Structural Versatility of Peptides from $C^{\alpha,\alpha}$-Dialkylated Glycines. An Infrared Absorption and ^1H Nuclear Magnetic Resonance Study of Homopeptides from 1-Aminocyclohexane-1-carboxylic Acid[1]

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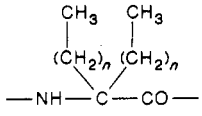
ABSTRACT: The preferred conformation of N- and C-protected homopeptide series of 1-aminocyclohexane-1-carboxylic acid from monomer to pentamer in chloroform solution was determined by using infrared absorption and ^1H nuclear magnetic resonance as a function of concentration, temperature, and addition of perturbing agents. The results obtained are strongly in favor of the onset of an incipient 3_{10} helix at the tripeptide level, as found in the crystal state. A comparison is also made with the conformational propensities of homopeptides of 1-aminocyclopentane-1-carboxylic acid and the $C^{\alpha,\alpha}$-dialkylated glycyl residues with linear side chains.

Introduction

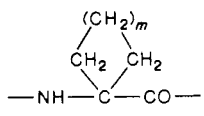
The development of peptides as potential therapeutic agents is a field of intense interest to many organic chemists. A primary pitfall in the use of many synthetic peptides is their rapid degradation in vivo by a number of peptidases. One current approach to avoiding the hydrolysis of the peptide bond is to incorporate in the main chain a structural restriction, e.g., a glycyl residue dialkylated at the α-carbon.[2]

In the preceding paper[1] we have shown by energy calculations that the conformational space available to the $C^{\alpha,\alpha}$-dialkylated, cyclic residue 1-aminocyclohexane-1-carboxylic acid (Acc^6) is sterically restricted and that the minimum energy conformation falls in the $\alpha/3_{10}$ helical region. This theoretical finding was confirmed by a crystal-state experimental investigation on two fully blocked Acc^6 homotetrapeptides, carried out by X-ray diffraction (formation of 3_{10} helices).[1]

We describe here the results of our conformational analysis in a solvent of low polarity (chloroform) of the $Z(Acc^6)_nO$-*t*-Bu (Z, benzyloxycarbonyl; n = 1–5; O-*t*-Bu, *tert*-butoxy) and *p*-BrBz$(Acc^6)_nO$-*t*-Bu (*p*-BrBz, *p*-bromobenzoyl; n = 3,4) homopeptides, as determined by IR absorption and ^1H NMR. Preliminary communications of part of these data have been presented.[3,4] A ^1H NMR study of *t*-Boc$(Acc^6)_3$OMe (*t*-Boc, *tert*-butoxycarbonyl; OMe, methoxy) has recently been published.[5] The results obtained will be compared with those of the homopeptides from the lower homologue Acc^5 (1-aminocyclopentane-1-carboxylic acid) and the $C^{\alpha,\alpha}$-dialkylated glycines with linear side chains Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine),[7–14] Deg ($C^{\alpha,\alpha}$-diethylglycine),[15] and Dpg ($C^{\alpha,\alpha}$-di-*n*-propylglycine).[14]



n = 0, Aib
n = 1, Deg
n = 2, Dpg



m = 2, Acc^5
m = 3, Acc^6

Experimental Section

Synthesis of Peptides. The synthesis and characterization of the intermediates $Z\text{–}Acc^6\text{–}OH$,[16–18] $(Z\text{–}Acc^6)_2O$,[18] $H\text{–}Acc^6\text{–}O$-*t*-Bu,[18] $Z(Acc^6)_2OH$,[18] and the oxazolone from $Z(Acc^6)_2OH$[18] have been described. In addition, the X-ray diffraction structure of the symmetrical anhydride $(Z\text{–}Acc^6)_2O$ has recently been reported.[19] Newly synthesized intermediates are as follows.

$Z\text{–}Acc^6\text{–}O\text{–}Piv$ (Piv, Pivalic). This compound was synthesized from $Z\text{–}Acc^6\text{–}OH$ and pivaloyl chloride in anhydrous benzene at 0–20 °C in the presence of triethylamine: yield 92%; mp 46–47 °C (from benzene–petroleum ether); R_{f_1} 0.95. Anal. Calcd for $C_{20}H_{27}NO_5$: C, 66.5; H, 7.5; N, 3.9. Found: C, 65.8; H, 7.6; N, 3.9. IR absorption (KBr) (ν_{max} 3389, 1812, 1749, 1719, 1521 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 [s, 9 H, $C(CH_3)_3$], 1.32–2.09 (m, 10 H, $Acc^6\text{–}CH_2$), 4.95 (s, 1 H, NH), 5.11 (s, 2 H, benzylic CH_2), 7.36 (s, 5 H, phenyl CH). Interestingly, under strictly comparable experimental conditions, Kenner and co-workers[18] isolated $(Z\text{–}Acc^6)_2O$ instead of $Z\text{–}Acc^6\text{–}O\text{–}Piv$. We were able to show that $Z\text{–}Acc^6\text{–}O\text{–}Piv$ is converted to $(Z\text{–}Acc^6)_2O$ by heating in anhydrous benzene (or toluene).

***p*-BrBz–Acc^6–OH.** This compound was prepared from *p*-BrBzCl and $H\text{–}Acc^6\text{–}OH$ in an aqueous acetone mixture: yield 87%; mp 233–234 °C (from ethyl acetate); R_{f_1} 0.25, R_{f_2} 0.90. Anal. Calcd for $C_{14}H_{16}NO_3Br$: C, 51.6; H, 4.9; N, 4.3; Br, 24.5. Found: C, 51.6; H, 5.0; N, 4.3; Br 24.3. IR absorption (KBr) ν_{max} 3294, 1710, 1632, 1592, 1547 cm^{-1}; ^1H NMR ($CDCl_3$–Me_2SO 2:1) δ 1.30–2.25 (m, 10 H, $Acc^6\text{–}CH_2$), 7.80 (m, 4 H, phenyl CH), 8.00 (s, 1 H, NH).

Oxazolone from *p*-BrBz–Acc^6–OH. This compound was synthesized from *p*-BrBz–Acc^6–OH in acetic anhydride at 120 °C for 20 min: yield 82%; mp 88–89 °C (from toluene–petroleum ether); R_{f_1} 0.95. Anal. Calcd for $C_{14}H_{14}NO_2Br$: C, 54.6; H, 4.6; N, 4.5; Br 25.9. Found: C, 54.5; H, 4.6; N, 4.6; Br, 25.8. IR absorption (KBr) ν_{max} 1813, 1651, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48–1.90 (m 10 H, $Acc^6\text{–}CH_2$) 7.76 (m, 4 H, phenyl CH). The X-ray diffraction structure of this compound has recently been solved.[19]

$Z(Acc^6)O$-*t*-Bu was prepared from $Z\text{–}Acc^6\text{–}OH$[16–18] and isobutene in the presence of a catalytic amount of concentrated H_2SO_4 in anhydrous methylene chloride. $Z(Acc^6)_2O$-*t*-Bu was synthesized from the mixed anhydride $Z\text{–}Acc^6\text{–}O\text{–}Piv$ and $H\text{–}Acc^6\text{–}O$-*t*-Bu[18] in anhydrous benzene at 0–20 °C. The other members of the homopeptide series $Z(Acc^6)_nO$-*t*-Bu (n = 3–5) were prepared from the oxazolone of $Z(Acc^6)_2OH$[18] and the appropriate $H(Acc^6)_{n-2}O$-*t*-Bu in anhydrous acetonitrile under reflux. $H\text{-}(Acc^6)_nO$-*t*-Bu (n = 2, 3) were prepared by catalytic hydrogenation

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Table I
Summary of Analytical Data and Physical Properties of the Acc^6 Homooligopeptides

compound	C, % calcd	C, % found	H, % calcd	H, % found	N, % calcd	N, % found	Br, % calcd	Br, % found	mp,[b] °C	recryst solvt[c]
$Z(Acc^6)_1O$-*t*-Bu[a]	68.4	68.2	8.2	8.3	4.2	4.1			59–060	AcOEt/EP
$Z(Acc^6)_2O$-*t*-Bu	68.1	67.6	8.4	8.5	6.1	6.0			150–151	AcOEt/EP
$Z(Acc^6)_3O$-*t*-Bu[a]	67.9	67.8	8.5	8.5	7.2	7.2			165–166	AcOEt/EP
$Z(Acc^6)_4O$-*t*-Bu	67.8	67.1	8.5	8.6	7.9	7.8			200–201	AcOEt
$Z(Acc^6)_5O$-*t*-Bu	67.7	67.2	8.6	8.7	8.4	8.3			221–222	AcOEt
p-BrBz$(Acc^6)_3O$-*t*-Bu	60.8	60.7	7.3	7.4	6.6	6.6	12.6	12.5	199–200	AcOEt
p-BrBz$(Acc^6)_4O$-*t*-Bu	61.8	61.8	7.6	7.6	7.4	7.5	10.5	10.5	224–225	AcOEt

[a] Reference 18. [b] Determined on a Leitz Model Laborlux 12 apparatus. [c] AcOEt, ethyl acetate; EP, petroleum ether.

of the corresponding Z-protected derivatives. *p*-BrBz$(Acc^6)_nO$-*t*-Bu (n = 3, 4) were synthesized from the oxazolone of *p*-BrBz-(Acc^6)OH and the appropriate $H(Acc^6)_{n-1}O$-*t*-Bu in anhydrous acetonitrile under reflux. A summary of the analytical data and physical properties of $Z(Acc^6)_nO$-*t*-Bu (n = 1–5) and *p*-BrBz-$(Acc^6)_nO$-*t*-Bu (n = 3, 4) is given in Table I.

Infrared Absorption. Infrared absorption spectra were recorded with a Perkin-Elmer Model 580 B spectrophotometer equipped with a Perkin-Elmer Model 3600 IR data station. The band positions are accurate to ±1 cm^{-1}. Cells with lengths of 0.1, 1.0, and 10 mm (with CaF_2 windows) were used. Spectrograde deuteriochloroform (99.8% *d*) was purchased from Merck.

^1H Nuclear Magnetic Resonance. The ^1H nuclear magnetic resonance spectra were recorded with a Bruker Model WP 200 SY spectrometer. Measurements were carried out in deuteriochloroform (99.96% *d*; Merck) and dimethyl-d_6 sulfoxide (99.96% d_6; Stohler) with tetramethylsilane as the internal standard.

The free radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) was purchased from Sigma.

Results and Discussion

The preferred conformations of the terminally blocked homopeptides $Z(Acc^6)_nO$-*t*-Bu (n = 1–5) and *p*-BrBz-$(Acc^6)_nO$-*t*-Bu (n = 3, 4) were investigated in a solvent of low polarity ($CDCl_3$) at various concentrations (in the range 10–0.1 mM) and temperatures by using IR absorption in the amide A and amide I regions and ^1H NMR.

The IR absorption spectra in the N–H stretching region (amide A) of the $Z(Acc^6)_nO$-*t*-Bu (n = 1–5) series (concentration 1 mM) is illustrated in Figure 1. The spectra of the higher oligomers (n = 3–5) are characterized by two bands near 3435 (free N–H groups[7–15,20]) and 3370 cm^{-1} (H-bonded N–H groups[7–15,20]). The relative intensity of the low-frequency band (A_H/A_F) ratio increases rapidly with increasing main-chain length; concomitantly, the absorption maximum shifts to lower wavenumbers (to 3360 cm^{-1} in the pentamer).

Using the Mizushima's dilution method,[20] we have been able to show that at 1 mM concentration self-association via intermolecular H bonding is negligible for all oligomers, but this phenomenon does occur, although to a low extent (≈10%), at 10 mM concentration for the pentamer. As a consequence, the H bonding observed at 1 mM concentration (Figure 1) should be interpreted as arising from intramolecular N–H···O=C interactions only. The behavior of the two *p*-BrBz-blocked peptides parallels that of the corresponding Z-protected homologues in terms of number and positions of absorption bands, but the A_H/A_F ratios are significantly higher (≈30%).

In the less informative C=O stretching region (amide I), in addition to an intense band at approximately 1725 cm^{-1}, due to overlapping of the urethane for *p*-bromobenzamide and *tert*-butyl ester carbonyl vibrators,[8,9,12,14,15] a broad band centered at 1680–1670 cm^{-1} (free and weakly H-bonded peptide carbonyl vibrators[8,9,12,14,15]) is seen for the higher oligomers (n = 3–5).

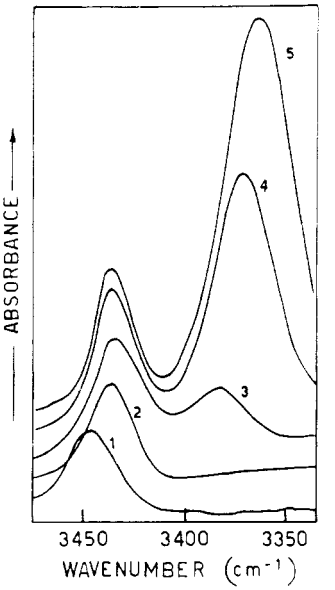

Figure 1. IR absorption spectra in the N–H stretching region of the $Z(Acc^6)_nO$-*t*-Bu (n = 1–5) homopeptides in $CDCl_3$ solution (concentration 1 mM).

The present IR absorption investigation has shown that intramolecular H bonding, the extent of which increases with increasing number of residues in the peptide chain, is the predominant factor for Acc^6 homopeptides in $CDCl_3$ solution. The tendency of these peptides to self-associate appears to be of marginal significance. However, on the basis of the IR absorption data only, it is not safe to discriminate unequivocally among the types of intramolecularly H-bonded species that are formed, although the observation of the 3370-cm^{-1} band in the trimer (which is absent in the dimer) would point to the onset of an intramolecularly H-bonded form of the C_{10} (β-bend) type[21–23] at n = 3.

To get additional information on the conformational preferences of the terminally protected Acc^6 homopeptides in chloroform, we carried out a ^1H NMR study. The analysis of inaccessible (or intramolecularly H bonded) NH groups was performed with use of temperature[11,24,25] and solvent (Me_2SO)[26] dependencies of NH chemical shifts and free radical (TEMPO)[27] induced line broadening of NH resonances. Figure 2 shows the spectra of the five Z-protected homopeptides in the NH region (4.5–7.3 ppm), while Figure 3 graphically describes the results for the pentamer, taken as a representative example.

The upfield NH resonance in all five peptides (4.84–5.38 ppm) is unambiguously assigned to the N(1)H (urethane) group.[5,6,9,11,24,25] The C-terminal N(2)H signal of the dimer,

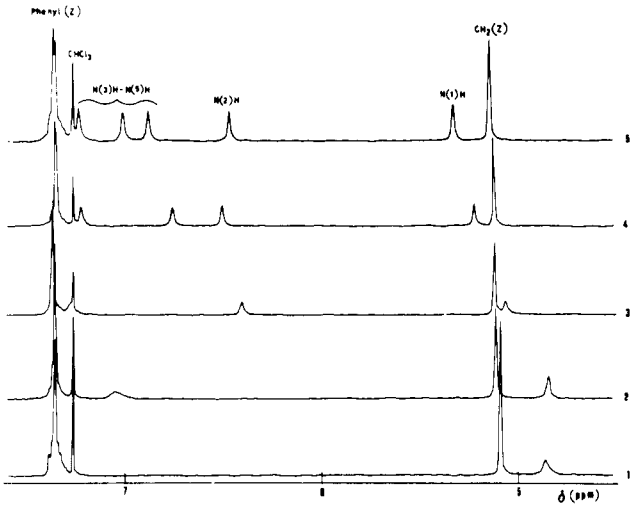

Figure 2. Partial ^{1}H NMR spectra (low-field region) of the Z(Acc^6)$_n$O-*t*-Bu (n = 1–5) homopeptides in $CDCl_3$ solution (concentration 2 mM, temperature 293 K).

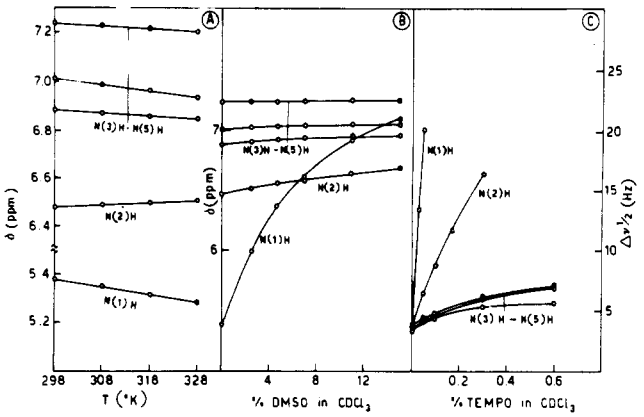

Figure 3. (A) Plot of NH chemical shifts in the ^{1}H NMR spectrum of Z(Acc^6)$_5$O-*t*-Bu versus temperature in $CDCl_3$. (B) Plot of NH chemical shifts of the same peptide versus increasing percentages of Me_2SO to the $CDCl_3$ solution (v/v). (C) Plot of bandwidth of the NH signals of the same peptide versus increasing percentages of TEMPO (w/v) in $CDCl_3$; peptide concentration 5 mM.

assigned by elimination, is found at 7.05 ppm. The two resonances at 6.41 and 7.28 ppm in the trimer have been related to the N(2)H and N(3)H groups, respectively, by analogy with Z(Aib)$_3$O-*t*-Bu[9,11] and *t*-Boc(Acc^6)$_3$OMe[5] (in the latter peptide the two resonances were unequivocally identified by nuclear Overhauser effect experiments). By analogy, we relate the resonance near 6.45 ppm in the tetramer and pentamer to the N(2)H group. Clear-cut assignments of the remaining NH resonances of Z-(Acc^6)$_n$O-*t*-Bu (n = 4, 5) are not possible at present. However, their occurrence in the narrow range of 6.76–7.23 ppm might be considered a first indication of a closely related structural environment for the N(3) and N(4) protons and N(3)–N(5) protons of the tetramer and pentamer, respectively. It is pertinent to mention here that we number the amino acid residues as usual,[5,6,8,9,11,24,25] i.e., from the N terminus of the peptide chain, so that the proton attached to the nitrogen of the N-terminal residue is labeled N(1)H.

An analysis of the spectra as a function of concentration (not shown) indicates that a 10-fold dilution (from 20 to 2 mM) produces a sizable variation (to higher fields) only of the chemical shift of the N(1)H proton of the pentamer.[8,9,11,24,25,28] For the N(2)H–N(5)H protons of the pentamer and all the protons of the lower oligomers, the concentration effect is negligible. In agreement with the IR absorption results discussed above, we conclude that at 20 mM concentration the pentamer is the only Acc^6 oligomer that self-associates and that in this process the urethane N(1)H group plays the role of the H-bonding donor. Interestingly, the self-association motif of the pentamer in concentrated $CDCl_3$ solution can be nicely correlated with the packing mode of the tetramer in the crystal state.[1]

In the Z(Acc^6)$_n$O-*t*-Bu (n = 3–5) oligomers in the absence of self-association two classes of NH protons were observed: (1) The first class [N(1)H and N(2)H protons] includes protons whose chemical shifts are sensitive to heating and addition of the strong H-bonding acceptor solvent Me_2SO[29] and whose resonances significantly broaden upon addition of TEMPO. Interestingly, the sensitivity of the N(1)H proton is always higher than that of the N(2)H proton. (2) The second class (all other NH protons) include those displaying a behavior characteristic of shielded protons (relative insensitivity of chemical shifts to temperature variation and solvent composition and of line widths to the presence of the paramagnetic agent TEMPO).

The present ^{1}H NMR data support the view that at low concentration (<10 mM) in $CDCl_3$ solution the N(3)H proton of the trimer, the N(3)H and N(4)H protons of the tetramer, and the N(3)H–N(5)H protons of the pentamer are inaccessible to solvent and perturbing agents and therefore, most probably, intramolecularly H bonded. The intramolecular H-bonding scheme of the pentamer does not appear to change upon self-association [involving the N(1)H proton as the donor of the intermolecular H bond]. Since all NH protons, beginning from the N(3)H proton, of Z(Acc^6)$_n$O-*t*-Bu (n = 3–5) form stable intramolecular H bonds, we are tentatively inclined to conclude that the structure predominantly adopted in chloroform by these peptides is the 3_{10} helix[30–32] (a series of consecutive, type-III β-bends[21–23]) rather than the α helix, which would require the NH protons involved in the intramolecular H bonding to begin from the N(4)H proton.[22,31,32] These more detailed conclusions are in full agreement with the preliminary indications extracted from the IR absorption study and discussed above.

Conclusions

In this work we have examined the preferred conformation of terminally blocked homopeptides to the pentamer derived from the C$^{\alpha,\alpha}$-dialkylated, cyclic amino acid residue Acc^6 in chloroform solution by using IR absorption and ^{1}H NMR. Due to the absence of the α-CH proton in the Acc^6 residue, in our ^{1}H NMR investigation we concentrated heavily on the analysis of the NH resonances. From the results obtained we are inclined to conclude that the trimer and the higher oligomers tend to form (incipient) 3_{10} helices.[30–32] The ability of an Acc^6 residue to drastically restrict the conformations explorable by the peptide backbone[1,5] is indicated by the similarity between the solution conformations and self-association modes of Z(Acc^6)$_n$O-*t*-Bu (n = 1–5) and *p*-BrBz(Acc^6)$_n$O-*t*-Bu (n = 3, 4) described here and the crystal structures and packing modes of Z(Acc^6)$_4$O-*t*-Bu, *p*-BrBz(Acc^6)$_4$O-*t*-Bu, and *t*-Boc(Acc^6)$_3$OMe already reported.[1,5] Therefore, it is not unexpected that the incorporation of Acc^6 residues into bioactive peptides have resulted in the stabilization of folded conformations.[33–36]

A comparison of the results obtained for Acc^6 (this study

and ref 5) with those already reported for the lower homologue Acc[5] (ref 6) and the $C^{\alpha,\alpha}$-dialkylated glycines with linear side chains Aib,[7-14] Deg,[15] and Dpg[14] allows us to conclude that cyclic residues (Acc[5] and Acc[6]) and the smallest acyclic residue (Aib) favor the formation of folded/helical structures, whereas the acyclic residues with C^{γ} atoms (Deg and Dpg) tend to form fully extended, multiple C_5 conformations.[22] The structural versatility of the family of conformationally restricted $C^{\alpha,\alpha}$-dialkylated glycines is expected to become an important component in the arsenal of synthetic chemists in planning agonists and antagonists of bioactive peptides.

Registry No. Z-Acc[6]-OBu-*t*, 4657-61-8; Z-(Acc[6])$_2$-OBu-*t*, 114532-71-7; Z-(Acc[6])$_3$-OBu-*t*, 4514-67-4; Z-(Acc[6])$_5$-OBu-*t*, 114532-72-8; *p*-BrBz-(Acc[6])$_3$-OBu-*t*, 114532-73-9; *p*-BrBz-(Acc[6])$_4$-OBu-*t*, 114273-56-2; Z-Acc[6]-OH, 17191-43-4; Piv-Cl, 3282-30-2; Z-Acc[6]-O-Piv, 114532-74-0; *p*-BrBzCl, 586-75-4; H-Acc[6]-OH, 2756-85-6; *p*-BrBz-Acc[6]-OH, 114532-75-1; *p*-BrBz-Acc[6]-OH (oxazolone), 114532-76-2; H-Acc[6]-OBu-*t*, 4507-58-8; H-(Acc[6])$_2$-OBu-*t*, 114532-77-3; H-(Acc[6])$_3$-OBu-*t*, 114532-78-4.

References and Notes

(1) This work is part 178 of the series "Linear Oligopeptides". Part 177: Pavone, V.; Barone, V.; Benedetti, E.; Di Blasio, B.; Lelj, F.; Pedone, C.; Santini, A.; Crisma, M.; Bonora, G. M.; Toniolo, C. *Macromolecules*, preceding paper in this issue.
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