone, V.; Lombardi, A.; Pedone, C.; Benedetti, E. *Biopolymers* **1993**, *33*, 1037. (b) Benedetti, E.; Pedone, C.; Pavone, V.; Di Blasio, B.; Saviano, M.; Fattorusso, R.; Crisma, M.; Formaggio, F.; Bonora, G. M.; Toniolo, C.; Kaczmarek, K.; Redlinski, A. S.; Leplawy, M. T. *Biopolymers* **1994**, *34*, 1409.

FIGURE 6. Backbone conformational preferences predicted for Ac-Db$_z$g-NHMe (red triangles), Ac-Dϕg-NHMe (blue diamonds), and Ac-Aib-NHMe (green circles) at the B3LYP/6-31+G(d,p) level. Only minimum energy conformations with relative energies (ΔE) lower than 5.0 kcal/mol are indicated.

experimental studies on Db$_z$g-containing peptides also indicated that the fully extended is the preferred conformation in solution.[6b]

Influence of the Benzyl Side Groups in the Conformational Properties. Figure 6 represents the position of all the minima with relative energy lower than 5 kcal/mol characterized at the B3LYP/6-31+G(d,p) level for Ac-Db$_z$g-NHMe, Ac-Dϕg-NHMe, and Ac-Aib-NHMe. As can be seen, the benzyl side groups restrict significantly the backbone conformation of Ac-Db$_z$g-NHMe. Thus, in spite of the latter dipeptides showing the highest number of low energy minima, only three backbone conformations are detected, i.e., C$_5$, C$_7$, and α'. In contrast, the low energy minima of Ac-Dϕg-NHMe and Ac-Aib-NHMe involve four (C$_5$, C$_7$, α, and P$_{II}$) and five backbone conformations (C$_5$, C$_7$, α, P$_{II}$, and α'), respectively.[5] Furthermore, it should be noted that when the whole set of energy minima is considered, the relative energy interval is 4.5, 9.8, and 21.4 kcal/mol for Ac-Aib-NHMe, Ac-Dϕg-NHMe, and Ac-Db$_z$g-NHMe. Thus, the unfavorable interactions between the backbone and the side groups, which are reflected in the spread out energy spectra, increase with the size of the substituents. On the other hand, our previous study on Ac-Dϕg-NHMe and Ac-Aib-NHMe observed that the phenyl in the substituents induce strain in the internal geometry of the peptide.[5]

In order to quantitatively evaluate the unfavorable interactions and the strain effects induced by benzyl substituents of Ac-Db$_z$g-NHMe, the isodesmic reactions displayed in Scheme 1 have been considered. For each optimized backbone conformation of Ac-Db$_z$g-NHMe, the energy associated with the benzyl substituents $G^{Bz/Bz}$ in reactions 1 and 2 was estimated according to eqs 1 and 2, respectively:

$$G^{Bz/Bz} = G^{Ac\text{-}Dbzg\text{-}NHMe} + 2G^{CH_3CH_3} - \\ (G^{Ac\text{-}Aib\text{-}NHMe} + 2G^{CH_3CH_2Ph}) \quad (1)$$

$$G^{Bz/Bz} = G^{Ac\text{-}Dbzg\text{-}NHMe} + 2G^{CH_3CH_3} - \\ (G^{Ac\text{-}D\varphi g\text{-}NHMe} + 2G^{CH_3CH_2CH_3}) \quad (2)$$

where $G^{Ac\text{-}Dbzg\text{-}NHMe}$ is the free energy of the conformation under study, obtained according to the Boltzmann population of all conformers with such backbone conformation; $G^{Ac\text{-}Aib\text{-}NHMe}$ and $G^{Ac\text{-}D\varphi g\text{-}NHMe}$ correspond to the free energies of the Aib and Dϕg homologues with similar backbone conformation; and $G^{CH_3CH_3}$, $G^{CH_3CH_2CH_3}$, and $G^{CH_3CH_2Ph}$ are the free energies of ethane,

TABLE 3. Free Energy Contribution Associated with the Benzyl Side Groups^a ($G^{Bz/Bz}$) for the Backbone Conformations of Ac-Db$_z$g-NHMe in the Gas Phase and Aqueous Solution

backbone conformation	$G^{Bz/Bz}$ (gas phase)	$G^{Bz/Bz}$ (aqueous solution)
	reaction 1^b	
C$_5$	5.3	0.1
C$_7$	5.3	7.1
α'	3.3	0.7
	reaction 2^b	
C$_5$	−5.8	−22.4
C$_7$	−6.6	−15.2
α'	−10.3	−23.1

^{a} In kcal/mol. $G^{Bz/Bz}$ was computed using eqs 1 and 2 (see text). ^{b} In Scheme 1.

propane, and ethylbenzene, respectively, calculated for the lowest minimum energy conformation at the B3LYP/6-31+G(d,p) level.

In eqs 1 and 2, $G^{Bz/Bz}$ provides an estimation of the energy value associated with the replacement of the methyl (reaction 1 in Scheme 1) and phenyl (reaction 2 in Scheme 1) side groups by benzyl for a given backbone conformation. Results obtained for the C$_5$, C$_7$, and α' backbone conformations of Ac-Db$_z$g-NHMe described are displayed in Table 3.

The positive values of $G^{Bz/Bz}$ derived from reaction 1 and eq 1 reveal significant unfavorable effects for all backbone conformations in both gas phase and aqueous solution, even though these are significantly lower in the latter environment. On the basis of our previous study on Ac-Dϕg-NHMe and Ac-Aib-NHMe,[5] this is an expected result that should be attributed to two different factors: First, the strain induced by the benzyl groups of Ac-Db$_z$g-NHMe in the internal geometry of the peptide. In addition, substitution of methyl by benzyl introduces the possibility of repulsive interactions between the π electron density of the phenyl groups and the lone pairs of the oxygen atoms contained in the backbone carbonyl groups. The strength of such unfavorable interactions is reduced considerably in aqueous solution for backbone conformations in which the oxygen atoms show a higher accessibility to the surrounding solvent, i.e. C$_5$ and α'.

On the other hand, all $G^{Bz/Bz}$ values obtained from reaction 2 and eq 2 are negative, illustrating that the unfavorable effects induced by the side groups are considerably higher in Ac-Dϕg-NHMe than in Ac-Db$_z$g-NHMe. This is because the conformational flexibility of the benzyl groups is higher than that of the phenyl ones, which contributes to reduce both the backbone strain and the repulsive electronic interactions induced by the side chains of Ac-Dϕg-NHMe. The reduction of the strain is indicated when comparing the $\angle N-C^\alpha-C$ and $\angle C^\beta-C^\alpha-C^\beta$ bond angles for the minimum energy conformations of Ac-Dϕg-NHMe[5] and Ac-Db$_z$g-NHMe (Table 1). The deviation with respect to the value ideally expected for these bond angles, i.e., 109.5°, is lower for the three backbone conformations of Ac-Db$_z$g-NHMe than for Ac-Dϕg-NHMe. Furthermore, it is worth noting that $G^{Bz/Bz}$ is notably lower in aqueous solution than in the gas phase, indicating than the benzyl side groups allows a more favorable interaction with the peptide backbone than the phenyl ones. This can be also justified by the higher conformational flexibility of the former with respect to the latter.

Conclusions

DFT calculations at the B3LYP/6-31+G(d,p) level have been used to analyze the conformational preferences of the Ac-Db$_z$g-

NHMe. A total of 46 different minimum energy conformations, which are stabilized by attractive backbone···backbone (hydrogen bonds) and backbone···side chain (N−H···π) interactions, have been identified. However, only nine such minima showed a relative energy lower than 5 kcal/mol. The conformational free energies have been calculated in five different environments. The results showed that four different minima, which correspond to C_5 and C_7 backbone conformations, are energetically accessible at room temperature. However, the energy of three such minima increases rapidly with the polarity of the environment, a structure with a C_5 backbone conformation being the only significant minimum (population= 100%) in both methanol and aqueous solutions.

Comparison with the results previously reported for Ac-Aib-NHMe and Ac-Dϕg-NHMe at the same level of theory has provided information about the effects produced by the side chain. Specifically, substitution of the methyl side groups of Ac-Aib-NHMe by benzyl produces, as expected, unfavorable strain effects in the internal geometry of the peptide and repulsive interactions between the π-aromatic rings and the carbonyl oxygen atoms. In contrast, these two effects are significantly reduced when the phenyl side groups of Ac-Dϕg-NHMe are replaced by benzyl, which should be attributed to the higher conformational flexibility of the latter.

Supporting Information Available: Coordinates and energy of the minimum energy conformations characterized for Ac-Db$_z$g-NHMe. This material is available free of charge via the Internet at http://pubs.acs.org.

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