Destabilization of the 3_{10}-Helix in Peptides Based on C^{α}-Tetrasubstituted α-Amino Acids by Main-Chain to Side-Chain Hydrogen Bonds

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Abstract: The homooligopeptide series based on *O*,*O*-isopropylidene-α-hydroxymethylserine from dimer through pentamer has been synthesized to examine the conformational preferences of this new C^{α}-tetrasubstituted α-amino acid characterized by concomitant $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$ cyclization and presence of two ether oxygen atoms in the γ-positions of the six-membered ring 1,3-dioxane system. To this aim we have exploited X-ray diffraction in the crystal state and FTIR absorption and ^{1}H NMR techniques in solution. The results obtained are compared with those of the homooligopeptides based on the related cyclohexane-containing C^{α}-tetrasubstituted residue. We conclude that in the former peptides a competition takes place between the classical intramolecular (peptide) C═O···H−N (peptide) H-bonds, stabilizing the β-bend/3_{10}-helical structures, and the newly discovered (peptide) N_{i+1}−H···O_i^{γ} (side-chain ether) intramolecular H-bonds. The extent of regular (incipient) 3_{10}-helix formation, where this latter type of H-bond is absent, tends to increase as peptide main-chain length increases. As a result of this intramolecular N−H···O^{γ} interaction, the critical main-chain length for 3_{10}-helix formation in the crystal state shifts from the shortest possible oligomer, the terminally protected trimer, in the cyclohexane series to the pentamer in the 1,3-dioxane series. Interestingly, a strict correlation has been found between the observed (peptide) N_{i+1}−H···O_i^{γ} (side-chain ether) intramolecular H-bond and (i) the backbone ψ torsion angle of the *i* residue (extended), and (ii) the disposition of the α-amino substituent in the 1,3-dioxane ring of the $i + 1$ residue (axial).

Introduction

As early as in 1971, by calculating the sterically allowed conformations of Ac-Aib-NHMe (Ac, acetyl; Aib, α-aminoisobutyric acid; NHMe, methylamino) for the first time (Figure 1), Marshall[1] clearly showed the striking effect of the C^{α}-methyl replacement (from Ala to Aib) responsible for a drastic (20 times) reduction of the total accessible φ,ψ area. In addition, he was able to demonstrate that the φ,ψ area explored by this achiral C^{α}-tetrasubstituted residue coincides with that where the right- and left-handed α-helices are found. The first unequivocal experimental proofs (by single-crystal X-ray diffraction) of Aib structural propensity were published in the late 1970s by Balaram and co-workers[2] who showed that the -Aib-Pro-Aib- and -$(Aib)_4$- sequences are folded in the 3_{10}-helical conformation (the α- and 3_{10}-helices have a close set of φ,ψ torsion angles, but clearly distinguishable C═O···H−N intramolecular H-bonding schemes).[3] These studies catalyzed an explosion of papers, where an impressive number of X-ray diffraction structures of Aib-containing peptides were reported.[4] It was concluded that Aib is the strongest known 3_{10}/α-helical former and that the tendency to adopt the 3_{10}-helix depends on several factors, including a combination of high Aib content (the optimum is obviously achieved with the homooligomers) and a short main-chain length (≤7 amino acid residues).[5] Interestingly, the highly conformationally restricted nature of this family of peptides is considered responsible for the

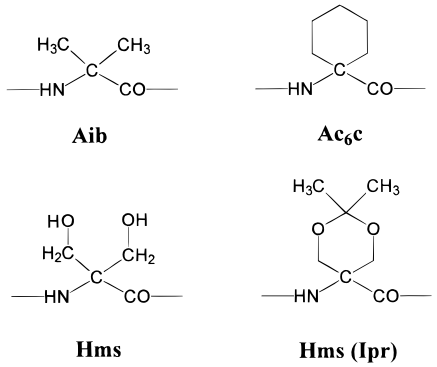

Figure 1. The four C^{α}-tetrasubstituted α-amino acids discussed in this work.

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observation that the conformation found in the crystal state is also that usually largely prevailing in solution.

More recently, this same conformational conclusion was also reported for peptides based on C^{α}-tetrasubstituted α-amino acids with $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$ cyclization, i.e., the Ac_nc (1-aminocycloalkane-1-carboxylic acid) residues with $n = 3-9$, 12 (Figure 1).[5b−d,6,7] Strictly comparable results were obtained with peptides rich in $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$ cyclized residues with a heteroatom (N, O, or S) in the δ-position of the six-membered ring system.[5e,7,8]

In this article we describe the results of our crystal-state (by X-ray diffraction) and solution (by FTIR absorption and ^{1}H NMR techniques) conformational analyses of the complete homooligopeptide series (to the pentamer) based on HmS(Ipr) (*O*,*O*-isopropylidene-α-hydroxymethylserine) (Figure 1), an amino acid of the Aib family, characterized by $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$ cyclization and presence of two ether oxygen atoms in the γ-positions of the six-membered ring system.[9] A comparison is also made with the data obtained under strictly analogous experimental conditions for the homooligopeptide series based on Ac_6c, the related cyclohexane-containing amino acid (a conformational study on the Ac_6c series using similar conditions but less sophisticated instrumentation has been reported previously).[10] For the short HmS(Ipr) homooligomers, unambiguous evidence is found in the crystal state that the 3_{10}-helix, typical of the Ac_6c peptides, is destabilized by the newly observed main-chain to side-chain intramolecular H-bond between an ether O^{γ} atom of the 1,3-dioxane moiety and an adjacent peptide N−H group. The HmS(Ipr) residue is a side-chain protected version of HmS (α-hydroxymethylserine) (Figure 1). This latter amino acid is of potential interest as it is known to characterize some naturally occurring peptide antibiotics and immunosuppressants and to behave as an excellent metal ion binder and galactose mimetic.[9b,11]

Materials and Methods

Synthesis and Characterization of Peptides. Melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was carried out on 250-μm silica gel GF precoated uniplates (Analtech) with the following solvent systems: **I** (ethyl acetate (AcOEt)/*n*-heptane, 1:1); **II** (AcOEt/*n*-heptane, 2:1). Spots were visualized by UV light, by chlorine followed by starch/KI spray, or by spraying with ninhydrin. HPLC was performed on a Thermoseparation instrument using a C_{18} Vydac (0.46 × 25 cm) column thermostated at 36 °C with flow rate 1 mL/min and UV detection at 220 nm. The following solvent system, with linear gradient change over 25 min, was used: A 0.05% TFA (trifluoroacetic acid) in water; B 0.038% TFA in acetonitrile/water, 90:10. Analytical ^{1}H NMR spectra were obtained on a 250 MHz instrument (Bruker Avance DPX) with tetramethylsilane as the internal standard. Chemical shifts are in ppm. FAB mass spectra were recorded on a APO Electron (Ukraine) model MI 12001E mass spectrometer equipped with a FAB ion source.

The synthesis and characterization of the homooligomers Z-$(Ac_6c)_n$-O*t*Bu (Z, benzyloxycarbonyl; O*t*Bu, *tert*-butoxy; $n = 1-5$)[10d] and Z-$[HmS(Ipr)]_n$-OMe (OMe, methoxy; $n = 1-3$)[9b] were already reported. However, it should be noted that in the original reference[9b] the tripeptide Z-$[HmS(Ipr)]_3$-OMe was prepared from Z-$[HmS(Ipr)]_2$-OH and H-HmS(Ipr)-OMe in the presence of TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate,[12a,b] as the activating agent, in 42% yield, whereas in the present work it was prepared from Z-HmS(Ipr)-OH[9b] and H-$[HmS(Ipr)]_2$-OMe in the presence of HATU, O−(7-aza-benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate,[12c] in 79% yield. The synthesis and characterization of Boc-$[HmS(Ipr)]_2$-OMe (Boc, *tert*-butyloxycarbonyl) will be reported elsewhere.[13]

Z-$[HmS(Ipr)]_4$-OMe. A solution of Z-$[HmS(Ipr)]_3$-OMe[9b] (0.160 g, 0.25 mmol) in methanol (20 mL) was hydrogenated for 2 h (at 4 kg/cm^2 on a Parr apparatus) in the presence of 10% Pd/charcoal catalyst (25 mg). After filtration and evaporation of the solvent, the TLC homogeneous, N^{α}-deprotected compound was dissolved in dichloromethane (DCM) (2 mL) and added to a stirred solution of Z-HmS-(Ipr)-OH (0.078 g, 0.25 mmol), HATU (0.105 g, 0.275 mmol), and di*iso*propylethylamine (DIEA) (0.088 mL, 0.5 mmol) in DCM (2 mL).

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After 24 h, the solvent was evaporated, and the residue was taken up in AcOEt and washed with 1 N $KHSO_4$, 1 N $NaHCO_3$, and brine. After the organic layer was dried and evaporated, the crude product was crystallized from DCM/*n*-hexane. Yield 0.118 g (60%); mp 185−187 °C; TLC 0.25 (**I**), 0.35 (**II**); HPLC R_t 9.391 min (60−90% **B**), R_t 14.798 min (45−75% **B**); ^{1}H NMR ($CDCl_3$) δ 1.38−1.54 (7s, 24H, Ipr CH_3); 3.71 (s, 3H, OMe CH_3); 3.99−4.41 (m, 16H, HmS βCH_2); 5.13 (s, 2H, Z CH_2); 5.99 (s, 1H, HmS NH), 7.39 (m, 5H, Z-phenyl CH); 7.61 (s, 1H, HmS NH); 7.65 (s, 1H, HmS NH); 7.71 (s, 1H, HmS NH). FAB-MS $C_{37}H_{54}N_4O_{15}$ (MW = 794.8), *m/z* 795 $(MH)^+$.

Z-[HmS(Ipr)]$_5$-OMe. After hydrogenation of Z-[HmS(Ipr)]$_4$-OMe (120 mg, 0.15 mmol), the N$^\alpha$-deprotected compound was coupled with Z-HmS(Ipr)-OH (47 mg, 0.15 mmol) in the presence of HATU (64 mg, 0.165 mmol) and DIEA (55 μL, 0.3 mmol) as reported above for the tetrapeptide. The crude product was crystallized from DCM/*n*-hexane. Yield 70 mg (49%); mp 209−211 °C; TLC 0.12 (**I**); R_t 11.291 min (60−90% **B**), R_t 17.807 min (50−80% **B**); ^{1}H NMR ($CDCl_3$) δ 1.30−1.51 (7s, 30H, Ipr CH_3); 3.75 (s, 3H, OMe CH_3); 3.95−4.32 (m, 20H, HmS βCH_2); 5.15 (s, 2H, Z CH_2); 6.03 (s, 1H, HmS NH), 7.37 (m, 5H, Z-phenyl CH); 7.38 (s, 1H, HmS NH); 7.46 (s, 1H, HmS NH); 7.49 (s, 1H, HmS NH); 7.62 (s, 1H, HmS NH). FAB-MS $C_{44}H_{65}N_5O_{18}$ (MW = 952.0), *m/z* 952 $(MH)^+$, 974 $[(M + Na)^+]$, 990 $[(M + K)^+]$.

FTIR Absorption. The FTIR absorption spectra were recorded using a Perkin-Elmer model 1720X 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 10.0 mm (with CaF_2 windows) were used. Spectrograde deuteriochloroform (99.8% D) was purchased from Fluka.

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) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) was purchased from Sigma.

X-ray Diffraction. Data collection was performed using a Philips PW1100 four-circle diffractometer for the di- and tetrapeptides, and a Kuma Diffraction KM4 automated diffractometer for the tri- and pentapeptides. The crystals of the tetrapeptide acetone solvate are stable only in the presence of the mother liquor. Therefore, for data collection of this compound, a crystal was sealed in a glass capillary with mother liquor. Cell parameters for the four structures were obtained by least-squares refinement of the angular settings of 48−90 carefully centered high angle reflections. The structures were solved by direct methods (SHELXS 86[14a] program for the di- and tetrapeptides, while SHELXS 90[14b] for the tri- and pentapeptides). Refinement was carried out on F^2, with all non-H atoms anisotropic, by application of either the SHELXL 93[14c] (di- and tetrapeptides) or the SHELXL 97[14d] (tri- and pentapeptides) program. In the structure of the dipeptide the cyclic side chain of the N-terminal residue is disordered. The O1$^{\gamma 2}$ atom and the two methyl groups were refined at two sets of positions, with occupancy factor of 0.50 each. H-atoms in the di- and tetrapeptide structures 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 methyl groups) times the U_{eq} of the carrying atom. H-atoms of the tripeptide were located on a difference Fourier map and they were isotropically refined. In the pentapeptide, the positions of the H-atoms of all five N−H groups were assessed from a difference Fourier map, and subsequently they were isotropically refined, while all other H-atoms were placed at geometrically calculated positions and allowed to ride on parent atoms with U_{iso} included in the refinement. The relatively large residual electron density peak (1.151 e $Å^{-3}$) in the pentapeptide structure is located in the proximity of the CH_2 group of the Z-protecting moiety, and may indicate some disorder in that part of the molecule. However, despite a number of attempts, no viable model for that disorder could be obtained.

Other relevant crystallographic data and diffraction parameters are listed in Table 1.

Results and Discussion

Crystal-State Conformational Analysis. The molecular and crystal structures of the four following HmS(Ipr) homopeptides were determined by X-ray diffraction: Boc-[HmS(Ipr)]$_2$-OMe and Z-[HmS(Ipr)]$_n$-OMe (n = 3−5). The molecular structures with the atomic numbering schemes are illustrated in Figures 2−5, respectively. Relevant N$^\alpha$-protecting group, main-chain, and side-chain torsion angles[15] are given in Table 2. In Table 3 the intra- and intermolecular H-bond parameters are listed.

Bond lengths and bond angles (deposited) are in general agreement with previously reported values for the geometry of the *tert*-butyloxycarbonylamino[16a] and benzyloxycarbonylamino[16b] moieties, the methyl ester group,[17] and the peptide unit.[18] The conformationally informative τ (N−C$^\alpha$−C′) bond angle[5b−d,18b,19] of the central residue of the tripeptide is very narrow, 105.2-(2)°, a preliminary indication of the onset of the fully extended (C_5) conformation[20] at the level of that amino acid.

Of the fourteen HmS(Ipr) residues included in the four peptides examined, nine populate the helical region (A or A*) of the conformational space,[21] whereas four of them are *semi*-extended (region F or F*) and one is fully extended (region E or E*). Each of the four compounds, having no chiral atoms, crystallizes with retention of the center of symmetry; thus, in each unit cell, molecules of both handedness simultaneously occur. The average values for the ϕ,ψ backbone torsion angles of the HmS(Ipr) residues forming helical turns are ±55.7°, ±36.0°, close to those expected for a regular 3_{10}-helix (±57°, ±30°).[3] In the only X-ray structure published to date for a HmS(Ipr) peptide, namely Z-HmS(Ipr)-L-Ala-OMe, the C$^\alpha$-tetrasubstituted residue is helical.[9b]

In the homo-dipeptide, despite the fact that its main-chain length would be too short for 1 ← 4 C═O···H−N intramolecularly H-bonded β-bend formation, the sets of ϕ,ψ torsion angles are close to those appropriate for a type-II (II′) β-bend structure.[20a,22] The only intramolecular H-bond observed is that between the peptide N2−H group of residue 2 and the side-chain ether O1$^{\gamma 2}$ atom of residue 1 with formation of a six-membered ring ("C_6") structure.

The three residues of the homo-tripeptide adopt three different conformations, i.e., helical, fully extended (C_5), and *semi*-extended, respectively. It is worth mentioning that this is the

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Table 1. Crystallographic Data and Diffraction Parameters for the HmS(Ipr) Homopeptides

parameter	dipeptide	tripeptide	tetrapeptide acetone solvate	pentapeptide
empirical formula	$C_{20}H_{34}N_2O_9$	$C_{30}H_{43}N_3O_{12}$	$C_{40}H_{60}N_4O_{16}$	$C_{44}H_{65}N_5O_{18}$
formula weight (amu)	446.5	637.7	852.9	952.0
color, habit	colorless, prisms	colorless, prisms	colorless, prisms	colorless, prisms
crystal system	monoclinic	triclinic	monoclinic	triclinic
space group	$P2_1/a$	$P\bar{1}$	$P2_1/c$	$P\bar{1}$
a (Å)	18.180(3)	11.243(2)	11.182(2)	11.632(2)
b (Å)	11.754(2)	11.451(2)	21.510(3)	11.945(2)
c (Å)	11.214(2)	13.122(3)	18.033(3)	19.100(4)
α (deg)	90.0	84.47(3)	90.0	98.73(3)
β (deg)	90.3(1)	80.20(3)	95.6(1)	102.87(3)
γ (deg)	90.0	77.90(3)	90.0	98.24(3)
V (Å^3)	2396.3(7)	1624.5(5)	4316.7(12)	2513.6(10)
Z (molecules/unit cell)	4	2	4	2
density (calc) (g/cm^3)	1.238	1.304	1.312	1.258
absorption coefficient (mm^{-1})	0.097	0.849	0.852	0.822
F(000)	960	680	1824	1016
collected reflections	6069	7653	6441	10387
independent reflections	5789 [$R_{int} = 0.014$]	6792 [$R_{int} = 0.042$]	6418 [$R_{int} = 0.098$]	9129 [$R_{int} = 0.028$]
observed reflections [$I \geq 2\sigma(I)$]	3837	4393	1587	5088
solved by	SHELXS 86	SHELXS 90	SHELXS 86	SHELXS 90
refined by	SHELXL 93	SHELXL97	SHELXL93	SHELXL 97
final R indices [$I \geq 2\sigma(I)$]	R1 = 0.0459, wR2 = 0.1358	R1 = 0.0467, wR2 = 0.1472	R1 = 0.0768, wR2 = 0.1588	R1 = 0.0802, wR2 = 0.2151
final R indices (all data)	R1 = 0.0965, wR2 = 0.1669	R1 = 0.0865, wR2 = 0.1716	R1 = 0.2948, wR2 = 0.2201	R1 = 0.1428, wR2 = 0.2485
temperature (K)	293(2)	293(2)	293(2)	293(2)
radiation (λ)	Mo Kα (0.71073 Å)	Cu Kα (1.54178 Å)	Cu Kα (1.54178 Å)	Cu Kα (1.54178 Å)
scan method	$\theta-2\theta$	$\omega-2\theta$	$\theta-2\theta$	$\omega-2\theta$
θ range (deg)	2.06−28.01	3.42−80.90	3.21−60.00	2.42−69.97
index ranges	$-23 < h < 24$, $0 < k < 15$, $0 < l < 14$	$-14 < h < 14$, $-14 < k < 14$, $-16 < l < -1$	$-12 < h < 12$, $0 < k < 24$, $0 < l < 20$	$-14 < h < 13$, $-14 < k < 14$, $-1 < l < 23$
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2	full-matrix block least-squares on F^2	full-matrix block least-squares on F^2
data/restraints/parameters	5783/4/307	6792/0/579	6412/13/529	9129/186/754
goodness of fit on F^2	1.064	1.026	0.742	1.054
crystallization solvent	ethyl acetate/*n*-hexane (vapor diffusion)	ethyl acetate/*n*-hexane (slow evaporation)	acetone/water (vapor diffusion)	ethanol/water (vapor diffusion)
crystal size (mm)	0.50 × 0.50 × 0.40	0.30 × 0.10 × 0.10	0.25 × 0.20 × 0.10	0.50 × 0.30 × 0.15
$\Delta\rho_{max}$ and $\Delta\rho_{min}$ (e Å^{-3})	0.452/−0.367	0.271/−0.321	0.236/−0.285	1.151/−0.436

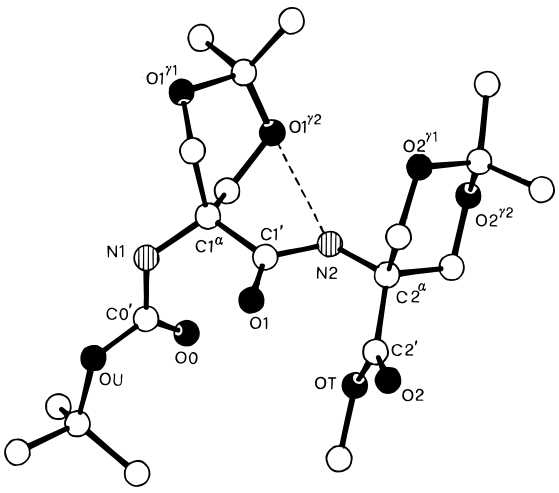

Figure 2. X-ray diffraction structure of Boc-[HmS(Ipr)]$_2$-OMe with numbering of the atoms. The intramolecular H-bond is represented by a dashed line.

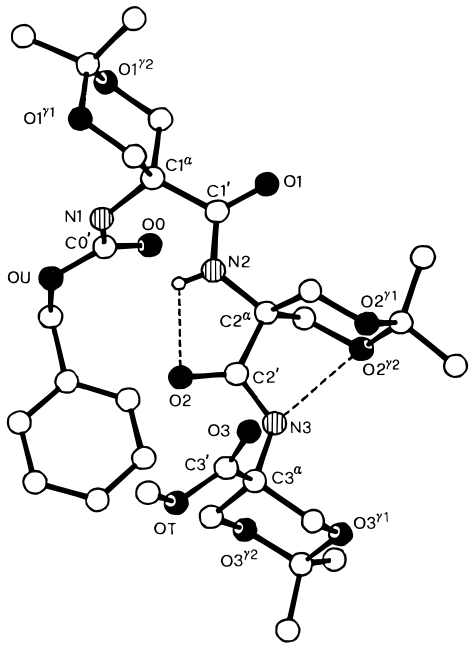

Figure 3. X-ray diffraction structure of Z-[HmS(Ipr)]$_3$-OMe with numbering of the atoms. The intramolecular H-bonds are represented by dashed lines.

first observation of a N−H···C═O intramolecularly H-bonded, fully extended (C_5) conformation[20] for a residue of the cycloaliphatic C^α-tetrasubstituted amino acid series (Ac_nc). In addition to the narrow τ bond angle mentioned above, two other typical features of the intramolecularly H-bonded C_5 conformation are: (i) the very short intramolecular N2···O2 separation, and (ii) the small N2−H···O2 angle. In homo-tripeptides and longer peptides of this family of amino acids the onset of the *semi*-extended conformation at the C-terminus of the backbone is not a rare event. The unusual structure of this tripeptide is further stabilized by an N−H···O$^{\gamma 2}$ (ether) intramolecular

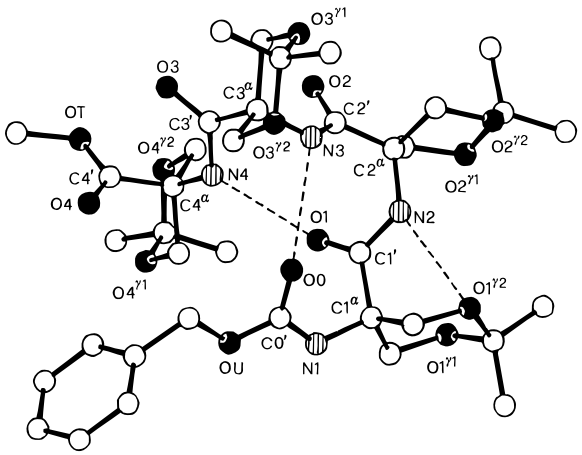

Figure 4. X-ray diffraction structure of Z-[HmS(Ipr)]$_4$-OMe with numbering of the atoms. The intramolecular H-bonds are represented by dashed lines.

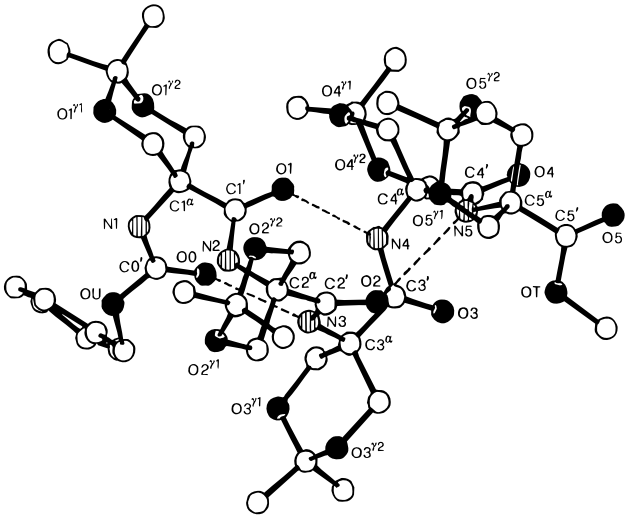

Figure 5. X-ray diffraction structure of Z-[HmS(Ipr)]$_5$-OMe with numbering of the atoms. The intramolecular H-bonds are represented by dashed lines.

H-bond of the same type as that discussed above for the dipeptide.

The 1−3 sequence of the homo-tetramer is folded in two consecutive 1 ← 4 C═O···H−N intramolecularly H-bonded β-bend conformations of the II (II′)−I′ (I) type, where residue 1 is *semi*-extended and residues 2 and 3 are helical, and the sign of the ψ torsion angle of residue 1 is opposite to that of residues 2 and 3. The C═O···H−N intramolecular H-bond producing the type-II (II′) C_{10} (β-bend) conformation is rather weak.[23] Again, the structure of this oligomer is characterized by an intramolecular main-chain (peptide N2−H group) to side-chain (ether O1$^{\gamma2}$ atom) H-bond of the "C_6" type.

The backbone of the homo-pentamer is folded in a regular right(left)-handed 3_{10}-helix. Peptide groups N3−H to N5−H and C′0═O0 to C′2═O2 participate in three consecutive 1 ← 4 intramolecular H-bonds of good geometry. No intramolecular main-chain to side-chain interaction characterizes this oligomer.

In the four compounds, few significant deviations of the ω torsion angles ($|\Delta\omega| > 10°$) from the ideal value of the *trans* planar urethane, peptide, and ester units (180°) are observed.

(23) (a) Ramakrishnan, C.; Prasad, N. *Int. J. Protein Res.* **1971**, *3*, 209−231. (b) Taylor, R.; Kennard, O.; Versichel, W. *Acta Crystallogr.* **1984**, *B40*, 280−288. (c) Görbitz, C. H. *Acta Crystallogr.* **1989**, *B45*, 390−395.

Table 2. Selected Protecting Group, Backbone and Side-Chain Torsion Angles[a] (deg) for the HmS(Ipr) Homopeptides

torsion angle	dipeptide	tripeptide	tetrapeptide	pentapeptide
θ^2		95.0(2)	178.5(6)	−106.2(6)
θ^1	173.3(1)	−173.1(2)	176.8(6)	176.8(4)
ω_0	162.5(1)	−176.0(2)	173.3(6)	−178.5(4)
ϕ_1	−63.8(2)	−57.4(2)	−51.9(9)	−54.4(5)
ψ_1	152.1(1)	−37.4(2)	143.5(6)	−39.9(5)
ω_1	172.6(1)	−179.8(2)	173.7(6)	−176.0(3)
ϕ_2	45.3(2)	−177.8(2)	53.6(9)	−53.2(5)
ψ_2	47.4(2)[b]	171.9(2)	36.3(9)	−38.7(5)
ω_2	−176.7(2)[c]	170.3(2)	175.9(6)	−172.4(4)
ϕ_3		−49.7(2)	56.2(8)	−59.9(5)
ψ_3		141.4(2)[b]	34.6(9)	−29.1(5)
ω_3		179.1(2)[c]	−172.5(6)	−179.5(4)
ϕ_4			−53.9(9)	−57.1(5)
ψ_4			125.7(6)[b]	−37.5(5)
ω_4			175.8(6)[c]	−164.3(4)
ϕ_5				−61.2(5)
ψ_5				−47.2(5)[b]
ω_5				−178.5(4)[c]
$\chi_1^{1,1}$	−102.8(2)[d], −163.2(2)[d]	63.9(2)	−175.0(6)	−65.0(4)
$\chi_1^{1,2}$	160.7(1)	−65.6(2)	169.8(5)	63.4(5)
$\chi_1^{2,1}$	−62.1(3)[d], 56.8(2)[d]	55.3(2)	58.1(8)	−56.0(5)
$\chi_1^{2,2}$	−62.5(2)	−59.1(2)	−57.0(8)	59.7(5)
$\chi_1^{3,1}$	45.0(3)[d], −64.6(2)[d]	−54.7(2)	−55.2(8)	54.3(5)
$\chi_1^{3,2}$	68.3(2)	56.5(2)	55.2(8)	−56.7(5)
$\chi_2^{1,1}$	−65.0(2)	167.6(2)	−73.7(7)	−65.6(4)
$\chi_2^{1,2}$	65.9(2)	−169.4(2)	73.8(7)	63.8(5)
$\chi_2^{2,1}$	−57.5(2)	−55.0(2)	−52.4(8)	−57.7(5)
$\chi_2^{2,2}$	58.5(2)	56.0(2)	56.0(7)	58.7(5)
$\chi_2^{3,1}$	58.0(2)	60.1(2)	58.4(7)	56.4(5)
$\chi_2^{3,2}$	−58.6(2)	−60.0(2)	−60.4(7)	−57.2(5)
$\chi_3^{1,1}$		−63.2(2)	−70.9(8)	−66.0(5)
$\chi_3^{1,2}$		61.0(2)	69.4(7)	64.1(5)
$\chi_3^{2,1}$		−56.7(2)	−57.6(8)	−56.8(6)
$\chi_3^{2,2}$		58.3(2)	57.6(7)	58.2(6)
$\chi_3^{3,1}$		52.8(2)	58.4(8)	56.6(7)
$\chi_3^{3,2}$		−53.9(2)	−58.2(8)	−57.9(7)
$\chi_4^{1,1}$			−172.5(6)	−70.5(4)
$\chi_4^{1,2}$			169.4(6)	68.7(4)
$\chi_4^{2,1}$			63.4(8)	−55.4(5)
$\chi_4^{2,2}$			−54.8(9)	56.3(5)
$\chi_4^{3,1}$			−58.4(9)	57.3(5)
$\chi_4^{3,2}$			52.9(9)	−58.0(5)
$\chi_5^{1,1}$				−63.4(4)
$\chi_5^{1,2}$				60.0(5)
$\chi_5^{2,1}$				−55.0(5)
$\chi_5^{2,2}$				59.1(5)
$\chi_5^{3,1}$				52.1(5)
$\chi_5^{3,2}$				−54.0(5)

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ,ψ,ω) and side chains (χ) are described in ref 15. For the torsion angles for rotation about bonds of the Boc- and Z-protecting groups (θ) see refs 16 a and b, respectively. [b] N−C$^{\alpha}$−C′−OT torsion angle. [c] C$^{\alpha}$−C′−OT−CT torsion angle. [d] The O1$^{\gamma2}$ atom is disordered over two sites.

In particular, the N-terminal urethane ω_0 torsion angle of the dipeptide and the peptide ω_4 torsion angle of the pentapeptide differ by about 17.5° and 15.7°, respectively, from the *trans* planar value. The *trans* arrangement of the θ^1 torsion angle of the Boc-NH- and Z-NH- moieties, found for all of the peptides investigated in this work, is that commonly observed for Boc-(Z)-protected amino acids and peptides (type-*b* conformation).[16] Not surprisingly,[16b] the values of θ^2 for the Z-peptides are concentrated in the three regions of ±90° and 180°. The methyl ester conformation with respect to the preceding C$^{\alpha}$−N bond is *synclinal* or intermediate between the *synperiplanar* and *synclinal* conformations in the homo-tetramer and homo-trimer, respectively, whereas it is close to the *anticlinal* conformation in the homo-dimer and homo-pentamer.[24]

Table 3. Intra- and Intermolecular H-Bond Parameters for the HmS(Ipr) Homopeptides

peptide	donor D−H	acceptor A	symmetry operation	distance (Å) D···A	distance (Å) H···A	angle (deg) D−H···A
Boc-[HmS(Ipr)]$_2$-OMe	N2−H	O1$^{\gamma 2}$	x, y, z	2.933(2)	2.29	132
	N1−H	O1	$-x, 2-y, 2-z$	2.905(2)	2.25	133
Z-[HmS(Ipr)]$_3$-OMe	N2−H	O2	x, y, z	2.580(2)	2.16(2)	113(2)
	N3−H	O2$^{\gamma 2}$	x, y, z	2.899(2)	2.23(3)	135(2)
	N1−H	O1$^{\gamma 1}$	$1-x, 1-y, -z$	3.004(2)	2.16(2)	165(2)
Z-[HmS(Ipr)]$_4$-OMe	N2−H	O1$^{\gamma 2}$	x, y, z	2.812(7)	2.19	129
	N3−H	O0	x, y, z	3.199(8)	2.63	125
	N4−H	O1	x, y, z	2.974(8)	2.30	135
	N1−H	O3	$x, {}^1/_2-y, -{}^1/_2+z$	3.074(7)	2.25	162
Z-[HmS(Ipr)]$_5$-OMe	N3−H	O0	x, y, z	2.998(5)	2.07(7)	154(5)
	N4−H	O1	x, y, z	3.064(4)	2.29(4)	156(5)
	N5−H	O2	x, y, z	3.090(5)	2.31(5)	153(5)
	N1−H	O4	$x, y-1, z$	3.034(5)	2.11(6)	165(5)
	N2−H	O5	$x, y-1, z$	3.089(5)	2.33(4)	167(4)

All of the six-membered 1,3-dioxane rings of the HmS(Ipr) residues are accommodated in the chair conformation, either of the $^4C_1$ or $^1C_4$ type.[25] In the former disposition the α-amino substituent is axial and the $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles are close to ±60°, while in the latter disposition the α-amino substituent is equatorial and the $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles have values close to 180°. Among the fourteen 1,3-dioxane rings studied ten (including all five rings of the homo-pentamer) are characterized by an axial α-amino substituent and four by an equatorial α-amino substituent. Interestingly, in the three cases where a (peptide) $N_{i+1}-H\cdots O_i^{\gamma}$ (ether) intramolecular H-bond is found, the α-amino substituent of the $i+1$ residue is always axial. The only distorted conformation for a 1,3-dioxane system is presented by one of the two conformers of residue 1 of the dipeptide, where the six-membered ring is intermediate between the (twist) $^2T_6$ and (boat) $^3B_6$ conformations. The *endo*cyclic 1,3-dioxane ring torsion angles have values in the range ±52.1-(5) to ±63.4(8)° (if the $\chi^{3,1}$ and $\chi^{3,2}$ torsion angles of the disordered residue 1 of the dipeptide are excluded), i.e., close to those expected for a chair conformation of a six-membered ring system.[25] The HmS(Ipr) side-chain conformation of the only structure of a peptide based on this amino acid solved to date by X-ray diffraction is a slightly distorted chair with the α-amino substituent occupying the axial position.[9b]

The crystal structure of the dipeptide is characterized by a single, strong intermolecular N−H···O═C H-bond between the urethane N1−H group and the peptide O1═C′1 group of a symmetry-related molecule, forming dimers. In the crystal packing mode of the tripeptide, the single intermolecular N−H···O H-bond observed links the urethane N1−H group and the ether O1$^{\gamma 1}$ atom of the same N-terminal residue, but of a centrosymmetric molecule. As in the di- and tripeptides, all of the H-atoms bound to nitrogens of the tetrapeptide are involved in H-bonding. In this latter peptide the intermolecular H-bonds involve the urethane N1−H group and the peptide O3═C′3 group of a symmetry-related molecule, generating rows of molecules along the *z*-direction. In the unit cell of the helical pentapeptide the molecules are held together in a head-to-tail fashion along the *y*-direction in rows stabilized by (urethane) N1−H···O4═C′4 (peptide) and (peptide) N2−H···O5═C′5 (ester) intermolecular H-bonds.

From the published X-ray diffraction data for the Ac_6c-containing derivatives and peptides it turns out that all of these residues are regular or slightly distorted helical (with the chair conformation and the axial disposition for the α-amino substituent largely prevailing) in the crystal state.[7,10a−c,e,f] More specifically, the shortest possible N$^{\alpha}$-acylated, Ac_6c-containing peptide capable of folding in a type-III (III′) β-bend conformation (the tripeptide) does indeed adopt this basic structural unit of the 3_{10}-helix. In contrast, from the present investigation it is clear that a regular 3_{10}-helical structure is adopted in the crystal state by the HmS(Ipr) homo-pentapeptide but not by its lower oligomers. Taken together, these results strongly support the view that the C$^{\alpha}$-tetrasubstituted HmS(Ipr) residue, as the other cyclic members of this family,[5b−d,6,7] does indeed have marked tendency to fold. However, in short peptides this tendency is counterbalanced by the property of the ether side chains to interact intra- and intermolecularly with appropriately positioned H-bonding donors. In any case, from our results this type of intermolecular interactions does not appear to be the dominating factor in biasing peptide conformation.

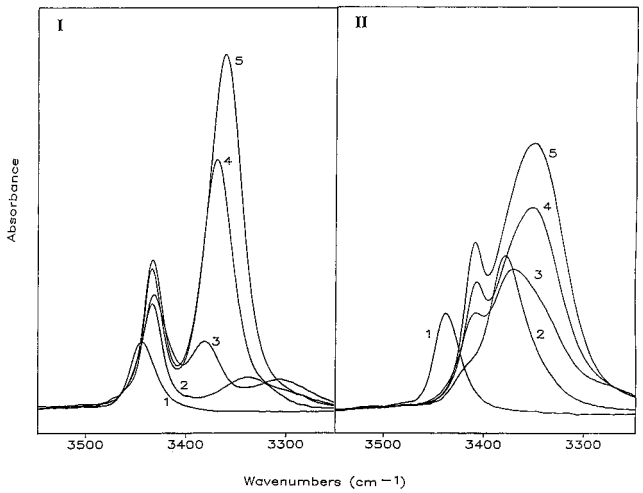

Figure 6. FTIR absorption spectra (N−H stretching region) of Z-$(Ac_6c)_n$-O*t*Bu ($n = 1-5$) (I) and Z-[HmS(Ipr)]$_n$-OMe ($n = 1-5$) (II) in $CDCl_3$ solution (peptide concentration 1 mM).

Solution Conformational Analysis. The preferred conformations of the terminally protected homopeptides Z-[HmS-(Ipr)]$_n$-OMe and Z-$(Ac_6c)_n$-O*t*Bu ($n = 1-5$) were investigated in a solvent of low polarity ($CDCl_3$) at various concentrations (in the range 10−0.1 mM) by using FTIR absorption in the N−H stretching (3500−3200 cm^{-1}) and C═O stretching (1750−1620 cm^{-1}) regions and ^{1}H NMR.

Figure 6 shows the FTIR absorption spectra of the two peptide series in the more informative 3500−3200 cm^{-1} region and Table 4 lists the absorption maxima as extracted from the inverted second-derivative spectra.

(24) Dunitz, J. D.; Strickler, P. In *Structural Chemistry and Molecular Biology*; Rich, A., Davidson, N., Eds.; Freeman: San Francisco, 1968; pp 595−602.

(25) Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354−1358.

Table 4. FTIR Absorption Data[a] for the Z-[HmS(Ipr)]$_n$-OMe and Z-(Ac$_6$c)$_n$-O*t*Bu (n = 1−5) Homopeptides

peptide		3500−3200 cm^{-1}	1750−1620 cm^{-1}
Z-[HmS(Ipr)]$_n$-OMe	n = 1	**3440**[b]	**1743**, **1719**
	n = 2	*3415*,[c] 3382	**1741**, 1712, **1670**
	n = 3	3412, **3375**, *3339*	**1740**, 1716, 1684, **1662**
	n = 4	3410, *3374*, *3366*, **3356**, 3347	1739, 1713, **1684**, 1660
	n = 5	3411, *3374*, *3358*, **3346**	1737, 1713, **1687**, 1661
Z-(Ac$_6$c)$_n$-O*t*Bu	n = 1	**3445**	**1731**, **1721**
	n = 2	**3433**, *3339*	**1729**, **1711**, *1701*, 1670
	n = 3	**3433**, 3384, *3306*	**1724**, 1685, 1670
	n = 4	3433, **3369**	**1722**, **1683**, 1652
	n = 5	3435, **3360**	**1723**, **1680**, 1650

[a] In $CDCl_3$ solution (peptide concentration 1 mM). [b] Strong band. [c] Very weak band.

Using the Mizushima's dilution method,[26a] we have been able to show that at 1 mM concentration self-association via intermolecular H-bonding is negligible for all oligomers (data not shown). However, this phenomenon does occur, although to a low extent (≅10%) at 10 mM concentration for the Ac$_6$c pentamer. Interestingly, it is not seen at all for the corresponding HmS(Ipr) oligomer. As a consequence, for all peptides investigated, the H-bonding observed at 1 mM concentration (Figure 6) should be interpreted as arising from *intra*molecular interactions only.

In the N−H stretching region the spectra of the higher Ac$_6$c oligomers (n = 4, 5) are typical of a peptide series forming multiple β-bends and 3_{10}-helical structures, being characterized by two strong bands at 3435−3433 cm^{-1} (free, solvated, amide NH groups) and 3369−3360 cm^{-1} (N−H···O═C H-bonded, amide NH groups).[26a−d] In this homopeptide series the intensity of the low-frequency band relative to the high-frequency band (A_H/A_F ratio) increases steadily with increasing main-chain length, and concomitantly the absorption maximum shifts to considerably lower wavenumbers. It is of interest that in all Ac$_6$c oligomers a band associated with free NH groups is consistently seen.

Although the general trend of the FTIR absorption curves of the HmS(Ipr) oligomers would be similar to that of their Ac$_6$c counterparts, the spectra of the two series are characterized by significant differences in many details. Similar to the spectra of the Ac$_6$c oligomers, those of the HmS(Ipr) oligomers exhibit an enhancement in the low-frequency band as n increases. However, in this latter peptide series (i) a band related to free NH groups (≥3425 cm^{-1}) is clearly seen in the amino acid derivative, but this band is absent in all homopeptides; (ii) a weak, but clearly discernible, band is present in the 3415−3410 cm^{-1} region of all homopeptides. There is ample evidence in the literature for a safe assignment of an absorption in the 3415−3390 cm^{-1} region in $CDCl_3$ solution to weak intramolecular H-bonds of the N−H···O═C type, indicative of the fully extended (C_5) peptide conformation;[26e,f] (iii) a broad band, resolved in the second-derivative spectra into multiple contributions, is found in the 3382−3346 cm^{-1} region, associated with stronger intramolecular H-bonds of the N−H···O═C type,[26a−d] as in the Ac$_6$c series, and, possibly, of the N−H···O$^{\gamma}$ (ether) type. It should be noted here that a HmS(Ipr) peptide NH group may be involved in two different kinds of intramolecular H-bonds with a side-chain O$^{\gamma}$ atom (i) of the same residue (with formation of a more strained five-membered ring structure) or (ii) of the preceding residue (with formation of a less strained six-membered ring structure).[27] It is also remarkable that solvated N−H stretching bands of a simple secondary amide in 1,4-dioxane, mimicking the situation characteristic of the 1,3-dioxane based HmS(Ipr) oligomers, may be found as low as 3350 cm^{-1} (data not shown).

In the less informative C═O stretching region, in addition to bands at 1743−1737 cm^{-1} (methyl ester), 1731−1722 cm^{-1} (*tert*-butyl ester), and 1724−1713 cm^{-1} (Z-urethane), the latter two absorptions partially overlapping each other in the Ac$_6$c higher oligomers, two bands, related to peptide carbonyl groups characterized by a different degree of H-bonding, are visible in the 1687−1650 cm^{-1} region.[26b−f]

The present FTIR absorption investigation has shown that N−H···O═C intramolecular H-bonding, the extent of which increases with increasing number of residues in the peptide chain, is the predominant factor for both Ac$_6$c and HmS(Ipr) homopeptides in $CDCl_3$ solution. However, the remarkable complexity of the spectra (including a substantial band broadening) of the HmS(Ipr) homo*peptides*, combined with the intriguing absence of free NH groups, points to a competition between N−H···O═C and N−H···O$^{\gamma}$ (side-chain ether) intramolecular H-bonds as a peculiar feature of this series.

To get more detailed information on the conformational tendencies of the terminally protected HmS(Ipr) and Ac$_6$c homopeptides in $CDCl_3$ solution, we carried out a ^{1}H NMR study. The analysis of inaccessible (or intramolecularly H-bonded) NH groups was performed with use of free radical (TEMPO) induced line broadening of NH resonances.[28a] Figure 7 graphically describes the results for the two homo-pentamers, taken as representative examples, in the presence of added TEMPO.

For both pentapeptides Z-(Ac$_6$c)$_5$-O*t*Bu and Z-[HmS(Ipr)]$_5$-OMe the assignment of the NH proton resonances was performed by 2D ^{1}H NMR ROESY experiments.[28b] This analysis showed the complete series of sequential NH$_i$ → NH$_{i+1}$ NOE interactions.[28c] Starting from the upfield *urethane* NH proton at position 1 of the peptide sequence, all other peptide NH resonances were unambiguously assigned.

The occurrence of the NH proton resonances in the narrow ranges 6.9−7.2 ppm (Ac$_6$c pentamer) and 7.4−7.6 ppm [HmS(Ipr) pentamer] (not shown) might be considered a first indication of closely related structural environments for the N(3)H−N(5)H protons of the Ac$_6$c pentamer and for the N(2)H−N(5)H protons of the HmS(Ipr) pentamer. In addition, it is worth noting that all of the NH proton resonances of the HmS(Ipr) pentamer, in particular that of the peptide N(2)H proton, are significantly shifted to lower fields, compared to the corresponding resonances of the Ac$_6$c pentamer. This finding tends to support the view that the NH protons of the 1,3-dioxane-containing pentamer are more perturbed by H-bonding, possibly with the side-chain O$^{\gamma}$ ether atoms, than the corresponding protons of the cyclohexane containing pentamer. Interestingly, addition of 40% 1,4-dioxane to a $CDCl_3$ solution

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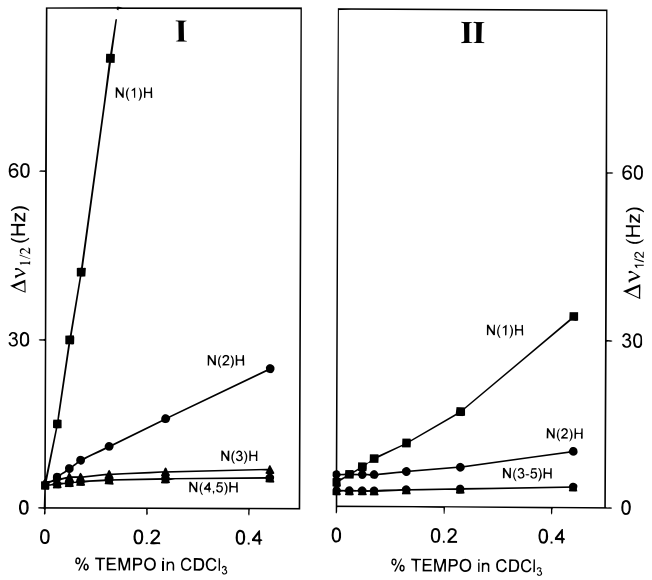

Figure 7. Plot of bandwidth of the NH signals in the ^{1}H NMR spectra of Z-$(Ac_6c)_5$-O*t*Bu (I) and Z-[HmS(Ipr)]$_5$-OMe (II) as a function of increasing percentages of TEMPO (*w*/*v*) in $CDCl_3$. Peptide concentration 1 mM.

of the model compound Ac-Aib-OMe induces a marked downfield shift (about 0.7 ppm) of its NH resonance (data not shown).

An analysis of the spectra as a function of concentration (not shown) indicates that a 10-fold dilution (from 10 to 1 mM) produces a sizable variation (to higher fields) only of the chemical shift of the N(1)H proton of the Ac_6c pentamer.[26d,28d] For all of the other NH protons of the two series, the concentration effect is negligible. In agreement with the FTIR absorption results discussed above, we conclude that at 10 mM concentration the Ac_6c pentamer is the only oligomer that self-associates and that in this process the urethane N(1)H group plays the role of the H-bonding donor.

In the Ac_6c pentamer in the absence of self-association (at 1 mM concentration), two classes of NH protons are observed. (i) The first class [N(1)H and N(2)H protons] includes protons whose resonances significantly broaden upon addition of TEMPO. The sensitivity of the N(1)H proton is much higher than that of the N(2)H proton. (ii) The second class (all other NH protons) includes those displaying a behavior characteristic of shielded protons (relative insensitivity of line widths to the presence of the paramagnetic agent TEMPO).

Also in the HmS(Ipr) pentamer the N(1)H and N(2)H resonances are more sensitive to the perturbing agent than the other NH resonances. Again, the N(1)H resonance is the most susceptible. Furthermore, the behavior of all NH resonances of the HmS(Ipr) pentamer in response to the addition of the perturbing agent is different from that of the Ac_6c pentamer (in general, the resonances of the Ac_6c pentamer are more sensitive).

The present ^{1}H NMR data strongly favor the conclusion that at low concentration (<10 mM) in $CDCl_3$ solution the N(3)H to N(5)H protons of the Ac_6c pentamer are inaccessible to the perturbing agent and therefore, most probably, intramolecularly H-bonded. The intramolecular H-bonding scheme of the Ac_6c 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, form stable intramolecular H-bonds, we are tentatively inclined to conclude that the structure predominantly adopted in $CDCl_3$ solution by this homopeptide is the 3_{10}-helix 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.[3] However, in the HmS(Ipr) pentamer this rather simple picture, emerging from the Ac_6c ^{1}H NMR data, is remarkably disturbed by additional sources of potential interactions with the NH protons. Clearly, there is no alternative possible than to identify the H-bonding acceptors with the O^γ atoms of the 1,3-dioxane side-chain moieties of the HmS(Ipr) residues. These more detailed conformational conclusions are in full agreement with the indications extracted from the results of the FTIR absorption investigation discussed above.

Conclusions

The pioneering theoretical and experimental works which emanated from the Marshall[1] and Balaram[2] groups, combined with the results of the systematic efforts produced by the Padova laboratory,[5b−d,6] clearly demonstrated the overwhelming preferences of Aib- and Ac_nc-rich *homo*peptides for the 3_{10}-helical structure. Recently, our group reported that the 3_{10}-helix of the homo-octapeptide from the related residue C^α-methylvaline can be effectively *stabilized* by intermolecular peptide···peptide self-association.[29] By contrast, in the present article we have shown that the peptide 3_{10}-helix can be significantly *destabilized* by competitive main-chain to side-chain H-bonding interactions. This conformational conclusion stands out clearly from the observed increase of the critical main-chain length for 3_{10}-helix formation in the crystal state [from the terminally protected homo-tripeptide in the Ac_6c series[10a−c,e,f] to the homo-pentapeptide in the present HmS(Ipr) series] and from the more complex FTIR absorption and ^{1}H NMR spectra of the HmS(Ipr) homopeptides with respect to those expected for a typical 3_{10}-helical (Ac_6c)[10d] series. In summary, the known structural versatility of the family of conformationally restricted C^α-tetrasubstituted α-amino acids,[5a−d,8e] further expanded by the experimental findings described here, is expected to become an important component in the arsenal of synthetic chemists in planning rigidified molecules of interest to the fields of biological and material sciences.

More specifically, in this first conformational investigation of an Ac_nc analogue, HmS(Ipr), with a ring heteroatom other than in *δ*-position, we have been able to find that a competition is operative between the intramolecular (peptide) N−H···O═C (peptide) H-bonds typical of the 3_{10}-helical structure[3] and the newly discovered intramolecular main-chain to side-chain H-bonding interactions of the (peptide) N_{i+1}−H···O_i^γ (ether) type. Of the two possible kinds of intramolecular (peptide) N−H···O^γ (ether) H-bonds in the HmS(Ipr) compounds only one, N_{i+1}−H···O_i^γ, giving rise to the "C_6" ring structure, has been unambiguously assessed in the crystal state. However, it should be mentioned that the experimentally determined intramolecular distances and angles between the N_i−H and O_i^γ atoms (not reported in Table 3), potentially involved in the alternative "C_5" ring structure, are not significantly outside the acceptable range for such a H-bond. As a consequence, with appropriate and limited spatial adjustments this favourable interaction might well be operative in solvents of low polarity, such as chloroform, where our FTIR absorption and ^{1}H NMR analyses have been performed. It is also of particular interest to note that the formation of the main-chain to side-chain "C_6" ring system (N_{i+1}−H···O_i^γ H-bond) requires an *extended* backbone ψ torsion angle for the *i*-residue that precludes the

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onset of a $3_{10}/\alpha$-helical conformation for the oligopeptide in that amino acid region. However, such an extended backbone ψ torsion angle is compatible with the $i + 1$ position of a type-II (II′) β-bend.[22] Conversely, the formation of the "C_5" ring system ($N_i-H\cdots O_i$ H-bond) is possible only if the backbone ϕ torsion angle for the *i*-residue falls in the *helical* region of the conformational space.[21]

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Supporting Information Available: Tables of crystal data and structure refinement, atomic coordinates, isotropic and anisotropic displacement parameters, bond lengths and angles, hydrogen displacement parameters, hydrogen coordinates, relevant backbone and side-chain torsion angles, and intra- and intermolecular H-bond parameters for **1** (53 pages, print/PDF). An X-ray crystallographic file, in CIF format, is available through the Web only. See any current masthead page for ordering information and Web access instructions.

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