Structural Versatility of Peptides from $C^{\alpha,\alpha}$-Disubstituted Glycines. Preferred Conformation of the $C^{\alpha,\alpha}$-Dibenzylglycine Residue

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The preferred conformation of the $C^{\alpha,\alpha}$-dibenzylglycine residue has been assessed in selected derivatives and small peptides by conformational energy computations, ^{1}H NMR spectroscopy, and X-ray diffraction. Conformational energy computations on the $C^{\alpha,\alpha}$-dibenzylglycine monopeptide, Ac-Dbz-NHMe, strongly support the view that this $C^{\alpha,\alpha}$-symmetrically disubstituted residue is conformationally restricted and that its minimum energy conformation falls in the fully-extended (C_5) region. The results of the theoretical analysis are in agreement with the solution and crystal-state structural propensity of Tfa-Dbz-Gly-DBH, Tfa-Dbz-L-Phe-OMe and its benzyl ester analogue, *m*-ClAc-Dbz-OH, Z-Gly-Dbz-Gly-OH and its t-butyl ester derivative. The implications for the use of the Dbz residue in designing conformationally constrained analogues of bioactive peptides are briefly discussed.

The significance of $C^{\alpha,\alpha}$-disubstituted glycines has been recently recognized in connection with the design and synthesis of conformationally restricted analogues of bioactive peptides.[1–4] Recent studies suggest that the $C^{\alpha,\alpha}$-dimethylglycine (α-aminoisobutyric acid, Aib) residue strongly prefers folded backbone conformations in the $3_{10}/\alpha$-helical region of the φ,ψ space (φ = ±60 ± 20°, ψ = ±30 ± 20°) (for a recent survey see ref. 3); by contrast, $C^{\alpha,\alpha}$-diethylglycine (Deg)[5–7] and $C^{\alpha,\alpha}$-di-n-propylglycine (Dpg)[5,8,9] residues, with longer side chains, preferentially adopt the fully-extended C_5 conformation (φ,ψ *ca.* 180°, 180°).[3]

As part of a programme aimed at investigating the conformational properties of $C^{\alpha,\alpha}$-symmetrically disubstituted glycyl residues we describe here the structural characterisation of $C^{\alpha,\alpha}$-dibenzylglycine (Dbz; earlier abbreviated as Bphe, Dbg, or Db_zg) in simple derivatives and peptides by using conformational energy computations, ^{1}H NMR spectroscopy and X-ray diffraction. In addition to model compounds, the general potential of this residue as a Phe replacement has been demonstrated by the synthesis of enkephalinamide, bradykinin, and substance P analogues.[10–15]

Experimental

Materials.—The synthesis and characterisation of Tfa-Dbz-L-Phe-OMe (**2**) (Tfa, trifluoroacetyl; OMe, methoxy),[14] *m*-ClAc-Dbz-OH (**4**) (*m*-ClAc, monochloroacetyl),[11] Z-Gly-Dbz-Gly-OH (**5**) (Z, benzyloxycarbonyl),[11] and Z-Gly-Dbz-Gly-OBu^t (**6**) (OBu^t, t-butoxy)[11] have already been reported.

Tfa-Dbz-Gly-DBH (**1**) (*DBH*, N′,N′-*Dibenzylacylhydrazido*).—Glycine (*N′,N′*-dibenzyl)hydrazide (0.714 g, 2.64 mmol)[16] and 2-trifluoromethyl-4,4-dibenzyloxazolin-5-one (1.0 g, 3 mmol)[13] were stirred in acetonitrile (20 cm^3) at 20 °C for 15 h. The solvent was removed and the residue taken up in ether and washed with 2 mol dm^{-3} citric acid (2 × 20 cm^3), water (20 cm^3), 1 mol dm^{-3} $NaHCO_3$ (30 cm^3), and water (20 cm^3). The ether solution was then dried ($MgSO_4$), filtered, and the solvent evaporated. The residual pale yellow oil (1.62 g) crystallised on standing. Recrystallisation from methanol–diethyl ether gave colourless crystals (1.35 g, 65%), m.p. 150–151 °C (Found: C, 68.0; H, 5.4; N, 9.4%. $C_{34}H_{33}F_3N_4O_3$ requires C, 67.8; H, 5.5; N, 9.3%).

Tfa-Dbg-L-Phe-OBzl (**3**) (*Bzl, benzyloxy*).—2-Trifluoromethyl-4,4-dibenzyloxazolin-5-one (1.0 g),[13] L-phenylalanine benzyl ester tosylate (1.60 g, 3.75 mmol), and triethylamine (0.38 g, 3.75 mmol) were stirred in CH_2Cl_2 (30 cm^3) for 2 days at 20 °C. Evaporation of the solvent gave a colourless glass which was taken up in ethyl acetate and the solution was washed as in the above preparation. The solution was dried and evaporated to leave a white solid (1.60 g). Recrystallisation from ethyl acetate–light petroleum (b.p. 60–80 °C) gave fine needles (1.44 g, 82%), m.p. 127–128 °C (Found: C, 69.5; H, 5.4; N, 4.7%. $C_{34}H_{31}F_3N_2O_4$ requires C, 69.4; H, 5.3; N, 4.8%).

Conformational Energy Computations.—The standard geometries of Scheraga and co-workers[17,18] were used for the acetamido and methylamido end groups, while the average X-ray data of the present work were the source of the geometrical parameters of the Dbz residue. Empirical two-body potential functions (AMBER[19] force field) were used for describing torsional, steric, electrostatic, and H-bond interactions. Steric and electrostatic interactions between 1–4 atoms were always halved.[17–19] A value for the relative permittivity, ε_r, of 1 was assumed in all calculations. The atomic charges are generally similar to the original AMBER ones but they are derived by empirical rules, thus avoiding any preliminary quantum-mechanical calculation.[20] In particular, following the suggestion of Lifson and co-workers[21] and some quantum mechanical

Table 1. Crystal data for Tfa-Dbz-Gly-DBH (1) methanol solvate, Tfa-Dbz-L-Phe-OMe (2), Tfa-Dbz-L-Phe-OBzl (3), *m*-ClAc-Dbz-OH (4), and Z-Gly-Dbz-Gly-OH (5).

	Compound				
	(1)	(2)	(3)	(4)	(5)
Molecular formula	$C_{34}H_{32}F_3N_4O_3{\cdot}CH_4O$	$C_{28}H_{27}F_3N_2O_4$	$C_{34}H_{28}F_3N_2O_4$	$C_{18}H_{17}ClNO_3$	$C_{28}H_{29}N_3O_6$
M(amu)	633.7	512.5	585.6	330.8	503.6
Crystallized from	MeOH–Et_2O	CH_3CO_2Et–light petroleum (b.p. 60–80 °C)	$(CH_3)_2CO$	$(CH_3)_2CO$–H_2O	$(CH_3)_2CO$–H_2O
D_{calc}/g cm^{-3}	1.24	1.26	1.30	1.29	1.28
Crystal system	Triclinic	Orthorhombic	Orthorhombic	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P2_12_12_1$	$P2_12_12_1$	$P\bar{1}$	$C2/c$
Z	2	4	4	2	8
a/Å	14.227(2)	19.374(2)	19.828(2)	12.669(2)	31.311(3)
b/Å	11.571(2)	12.974(2)	12.876(2)	9.628(1)	10.869(1)
c/Å	11.351(2)	10.756(2)	11.763(2)	7.683(1)	17.949(2)
α/°	111.3(10)			102.0(1)	
β/°	76.1(1)			96.1(1)	121.6(1)
γ/°	106.3(1)			109.4(1)	
V/Å	1 651.0	2 703.6	3 003.2	848.9	5 202.7
μ(Mo-K_α)/cm^{-1}	0.56	0.61	0.60	1.96	0.55
Solved by	MULTAN 80[27]	SHELX S-86[28]	MULTAN 80	MULTAN 80	SHELX S-86
No. of unique reflections	7 706	2 699	4 046	4 102	4 984
No. of observed[a] reflections	4 211	1 614	2 191	2 384	1 825
R	0.062	0.054	0.058	0.064	0.098
R_w[b]	0.073	0.059	0.066	0.064	0.112
S	1.37	1.01	0.96	0.75	1.40
H-atoms	Not refined	Refined	Refined	Not refined	Not refined

[a] $F \geqslant 7\sigma(F)$ for (1), (2), and (5); $F \geqslant 6\sigma(F)$ for (3) and (4). [b] $w = 1/[\sigma^2(F) + XF^2]$, where $X = 0.003$ for (1), 0.004 for (2), 0.0066 for (3), and 0.001 for (5). For (4), $w = 1$.

computations,[22] electroneutrality was imposed on the carbonyl and methylamino moieties and a constant charge of 0.03 was attributed to all the aliphatic hydrogen atoms. The charge on the oxygen (−0.5) was the same as in AMBER, but the charge on the nitrogen was slightly reduced from the original value in order to obtain a better relative stability of the fully extended conformation.[23] Within the aromatic moieties all the C-H units were assumed to be electroneutral with charges of 0.12 for the hydrogen atoms and −0.12 for the carbon atoms. Charges of −0.10 and 0.05 were adopted for the C^β atoms and *H*-C^β atoms, respectively, while that for the C^α atom, computed in order to achieve electroneutrality, turned out to be 0.04, close to the AMBER value for glycine. Moreover, a charge of 0.05 was assumed for the C^γ atoms.

Conformational energies are expressed as $\Delta E = E - E_0$, where E_0 is the energy of the most stable conformation. All computations were performed using the efficient package ICER[24] which is able to significantly reduce the computational time by means of a preliminary topological analysis. In fact, only energy differences between successive points were computed, taking into account only interactions between atoms whose relative positions are modified. Furthermore, interactions depending on a single dihedral angle (*e.g.*, torsional terms and 1,4-interactions) were calculated at the beginning of the computation only and stored for subsequent use. Finally, the program has a user friendly interface for the input of the polypeptide sequences and allows for the use of several force fields. The sign definition of the torsion angles is in agreement with the IUPAC-IUB rules.[25]

The conformational space was mapped in intervals of 30° for the φ, ψ, and χ torsion angles with ω angles fixed at 180°. The energy minima were fully optimized in the torsional subspace using the Newton–Raphson method[26] implemented in the ICER package.

^{1}H *NMR Spectra.*—^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer. Measurements were carried out in deuteriochloroform (99.96% ^{2}H; Merck) and in dimethyl sulphoxide (99.96% $^2H_6$; Fluka) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) was purchased from Sigma.

X-Ray Diffraction.—X-Ray diffraction data for compounds (1)–(5) were collected with a Philips PW1100 four-circle diffractometer using graphite-monochromatised Mo-K_α radiation (λ = 0.7107 Å). The θ–2θ scan mode up to 2θ = 56° was used. Intensities were corrected for Lorentz and polarization effects and put on an absolute scale. No absorption corrections were applied. The crystallographic data are summarized in Table 1. Lists of bond lengths, bond angles and torsion angles, the final positional parameters of the non-hydrogen atoms along with equivalent and anisotropic thermal factors have been deposited at the Cambridge Crystallographic Data Centre.*

Results and Discussion

Conformational Energy Computations.—Table 2 gives the conformational energy computation data for the Dbz monopeptide, Ac-Dbz-NHMe (Ac, acetyl; NHMe, methylamino). The most stable conformation (**I**) is the flat, fully-extended (C_5) structure[3,29] ($\varphi_1 = 180.0°$, $\psi_1 = 180.0°$) with the side-chain torsion angles $\chi^{1,1}$, $\chi^{2,1}$, $\chi^{1,2}$, and $\chi^{2,2}$ having values of −40.6°, −73.7°, 40.6°, and 73.7°, respectively (the two side chains of each residue are each the mirror image of the other). Higher energy (ΔE = 4.0–8.3 kcal mol^{-1}) minima occur in the $\alpha/3_{10}$ helical [3] ($\varphi_1 = -60.0°$, $\psi = -33.0°$) and distorted C_7[29] (φ_1 =

* For details, see 'Instructions for Authors (1990),' *J. Chem. Soc., Perkin Trans. 2*, in the January issue.

Table 2. Computed torsion angles/° and energies/kcal mol^{-1} for Ac-Dbz-NHMe.

Conformation	φ_1	ψ_1	ω_0	ω_1	$\chi^{1,1a}$	$\chi^{2,1a}$	$\chi^{1,2a}$	$\chi^{2,2a}$	ΔE
(I)	180.0	180.0	180.0	180.0	−40.6	−73.7	40.6	73.7	0.0
(II)	−60.0	−33.0	−179.0	177.0	146.4	82.3	49.0	87.5	4.0
(III)	179.5	−35.4	−179.9	180.0	−35.5	−73.7	51.7	85.0	5.0
(IV)	−59.4	102.6	−175.6	170.8	63.3	−72.2	17.4	86.7	8.3

[a] On $\chi^{i,j}$ the index i refers to the bond about which the torsion angle is calculated (i = 1 indicates the C_1^{α}–C_1^{β} bond, while i = 2 indicates the C_1^{β}–C_1^{γ} bond). On the other hand, the index j refers to benzylic side chain 1 or 2 bonded to the same C_1^{α}; on the two *ortho* (C^6) carbon atoms in each ring, the one chosen is that which gives an absolute value of <90° for the torsion angle C_1^{α}–C_1^{β}–C_1^{γ}–C_1^{δ}.

Table 3. NH chemical shifts and temperature coefficients[a] of Dbz peptides in $CDCl_3$ solution.

Compound	Concentration/ mmol dm^{-3}	N-terminal NH[b]	$\Delta\delta/\Delta T$[c]	Internal NH[b]	$\Delta\delta/\Delta T$[c]	C-terminal NH[b]	$\Delta\delta/\Delta T$[c]
Tfa-Dbz-L-Phe-OMe	11.1	7.63				6.25	
	1.4	7.62	−2.1			6.19	−0.5
Tfa-Dbz-Gly-DBH	10.4	7.62		6.80		6.87[d]	
	0.9	7.61	−0.3	6.67	−3.2	6.31	−3.2
Z-Gly-Dbz-Gly-OBu^t	10.4	5.25		6.69		6.54	
	1.1	5.24	−3.3	6.69	−1.0	6.53	−2.4

[a] Temperature range 25–55 °C. [b] In ppm (with tetramethylsilane as the internal standard). [c] In 10^3 ppm k^{-1}. [d] Approximate value, due to overlapping of the aromatic signals.

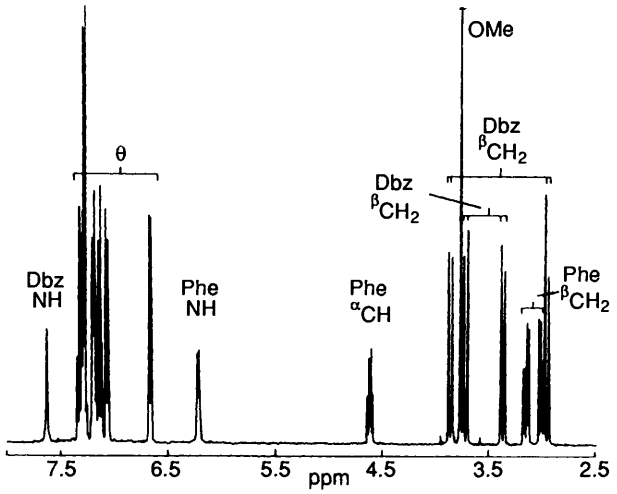

Figure 1. Complete (400 MHz) ^{1}H NMR spectrum of Tfa-Dbz-L-Phe-OMe (**2**) in $CDCl_3$ solution (1.4 × 10^{-3} mol dm^{-3}).

−59.4°, ψ_1 = 102.6°) regions [conformations (**II**) and (**IV**), respectively].

The relative stability of the different conformations is governed by interactions between arylalkyl side chains and the backbone. In particular, the H-bond contribution, operative in the C_7 structure, is not sufficient to overcome the destabilization of this conformation.

It may be concluded that the conformational space explorable by the Dbz residue is strongly reduced with respect to C^{α}-monoalkylated α-amino acids[17,18] and it is similar to that of other $C^{\alpha,\alpha}$-symmetrically disubstituted glycyl residues with bulky acyclic side chains (*e.g.*, Deg[6] and Dpg[8] residues).

Solution Conformation.—The solution conformational preferences of three selected Dbz peptides were examined in a solvent of low polarity ($CDCl_3$) at two concentrations (*ca.* 10 and 1 × 10^{-3} mol dm^{-3}) by using ^{1}H NMR spectroscopy. At 1 × 10^{-3} mol dm^{-3} concentration the effect on NH resonances of heating and addition of dimethyl sulphoxide (DMSO) and TEMPO was also examined in order to delineate NH proton solvent accessibilities. The polar solvent DMSO is expected to interact strongly with the exposed amide NH protons *via* N–H ··· O=S hydrogen bonds, thus inducing a downfield shift in their resonances.[30,31] The paramagnetic free radical TEMPO, on the other hand, is known to perturb the ^{1}H NMR spectra of compounds containing exposed –CONH– groups by broadening the resonances of their NH protons by virtue of nitroxide radical-amide interactions of the N–H ··· O–N= type.[32] As a representative example, Figure 1 shows the complete spectrum of Tfa-Dbz-L-Phe-OMe (**2**). The NH chemical shifts and temperature coefficients[33] of the three peptides are listed in Table 3. The effect of the perturbing agents DMSO and TEMPO on the NH resonances of a representative peptide Z-Gly-Dbz-Gly-OBu^t (**6**), is illustrated in Figure 2. All NH signals are unambiguously identified by virtue of their position (low field, *ca.* 7.6 ppm, for trifluoroacetamido NH;[7,9] high field, *ca* 5.2 ppm, for urethane NH[33]), peak multiplicity, and homonuclear spin-decoupling.

All Dbz NH protons are essentially concentration independent[34] and exhibit remarkably small variations as the temperature is raised and DMSO and TEMPO are added. Conversely, the L-Phe, Gly, and DBH NH protons are much more sensitive to DMSO and TEMPO; some of them are also sensitive, although not dramatically, to changes in concentration and temperature. On the basis of these data and of the position of the Dbz residues in the dipeptide chains (N-terminal) we are inclined to conclude that in $CDCl_3$ solution the incorporation of this $C^{\alpha,\alpha}$-disubstituted glycine might favour the onset of an intramolecularly H-bonded C_5 conformation, if no other effects eventually taking place are responsible for this behaviour.

Other relevant features of the ^{1}H NMR spectra are the following. (*a*) In Tfa-Dbz-L-Phe-OMe (**2**) and Tfa-Dbz-Gly-DBH (**1**) two and four aromatic CH protons, respectively, are significantly upfield shifted if compared to the other aromatic protons. We suggest a ring-current effect[35] between phenyl groups of the benzyl moieties of Dbz, Phe, and DBH residues as a reasonable interpretation of this phenomenon. (*b*) The chemical shifts of the signals for the methylene group of a Gly

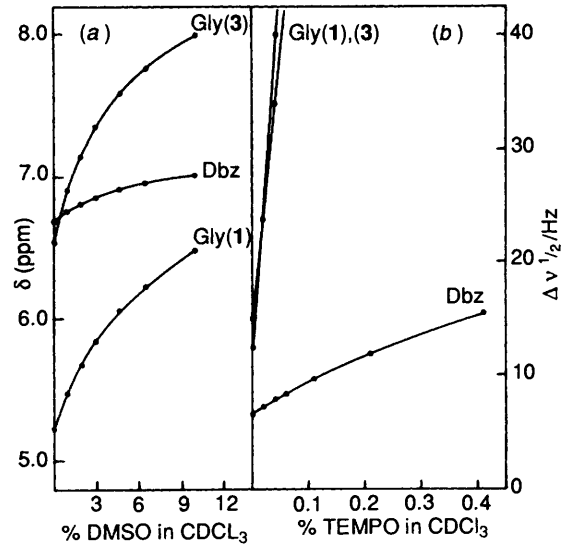

Figure 2. (*a*) Plot of NH chemical shifts in the ^1H NMR spectra of Z-Gly-Dbz-Gly-OBu^t (**6**) *versus* increasing percentages of DMSO in the $CDCl_3$ solution (v/v). (*b*) Plot of the bandwidth of the NH protons of the same peptide *versus* increasing percentages of TEMPO (w/v) in $CDCl_3$ (1.1×10^{-3} mol dm^{-3}).

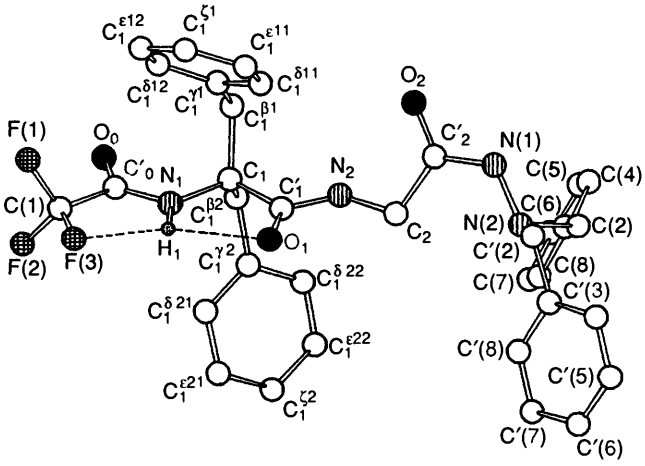

Figure 3. Molecular structure of Tfa-Dbz-Gly-DBH (**1**) with numbering of the atoms. The intramolecular H-bonds are shown as dashed lines.

residue at the N-terminus of a Dbz residue are confirmed to appear at values (3.7–3.8 ppm) *ca.* 0.2 ppm lower than those typically found for Gly-containing compounds (3.9–4.0 ppm).[15]

Crystal-state Conformation.—We have determined by X-ray diffraction the molecular and crystal structures of five terminally blocked Dbz derivatives and small peptides, namely Tfa-Dbz-Gly-DBH (**1**), Tfa-Dbz-L-Phe-OMe (**2**), Tfa-Dbz-L-Phe-OBzl (**3**), *m*-ClAc-Dbz-OH (**4**), and Z-Gly-Dbz-Gly-OH (**5**). The five molecular structures with the atomic numbering schemes are shown in Figures 3–7, respectively. Table 4 lists bond lengths, bond angles, and torsion angles characterizing the conformation of the Dbz residues.

The Dbz residues in the five compounds adopt an almost ideal intramolecularly H-bonded fully-extended C_5-ring structure. In fact the φ_i, ψ_i torsion angles[25] are very close to the expected (180°, 180°) values.[3] The critical intra-ring N_i–C_i^{α}–C_i' (τ) bond angles are remarkably compressed with respect to the tetrahedral value, as expected for a pentagonal form.[3,36] The $O_i \cdots N_i$ intramolecular separations, ranging from 2.651(8)–2.520(5) Å, and the corresponding $O_i \cdots H_i$–N_i separations, ranging from 2.157–1.936(64) Å, are typical for a C_5 conformation.[3,29,37] In the case of the three Tfa-protected dipeptides the C_5 conformation is additionally stabilized by an intramolecular $F(3) \cdots H_1$–N_1 interaction ('C_5' form), the $F(3) \cdots N_1$ distances ranging from 2.669(4)–2.633(6) Å and the $F(3) \cdots H_1$–N_1 distances from 2.287(64)–2.118(59) Å. The presence of the three-centre doubly intramolecular (bifurcated)[38] H-bonded 'C_5', C_5 conformation is corroborated by the values of the F(3)–C(1)–C'_0–N_1 and C(1)–C'_0–N_1–C_1^{α} (ω_0) torsion angles, reasonably close to the ideal 0° (*cis*) and 180° (*trans*) values, respectively.[29]

The $\chi^{1,1}$ (N–C^{α}–$C^{\beta 1}$–$C^{\gamma 1}$) and $\chi^{1,2}$ (N–C^{α}–$C^{\beta 2}$–$C^{\gamma 2}$) torsion angles of the Dbz residue are −70.9(5)° and 48.7(5)° for Tfa-Dbz-Gly-DBH (**1**), −44.5(5)° and 49.0(5)° for Tfa-Dbz-L-Phe-OMe (**2**), −49.7(5)° and 40.4(5)° for Tfa-Dbz-L-Phe-OBzl (**3**), −64.7(6)° and 48.2(6)° for *m*-ClAc-Dbz-OH (**4**), and −63.9(9)° and 52.2(9)° for Z-Gly-Dbz-Gly-OH (**5**).

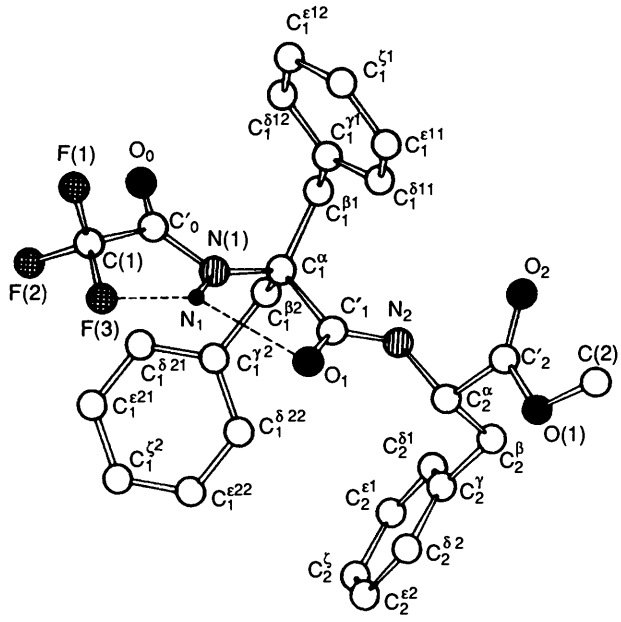

Figure 4. Molecular structure of the Tfa-Dbz-L-Phe-OMe (**2**) with numbering of the atoms. The intramolecular H-bonds are shown as dashed lines.

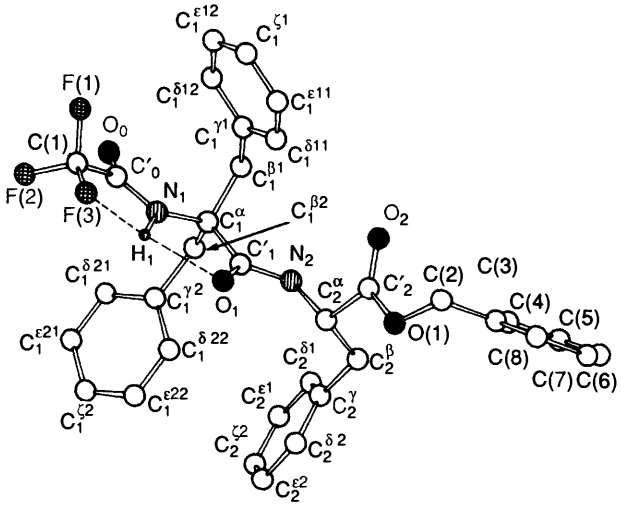

Figure 5. Molecular structure of Tfa-Dbz-L-Phe-OBzl (**3**) with numbering of the atoms. The intramolecular H-bonds are shown as dashed lines.

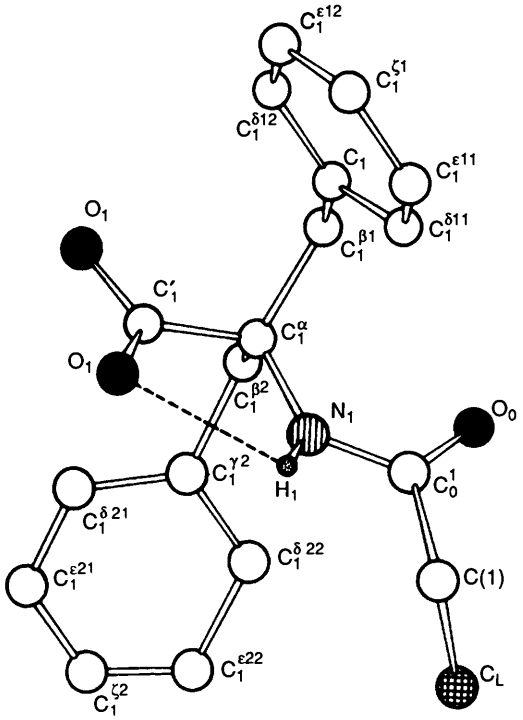

Figure 6. Molecular structure of *m*-ClAc-Dbz-OH (**4**) with numbering of the atoms. The intramolecular H-bond is shown as a dashed line.

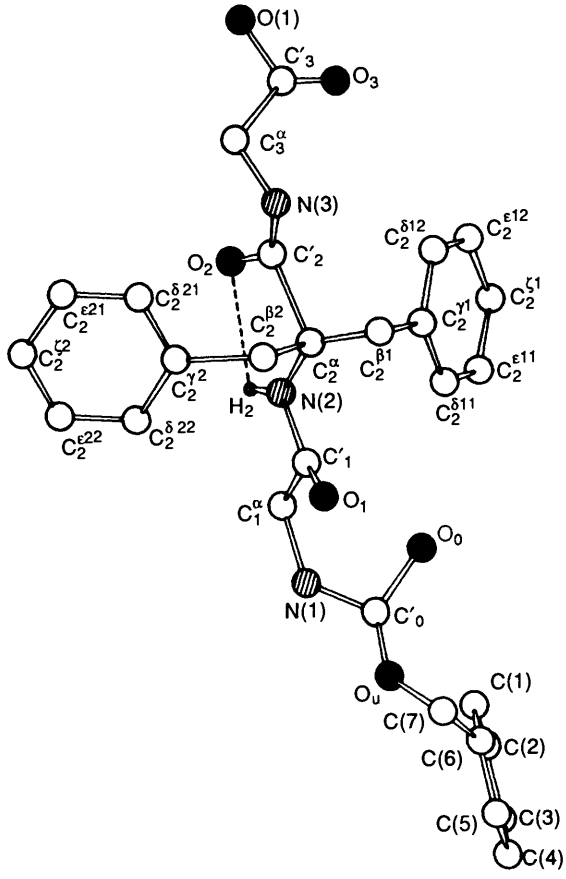

Figure 7. Molecular structure of Z-Gly-Dbz-Gly-OH (**5**) with numbering of the atoms. The intramolecular H-bond is shown as a dashed line.

In Tfa-Dbz-Gly-DBH (**1**) the peptide bond (ω_1) is *trans* [175.3(4)°],[39] while the C-terminal acylhydrazido –CO–NH– bond (ω_2) is *cis*, −1.4(7)°.[8] The Gly residue is partially extended [$\varphi_2 = -121.8(5)°$, $\psi_2 = -165.9(4)°$]. In the DBH moiety the torsion angles about the N(1)–N(2) bond have values of 127.2(5)° and −112.5(5)°, and the two benzyl groups assume the (*t*, *g*$^-$) conformation, the N(1)–N(2)–C′(2)–C′(3) and N(1)–N(2)–C(2)–C(3) torsion angles being 170.4(4)° and −72.7(5)°, respectively.[8] As expected for an acylhydrazido group,[8] the N(1) atom has sp^2 character [the C'_2–N(1)–N(2) bond angle is 122.2(4)°], while the N(2) atom has sp^3 character [the N(1)–N(2)–C(2) and N(1)–N(2)–C′(2) bond angles are 108.5(3)° and 108.3(4)°, respectively.

In Tfa-Dbz-L-Phe-OMe (**2**) the peptide (ω_1) and ester (ω_2)[40] bonds are both *trans* [−179.4(4)° and 172.9(5)°, respectively]. The L-Phe residue is *semi*-extended [$\varphi_2 = -96.0(5)°$, $\psi_T = 162.0(4)°$]. The conformation of the benzyl side chain of the L-Phe residue is *g*$^-$, the χ^1_2 torsion angle being −64.7(6)°.[36,41,42] Also, in the dipeptide benzyl ester analogue (**3**) the peptide (ω_1) and ester (ω_2) bonds are both *trans* [−179.2(4) and 174.0(5)°, respectively]. Again, the L-Phe residue is *semi*-extended [$\varphi_2 = -93.9(5)°$, $\psi_T = 170.8(4)°$] and the benzyl side chain takes the *g*$^-$ conformation [$\chi^1_2 = 61.9(6)°$]. The torsion angle characterizing the conformation of the C-terminal benzyl ester group, C'_2–O(1)–C(2)–C(3), has a value of −106.9(7)°.[40]

In *m*-ClAc-Dbz-OH (**4**) the amide bond (ω_0) is *trans*, −171.1(4)°, but the Cl–C(1)–C'_0–N_1 torsion angle is 116.4(5)°, thus precluding the onset of the 'C_5' form. The C'_1–O(1) and C'_1–O_1 bond lengths are 1.318(5) and 1.198(6) Å, respectively.

In Z-Gly-Dbz-Gly-OH the urethane (ω_0)[43] group and the peptide (ω_1 and ω_2) groups are all *trans*, but the ω_2 torsion angle deviates markedly from planarity [170.5(8)°, −174.3(8)°, and −165.5(7)°, respectively]. The Gly residues are both *semi*-extended [$\varphi_1 = -80.3(11)°$, $\psi_1 = 175.1(8)°$; $\varphi_3 = -96.6(10)°$, $\psi_T = 176.6(8)°$]. The torsion angles, characterizing the conformation of the benzyloxycarbonyl N^{α}-protecting group [θ^1, θ^2, and θ^3, about the C'_0–O_u, O_u–C(7), and C(7)–C(6) bonds, respectively],[43] are −175.3(10)°, −98.4(12)°, and 54.5(14)°, respectively. The C'_3–O(1) and C'_3–O_3 bond lengths are 1.290(10) and 1.205(9) Å, respectively.

Further, although indirect, support for the occurrence of the intramolecularly H-bonded conformation in these five compounds is given by the observation that the pertinent N–H and C=O groups are not involved in the intermolecular H-bonding schemes[6,8] (with one exception, see below). In fact, in Tfa-Dbz-Gly-DBH (**1**) methanol solvate we observe the formation of two intermolecular H-bonds, (acylhydrazido) N(1)···O_2 (acylhydrazido) (1 − *x*, 2 − *y*, 1 − *z*) and O_M···O_0(amide) (−*x*, 2 − *y*, 1 − *z*). The two heteroatomic distances are 2.906(5)[44,45] and 2.800(6) Å,[46,47] respectively. The N_2 atom is not involved in the H-bonding scheme.

The packing mode of both Tfa-Dbz-L-Phe-OMe (**2**) and Tfa-Dbz-L-Phe-OBzl (**3**) molecules is characterized by a (peptide) N–H···O=C (amide) intermolecular H-bond [the N_2···O_0 (−*x*, *y* + 1/2, −1/2 − *Z*) distance in (**2**) is 3.021(5) Å and the N_2···O_0 (−*x*, *y* − 1/2, −3/2 − *z*) distance in (**3**) is 3.041(5) Å] forming rows along the *y*-direction.

The only intermolecular H-bond in the mode of packing of the *m*-ClAc-Dbz-OH (**4**) molecules is seen between the (carboxylic acid) O–H···O=C (amide) groups [the O(1)···O_0 (*x*, *y*, 1 + *z*) distance is 2.577(4) Å], forming rows along the *y*-direction.

A more complex intermolecular H-bonding pattern occurs in the crystals of Z-Gly-Dbz-Gly-OH (**5**). The (peptide) C'_2=O_2 group plays the role of a double acceptor,[38] of the intramolecular H-bond with the (peptide) N_2–H_2 group (see above) and of an intermolecular H-bond with the (carboxylic acid) O–H group [the O(1)···O_2 (1/2 − *x*, 3/2 − *y*, 1 − *z*) separation is 2.661(10) Å]. In addition, rows along the *y*-direction

Table 4. Bond distances/Å, bond angles/° and torsion angles/° characterizing the 'C_5', C_5 and C_5 conformations of Tfa-Dbz-Gly-DBH (**1**) methanol solvate, Tfa-Dbz-L-Phe-OMe (**2**), Tfa-Dbz-L-Phe-OBzl (**3**), *m*-ClAc-Dbz-OH (**4**), and Z-Gly-Dbz-Gly-OH (**5**).

			(1)	(2)	(3)	(4)	(5)
'C_5'	$F(3)$–$C(1)$–C'_0–N_1		0.9(7)	−4.4(8)	−3.9(6)		
	$C(1)$–C'_0–N_1–$C^{\alpha}{}_1$	(ω_0)	−171.3(4)	178.9(5)	−165.6(4)		
	$F(3)\cdots N_1$		2.669(4)	2.633(6)	2.634(5)		
	$F(3)\cdots H_1$–N_1		2.238	2.118(59)	2.287(64)		
C_5	C'_0–N_i–$C^{\alpha}{}_i$–C'_i [a]	(φ_i)	177.9(4)	179.7(4)	175.9(4)	−179.2(5)	−178.9(8)
	N_i–$C^{\alpha}{}_1$–C'_i–N_{i+1}	(ψ_i)	176.4(4)	178.2(4)	180.0(4)	−173.6(4) [b]	179.3(7)
	N_i–$C^{\alpha}{}_i$–C'_i	(τ_i)	104.8(2)	103.5(4)	103.4(3)	104.6(4)	105.6(7)
	$O_i\cdots N_i$		2.587(4)	2.520(5)	2.542(5)	2.610(4)	2.651(8)
	$O_i\cdots H_i$–N_i		2.097	2.072(57)	1.936(64)	2.130	2.157

[a] The *i* residue refers to Dbz. [b] N_i–$C^{\alpha}{}_i$–C'_i–O(1).

are formed *via* intermolecular H-bonds between (peptide) N–H···O=C (peptide) groups and (urethane) N–H···O=C (carboxylic acid) group. The $N_3\cdots O_1$ (1/2 − *x*, 1/2 + *y*, 3/2 − *z*) and $N_1\cdots O_3$ (*x*, 1 − *y*, *z*) distances are 2.856(11) and 3.061(10) Å, respectively.

Conclusions

The results of the present analysis are in favour of the thesis that the Dbz residue, with symmetrically disubstituted, bulky side chains, preferentially adopts in the crystal state the intramolecularly H-bonded C_5-ring structure, in the (φ = 180°, ψ = 180°) region of the conformational space; with regard to the solution-preferred conformation the same conclusion might be assumed, although in this case the evidence is less convincing than that obtained in the crystal state. Therefore, the incorporation of a Dbz residue into a bioactive linear peptide might result in a significant stabilization of the fully-extended conformation. However, no detailed conformational analysis has been performed on the Dbz-containing analogues of the bioactive peptides synthesized so far.[10,12,14]

In previous studies from our laboratories it has been shown that the most populated conformation for the Deg[5–7] and Dpg[5,8,9] residues, also characterized by symmetrically disubstituted, bulky side chains, is the C_5-conformation. On the other hand, Aib, the prototype of this family of $C^{\alpha,\alpha}$-disubstituted achiral residues, strongly prefers $3_{10}/\alpha$-helical structures.[3] Therefore, from this study it is confirmed that in the crystal state and possibly also in solution the fully-extended conformation becomes more stable than the helical structures when both side-chains C^{β} atoms are symmetrically substituted but not interconnected in a cyclic system.

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