again slightly different, in the sense that theoretical studies on Aib derivatives and homopeptides have provided strong support for the preference of this residue for ϕ,ψ values in the 3_{10}- and α-helical regions of the Ramachandran map.[2,3,5-8]

We have also been able to demonstrate that the results of our theoretical analysis of the conformation of the Dpg derivative and homodipeptide are in excellent accord with their conformational preferences in the solid state, determined by X-ray diffraction and also described in this paper; in particular, one intramolecularly H-bonded C_5 conformation is present in the amino acid derivative, while two such forms are found consecutively in the dipeptide. It seems, therefore, that the influence of the N- and C-blocking groups used in this work on the conformational preferences of the Dpg residue is negligible. In particular, the trifluoromethyl group of the Tfa moiety is not rigid, but rather it shows rotational isomerization even in the solid state. In addition, we are tempted to exclude an influence of the DBH blocking group in view of a comparison with the infrared absorption and ^{1}H NMR results on the corresponding *tert*-butyl ester peptides.[62]

The only Iva-containing compound so far investigated by X-ray diffraction, mClAc-D-Iva-OH,[12] also adopts a fully extended C_5 conformation in the solid state, that is, one of the minimum energy conformations calculated in this work for this residue. Clearly, however, the structures of several carefully selected Iva-containing peptides should be solved before we could have in hand a complete picture of the conformations adopted by this residue in the solid state. Suitable candidates for this investigation appear to be peptides long enough (N- and C-blocked tripeptides and longer peptides) to assume a 3_{10}- or an α-helical structure, particularly with sequences corresponding to segments of the Iva-containing peptaibol antibiotics.[4,9-11] This study will also provide an experimental support to the preferred screw sense of the helical structures of Iva-containing peptides suggested by the theoretical analysis described here.

Finally, the conformational properties of a number of Aib derivatives and linear homo- and co-oligopeptides have been determined in the solid state by X-ray diffraction.[4,14-36] As expected from the conformational energy computations,[2,3,5-8] all sets of ϕ,ψ angles (but one) are found in the region of the Ramachandran map that includes both the 3_{10}-helical and α-helices.

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Registry No. Ac-Dpg-NHMe, 93039-01-1; Ac-(Dpg)$_2$-NHMe, 93000-44-3; Ac-D-Iva-NHMe, 93000-45-4; Tfa-Dpg-DBH, 87112-93-4; Ifa-(Dpg)-DBH, 87112-98-9.

Supplementary Material Available: Listings of positional parameters for Tfa-Dpg-DBH and Tfa(Dpg)$_2$DBH (4 pages). Ordering information is given on any current masthead page.

(62) Bonora, G. M.; Toniolo, C.; Di Blasio, B.; Pavone, V.; Pedone, C.; Benedetti, E.; Lingham, I.; Hardy, P. *J. Am. Chem. Soc.*, following paper in this issue.

Folded and Extended Structures of Homooligopeptides from α,α-Dialkylated α-Amino Acids. An Infrared Absorption and ^{1}H Nuclear Magnetic Resonance Study

Gian Maria Bonora,[1a] Claudio Toniolo,*[1a] Benedetto Di Blasio,[1b] Vincenzo Pavone,[1b] Carlo Pedone,[1b] Ettore Benedetti,*[1b] Ian Lingham,[1c] and Paul Hardy*[1c]

Contribution from the Biopolymer Research Centre, C.N.R., Institute of Organic Chemistry, University of Padova, 35100 Padova, Italy, the Dipartimento Chimico, University of Naples, 80134 Naples, Italy, and the Department of Chemistry, University of Exeter, Exeter EX4 4QD, Devon, England. *Received January 3, 1984*

Abstract: The conformational preferences of the N- and C-protected homopeptides from α,α-di-*n*-propylglycine (to the pentapeptide) in the solid state and in chloroform solution have been assessed by using infrared absorption and ^{1}H nuclear magnetic resonance. A comparison is made with the conformations adopted by the corresponding series from α-aminoisobutyric acid, also dialkylated at the α-carbon, and from L-norvaline, in which the single alkyl side chain is the same as those in α,α-di-*n*-propylglycine. The highest L-norvaline homopeptides exhibit a significant tendency for adopting an *intermolecularly* H-bonded β-structure, in contrast to the α,α-di-*n*-propylglycine and α-aminoisobutyric acid peptides where *intramolecular* H-bonding is the dominating factor. The likely absence of a conformational transition with increasing main-chain length and the exceptional structural stability upon heating of *all* the α,α-di-*n*-propylglycine homopeptides represent an additional relevant finding of the present work.

Structural restrictions of bioactive peptides via backbone modifications reduce the difficulties of the determination of the relationships between conformation and activity, an important goal of contemporary biochemistry.

In the preceding paper[2] we have shown by conformational energy computations that the preferred conformational space for the α,α-di-*n*-propylglycine (Dpg) residue occurs in the region of the fully extended structure. This theoretical result has been confirmed by an experimental investigation in the solid state on a Dpg derivative and a fully protected homodipeptide carried out by X-ray diffraction. In contrast, the α,α-dimethylglycine, or α-aminoisobutyric acid (Aib), residue, is known to prefer a conformation close to the region where the 3_{10}- and α-helices are found.[3]

(1) (a) University of Padova. (b) University of Naples. (c) University of Exeter.

(2) Benedetti, E.; Toniolo, C.; Hardy, P. M.; Barone, V.; Bavoso, A.; Di Blasio, B.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C., Bonora, G. M.; Lingham, I. N., preceding paper in this issue.

(3) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. *Biopolymers* **1983**, *22*, 205-215.

0002-7863/84/1506-8152$01.50/0

Table I. Summary of Analytical Data[a] and Physical Properties of the N-Trifluoroacetylated (L-Nva)$_n$ Peptides

compd	C, % calcd	C, % found	H, % calcd	H, % found	N, % calcd	N, % found	mp,[b] °C	$[\alpha]^{20}_D$, deg[f]	R_f[g]
Tfa-L-Nva-OMe	42.3	41.8	5.3	5.2	6.2	6.1	oil[c]	−18.9	0.75
Tfa(L-Nva)$_2$OMe	47.9	48.3	6.5	6.4	8.6	8.5	117–117.5[d]	−51.3	0.70
Tfa(L-Nva)$_3$OMe	50.8	50.2	7.1	7.0	9.9	9.8	188–189[d]	−66.0	0.50
Tfa(L-Nva)$_4$OMe	52.7	52.1	7.5	7.4	10.7	10.6	>265[e]	−80.2	0.35
Tfa(L-Nva)$_5$OMe	53.9	53.2	7.8	7.7	11.2	11.1	>265[e]	−84.3	0.15

[a] The fluorine percentages were not determined. [b] Determined on a Kofler Model RCH apparatus (Reichert, Wien). [c] From ethyl ether–petroleum ether. [d] From ethyl acetate–ethyl ether. [e] From *N*,*N*-dimethylformamide–ethyl ether. [f] The concentration range used was 0.25–0.05 (in 2,2,2-trifluoroethanol). The polarimetric measurements were performed on a Perkin Elmer Model 241 polarimeter equipped with a Haake Model L thermostat. [g] Thin-layer chromatography (silica gel plates 60F–254, Merck) was performed in the chloroform–ethanol 95:5 solvent mixture. The compounds were revealed by the hypochlorite–starch–iodide chromatic reaction. A single spot was observed in each case.

Table II. Summary of Analytical Data[a] and Physical Properties of the N-Trifluoroacetylated (Aib)$_n$ Peptides

compd	C, % calcd	C, % found	H, % calcd	H, % found	N, % calcd	N, % found	Mp,[c] °C	R_f[j]
Tfa-Aib-O-*t*-Bu	47.1	46.5	6.3	6.4	5.5	5.6	oil[d]	0.90
Tfa(Aib)$_2$O-*t*-Bu[b]	49.4	49.0	6.8	6.7	8.2	8.1	113–114[e]	0.85
Tfa(Aib)$_3$O-*t*-Bu	50.8	51.1	7.1	7.0	9.9	9.9	151–152[f]	0.55
Tfa(Aib)$_4$O-*t*-Bu	51.8	51.6	7.3	7.4	11.0	11.1	208–209[g]	0.35
Tfa(Aib)$_5$O-*t*-Bu	52.4	51.8	7.4	7.3	11.7	11.6	>265[g]	0.40
Tfa(Aib)$_2$OH	42.0	42.1	5.3	5.4	9.9	9.8	181–182[h]	0.10
Tfa(Aib)$_2$OH oxazolone	45.1	44.8	4.9	4.8	10.5	10.4	77–78[i]	0.85

[a] The fluorine percentages were not determined. [b] See also ref 9. [c] Determined on a Kofler Model RCH apparatus (Reichert, Wien). [d] From ethyl ether–petroleum ether. [e] From ethyl acetate–petroleum ether. [f] From preheated ethyl acetate–petroleum ether. [g] From acetonitrile. [h] From ethyl acetate. [i] Evaporated from a toluene solution. [j] Thin-layer chromatography was performed as described in Table I. The eluent used was chloroform–ethanol 9:1.

In this work we compare the conformational preferences of the Tfa(Dpg)$_n$O-*t*-Bu (Tfa, trifluoroacetyl; O-*t*-Bu, *tert*-butoxy; n = 2–5) series in the solid state and in a solvent of low polarity (chloroform), as determined by infrared (IR) absorption and ^1H nuclear magnetic resonance (NMR), with the conformations adopted by the Tfa(Aib)$_n$O-*t*-Bu (n = 1–5) series, also α,α-dialkylated, and the α-monoalkylated series Tfa(L-Nva)$_n$OMe (Nva, norvaline; OMe, methoxy; n = 1–5) in which the single alkyl side chain (*n*-propyl) is the same as those in Dpg.

Experimental Section

Synthesis of Peptides. The syntheses and characterization of Tfa-(Dpg)$_n$O-*t*-Bu (n = 2–5) have been described.[4-6]

The Tfa(L-Nva)$_n$OMe (n = 1–5) series was prepared from the corresponding HCl·H(L-Nva)$_n$OMe[7] by treatment with trifluoroacetic anhydride in anhydrous chloroform in the presence of *N*-methylmorpholine. A summary of the analytical data and physical properties of the N-trifluoroacetylated (L-Nva)$_n$ peptides is reported in Table I.

Tfa(Aib)$_n$O-*t*-Bu (n = 2, 4, 5) were synthesized from Tfa-Aib-OH oxazolone[8,9] and the pertinent H(Aib)$_{n-1}$O-*t*-Bu[10] in anhydrous acetonitrile under reflux as described by Jones et al.[10] To avoid the formation of *c*(Aib)$_2$,[11] the synthesis of Tfa(Aib)$_3$O-*t*-Bu was performed as reported above by using Tfa(Aib)$_2$OH oxazolone and H-Aib-O-*t*-Bu.[10] The synthesis of Tfa-Aib-O-*t*-Bu was obtained by treatment of H-Aib-O-*t*-Bu[10] with ethyl thiotrifluoroacetate. The C-deprotected derivative Tfa-(Aib)$_2$OH was prepared from the corresponding *tert*-butyl ester[9] using trifluoroacetic acid.[10] The Tfa(Aib)$_2$OH oxazolone was synthesized from Tfa(Aib)$_2$OH using acetic anhydride as the dehydrating agent under the conditions described by Leplawy et al.[8] and Jones et al.[10] A summary of the analytical data and physical properties of the N-trifluoroacetylated (Aib)$_n$ peptides is reported in Table II.

Table III. Infrared Absorption Frequencies (cm^{-1}) of the NH and C=O Stretching Bands of Three Tfa(X)$_n$OR Series in the Solid State

compd	n	3450–3250	1800–1600
Dpg	2	3385, 3325	1724,[d] 1664[d]
	3	3390, 3330,[d] 3290[d]	1728,[c] 1664, 1652[c]
	4	3392, 3346,[d] 3312	1725,[d] 1678,[d] 1658[c]
	5	3398, 3350[d]	1726, 1678, 1655[c]
Aib	1	3373,[a] 3320	1728,[a] 1713,[d] 1630[a]
	2	3376,[d] 3275	1724,[d] 1714,[a] 1642
	3	3384, 3292, 3248[d]	1732,[d] 1721, 1600, 1631[c]
	4	3408,[e] 3360,[d] 3334	1726, 1710,[d] 1680,[b] 1666,[b] 1658[a]
	5	3360,[d] 3334	1719, 1704,[a] 1684, 1671,[d] 1651[d]
Nva	1	3330	1746,[a] 1727[b,d]
	2	3319, 3287	1737,[d] 1698,[d] 1649[d]
	3	3325,[a] 3273[d]	1735,[d] 1708,[d] 1672,[a] 1636[c]
	4	3335,[a] 3276[d]	1748, 1712,[d] 1677, 1656, 1630[b,c]
	5	3335,[a] 3277[d]	1748, 1711,[d] 1673, 1653, 1628[c]

[a] Shoulder. [b] Broad band. [c] Very strong band. [d] Strong band. [e] Weak band.

Infrared Absorption. Infrared absorption spectra were recorded with a Perkin-Elmer Model 580 spectrophotometer. For the solution measurements, cells with 0.1- and 1.0-mm path lengths and CaF_2 windows were employed at 10^{-2}–5×10^{-4} M concentrations, whereas a 10-cm path-length cell with KBr windows was used at 10^{-4} M concentration. Spectrograde deuteriochloroform (99.8% *d*) was purchased from Merck (Darmstadt, West Germany). For the solid-state measurements the KBr disk technique was used. The band positions are accurate to ±1 cm^{-1}.

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

Results and Discussion

Solid-State Analysis. The solid-state conformational analysis of the homopeptide series from Dpg, Aib, and L-Nva, all three N_α-trifluoroacetylated, has been performed by using IR absorption in the NH (3450–3250 cm^{-1}) and C=O (1800–1600 cm^{-1}) stretching regions (Table (III).

In the L-Nva series, monoalkylated at the α-carbon, two bands are seen in the N–H stretching region: the high-frequency band

(4) Hardy, P. M.; Lingham, I. N. In "Peptides 1980"; Brunfeldt, K., Ed.; Scriptor: Copenhagen, 1981; pp 180–184.
(5) Hardy, P. M.; Lingham, I. N. *Int. J. Pept. Protein Res.* **1983**, *21*, 392–405.
(6) Hardy, P. M.; Lingham, I. N. *Int. J. Pept. Protein Res.* **1983**, *21*, 406–418.
(7) Bonora, G. M.; Maglione, A.; Fontana, A.; Toniolo, C. *Bull. Soc. Chim. Belg.* **1975**, *84*, 299–304.
(8) Leplawy, M. T.; Jones, D. S.; Kenner, G. W.; Sheppard, R. C. *Tetrahedron*, **1960**, *11*, 39–51.
(9) Leibfritz, D.; Haupt, E.; Dubischar, N.; Lachmann, H.; Oekonomopulos, R.; Jung, G. *Tetrahedron* **1982**, *38*, 2165–2181.
(10) Jones, D. S.; Kenner, G. W.; Preston, J.; Sheppard, R. C. *J. Chem. Soc.* **1965**, 6227–6239.
(11) Nagaraj, R.; Balaram, P. *Heterocycles* **1977**, *7*, 885–890.

Table IV. Infrared Absorption Frequencies (cm^{-1}) of the NH and C=O Stretching Bands of Three $Tfa(X)_n$ OR Series in $CDCl_3$ Solution (Concentration 10^{-3} M)

compd	n	3600–3200	1800–1600
Dpg	2	3445,[g] 3392,[f] 3330[f]	1723,[f] 1670
	3	3444,[g] 3394, 3340[f]	1721,[f] 1674,[a] 1660
	4	3445,[g] 3395, 3348[b,f]	1721,[f] 1678, 1655[f]
	5	3445,[g] 3392, 3350[b,f]	1721,[f] 1678, 1654[f]
Aib	1	3438, 3380[f]	1728,[f] 1713[f]
	2	3454, 3398, 3344[f]	1730,[a] 1722,[e], 1660[b]
	3	3454,[g] 3400, 3352[b,f]	1730,[a,g] 1722,[e] 1678,[a] 1658[f]
	4	3440,[a] 3390,[f] 3370,[f] 3346[a]	1722,[f] 1681,[f] 1665
	5[c]	3430,[b] 3364[b,f]	1730,[a] 1722, 1678,[f] 1662[a]
Nva	1	3416	1738,[a] 1724
	2	3428	1728,[b,f] 1682[f]
	3	3420	1734,[a,g] 1726,[f] 1680,[a] 1672[f]
	4[d]	3336, 3274[e]	1752,[g] 1716, 1680, 1668, 1628[e]
	5[d]	3340, 3276[e]	1751,[g] 1716, 1676, 1655, 1627[e]

[a] Shoulder. [b] Broad band. [c] Concentration 5×10^{-4} M. [d] The solution is slightly opalescent. [e] Very strong band. [f] Strong band. [g] Weak band.

(3335–3319 cm^{-1}), the intensity of which decreases with increasing peptide chain, is assigned to N–H groups of irregularly associated peptide species,[12,13] while the low-frequency band (3287–3273 cm^{-1}), the intensity of which is enhanced with increasing peptide chain, is assigned to strongly H-bonded N–H groups of regularly associated peptide structures.[12–15] In the C=O stretching region several absorptions are visible. We assign (i) the absorption at a frequency >1735 cm^{-1} to the carbonyl mode of the –COOMe ester group;[12,13,16] (ii) the absorption at 1727–1698 cm^{-1} to the carbonyl mode of the N_α-blocking trifluoroacetamido group;[17–19] (iii) the absorption(s) at 1667–1628 cm^{-1} to free and H-bonded peptide groups.[12,13]

The intense, low-frequency carbonyl absorption of the tri-, tetra-, and pentapeptides (1636–1628 cm^{-1}) is typical of the β-structure.[20] We are unable to determine unambiguously the type of β-structure formed by the $Tfa(L\text{-}Nva)_nOMe$ (n = 3–5) peptides, since the weak band at about 1700 cm^{-1}, diagnostic of the antiparallel-chain arrangement,[20] is overlapped by the band associated with the trifluoroacetamido moiety. Our results on Tfa-L-Nva-OMe agree well with those reported on other N-trifluoroacetylated amino acids in the solid state.[17,18] The formation of the intermolecularly H-bonded β-structure in the solid state at the tetrapeptide level has already been reported for the t-$Boc(L\text{-}Nva)_nOMe$ (t-Boc, *tert*-butyloxycarbonyl) series.[14]

The IR absorption spectra of the highest homopeptides (tetra- and pentapeptides) from Dpg and Aib are dramatically different from those discussed above for the L-Nva series. In particular, there is no evidence for the formation of a regular β-structure, even if bands of NH and C=O groups, forming H-bonds of weak and moderate strengths, occur. Analogous results have already been reported for $Z(Aib)_nO$-t-Bu (Z, benzyloxycarbonyl; n = 3–5).[21] Unfortunately, a complete theoretical and experimental characterization of the IR absorption bands characteristic of the fully extended multiple-C_5 conformation[22] (probably adopted by Dpg homopeptides[2]) and the incipient 3_{10}-helix (Aib homopeptides[3,21]) has not yet been performed.

Table V. NH Chemical Shifts and Temperature Coefficients[a] of Three $Tfa(X)_nOR$ Series

compd	n	N-terminal[b] NH	$\Delta\delta/\Delta T$[c]	other[b] NH's	$\Delta\delta/\Delta T$[c]
Dpg	2	8.01	1.6	6.88	1.2
	3	8.04	1.5	7.40, 6.85	1.0, 1.1
	4	8.05	1.5	7.42, 7.34, 6.84	0.8, 0.9, 0.9
Aib	1	7.30	3.2		
	2	7.88	3.9	6.66	1.3
	3	7.73	3.7	7.02, 6.70	2.3, 1.3
	4[d]	7.19	−1.2	6.91, 6.80, 6.45	2.9, 0.3, −2.1
	5[e]	7.32	2.2	7.03, 6.83, 6.75	3.0, 3.3, 1.8, 0.8
L-Nva	1	6.83	3.1		
	2	7.11	4.2	6.22	3.9
	3	7.22	7.3	6.47, 6.40	7.2, 6.7
	3′[e]	7.04	4.6	6.26, 6.20	4.2, 3.1

[a] In $CDCl_3$ solution (concentration 5×10^{-3} M). Temperature range = 25–55 °C. [b] In ppm (with Me_4Si as the internal standard). [c] In ppm $\times 10^3$/K. [d] Concentration 10^{-3} M. [e] Concentration 5×10^{-4} M.

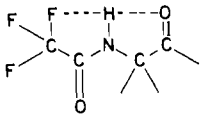
Solution Analysis. The conformational preferences of the $Tfa(X)_nOR$ (X = Dpg, Aib, L-Nva; R = O-t-Bu, OMe) homopeptide series have been examined in a solvent of low polarity (chloroform) at various concentrations (in the range 10^{-2}–10^{-4} M) using IR absorption and ^1H NMR (Tables IV and V).

In the L-Nva series (concentration 10^{-3} M), the IR absorption at 3428–3416 cm^{-1}, present in the lowest oligopeptides (Table IV), is attributed to free (solvated) peptide NH groups[12–14,21] or to amide NH groups with a weak intramolecular H-bond of the C–F···H–N type.[17,23] The opposite effect induced by the intramolecular H-bond, present in specific rotational isomers, and the electronegative fluorine substituents on the frequency of the N–H absorption of trifluoroacetamido derivatives has been discussed.[17,23] In the tetra- and pentapeptides, however, two bands are seen, associated with NH groups involved in H-bonds of moderate strength (3340–3336 cm^{-1})[12–14,21] and with strongly H-bonded NH groups (3276–3274 cm^{-1}).[12–15] In parallel to the onset of the intense 3275 cm^{-1} band, a strong band at 1628–1627 cm^{-1} appears in the C=O stretching region; as discussed above for the solid-state spectra, the two bands at about 3275 and 1630 cm^{-1} are diagnostic of β-structure formation.[12–15,20] Self-association in the $Tfa(L\text{-}Nva)_nOMe$ (n = 3, 4) peptides has been assessed from the onset of a weak band at about 3330 cm^{-1} in the tripeptide at 10^{-2} M concentration, and from the disappearance of the 3274-cm^{-1} band in the tetrapeptide at 10^{-4} M concentration (data not listed in Table IV). An IR absorption study of the t-$Boc(L\text{-}Nva)_nOMe$ series in the same solvent has been reported.[14,24,25]

As in the L-Nva series, the IR absorption properties of the Dpg and Aib series in chloroform (Table IV) reflect the corresponding properties observed in the solid state. In the concentration range examined (5×10^{-3}–3×10^{-4} M) there is clear evidence neither of dissociation of intermolecularly H-bonded species[26] nor of formation of a stable β-structure. In addition, the positions of the maxima in the spectra of all Dpg homopeptides appear to be only slightly sensitive to chain-length elongation. A reasonable, although tentative, explanation for this finding (an unusual observation for a homopeptide series[12,14,24]) is the absence of a dramatic conformational transition with increasing main-chain length (at least to the pentapeptide level, which is, however, in general, a significant level). In any case, the regularly increasing (with increasing main-chain length) absorptions of the Dpg and Aib series at 3370–3330 and 1665–1654 cm^{-1}, respectively, can be associated with H-bonded groups.[12–14,21] On the basis of the

(12) Baron, M. H.; de Lozé, C.; Toniolo, C.; Fasman, G. D. *Biopolymers* **1978**, *17*, 2225–2239.
(13) Baron, M. H.; de Lozé, C.; Toniolo, C.; Fasman, G. D. *Biopolymers* **1979**, *18*, 411–424.
(14) Palumbo, M.; Da Rin, S.; Bonora, G. M.; Toniolo, C. *Makromol. Chem.* **1976**, *177*, 1477–1492.
(15) Toniolo, C.; Palumbo, M. *Biopolymers* **1977**, *16*, 219–224.
(16) Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Int. J. Pept. Protein Res.* **1982**, *20*, 312–319.
(17) Nyquist, R. A. *Spectrochim. Acta* **1963**, *19*, 509–519.
(18) Moussebois, C. H.; Heremans, J. F.; Osinski, P.; Rennerts, W. *J. Org. Chem.* **1976**, *41*, 1340–1343.
(19) Tesch, H. W.; Schulz, R. C. *Makromol. Chem.* **1981**, *182*, 2981–2988.
(20) Miyazawa, T. In "Poly-α-Amino Acids"; Fasman, G. D., Ed.; Dekker: New York, 1967; Vol. 1, pp 69–103.
(21) Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1982**, *104*, 2437–2444.
(22) Toniolo, C. *CRC Crit. Rev. Biochem.* **1980**, *9*, 1–44.
(23) Krueger, P. J.; Smith, D. W. *Can. J. Chem.* **1967**, *45*, 1611–1618.
(24) Toniolo, C.; Bonora, G. M.; Crisma, M.; Bertanzon, F.; Stevens, E. S. *Makromol. Chem.* **1981**, *182*, 3149–3162.
(25) Sugawara, N.; Stevens, E. S.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7044–7047.
(26) Wilkening, R. R.; Stevens, E. S.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1983**, *105*, 2560–2561.

IR absorption data alone, it is not safe to assign specific intramolecularly H-bonded conformations to the Dpg and Aib homopeptides. In the 3400–3380- and 1730–1710-cm^{-1} regions it is possible that an absorption associated with a conformation of the N-terminal trifluoroacetyl–aminoacryl moiety of type **1** would



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also contribute to the observed spectra.[27] As expected, the absorption of the –COO-*t*-Bu ester group (1730–1710 cm^{-1}),[21] overlapping that of form **1**, is found at a frequency lower than that of the –COOMe ester group (1750–1730 cm^{-1}).[12,13,16] The IR absorption data of the Tfa(Aib)$_n$O-*t*-Bu peptides (n = 1–5) in chloroform reported in this work are in substantial agreement with those of *Z*(Aib)$_n$O-*t*-Bu (n = 1–5),[21,26] Ac(Aib)$_n$NHMe (NHMe, methylamino) (n = 1–3),[28,29] Ac(Aib)$_3$OMe,[28] *Z*-(Aib)$_n$OMe (n = 2, 3),[30,31] Tfa-Aib-O-*n*-m (O-*n*-m = *n*-amyloxy),[32] and Ac-Aib-NMe$_2$ (NMe$_2$, dimethylamino),[29] already described.

In order to obtain additional information on the conformational preferences of the homopeptides from Dpg, Aib, and L-Nva, the NH chemical shifts in their ^{1}H NMR spectra have been investigated in deuteriochloroform (concentration 5 × 10^{-3} M) as a function of temperature[33] (Table V). The spectra of (Aib)$_n$O-*t*-Bu (n = 4, 5) have been recorded at a concentration of 10^{-3} and 5 × 10^{-4} M, respectively, for solubility reasons. To confirm the occurrence of self-association in Tfa(L-Nva)$_3$OMe, the ^{1}H NMR spectrum of this tripeptide has been recorded also at 5 × 10^{-4} M concentration. Tfa(L-Nva)$_n$OMe (n = 4, 5) do not give solutions clear enough for a ^{1}H NMR measurement to a concentration as low as 5 × 10^{-4} M. The trifluoroacetamido NH signal of the various peptides is unambiguously identified by virtue of its low-field position.[34] The peptide NH signal of the dipeptides is then assigned by elimination. Unequivocal assignments of the remaining NH signals of tri-, tetra-, and pentapeptides are not possible at present.

The upfield shift (about 0.2 ppm) of all the NH resonances of Tfa(L-Nva)$_3$OMe upon decreasing the concentration from 5 × 10^{-3} M to 5 × 10^{-4} M is evidence of intermolecular H-bond formation,[35] a finding in agreement with the IR absorption results discussed above for this tripeptide. The dominant role of self-association in Tfa(L-Nva)$_3$OMe is also apparent from a comparison of the temperature coefficients of chemical shifts of all its NH resonances at the two concentrations. The marked increase in the temperature coefficients upon 10 times increasing concentration reflects the extensive formation of associated species which melt out as the temperature is raised.[33] A ^{1}H NMR investigation of *t*-Boc(L-Nva)$_n$OMe (n = 1–4) in the same solvent has been reported.[33,35]

The ^{1}H NMR data of the Dpg and Aib homopeptides confirm the IR absorption results discussed above, in the sense that all NH chemical shifts are essentially concentration independent in the 5 × 10^{-3}–3 × 10^{-4} M range (data not shown in Table V). In addition, it is worth noting that in the Dpg series *all* NH chemical shifts exhibit remarkably small variations either as the main-chain length is increased or as the temperature is raised. When the temperature dependence of NH chemical shifts in chloroform is small, then either the NH protons are initially exposed to solvent or they are initially shielded (H-bonded or buried) and remain shielded over the course of temperature variation.[33] On the basis of the results of the preceding paper[2] and the IR absorption data reported in this work, we favor the latter explanation. Finally, an unequivocal interpretation of the NH temperature coefficients of the Aib homopeptides in terms of the type of intramolecularly H-bonded species formed is, in our view, not possible.[28]

Conclusion

In this paper we examined the preferred conformations of the homopeptide series derived from the α,α-dialkylated glycyl residues Dpg and Aib and the α-monoalkylated glycyl residue L-Nva (with the same side chain as those of Dpg), having the general formula Tfa(X)$_n$OR (n = 1–5) in the solid state and in chloroform solution using IR absorption and ^{1}H NMR. Due to the absence of the α-CH proton in the Dpg and Aib residues, in our ^{1}H NMR study we concentrated heavily on the analysis of the NH resonances.

The results obtained point to the following conclusions.

(i) The highest Tfa(L-Nva)$_n$OMe homopeptides (the tetra- and pentapeptides) exhibit a strong tendency for assuming an *intermolecularly* H-bonded β-structure, in agreement with previous results of the Padova group.[14,15,24,36–38] When association is prevented by dilution, the extent of intramolecularly H-bonded species formed is surprisingly low (data not shown in Table IV). This latter finding, in contrast to the previously reported results for the *t*-Boc(L-Nva)$_n$OMe (n = 1–5) peptides,[14,24,35] underlines the marked effect of the N$_\alpha$-blocking trifluoroacetamido group in this series.

(ii) In the homopeptides from the α,α-dialkylated Dpg and Aib residues, however, *intramolecular* H-bonding is the dominating factor. The likely absence of a conformational transition with increasing main-chain length and the exceptional structural stability upon heating of *all* the Dpg homopeptides is another interesting result of this investigation. The restricted mobility of the Dpg side chains is demonstrated by the magnetic nonequivalence of the β-CH$_2$ protons in the ^{1}H NMR spectra of all Dpg homopeptides (data not shown in Table V). This latter result was previously reported for Dpg derivatives.[5] In summary, on the basis of the data in the literature on the Aib homopeptides[3,21,28,31] and those reported in the preceding paper on Dpg homopeptides,[2] it seems reasonable to interpret the present IR absorption and ^{1}H NMR results of the peptide series from these two conformationally restricted residues as arising from incipient 3_{10}-helices (Aib peptides) and multiple-C$_5$ conformations (Dpg peptides), respectively. It is our contention that a more satisfactory picture of the solid state and solution conformational preferences of the homopeptides derived from α,α-dialkylated glycyl residues will be obtained only after the completion of a detailed investigation of the homopeptides derived from the optically active Iva residue.[2]

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Registry No. Tfa-L-Nva-Ome, 74538-84-4; Tfa-(L-Nva)$_2$-OMe, 92844-84-3; Tfa-(L-Nva)$_3$-OMe, 92844-85-4; Tfa-(L-Nva)$_4$-OMe, 92844-86-5; Tfa-(L-Nva)$_5$-Ome, 92900-54-4; Tfa-Aib-O-*t*-Bu, 92900-55-5; Tfa-(Aib)$_2$-O-*t*-Bu, 84758-72-5; Tfa-(Aib)$_3$-O-*t*-Bu, 92844-87-6; Tfa-(Aib)$_4$-O-*t*-Bu, 92844-88-7; Tfa-(Aib)$_5$-O-*t*-Bu, 92844-89-8; Tfa-(Aib)$_2$-OH, 92844-90-1; Tfa-(Aib)$_2$-OH oxazolone, 92900-56-6; Tfa-

(27) Bosch, R.; Brückner, H.; Jung, G.; Winter, W. *Tetrahedron* **1982**, *38*, 3579–3583.
(28) Paterson, Y.; Stimson, E. R.; Evans, D. J.; Leach, S. J.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1982**, *20*, 468–480.
(29) Aubry, A.; Protas, J.; Boussard, G.; Marraud, M.; Néel, J. *Biopolymers* **1978**, *17*, 1693–1711.
(30) Rao, C. P.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *FEBS Lett.* **1979**, *100*, 244–248.
(31) Rao, C. P.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *Biochemistry* **1980**, *19*, 425–431.
(32) Dorlet, C.; Toussaint, A. *Bull. Soc. Chim. Belg.* **1964**, *73*, 127–131.
(33) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050.
(34) Loftus, P.; Bernstein, P. R. *J. Org. Chem.* **1983**, *48*, 40–44.
(35) Pysh, E. S.; Toniolo, C. *J. Am. Chem. Soc.* **1977**, *99*, 6211–6214.
(36) Toniolo, C.; Bonora, G. M.; Fontana, A. *Bull. Soc. Chim. Belg.* **1975**, *84*, 305–312.
(37) Toniolo, C.; Bonora, G. M. In "Peptides: Chemistry, Structure and Biology"; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science: Ann Arbor, MI, 1975; pp 145–150.
(38) Balcerski, J. S.; Pysh, E. S.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1976**, *98*, 3470–3473.

(Dpg)$_2$-O-*t*-Bu, 87113-51-7; Tfa-(Dpg)$_3$-O-*t*-Bu, 92844-91-2; Tfa-(Dpg)$_4$-O-*t*-Bu, 87112-71-8; Tfa-(Dpg)$_5$-O-*t*-Bu, 87112-73-0; H-L-Nva-OMe·HCl, 56558-30-6; H-(L-Nva)$_2$-OMe·HCl, 92844-92-3; H-(L-Nva)$_3$-OMe·HCl, 79780-94-2; H-(L-Nva)$_4$-OMe·HCl, 92844-93-4; H-(L-Nva)$_5$-OMe·HCl, 92900-57-7; Tfa-Aib-OH oxazolone, 705-20-4; H-(Aib)-O-*t*-Bu, 4512-32-7; H-(Aib)$_3$-O-*t*-Bu, 92844-94-5; H-(Aib)$_4$-O-*t*-Bu, 92844-95-6; trifluoroacetic anhydride, 407-25-0; chloroform, 67-66-3; *N*-methylmorpholine, 109-02-4; acetonitrile, 75-05-8; ethyl thiotrifluoroacetate, 383-64-2; trifluoroacetic acid, 76-05-1; acetic anhydride, 108-24-7.

^{18}O Isotope Effect in ^{13}C Nuclear Magnetic Resonance Spectroscopy. 9. Hydrolysis of Benzyl Phosphate by Phosphatase Enzymes and in Acidic Aqueous Solutions[1,2]

Jean E. Parente, John M. Risley, and Robert L. Van Etten*

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907. *Received January 30, 1984*

Abstract: The ^{18}O isotope-induced shifts in ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectroscopy were used to establish the position of bond cleavage in the phosphatase-catalyzed and acid-catalyzed hydrolysis reactions of benzyl phosphate. The application of the ^{18}O-isotope effect in NMR spectroscopy affords a continuous, nondestructive assay method for following the kinetics and position of bond cleavage in the hydrolytic process. The technique provides advantages over most discontinuous methods in which the reaction components must be isolated and converted to volatile derivatives prior to analysis. In the present study, [α-^{13}C,*ester*-^{18}O]benzyl phosphate and [*ester*-^{18}O]benzyl phosphate were synthesized for use in enzymatic and nonenzymatic studies. Hydrolysis reactions catalyzed by the alkaline phosphatase from *E. coli* and by the acid phosphatases isolated from human prostate and human liver were all accompanied by cleavage of the substrate phosphorus–oxygen bond consistent with previously postulated mechanisms involving covalent phosphoenzyme intermediates. An extensive study of the acid-catalyzed hydrolysis of benzyl phosphate at 75 °C revealed that the site of bond cleavage is dependent on pH. At pH $\leq$1.3, the hydrolysis proceeds with C–O bond cleavage; at 1.3 < pH < 2.0, there is a mixture of C–O and P–O bond scission, the latter progressively predominating as the pH is raised; at pH $\geq$ 2.0, the hydrolysis proceeds with exclusive P–O bond scission. (*S*)-(+)-[α-^2H]Benzyl phosphate was also synthesized. Hydrolysis of this chiral benzyl derivative demonstrated that the acid-catalyzed C–O bond scission of benzyl phosphate proceeds by an A-1 (S_N1) mechanism with 70% racemization and 30% inversion at carbon.

The hydrolysis reactions of organic esters of phosphoric acid are of considerable chemical, biological, and technical importance in chemistry and enzymology.[3,4] The hydrolysis of a phosphate monoester (R–O–PO_3H_2) involves the scission of a bond to oxygen: either the P–O bond of the phosphate moiety or the C–O bond to the substituent (R) group. The position of bond cleavage, therefore, can be of key importance in studying the reaction mechanism.

The hydrolysis reactions of (primary) monoalkyl phosphate esters have been studied most extensively under three sets of experimental conditions: as the monoanion, as the neutral species in dilute acid, and in solutions of strong acid where further protonation of the neutral ester may occur. Most esters exhibit a similar reaction pattern under these conditions.[5-7] The pH-rate profile for hydrolysis of the monoanion generally shows a simple kinetic form with a rate maximum at pH 4–5; only P–O bond scission is observed for the monoanion. In solutions of acid to 1 M, the hydrolysis of the neutral species proceeds by C–O bond scission exclusively (at least within the often large limits of experimental error for isotopic analysis). In strong acid solutions ($\geq$1 M), hydrolysis of the conjugate acid of the neutral species can occur with the simultaneous operation of more than one mechanism such that both C–O and P–O bond cleavage patterns are found. Concise mechanistic explanations for many of these experimental observations are still lacking, and additional experimental evidence would be valuable.

One phosphomonoester where such data are of particular interest is benzyl phosphate. The acid-catalyzed hydrolysis of benzyl phosphate at 75 °C was initially studied by Kumamoto and Westheimer.[8] They found that the hydrolysis pH-rate profile differed markedly from that exhibited by simple alkyl monoesters.[5-7] In particular, whereas the rate of hydrolysis of simple alkyl monoesters, such as glycerol or ethyl phosphate,[9] generally displays a maximum at pH 4, the rate of hydrolysis of benzyl phosphate remains constant in the pH range 2–5. Tentative conclusions regarding the hydrolysis reaction of benzyl phosphate have been presented: (1) in strong acid, C–O bond cleavage is "probably" observed,[6] which would result in the formation of the moderately stable carbonium ion; (2) the neutral phosphate ester may (or must)[6,8] cleave at the C–O bond; and (3) the phosphate monoester monoanion was assumed[6] to display P–O bond cleavage by formation of a metaphosphate ion. These conclusions were based on the rate data, on the properties of the benzyl group,[10] and on extrapolations from the hydrolysis of other phosphate monoesters, but direct experimental evidence has not been presented to support or to refute these assertions. Moreover, conclusions about the involvement of metaphosphate ion in the hydrolysis of typical phosphate monoesters have been called into question as the result of a study of the stereochemistry of alcoholysis reactions.[11] A thorough understanding of the benzyl

(1) Part 8: Risley, J. M.; Van Etten, R. L.; Uncuta, C.; Balaban, A. T. *J. Am. Chem. Soc.* **1984**, *106*, 7836-7840.

(2) Taken in part from the M.S. thesis of J. E. Parente, Purdue University, 1982. Preliminary communications of portions of this work have been presented: (a) Parente, J. E.; Risley, J. M.; Van Etten, R. L. *Proc. Indiana Acad. Sci.* **1981**, *91*, 178. (b) Risley, J. M.; Parente, J. E.; Van Etten, R. L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 906. (c) Van Etten, R. L. *Ann. N.Y. Acad. Sci.* **1982**, *390*, 27-51.

(3) Bruice, T. C.; Benkovic, S. J. "Bioorganic Mechanisms"; Benjamin: New York, 1966; Vol. 2, Chapter 5.

(4) Benkovic, S. J.; Schray, K. J. *Enzymes* **1970**, *8*, 201-238.

(5) Bunton, C. A. *Acc. Chem. Res.* **1970**, *3*, 257-265.

(6) Cox, J. R., Jr.; Ramsay, O. B. *Chem. Rev.* **1964**, *64*, 317-352.

(7) Barnard, P. W. C.; Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Silver, B. L.; Vernon, C. A. *Chem. Ind. (London)* **1955**, 760-763.

(8) Kumamoto, J.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2515-2518.

(9) Bailly, M. C. *Bull. Soc. Chim.* **1942**, *9*, 314-339, 340-350. Desjobert, A. *Bull. Soc. Chim.* **1947**, *14*, 809-812.

(10) Hammett, L. P. "Physical Organic Chemistry"; McGraw-Hill: London, 1940; p 154. Lowry, T. H.; Richardson, K. C. "Mechanism and Theory in Organic Chemistry"; Harper & Row: New York, 1976; p 179.

0002-7863/84/1506-8156$01.50/0