Flat Peptides

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Abstract: We have synthesized by solution methods the first homopeptide series, *p*BrBz-$(\Delta Ala)_n$-OMe (n = 1−6), based on a $C^{\alpha,\beta}$-didehydro-α-amino acid, to determine the preferred conformation of this residue, characterized by an sp^2 α-carbon atom and the smallest side chain. To this aim, we have exploited FTIR absorption and ^1H NMR techniques in solution and X-ray diffraction in the crystal state. Our investigation shows that a multiple, consecutive, fully extended conformation (2.0_5-helix) largely predominates for all oligomers in deuteriochloroform solution and occurs in the crystal state for the monomer, dimer, and trimer as well. These peptide molecules are completely flat, including the amino acid side chains, and form planar sheets. This novel peptide structure is stabilized by two types of intramolecular H-bonds, $N_i-H\cdots O_i{=}C'_i$ (typical of the 2.0_5-helix) and $C^{\beta}_{i+1}-H\cdots O_i{=}C'_i$ (characteristic of ΔAla peptides). The results obtained are compared with those of the oligopeptides based on the related C^{β}-substituted, $C^{\alpha,\beta}$-didehydro-α-amino acid residues.

Introduction

Identification of peptide backbones with new, well-defined, regular secondary structural elements (helices, sheets, and turns) is of outmost importance in the design of predetermined, simple structural and functional motifs with potential applications in biochemistry and materials science.[1]

Among noncoded α-amino acids, the class of $C^{\alpha,\beta}$-didehydro-α-amino acids (ΔAAs) is of particular interest. Both electronic and steric factors play important roles in directing the conformational properties of didehydropeptides.[2] More specifically, a variety of recent studies has unambiguously recognized the strong tendency of the conformationally constrained, C^{β}-substituted, γ-branched residues of this class Δ^ZPhe and Δ^Z-Leu to stabilize β-turns[3] in short model compounds and to nucleate the 3_{10}-helical structure[4] in longer peptides.[2,5] On the other hand, only few and nonsystematic crystal-state[2,6] and solution[2,7] *experimental* investigations have been devoted to the conformational preference of the simplest residue of this class, ΔAla. In addition, they have been restricted to small compounds such as *linear* derivatives ("monopeptides") and dipeptides, the latter, however, containing only a *single* ΔAla residue [the only exception is represented by the X-ray diffraction analysis of the *cyclic* homodipeptide $c(\Delta Ala)_2$,[6a] the conformation of which, however, is strongly forced to be folded by the constraints imposed by the small ring size]. All of these studies provide evidence that the fully extended (C_5) conformation[3b,8] is preferred by a single ΔAla residue. A number of theoretical analyses confirmed this conclusion for short (n < 6) ΔAla homooligomers.[2,9] However, recent conformational energy calculations predicted that the 3_{10}-helix is the most stable structure in longer homooligopeptides of this family.[9a−c]

In an attempt to contribute to solving this issue, we have synthesized a terminally blocked, complete, monodispersed ΔAla homooligomeric series, namely *p*BrBz-$(\Delta Ala)_n$-OMe (where *p*BrBz is *p*-bromobenzoyl and OMe is methoxy), from monomer to hexamer (n = 1−6) (Figure 1). We have carried out a conformational analysis in solution using FTIR absorption and ^1H NMR on the complete series and a X-ray diffraction

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Table 1. Physical and Analytical Properties of the A_2pr(Z) and ΔAla Homopeptides

compd	yield (%)	melting point (°C)	recryst solvent [b]	$[\alpha]^{25}_D$ (deg)[c]	FAB-MS (*m*/*z*) [e]
Boc-[A_2pr(Z)]$_2$-OMe	83	119−122	EtOAc/hexane	−0.6	573[f]/573.3[g]
Boc-[A_2pr(Z)]$_3$-OMe	84	153−155	$CHCl_3$/hexane	−18.0	793/793.3
Boc-[A_2pr(Z)]$_4$-OMe	86	193−196	$CHCl_3$/hexane	−23.1[d]	1013/1013.4
Boc-[A_2pr(Z)]$_5$-OMe	99	218−220[a]	DMF/H_2O	−25.8[d]	1234/1233.5
Boc-[A_2pr(Z)]$_6$-OMe	99	236−239[a]	DMF/H_2O	−20.6[d]	1454/1453.6
*p*BrBz-A_2pr(Z)-OMe	92	113−116	EtOAc/Et_2O/hexane	+13.4	435/435.1
*p*BrBz-[A_2pr(Z)]$_2$-OMe	81	195−197	DMF/H_2O	−24.3[d]	655/655.1
*p*BrBz-[A_2pr(Z)]$_3$-OMe	93	213−215	DMF/H_2O	−34.9[d]	875/875.1
*p*BrBz-[A_2pr(Z)]$_4$-OMe	88	234−242[a]	DMF/H_2O	−38.2[d]	1095/1095.2
*p*BrBz-[A_2pr(Z)]$_5$-OMe	97	250−259[a]	DMF/H_2O	−38.1[d]	1315/1315.3
*p*BrBz-[A_2pr(Z)]$_6$-OMe	90	255−264[a]	DMF/H_2O	−35.6[d]	1535/1535.4
*p*BrBz-ΔAla-OMe	74	94−96	EtOAc/hexane	−	284/284.2
*p*BrBz-(ΔAla)$_2$-OMe	65	119−122	EtOAc/hexane	−	353/353.2
*p*BrBz-(ΔAla)$_3$-OMe	62	>210[a]	$CHCl_3$/hexane	−	422/422.2
*p*BrBz-(ΔAla)$_4$-OMe	58	>210[a]	$CHCl_3$/hexane	−	491/491.1
*p*BrBz-(ΔAla)$_5$-OMe	30	>210[a]	$CHCl_3$/hexane	−	560/560.1
*p*BrBz-(ΔAla)$_6$-OMe	22	>210[a]	DMF/H_2O	−	629/629.1

[a] With decomposition. [b] EtOAc, ethyl acetate; DMF, *N*,*N*′-dimethylformamide; Et_2O, diethyl ether. [c] *c* 1.0, $CHCl_3$. [d] *c* 1.0, DMF. [e] $(M + H)^+$. [f] Found. [g] Calculated.

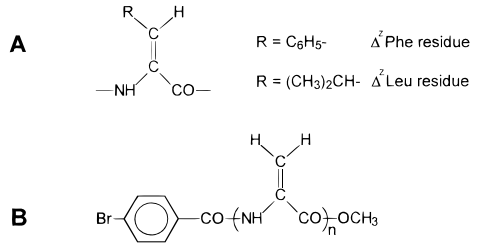

Figure 1. (A) Δ^ZPhe and Δ^ZLeu residues. (B) *p*BrBz-(ΔAla)$_n$-OMe ($n = 1-6$) homopeptides investigated in this work.

investigation on the crystalline monomer, dimer, and trimer. The data obtained strongly support the view that the fully extended, 2.0_5-helix is the conformation preferred by the ΔAla homopeptides to the hexamer level. A preliminary account of the results of this work has been reported.[10]

It is also worth mentioning that these findings may have additional implications as ΔAA residues are frequently found in naturally occurring peptides of microbial and fungal metabolite origins,[11,12] and they are also constituents of a few proteins. In particular, it has been shown that ΔAla is posttranslationally formed from Ser in the active site of the enzyme histidine ammonia-lyase,[13] and segments of consecutive 1−4 ΔAla residues are found in a number of thiopeptide antibiotics.[12]

Materials and Methods

Synthesis and Characterization of Peptides. Melting points were determined with a Yanaco model MP-J3 (Kyoto, Japan) apparatus and are uncorrected. Silica gel column chromatography was carried out with Merck (Darmstadt, Germany) silica gel 60 (70−230 mesh). Analytical ^{1}H NMR spectra were recorded on a Jeol model JNM-EX (Tokyo, Japan) 270-MHz or a Bruker model AM 400 (Karlsruhe, Germany) spectrometer. Peptide mass numbers were determined by fast-atom bombardment mass spectrometry (FAB-MS) using a Jeol model JMS-HX 100 spectrometer. Specific optical rotations were measured with a Horiba model SEPA-200 polarimeter (Kyoto, Japan).

The physical and analytical properties of the newly synthesized A_2pr(Z) (A_2pr, α,β-diaminopropionic acid; Z, benzyloxycarbonyl) and ΔAla homopeptides are listed in Table 1. All peptides were also chemically homogeneous by ^{1}H NMR spectrometry. Typical procedures were the following.

***p*BrBz-A_2pr(Z)-OMe.** To a solution of HCl·H-A_2pr(Z)-OMe (1.20 g, 4.15 mmol) in *N*,*N*′-dimethylformamide (DMF) (5 mL) were added *p*BrBz-Cl (0.913 g, 4.15 mmol) and triethylamine (TEA) (1.27 mL, 9.13 mmol) in 10 equal and alternate portions over 100 min at 0 °C. The mixture was stirred for 2 h at 0 °C and then overnight at room temperature. The precipitated TEA·HCl was filtered off, and the filtrate was concentrated in vacuo. The residue was triturated with water, and the resulting crystalline product was collected by filtration, followed by recrystallization from ethyl acetate (EtOAc)−diethyl ether (Et_2O)−hexane.

***p*BrBz-ΔAla-OMe**. To a solution of *p*BrBz-A_2pr(Z)-OMe (0.750 g, 1.72 mmol) in acetic acid (AcOH) (2 mL) was added 25% HBr/AcOH (5 mL) at room temperature. After being stirred for 2 h, the reaction mixture was concentrated in vacuo. The residue was crystallized by trituration with Et_2O, and the *p*BrBz-A_2pr-OMe hydrobromide was used for the subsequent reaction without further purification. To a solution of the above-mentioned hydrobromide (0.640 g, 1.67 mmol) in a methanol (MeOH)/DMF (1:3) mixture (64 mL) were added CH_3I (4.0 mL, 64.2 mmol) and $KHCO_3$ (3.21 g, 32.1 mmol). After the reaction mixture was stirred for 6 h at room temperature, EtOAc (200 mL) was added. The precipitated insoluble material was filtered off, and the filtrate was concentrated in vacuo. EtOAc (100 mL) and water (20 mL) were added to the residue. The EtOAc layer was washed with water (20 mL × 3), 10% aqueous citric acid (20 mL × 3), saturated aqueous $NaHCO_3$ (20 mL × 3), and brine (20 mL × 3). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo below 25 °C. The residue was crystallized by trituration with hexane. The crystalline product was collected by filtration and recrystallized from EtOAc/hexane.

Boc-[A_2pr(Z)]$_2$-OMe. To a stirred solution of Boc-A_2pr(Z)-OSu (Boc, *tert*-butyloxycarbonyl; OSu, *N*-hydroxysuccinimido)[14] (1.50 g, 3.44 mmol) and HCl·H-A_2pr(Z)-OMe (0.994 g, 3.27 mmol) in DMF (15 mL) was added TEA (0.474 mL, 3.44 mmol) at 0 °C. After the solution was stirred for 1 h at 0 °C and overnight at room temperature, the precipitated TEA·HCl was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in $CHCl_3$ (10 mL) and washed with 10% aqueous citric acid (5 mL × 3), brine (5 mL × 3), saturated aqueous $NaHCO_3$ (5 mL × 3), and brine (5 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was crystallized by trituration with hexane. The crystalline product was collected by filtration and recrystallized from EtOAc/hexane.

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Table 2. Crystallographic Data and Diffraction Parameters for the *p*BrBz-(ΔAla)$_n$-OMe ($n = 1-3$) Homopeptides

parameter	monopeptide	dipeptide	tripeptide
empirical formula	$C_{11}H_{10}NO_3Br$	$C_{14}H_{13}N_2O_4Br$	$C_{17}H_{16}N_3O_5Br$
formula weight (amu)	284.1	353.2	422.2
color, habit	colorless, plates	colorless, plates	colorless, plates
crystal system	triclinic	triclinic	monoclinic
space group	$P\bar{1}$	$P\bar{1}$	$P2_1/c$
a (Å)	8.899(1)	9.937(2)	9.885(1)
b (Å)	13.709(2)	11.187(2)	6.779(1)
c (Å)	4.895(1)	6.743(1)	26.339(2)
α (deg)	96.2(1)	102.3(1)	90.0
β (deg)	89.9(1)	94.2(1)	98.3(1)
γ (deg)	107.2(1)	93.7(1)	90.0
V (Å^3)	566.8(2)	727.9(2)	1746.5(3)
Z (molecules/unit cell)	2	2	4
density (calcd) (g/cm^3)	1.665	1.611	1.606
absorption coefficient (mm^{-1})	3.616	2.840	2.388
F(000)	284	356	856
collected reflections	2752	3525	4316
independent reflections	2749 [R(int) = 0.0164]	3513 [R(int) = 0.0188]	4207 [R(int) = 0.0291]
observed reflections [$I \geq 2\sigma(I)$]	1170	1190	2071
solved by	SHELXS 86	SHELXS 86	SHELXS 86
refined by	SHELXL 93	SHELXL 93	SHELXL 93
final R indices [$I \geq 2\sigma(I)$]	$R1 = 0.0562$, $wR2 = 0.1383$	$R1 = 0.0668$, $wR2 = 0.1620$	$R1 = 0.0432$, $wR2 = 0.1069$
final R indices (all data)	$R1 = 0.1596$, $wR2 = 0.1838$	$R1 = 0.2254$, $wR2 = 0.2386$	$R1 = 0.1214$, $wR2 = 0.1390$
temperature (K)	293(2)	293(2)	293(2)
radiation (λ)	Mo Kα (0.71073 Å)	Mo Kα (0.71073 Å)	Mo Kα (0.71073 Å)
scan method	$\theta-2\theta$	$\theta-2\theta$	$\theta-2\theta$
θ range (deg)	2−28	2−28	2−28
index ranges	$-11 \leq h \leq 11$, $-18 \leq k \leq 17$, $0 \leq l \leq 6$	$-13 \leq h \leq 13$, $-14 \leq k \leq 14$, $-1 \leq l \leq 8$	$-13 \leq h \leq 12$, $0 \leq k \leq 8$, $0 \leq l \leq 34$
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2	full-matrix least-squares on F^2
data/restraints/parameters	2749/0/133	3510/0/179	4207/0/223
goodness of fit on F^2	0.940	0.854	0.869
crystallization solvent	acetone	acetone	acetone
crystal size (mm)	0.2 × 0.2 × 0.1	0.4 × 0.4 × 0.2	0.4 × 0.2 × 0.1
$\Delta\rho_{max}$ and $\Delta\rho_{min}$ (e·Å)	0.478/−0.760	0.673/−0.698	0.530/−0.416

HCl·H-[A$_2$pr(Z)]$_2$-OMe. To a solution of Boc-[A$_2$pr(Z)]$_2$-OMe (1.53 g, 2.67 mmol) in EtOAc (25 mL) was added 4 M HCl/EtOAc (20 mL) at room temperature. After being stirred for 1 h, the mixture was concentrated in vacuo, and the residue was crystallized by trituration with Et$_2$O. The resulting ester hydrochloride was used for the subsequent reaction without further purification.

FTIR Absorption. The FTIR absorption spectra were recorded using a Perkin-Elmer model 1720 X (Norwalk, CT) FTIR spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were obtained under the same conditions.Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF_2 windows) were used. Spectrograde deuteriochloroform (99.8% D) was purchased from Fluka (Buchs, Switzerland).

NMR Spectroscopy. The ^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer. Measurements were carried out in deuteriochloroform (99.96% D; Acros, Geel, Belgium) and deuterated dimethyl sulfoxide (99.96% D_6; Stohler, Waltham, MA) with tetramethylsilane as the internal standard. The free radical 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) was purchased from Sigma (St. Louis, MO).

X-ray Diffraction. Data collection was performed using a Philips PW 1100 (Eindhoven, The Netherlands) four-circle diffractometer. Unit cell determination was carried out by least-squares refinement of the setting angles of 25 high-angle reflections accurately centered using Mo Kα radiation. All of the structures were solved by direct methods, using the SHELXS 86[15a] program. Refinement was carried out on F^2, with all non-H atoms anisotropic, by application of the SHELXL 93[15b] program. The H atoms of the three peptides were calculated at idealized positions, and during the refinement they were allowed to ride on their carrying atom, with U_{iso} set equal to 1.2 (or 1.5 for the methyl groups) times the U_{eq} of the attached atoms. Crystal data and diffraction parameters are listed in Table 2.

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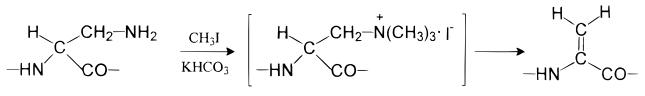
Results and Discussion

Peptide Synthesis. Since ΔAAs are relatively unstable compounds, their incorporation at an early stage in peptide synthesis may limit later reactions.[11] Therefore, the methodology we report here allows conversion to the ΔAAs on completion of peptide synthesis. Saturated α-amino acids such as Ser, Cys, and A$_2$pr are commonly exploited as precursors of ΔAla residues.[11]

In the synthesis of our (ΔAla)$_n$ homopeptides, we took advantage of Shiba's procedure[16a] based on the Hofmann degradation of A$_2$pr as shown below:



The Z-protecting group for the β-amino function of A$_2$pr was removed from the intermediate A$_2$pr(Z) homopeptide with 25% HBr/AcOH[16b] prior to the Hofmann degradation. The resulting free side-chain amino group was then quaternized according to the Chen−Benoiton method ($CH_3I/KHCO_3$).[16c] The intermediate N$^\beta$-trimethyl-A$_2$pr peptides were not isolated during the synthetic procedure.

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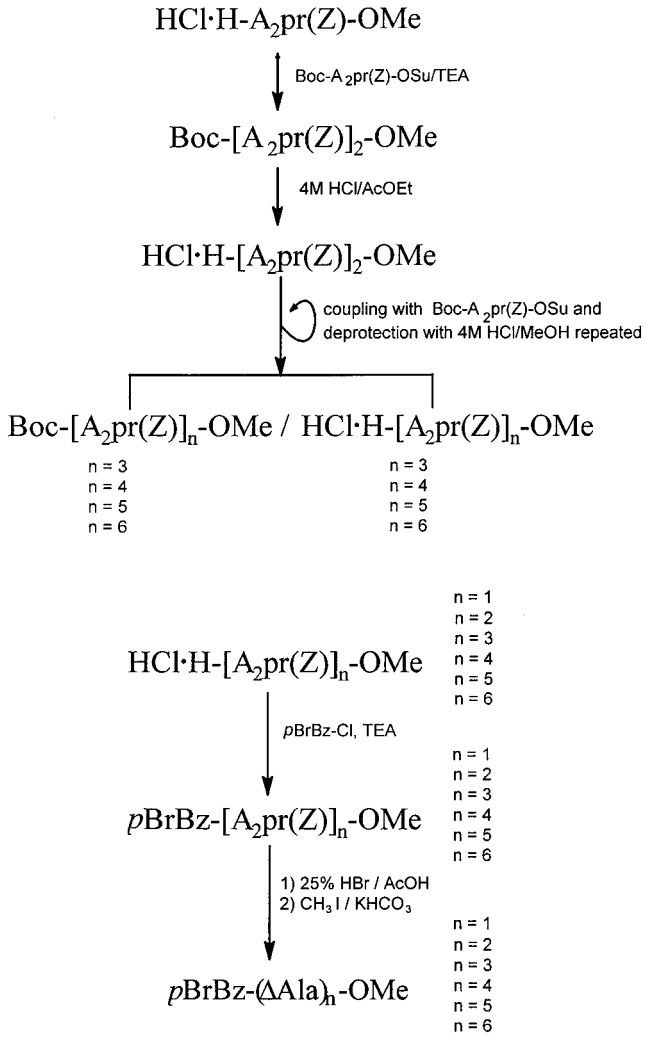
Chart 1



The preparation of the $A_2pr(Z)$ homopeptides was achieved by using the C-activated Boc-$A_2pr(Z)$-OSu derivative.[14] Removal of the N^{α}-protecting Boc group was obtained by mild acidolysis. Blocking of the N^{α}-amino function with the *p*BrBz moiety was achieved with *p*BrBz-Cl in the presence of a base (TEA). The *p*BrBz group is useful in that it contains a heavy atom (Br) which helps crystallographers in solving the phase problem during the X-ray diffraction analysis. The final products were characterized by melting point determination, polarimetry, ^{1}H NMR, and mass spectrometry. The synthetic procedures are schematically outlined in Chart 1. An alternative synthesis of a stretch of four consecutive ΔAla residues by repetition of stepwise elongation of a Ser derivative and subsequent β-elimination has been reported by Shin and co-workers in connection with the synthesis of thiopeptide antibiotics.[16d,e]

Solution Conformational Analysis. The preferred conformation adopted by the terminally blocked $(\Delta Ala)_n$ ($n = 1-6$) homopeptide series was determined in a solvent of low polarity ($CDCl_3$) by FTIR absorption and ^{1}H NMR techniques.

Figure 2 illustrates the FTIR absorption spectra in the conformationally informative N−H stretching region. Using Mizushima's dilution technique,[17a] we have been able to demonstrate that, at 1 mM concentration, intermolecular H-bonding is negligible for all oligomers (not shown). At 1 mM concentration or below, a very weak band is seen at about 3450 cm^{-1} (free, solvated NH groups), followed for all oligomers by a broad and intense absorption centered in the 3405−3380-cm^{-1} region (weakly intramolecularly H-bonded NH groups). This latter spectral region is characteristic of the fully extended (C_5) peptide conformation.[17b,c] As the peptide main-chain length is enhanced, (i) the frequency of the absorption maximum of the 3400-cm^{-1} band tends to decrease slightly and (ii) the intensity of this band, relative to that of the 3450-cm^{-1} band, tends to increase regularly. The effect of heating to 55 °C does not alter the overall pattern (not shown).

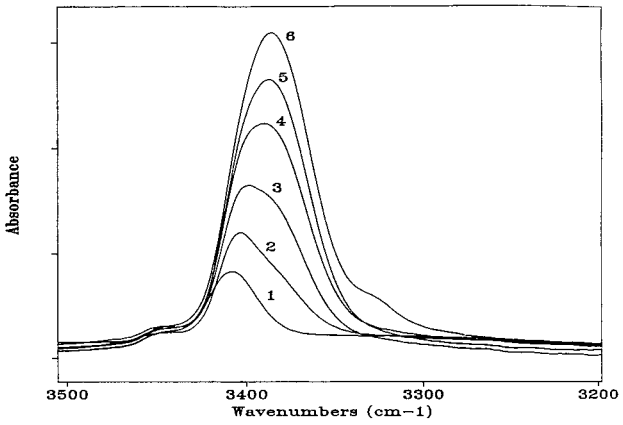


Figure 2. FTIR absorption spectra in the N−H stretching region for the *p*BrBz-$(\Delta Ala)_n$-OMe ($n = 1-6$) homopeptides in $CDCl_3$ solution. Peptide concentration, 1 mM.

Table 3. Chemical Shifts Values for the Amide/Peptide NH Proton Resonances of the *p*BrBz-$(\Delta Ala)_n$-OMe ($n = 1-6$) Homopeptides (1 mM Concentration in $CDCl_3$ Solution)

	NH protons					
	internal residues				N-terminal residue	C-terminal residue
n						
1						8.48
2					8.85	8.58
3				8.96	8.85	8.56
4			8.97	8.95	8.86	8.56
5		8.97	8.95	8.95	8.86	8.56
6	8.97	8.95	8.95	8.95	8.86	8.56

The present FTIR absorption investigation has provided convincing evidence that *intra*molecular H-bonding of the C_5-type is the most important factor influencing the thermally stable conformation of $(\Delta Ala)_n$ homooligomers in $CDCl_3$ solution.

To get more detailed information on the preferred conformation of the $(\Delta Ala)_n$ homooligomers in $CDCl_3$ solution, we carried out a 400-MHz ^{1}H NMR investigation. The delineation of inaccessible (or intramolecularly H-bonded) amide/peptide NH groups was performed by using (i) solvent dependence of NH chemical shifts by adding increasing amounts of the H-bonding acceptor solvent DMSO[18a] to the $CDCl_3$ solution, (ii) free-radical TEMPO-induced line broadening of NH resonances,[18b] and (iii) temperature dependence of NH chemical shifts in $CDCl_3$ solution.[18c]

A complete assignment has been achieved for the proton resonances of the monomer, dimer, and trimer by means of homonuclear decoupling and NOE experiments. A partial

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(18) (a) Kopple, K. D.; Ohnishi, M. *Biochemistry* **1969**, *8*, 4087−4095. (b) Kopple, K. D.; Schamper, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 3644−3646. (c) Bonora, G. M.; Mapelli, C.; Toniolo, C.; Wilkening, R. R.; Stevens, E. S. *Int. J. Biol. Macromol.* **1984**, *6*, 179−188.

Table 4. Chemical Shifts Values for the Vinyl C$^{\beta}$H Proton Resonances of the *p*BrBz-(ΔAla)$_n$-OMe ($n = 1-6$) Homopeptides (1 mM Concentration in $CDCl_3$ Solution)

	H-bonded vinyl CH protons						free vinyl CH protons					
n	N-terminal residue	other residues					C-terminal residue	other residues				
1	6.80						6.01					
2	6.79					6.65	6.03					5.50
3	6.80				6.66	6.65	6.04				5.55	5.51
4	6.80			6.66	6.65	6.65	6.04			5.64	5.56	5.52
5	6.80		6.67	6.66	6.65	6.65	6.04		5.57	5.56	5.56	5.52
6	6.80	6.67	6.67	6.66	6.66	6.65	6.04	5.57	5.57	5.56	5.55	5.53

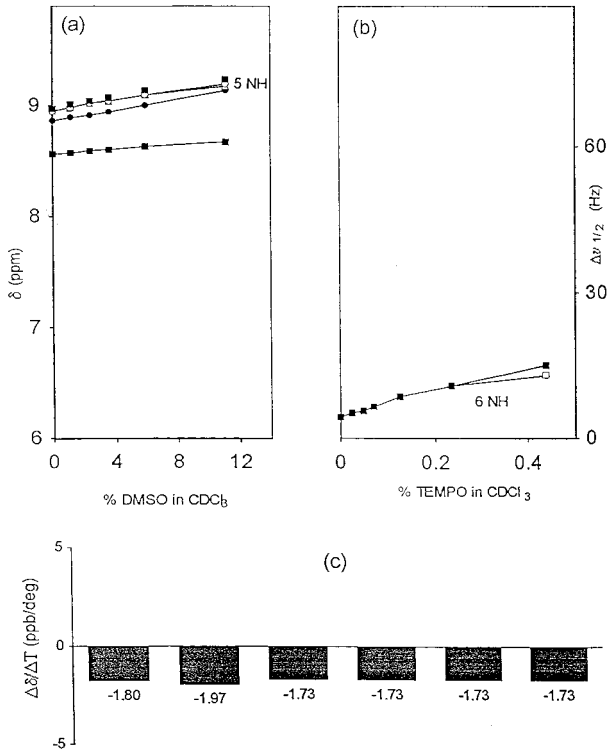

Figure 3. (a) Plot of NH chemical shifts in the ^{1}H NMR spectra of *p*BrBz-(ΔAla)$_6$-OMe as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (b) Plot of the bandwidths of the NH protons of the same homopeptide as a function of increasing percentages of the free radical TEMPO (w/v) in $CDCl_3$ solution. (c) Plot of temperature coefficients for the amide NH protons of the same homopeptide in $CDCl_3$ solution. Peptide concentration, 1 mM.

tentative assignment of the NH and vinyl C$^{\beta}$H proton resonances of the N- and C-terminal ΔAla residues of the longer oligomers has been performed by analogy with the corresponding values for the shorter oligomers. From an analysis of the spectra of the more soluble oligomers (from monomer to tetramer) in the 10−1 mM concentration range (results not shown), we have been able to conclude that dilution does not induce any significant shift of any of the proton resonances.

Tables 3 and 4 list the chemical shifts of the amide/peptide NH and vinyl C$^{\beta}$H protons, respectively, for all oligomers in $CDCl_3$ solution, while Figure 3 shows the effects of the added perturbing agents DMSO and TEMPO and heating on the NH resonances of the hexamer, selected as a typical example. An inspection of the values in Table 3 clearly indicates that *all* of the NH protons are at low fields compared to usual peptide NH protons and are of three different types: (i) the least deshielded peptide NH proton (8.48−8.58 ppm) of the C-terminal residue at highest fields, (ii) the amide NH proton of the N-terminal residue at intermediate fields (8.85−8.86 ppm), and (iii) a clustering of the most deshielded peptide NH protons of *all* of the internal residues in a very narrow range at lowest fields (8.95−8.97 ppm). On the other hand, there are two groups (50% each) of vinyl C$^{\beta}$H protons (Table 4): (i) deshielded resonances (6.65−6.80 ppm), which we assign to intramolecularly H-bonded C$^{\beta}$H protons, and (ii) shielded resonances (5.50−6.04 ppm), which we assign to free C$^{\beta}$H protons. Within the former group, the resonance corresponding to the N-terminal residue is that found at lowest fields, whereas within the latter group, the resonance corresponding to the C-terminal residue is that found at lowest fields.

Furthermore, from the data illustrated in Figure 3, it is evident that *none* of the proton NH chemical shifts is markedly sensitive to the addition of DMSO and heating, nor do their resonances broaden significantly upon addition of TEMPO. This behavior is characteristic of intramolecularly H-bonded NH protons.

Taken together, these ^{1}H NMR results fit nicely with the FTIR absorption data discussed above, in that a multiple, consecutive C_5 conformation seems to largely prevail for *all* of the (ΔAla)$_n$ homooligomers in $CDCl_3$ solution. In this conformation, *all* of the NH groups are *intra*molecularly H-bonded, whereas *inter*-molecular H-bonds do not play a significant role, even at high concentrations. These fully extended structures appear to be further stabilized by *intra*molecular H-bonds involving *one* vinyl C$^{\beta}$H proton from *each* residue as donor.

Crystal-State Conformational Analysis. The molecular structures of the *p*BrBz-(ΔAla)$_n$-OMe ($n = 1-3$) homopeptides with the atomic numbering schemes are illustrated in Figure 4. Average bond distances and bond angles characterizing the ΔAla residue are shown in Figure 5A,B. Relevant backbone torsion angles[19] are presented in Table 5. In Table 6, the inter- and intramolecular H-bond parameters are listed.

Interestingly, in peptides from protein amino acids, where the α-carbon is sp^3 hybridized, the C$^{\alpha}$−N (1.45 Å) and C$^{\alpha}$−C′ (1.52 Å) bond lengths[20] are significantly longer than those in ΔAla peptides, as expected, since in the latter compounds a resonance effect with the C$^{\alpha}$=C$^{\beta}$ bond is operative. Furthermore, the bond angle at the nitrogen is much wider than the corresponding parameter in *normal* peptides,[20] and the conformationally informative τ (N−C$^{\alpha}$−C′) bond angle[20] is greatly narrowed compared to that typical of an sp^2-hybridized atom (120°). This latter finding may be considered a preliminary indication of the onset of the fully extended (C_5) structure[3b,8] for the ΔAla homooligomers in the crystal state.

The three peptides examined form single (monomer) or multiple and consecutive (dimer and trimer) fully extended (C_5) conformations. The resulting helical structure (2.0_5-helix) has been experimentally verified so far only in the terminally blocked (Deg)$_{2-5}$ and (Dpg)$_2$ (Deg, C$^{\alpha,\alpha}$-diethylglycine; Dpg, C$^{\alpha,\alpha}$-di-*n*-propylglycine) homooligomers.[8] Thus, our (ΔAla)$_n$ dimer and trimer are the first 2.0_5-helical peptides not based on

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(20) Benedetti, E. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol. 6; Weinstein, B., Ed.; Dekker: New York, 1983; pp 105−184.

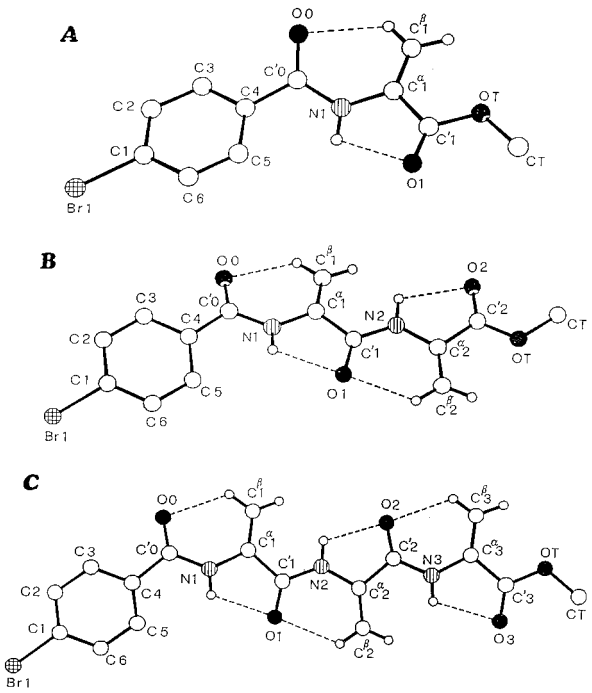

Figure 4. X-ray diffraction structures of (A) *p*BrBz-ΔAla-OMe, (B) *p*BrBz-(ΔAla)$_2$-OMe, and (C) *p*BrBz-(ΔAla)$_3$-OMe with numbering of the atoms. The N−H···O=C′, C′=O···H−C$^\beta$, and C$^\beta$−H···OT intramolecular H-bonds are represented by dashed lines.

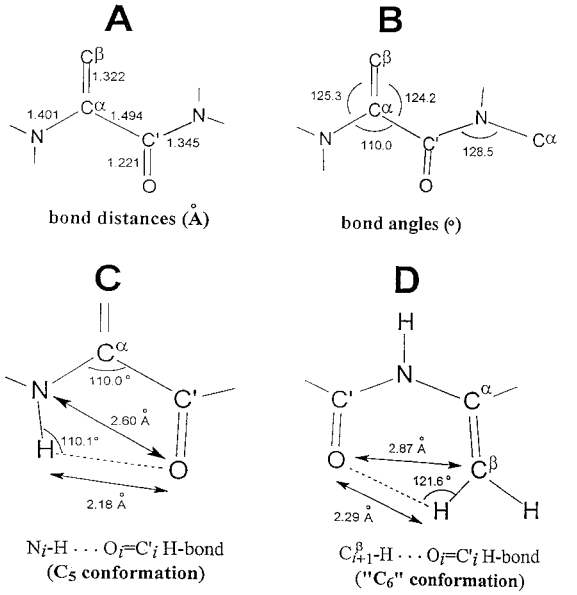

Figure 5. Average bond distances (A) and bond angles (B) for the ΔAla residue and average parameters for the N$_i$-H···O$_i$=C′$_i$ intramolecularly H-bonded C$_5$ conformation (C), further stabilized by intramolecular C$^\beta_{i+1}$−H···O$_i$=C′$_i$ H-bonds (D), of the ΔAla homopeptides.

C$^\alpha$-tetrasubstituted α-amino acids. An additional interesting observation is that the ΔAla homooligomers are *flat* molecules, *including* the amino acid *side* chains. The dihedral angle between adjacent C$_5$ rings is in the range 3.5−8.0°. This property is obviously not shared by the Deg and Dpg homooligomers. The average parameters for the C$_5$ conformation in ΔAla peptides are given in Figure 5C. Interestingly, the τ bond angle is greatly expanded compared to the corresponding bond angle for the C$_5$ conformation of Deg and Dpg homooligomers (102−103°).[8] This difference may be explained, at least in part, on the basis of the different type of hybridization for the α-carbon atom (sp^2 in ΔAla peptides, sp^3 in Deg and Dpg peptides). The parameters characterizing the N$_i$−H···O$_i$=C$_i$′ intramolecular H-bond shown in Figure 5C should be compared to the corresponding ones in Deg and Dpg peptides: N···O and H···O distances 2.54 and 2.00 Å, respectively, and N−H···O angle 110.6°.[8] Overall, it seems that the pentagonal ring of the C$_5$ structure of ΔAla peptides would be somewhat more expanded than the analogous C$_5$ ring structure of Deg and Dpg peptides.

Table 5. Selected Backbone Torsion Angles[a] (deg) for the *p*BrBz-(ΔAla)$_n$-OMe (n = 1−3) Homopeptides

torsion angle	monopeptide	dipeptide	tripeptide
ω_0	−178.0(5)	175.7(6)	170.3(3)
ϕ_1	156.2(5)	179.5(7)	−178.2(3)
ψ_1	173.1(5)[b]	−170.8(6)	−172.3(3)
ω_1	−179.5(5)[c]	−169.7(7)	179.9(3)
ϕ_2		170.3(7)	−178.5(3)
ψ_2		178.5(6)[b]	176.8(3)
ω_2		177.9(6)[c]	172.6(3)
ϕ_3			−175.8(3)
ψ_3			−179.9(3)[b]
ω_3			−178.8(3)[c]

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, ω) are described in ref 19. [b] N−C$^\alpha$−C′−OT torsion angle. [c] C$^\alpha$−C′−OT−CT torsion angle.

The φ,ψ,ω backbone torsion angles are all very close to the trans disposition (180° ± 10°), as expected for a fully extended (C$_5$) conformation.[3b,8] The only significant exception is represented by the φ_1 torsion angle of the monomer, where the C$_5$ structure seems to be slightly distorted. In the *fully extended* structure of the ΔAla dimer and trimer, the C$^\alpha_i$···C$^\alpha_{i+1}$ distance is 3.77 Å, while in the antiparallel pleated-sheet β-structure, 3$_{10}$-helix, and α-helix, this same distance is 3.47, 1.94, and 1.56 Å, respectively.[4]

The structure of these peptides is further stabilized by C$^\beta_{i+1}$−H···O$_i$=C′$_i$ *intra*molecular H-bonds.[21a] The average parameters characterizing this hexagonal (C$_6$) ring structure are given in Figure 5D. An additional H-bond is observed between the C$^\beta$-H2 group of the C-terminal ΔAla residue and the OT oxygen atom of the methyl ester group. These findings closely parallel the ^{1}H NMR observations about the chemical shifts of the C$^\beta$H protons in solution.

The most striking feature in the packing mode of these ΔAla homopeptides is their arrangement in planar, parallel layers, with an approximate interlayer separation of 3.30 Å (for the trimer, see Figure 6A). There are no N−H···O=C′ intermolecular H-bonds, with the single exception of the monomer, in which the slight warping of the molecule allows the N1−H group to approach the C′0=O0 group of a symmetry-related molecule in an adjacent layer, to form a H-bond of normal strength.[21b] To our knowledge, this unusual lack of N−H···O=C′ intermolecular H-bonds is shared only by the 2.0$_5$-helix forming Deg and Dpg homopeptides.[8] A significant contribution to the stability of the planar arrangement of the molecules might be ascribed to the intermolecular (aryl) C−H···O=C′ short contacts listed in Table 6 and illustrated for the tripeptide in Figure 6B. It has also to be mentioned that, in all of the three structures, the distances between the Br atoms of centrosymmetric pairs of molecules (within the same layer) are in the range 3.556(3)−3.681(1) Å, in which dispersion effects between the bromines may be operative.

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Table 6. Intra- and Intermolecular H-Bond Parameters for the *p*BrBz-(ΔAla)$_n$-OMe (n = 1−3) Homopeptides

peptide	donor D−H	acceptor A	symmetry operation	distance (Å) D ···A	distance (Å) H···A	angle (deg) D−H···A
*p*BrBz-ΔAla-OMe	N1−H	O1	x, y, z	2.687(7)	2.38	101
	C$^\beta$1−H1	O0	x, y, z	2.869(7)	2.35	115
	C$^\beta$1−H2	OT	x, y, z	2.730(8)	2.41	100
	N1−H	O0	$x, y, 1 + z$	3.005(6)	2.31	139
	C2−H	O1	$-x, 2 - y, 1 - z$	3.386(6)	2.47	170
	C5−H	O0	$1 - x, 2 - y, -1 - z$	3.386(7)	2.61	141
*p*BrBz-(ΔAla)$_2$-OMe	N1−H	O1	x, y, z	2.599(8)	2.17	110
	N2−H	O2	x, y, z	2.624(8)	2.22	109
	C$^\beta$1−H1	O0	x, y, z	2.855(11)	2.25	122
	C$^\beta$2−H1	O1	x, y, z	2.910(10)	2.32	121
	C$^\beta$2−H2	OT	x, y, z	2.737(10)	2.43	99
	C2−H	O0	$x, y, 1 + z$	3.147(8)	2.50	127
*p*BrBz-(ΔAla)$_3$-OMe	N1−H	O1	x, y, z	2.596(4)	2.16	111
	N2−H	O2	x, y, z	2.583(4)	2.14	111
	N3−H	O3	x, y, z	2.624(4)	2.21	109
	C$^\beta$1−H1	O0	x, y, z	2.850(5)	2.26	121
	C$^\beta$2−H1	O1	x, y, z	2.870(4)	2.27	122
	C$^\beta$3−H1	O2	x, y, z	2.895(5)	2.30	121
	C$^\beta$3−H2	OT	x, y, z	2.757(5)	2.45	99
	C2−H	O0	$x, -1 + y, z$	3.075(3)	2.40	129

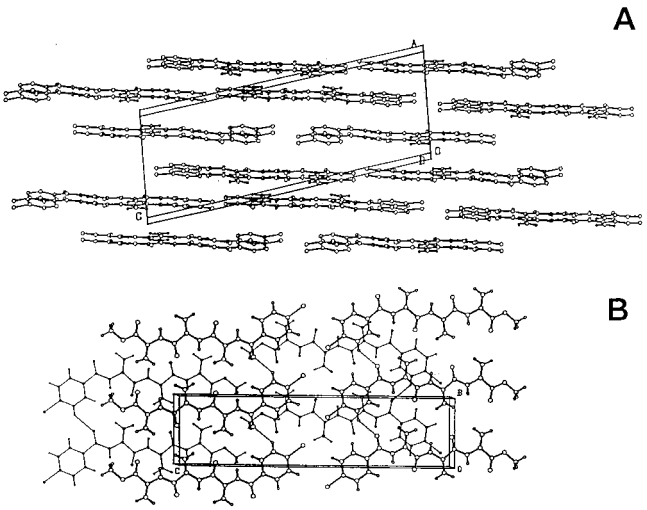

Figure 6. Packing mode of the *p*BrBz-(ΔAla)$_3$-OMe molecules in the crystal as viewed (A) along the *b* direction and (B) along the *a* direction. For clarity, in the latter view, only the molecules belonging to two adjacent layers are shown with different bond thickness. Intermolecular C−H···O═C′ contacts are represented by dashed lines.

Conclusions

The experimental results reported by other groups[2,5−7] and in the present work strongly support the view that γ-branched ΔAAs (Δ^ZPhe and Δ^ZLeu) are very effective β-turn/3$_{10}$-helix formers, whereas ΔAla, with no C$^\beta$-substituents, behaves quite differently, overwhelmingly preferring the fully extended (2.0$_5$-helical) conformation. Steric effects that might cause warping of a C$^\beta$-substituted ΔAA peptide main chain are absent in ΔAla peptides. Thus, these findings emphasize the need for carefully taking into account C$^\beta$-substitution in ΔAAs for a correct peptide design. It still remains to be seen whether longer ΔAla stretches would fold into the 3$_{10}$-helical structure, as suggested by recent theoretical studies.[9a−c]

Exciting, new features of ΔAla fully extended peptides are the following. (i) These molecules are flat, including the amino acid side chains. (ii) The molecules pack in layers, without any significant contribution from *inter*molecular N−H···O═C H-bonds. (iii) The average *intra*molecular $C^\alpha_i \cdots C^\alpha_{i+1}$ distance (3.77 Å) is the largest known separation of this type for any peptide structure experimentally found to date. (iv) This novel peptide structure is stabilized by two types of *intra*molecular H-bonds, $N_i-H\cdots O_i{=}C'_i$, involving *all* of the NH groups of the molecule and typical of the five-membered ring C_5 form, and $C^\beta_{i+1}-H\cdots O_i{=}C'_i$, characteristic of ΔAla peptides and giving rise to a six-membered ring system. In this connection, it is gratifying to note that the structure found in the crystal state corresponds to the most populated conformation in a poorly interacting solvent such as deuteriochloroform.

In summary, in the present conformational study of (ΔAla)$_{1-6}$ homopeptides, we have identified for the first time a completely flat peptide structure. For these striking planar peptide sheets, we foresee a bright future in biochemical and materials science applications.

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Supporting Information Available: Experimental details for the synthesis and characterization of peptides, and tables of crystal data and structure refinement, atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and isotropic displacement parameters, and selected torsion angles for *p*BrBz-ΔAla-OMe, *p*BrBz-(ΔAla)$_2$-OMe, and *p*BrBz-(ΔAla)$_3$-OMe (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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