Table IV; their bond angles are available in the supplementary material. These Na-O or Na-N distances are normal compared to those found in other sodium-coordinated compounds.[15] Each sodium ion coordinates with six (Na4) or seven (Na1-Na3) surrounding ligands. The coordination geometry could be essentially described as a tetragonally distorted octahedron, being bound to four (Na4) or five (Na1-Na3) ligands in an approximately equatorial plane and two others as a distorted axial coordination.

The other significant feature of this structure is the very elaborate network of hydrogen bonds formed between the PQQ and water molecules, and the water molecules combined with the sodium ions form an extremely tightknit molecular packing of PQQ. The schematic hydrogen-bonding network is shown in Figure 7; possible hydrogen bonds are listed in Table V, as judged from their bond distances. Each of the 10 independent water molecules, as the electron-donor or -acceptor atom, participates in three to five hydrogen-bond formations with the polar atoms of neighboring PQQ or water molecules. As is obvious from Figures 5-7, the PQQ molecules exist in the heavily hydrated and sodium-coordinated state. Since half the volume of the unit cell is occupied by these sodium ions and water molecules, it would be reasonable to some extent to consider that the PQQ molecules also exhibit a similar environment in the solution state.[27] Thus, the packing mode of the PQQ molecules observed in this crystal structure may be useful when various behaviors of the molecules in aqueous solutions are being considered.

Supplementary Material Available: Tables of anisotropic thermal parameters of non-hydrogen atoms, atomic coordinates and isotropic thermal parameters of hydrogen atoms, bond lengths and angles, torsion angles, equations of least-squares best planes along with the atomic deviations from them and the dihedral angles between them, coordination angles concerning sodium ions, and short contacts less than 3.4 Å (11 pages), Ordering information is given on any current masthead page,

(27) Preliminary ^{1}H NMR experiments of PQQ in ^2H_2O solution have shown the existence of the stacking interaction, judging from the prominent upfield shifts of aromatic protons with increasing of concentration.

First Observation of a Helical Peptide Containing a Chiral Residue without a Preferred Screw Sense

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Abstract: The molecular and crystal structure of the fully blocked, *chiral* pentapeptide Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe was determined by X-ray diffraction. It was found that the two crystallographically independent molecules (A and B), aligned in an antiparallel arrangement in the asymmetric unit, differ essentially by the *handedness* of their 3_{10}-helical structure, left-handed for A while right-handed for B. To our knowledge, this is the first observation of a helical peptide containing a chiral residue without a preferred screw sense. Conformational energy computations on the same peptide support the view that various helical structures, including two close to those found in the crystal state, have comparable stabilities.

The C^α,C^α-*di*alkylated chiral α-amino acid isovaline (Iva)[2] is a constituent of the naturally occurring peptaibol mycotoxins where it is present either as the *R* (D) or as the *S* (L) enantiomer.[3-6] In this work, the *R*,*S* nomenclature has been used throughout to avoid ambiguities, since *S*-(+)-Iva can be formally derived from either L-Abu or D-Ala,[7,8] as illustrated in Figure 1.

Conformational energy calculations of Ac-*R*-Iva-NHMe have shown that several conformations, including the fully extended (C_5) and the *right*-handed $3_{10}/\alpha$-helical conformations, represent local minima of comparable energy.[9] These results are of interest for the following reasons: (i) peptides containing Iva, with its side chains of bulkiness intermediate between those of Aib and Deg, can adopt either the helical conformations characterizing Aib peptides[10-15] or the C_5 conformation typical of Deg peptides.[16] (ii) The more stable helical conformation for the *R*-Iva enantiomer has the same handedness (right-handed) as that exhibited by the C^α-*mono*alkylated *protein* α-amino acids with *S* chirality. Only a few X-ray diffraction structures were reported so far for open-chain derivatives: mClAc-*R*-Iva-OH, which adopts the C_5 conformation (additionally stabilized by a Cl···H-N intramolecular H-bond),[17] and Boc-*R*-Iva-*S*-Hyp(Bzl)-*S*-Ala-*S*-Phol and Boc-

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(2) Abbreviations: Abu, α-aminobutyric acid; Aib, α-aminoisobutyric acid or C^α,C^α-dimethylglycine; Iva, isovaline or C^α-methyl-C^α-ethylglycine; Deg, C^α,C^α-diethylglycine; Phol, phenylalaninol; Ac, acetyl; mClAc, monochloroacetyl; Boc, (*tert*-butyloxy)carbonyl; *p*BrBz, *p*-bromobenzoyl; PChd, 3,5-di-*tert*-butyl-4-oxo-1-phenyl-2,5-cyclohexadien-1-yl; Bzl, benzyl; OMe, methoxy; NHMe, methylamino; Tfa^-, trifluoroacetate; HOBt, 1-hydroxybenzotriazole; DCC, *N*,*N*′-dicyclohexylcarbodiimide.

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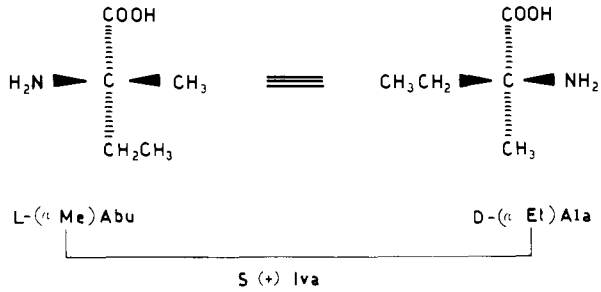

Figure 1. Configuration of Iva can be assigned to the L or D series depending upon the choice of the amino acid (Abu or Ala) from which it is formally derived. However, according to the rules of the Fischer projections, the correct designation for *S*-Iva is L.

R-Iva-*S*-Hyp(Bzl)-Aib-Phol, which fold into two consecutive type III β-bends.[18] In addition, in a *solution* conformational study on host-guest peptides it was recently shown that Iva exerts a strong helix-inducing effect, although lower than that of Aib.[19]

In this work we describe synthesis, X-ray diffraction investigation, and conformational energy calculations of Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe with the aim of (i) determining whether a guest Iva residue might be accommodated into the helical structure formed by a host $(Aib)_n$ chain and, if so, (ii) establishing a relationship between Iva α-carbon chirality and helix screw sense of the fully blocked pentapeptide.

Experimental Section

Synthesis of Peptides. *R,S*-Iva was prepared according to a modified Strecker synthesis,[20,21] and the enantiomers were resolved by enzymatic cleavage of mClAc-*R,S*-Iva-OH.[22] PChd-Aib-OH was synthesized by saponification of its methyl ester, which, in turn, was prepared electrochemically.[23] The synthesis and characterization of Ac-Aib-OH,[24-28] HCl·H-Aib-OMe,[26,28-32] and HCl·H-*S*-Iva-OMe[33] have been reported.

PChd-Aib-Aib-OMe. PChd-Aib-OH (3.0 g, 7.8 mmol) in a mixture of dichloromethane (90 mL) and *N,N*-dimethylformamide (45 mL) was activated at 0 °C by addition of HOBt (1.12 g, 8.3 mmol) and a 1 N DCC solution in dichloromethane (8 mL). After 2 h at room temperature the solution was filtered through a glass filter disk and added to a solution of HCl·H-Aib-OMe (1.20 g, 7.8 mmol) in *N,N*-dimethylformamide (20 mL). After addition of *N*-methylmorpholine to adjust the pH to 7.9–8.5 and stirring for 24 h, acetic acid was added, and the solvents were evaporated to dryness. The residue was extracted with ethyl acetate, and after filtration, the extract was washed twice each with 5% citric acid, water, and 5% $NaHCO_3$. After being dried over Na_2SO_4 and evaporation, the residue was crystallized from light petroleum–ethyl acetate: yield 3.22 g (85%); mp 123–125 °C; R_f^{II} 0.83.

Anal. Calcd for $C_{29}H_{42}N_2O_4$ (482.7): C, 72.2; H, 8.8; N, 5.8. Found: C, 72.5; H, 9.0; N, 5.8. MS (FD), *m/z* 482/483 (M^+); ^1H NMR ($CDCl_3$) δ 1.20 (s, 18 H, *t*-Bu, PChd), 1.33 (s, 6 H, CH_3, Aib^1), 1.59 (s, 6 H, CH_3, Aib^2), 1.8 (s, br, 1 H, NH, Aib^1), 3.74 (s, 3 H, OCH_3), 6.67 (s, 2 H, CH-2/6, PChd), 7.28–7.47 (m, 5 H, phenyl, PChd), 8.20 (s, br, 1 H, NH, Aib^2); ^{13}C NMR ($CDCl_3$) δ 24.77 (C^β, Aib^2), 27.49 (C^β, Aib^1), 29.14 [$C(CH_3)_3$, PChd], 34.83 [$C(CH_3)_3$, PChd], 52.50 (OCH_3), 56.48 (C^α, Aib), 60.18 (C^α, Aib), 60.40 (C-1, PChd), 125.71 (C-2′/6′, phenyl, PChd), 127.59 (C-4′, phenyl, PChd), 128.84 (C-3′/5′, phenyl, PChd), 142.76 (C-1′, phenyl, PChd), 175.10 (CO, Aib), 176.98 (CO, Aib), 186.47 (C-4, PChd); IR (KBr) 3380 (NH), 3324 (NH), 1740 (CO, Aib^2, ester), 1665 (CO, Aib^1, amide I), 1655, and 1633 (CO/C=C, quinone) cm^{-1}.

Tfa⁻·H_2^+-Aib-Aib-OMe. PChd-Aib-Aib-OMe (4.0 g, 8.3 mmol) was deprotected in a trifluoroacetic acid–dichloromethane (1:1) solution at room temperature for 20 min. The reaction mixture was extracted three times with water (40 mL). The aqueous solution was evaporated in vacuo at 50 °C, and the residue was stirred in cold diethyl ether: yield 1.99 g (76%); mp 130–131 °C; R_f^{III} 0.27; MS (FD), *m/z* 203 [(M − TFA + H)$^+$]; ^1H NMR (CD_3OD) δ 1.47 (s, 6 H, CH_3, Aib), 1.56 (s, 6 H, CH_3, Aib), 3.66 (s, 3 H, OCH_3); ^{13}C NMR (CD_3OD) δ 23.87 (C^β, Aib), 25.14 (C^β, Aib), 52.89 (OCH_3), 57.71 (C^α, Aib), 58.10 (C^α, Aib), 172.63 (CO, Aib), 176.21 (CO, Aib).

Ac-Aib-Aib-OMe. Ac-Aib-OH (9.46 g, 65.0 mmol) in *N,N*-dimethylformamide (100 mL) was activated by addition of HOBt (10.5 g, 78 mmol) and a 1 N DCC solution in dichloromethane (82 mL). After 1 h, HCl·H-Aib-OMe (10.01 g, 65.0 mmol) in *N,N*-dimethylformamide (40 mL) and *N*-methylmorpholine (13.2 g, 130 mmol) were added, followed by an additional 130 mmol of the base during the reaction. After 100 h of stirring at room temperature, acetic acid was added to adjust the pH to 6. The solvents were removed in vacuo; the residue was taken up in dichloromethane and washed with 5% citric acid, water, 5% $NaHCO_3$, and water (three times each). After evaporation of the solvent, the product was crystallized from methanol–diethyl ether: yield 4.43 g (28%); mp 146–147 °C; R_f^{II} 0.43.

Anal. Calcd for $C_{11}H_{20}N_2O_4$ (244.3): C, 54.1; H, 8.3; N, 11.5. Found: C, 54.3; H, 8.4; N, 11.7. MS (FD), *m/z* 244 (M^+); ^1H NMR (CD_3OD) δ 1.42 (s, 6 H, CH_3, Aib), 1.44 (s, 6 H, CH_3, Aib), 1.94 (s, 3 H, CH_3, Ac), 3.66 (s, 3 H, OCH_3), 4.80 (s, ~2 H, H_2O or NH); ^{13}C NMR (CD_3OD) δ 23.06 (CH_3, Ac), 25.05 (C^β, Aib), 25.33 (C^β, Aib), 52.76 (OCH_3), 57.31 (C^α, Aib), 57.76 (C^α, Aib), 172.86 (CO, Ac), 176.21 (CO, Aib), 176.72 (CO, Aib); IR (KBr) 3400/3300 (NH), 1725 (CO, Aib^2, ester), 1680/1655 (CO, Ac and Aib^1, amide I), 1513 (amide II) cm^{-1}.

Ac-Aib-Aib-OH. Ac-Aib-Aib-OMe (3.4 g, 13.9 mmol) in methanol (50 mL) was saponified by addition of 1 N NaOH (35 mL). After 6 h at room temperature, the solution was neutralized, evaporated in vacuo to dryness, and acidified to pH 3. The product precipitates out of the solution at −10 °C: yield 2.8 g (89%); mp 234–236 °C; R_f^1 0.71.

Anal. Calcd for $C_{10}H_{18}N_2O_4$ (230.3): C, 52.4; H, 7.5; N, 12.2. Found: C, 52.1; H, 7.6; N, 12.1. MS (FD), *m/z* 231 [(M + H)$^+$]; ^1H NMR (CD_3OD), δ 1.42 (s, 6 H, CH_3, Aib), 1.49 (s, 6 H, CH_3, Aib), 1.94 (s, 3 H, CH_3, Ac), 9.89 (s, br, incomplete H → D exchange, 0.4 H, OH); ^{13}C NMR (CD_3OD) δ 22.95 (CH_3, Ac), 24.81 (CH_3, Aib), 25.33 (CH_3, Aib), 57.31 (C^α, Aib), 57.85 (C^α, Aib), 172.95 (CO, Ac), 176.07 (CO, Aib), 178.32 (CO, Aib); IR (KBr) 3390/3285 (NH), 1715 (COOH), 1663/1635 (CO, Ac and Aib^1, amide I), 1555 (amide II) cm^{-1}.

Ac-Aib-Aib-*S*-Iva-OMe. Ac-Aib-Aib-OH (2.3 g, 10.0 mmol) was coupled to HCl·H-*S*-Iva-OMe (1.7 g, 10.1 mmol) in *N,N*-dimethylformamide (80 mL) by addition of HOBt (1.6 g, 11.9 mmol) and a 1 N DCC solution in dichloromethane (15 mL), followed by *N*-methylmorpholine to adjust the pH to 8.0–8.5. After 3 days at 50 °C the crude product was isolated as described above for Ac-Aib-Aib-OMe. In order to remove residual *N,N*′-dicyclohexylurea, it was suspended in diethyl ether, filtered, and crystallized from chloroform: yield 1.3 g (38%); mp 159 °C; R_f^{II} 0.33, R_f^{III} 0.63.

Anal. Calcd for $C_{16}H_{29}N_3O_5$ (343.4): C, 56.0; H, 8.5; N, 12.2. Found: C, 56.3; H, 8.4; N, 12.0. MS (FD), *m/z* 343 (M^+), 344 [(M + H)$^+$]; ^1H NMR (CD_3OD) δ 0.85 (t, 3 H, CH_2CH_3, Iva), 1.39 (s, 6 H, CH_3, Aib), 1.40 (s, 6 H, CH_3, Aib), 1.42 (s, 3 H, CH_3, Iva), 1.79–1.97 (2 m, due to magnetic nonequivalence, 1 H each, CH_2–CH_3, Iva), 1.97 (s, 3 H, CH_3, Ac), 3.65 (s, 3 H, OCH_3); ^{13}C NMR (CD_3OD) δ 8.38 (CH_2–CH_3, Iva), 21.95 (CH_3, Iva), 23.15 (CH_3, Ac), 25.14$_4$* (CH_3, Aib^1), 25.16$_4$* (CH_3, Aib^1), 25.48* (CH_3, Aib^2), 25.72* (CH_3, Aib^2), 30.95 (CH_2–CH_3, Iva), 52.42 (OCH_3), 57.58** (C^α, Aib), 57.69** (C^α, Aib), 60.76** (C^α, Iva), 173.09*** (CO, Ac), 175.77*** (CO, Iva), 176.17*** (CO, Aib), 176.33*** (CO, Aib) [(*) denotes the assumption that magnetic nonequivalence of Aib-C^β decreases with decreasing distance to Iva; (**) denotes comparison with Ac-Aib-Aib-*S*-Iva-OH; (***) denotes comparison with Ac-Aib-Aib-OMe]; IR (KBr) 3400/3365/3315/3290 (NH), 1730 (CO, ester), 1665/1645 (amide I), 1553 (amide II) cm^{-1}.

Ac-Aib-Aib-*S*-Iva-OH. Ac-Aib-Aib-*S*-Iva-OMe (1.38 g, 4.03 mmol) in methanol (15 mL) was saponified by addition of 1 N NaOH (12 mL).

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After 22 h at 20 °C and 2 h at 50 °C, the solution was acidified and extracted with ethyl acetate: yield 840 mg (63%); mp 205–208 °C; R_f^{I} 0.52, R_f^{III} 0.60.

Anal. Calcd for $C_{15}H_{27}N_3O_5$ (329.4): C, 54.7; H, 8.3; N, 12.8. Found: C, 54.6; H, 8.0; N, 12.6. MS (FD), m/z 330 [$(M + H)^+$], 659 [$(2M + H)^+$]; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, CH_2–CH_3, Iva), 1.40 (s, 6 H, CH_3, Aib), 1.41 (s, 6 H, CH_3, Aib), 1.45 (s, 3 H, CH_3, Iva), 1.84–2.09 (m, 2 H, CH_2–CH_3, Iva), 2.00 (s, 3 H, CH_3, Ac), 7.52 (br, NH), 7.65 (br, NH), 8.12 (br, NH); ^{13}C NMR (CD_3OD) δ 8.52 (CH_2–CH_3, Iva), 22.25 (CH_3, Iva), 23.10 (CH_3, Ac), 25.18$_9$* (CH_3, Aib^1), 25.22$_3$* (CH_3, Aib^1), 25.47* (CH_3, Aib^2), 25.58* (CH_3, Aib^2), 30.63 (CH_2–CH_3, Iva), 57.65 (C^α, Aib), 57.88 (C^α, Aib), 60.93 (C^α, Iva), 173.09 (CO, Ac), 176.06 (CO, Aib), 176.13 (CO, Aib), 177.65 (CO, Iva) [(*) denotes the assumption that the splitting of the geminal Aib methyl groups is larger for the Aib linked to Iva]; IR (KBr) 3315 (NH, OH), 1735 (COOH), 1660 (amide I), 1530 (amide II) cm^{-1}.

Ac-Aib-Aib-*S*-Iva-Aib-Aib-OMe. Ac-Aib-Aib-*S*-Iva-OH (440 mg, 1.33 mmol) was coupled to $Tfa^-{\cdot}H_2^+$-Aib-Aib-OMe (420 mg, 1.33 mmol) in a dichloromethane–*N*,*N*-dimethylformamide (9:1) solution (40 mL) by addition of HOBt (200 mg, 1.5 mmol) and a 1 N DCC solution in dichloromethane (1.7 mL). The pH was adjusted to 7.5–8.0 with *N*-methylmorpholine. After 6 days at 45 °C the product was isolated as described for Ac-Aib-Aib-OMe, chromatographed on Sephadex LH20 with methanol, and crystallized from chloroform: yield 94 mg (18%); mp 261–264 °C; R_f^{II} 0.62, R_f^{IV} 0.25.

Anal. Calcd for $C_{24}H_{43}N_5O_7$ (513.6): N, 13.6. Found: N, 13.5. The determination of the optical purity by the *o*-phthalaldehyde–Boc-*S*-Cys(H)-OH method of the hydrolysate revealed no *R*-Iva. MS (FD), m/z 514 (M^+), 369 (A_4), 284 (A_3), 229 (M – A_3), 185 (A_2); ^{1}H NMR ($^{12}CDCl_3/^{12}CD_3OD$, 1:1) δ 0.84 (t, 3 H, CH_2–CH_3, Iva), 1.36$_5$ and 1.37$_2$, 1.44$_5$ and 1.45$_3$, and 1.46$_7$ and 1.47$_5$ (6 s, 3 H each, CH_3, Aib), 1.40$_4$ (s, 6 H, CH_3, Aib), 1.38$_7$ (s, 3 H, CH_3, Iva), 1.71 and 1.97 (2 m, due to magnetic nonequivalence, 1 H each, CH_2–CH_3, Iva), 1.98 (s, 3 H, CH_3, Ac), 3.65 (s, 3 H, OCH_3), 7.63 (s, 1 H, NH), 7.67 (s, 1 H, NH), 7.72 (s, 1 H, NH); ^{13}C NMR ($^{12}CDCl_3/^{12}CD_3OD$, 1:1) δ 8.22 (CH_2–CH_3, Iva), 21.71 (CH_3, Iva), 23.18 (CH_3, Ac), 24.81, 24.90, 24.99, 25.04, 25.36, 25.45, 25.66, and 26.14 (8 s, 3 H each, CH_3, Aib), 30.71 (CH_2–CH_3, Iva), 52.45 (OCH_3), 56.70, 57.15, 57.34, and 57.43 (4 C^α, Aib), 60.70, 60.79 (C^α, Iva), 172.67 (CO, Ac), 175.55, 175.73, 176.33, 176.43, 176.63 (CO, Aib and Iva); IR (KBr) 3420/3312/3271 (NH), 1733 (CO, ester), 1665/1647 (amide I), 1539 (amide II) cm^{-1}.

Analytical Procedures. Thin-layer chromatography was performed on silica gel plates 60 F_{254} (Merck) in the following solvent systems: (I) chloroform–methanol–acetic acid–water, 65:25:3:4; (II) chloroform–methanol, 8:1; (III) 1-butanol–acetic acid–water, 3:1:1; (IV) acetone. *N*,*N*′-Dicyclohexylurea was used as standard: R_f^{I} 0.83; R_f^{II} 0.65; R_f^{III} 0.91; R_f^{IV} 0.64. The chromatograms were developed with ninhydrin and chlorine–4,4′-bis(dimethylamino)diphenylmethane. Melting points were determined in a Tottoli apparatus. C, H, and N elemental analyses were carried out on a Perkin-Elmer Model 240 elemental analyzer. The optical purity was determined by HPLC with the *o*-phthalaldehyde–Boc-*S*-Cys(H)-OH method.[34,35]

Proton (400 MHz) and carbon-13 (100.6 MHz) nuclear magnetic resonance spectra were obtained on a Bruker WM 400 spectrometer. IR absorption spectra were measured on a Perkin-Elmer Model 21 IR spectrophotometer. Field-desorption mass spectra were recorded with a Varian MAT 711 mass spectrometer.

X-ray Diffraction. Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe crystallizes (from an acetone solution by slow evaporation) in the triclinic crystal system (space group *P*1) with a = 14.767 (2) Å, b = 11.548 (2) Å, c = 8.735 (2) Å, α = 93.8 (2)°, β = 90.3 (2)°, γ = 93.7 (2)°, V = 1483 Å^3, Z = 2, μ = 0.8 cm^{-1}, and d_c = 1.148 g·cm^{-3}.

A total of 7171 independent reflections were measured on a Philips PW 1100 four-circle diffractometer operating in the $\theta/2\theta$ scan mode to 2θ = 56° (scan width 1.2° and scan speed 0.03° s^{-1}) with graphite monochromatized Mo Kα radiation (λ = 0.7107 Å). No absorption correction was applied. During data collection, three standard reflections with 10% intensity variation were measured every 180 min to check the stability of the crystal and the electronics.

The structure was solved by centrosymmetric direct methods with MULTAN 80;[36] however, the C-13 atom was shown as linked to the C-14 and to the C-12 atoms as well with 50:50 statistical weights. Once the *S* chirality of the title compound was imposed, the structure was refined as *P*1 with anisotropic thermal parameters for all non-hydrogen atoms (w = 1). All H atoms were calculated and not refined. All calculations were performed on the IBM 370/158 computer of the University of Padova with SHELX 76[37] and its atomic scattering values. The final conventional R factor for 4752 reflections considered observed [$I \geq 3\sigma(I)$] was 0.084. Tables of atomic positional and thermal parameters, bond lengths, and bond angles have been deposited as supplementary material.

Conformational Energy Calculations: Bond Lengths and Bond Angles. The standard geometry of ECEPP/2[38–40] was used for the N- and C-terminal groups. The two geometries adopted for the Aib residues were those reported in ref 15. For Iva, the angles between the bonds to the substituents on the C^α atom and the τ (C^α–C^β–C^γ) angle in the ethyl side chain were taken from the crystal structure of mClAc-*R*-Iva-OH.[17] All other bond angles and all bond lengths correspond to ECEPP/2 geometries of amino acid residues.

Potentials and Energy Parameters. Conformational energies were calculated with a modified version of ECEPP/2. The potential-energy functions and energy parameters of Scheraga and co-workers[39,40] were used without change. Partial charges of the Aib residue were taken from ref 15; those of the Iva residue were obtained from CNDO/2 calculations, performed on Ac-Iva-NHMe. To be consistent with the ECEPP/2 procedure, partial charges were calculated as "overlap normalized" electron densities [CNDO(ON)].[39] Partial charges for the backbone atoms, except for C^α, were close to those derived for other amino acid residues.[39] Therefore, ECEPP standard values for these atoms were used. For C^α and the side-chain carbon atoms, the values were as follows: C^α, 0.104 ecu; C^β_{methyl}, −0.106 ecu; C^β_{ethyl}, −0.042 ecu; H, 0.031 ecu (ecu = electronic charge unit).

Location of Low-Energy Conformations. Conformational energies are generally expressed as $E - E_o$, where E_o is the energy of the lowest energy conformation. Starting positions for the exploration of the minimum energy conformations of the pentapeptide were derived from calculations on Ac-$(Aib)_2$-*R*-Iva-OMe, Ac-$(Aib)_2$-*R*-Iva-NHMe, and Ac-$(Aib)_2$-NHMe.[41] These starting conformations were minimized with respect to dihedral angles with ECEPP/2 in conjunction with a minimizing (numerically differentiating) subroutine.[42] The process was terminated when the conformational energy changed by less than 0.001 kcal/mol between two subsequent calculations.

Results and Discussion

Despite their potential interest in pharmacology and biophysics, only a very limited number of papers have dealt with the synthetic aspects of Iva-containing peptides.[18,19,33,43,44] We have synthesized the pentapeptide Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe by reacting Ac-$(Aib)_2$-*S*-Iva-OH with H-$(Aib)_2$-OMe in the presence of DCC/HOBt. The N-blocked tripeptide Ac-$(Aib)_2$-*S*-Iva-OH, in turn, was prepared by saponification of its methyl ester derivative, while the C-protected dipeptide H-$(Aib)_2$-OMe was obtained by trifluoroacetolytic cleavage of the PChd group from PChd-$(Aib)_2$-OMe. The latter, fully protected dipeptide was synthesized from PChd-Aib-OH and H-Aib-OMe, as described above for the pentapeptide. The PChd group is effectively favoring the coupling reaction with H-Aib-OMe (PChd-Aib-Aib-OMe, yield 85%) with respect to the acetyl group (Ac-Aib-Aib-OMe, yield 28%). The chemical purity of the final compounds and the various synthetic intermediates was analyzed by ^{1}H and ^{13}C nuclear magnetic resonance, infrared absorption spectroscopy, and mass spectrometry.

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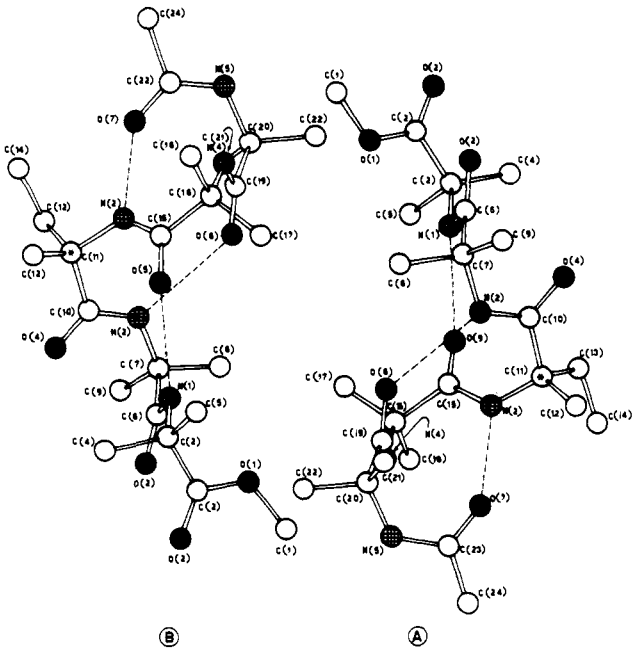

Figure 2. A perspective view of the antiparallel alignment of the two independent molecules (A and B) in the asymmetric unit of Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe with numbering of the atoms. The chiral α-carbons are starred. Intramolecular H-bonds are indicated as dashed lines.

A perspective view of the two independent molecules (A and B) aligned in an antiparallel arrangement in the asymmetric unit of Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe, together with the numbering of the atoms, is illustrated in Figure 2. Table I lists the torsion angles.

The geometrical parameters of acetamido[45-47] and methyl ester[48] groups, Aib[15,46,49] and Iva[17,18] residues, and peptide units[50,51] compare well with literature values. In particular, we confirm the asymmetry of the bond angles at the C^α atom of the Aib residues.[15,46,49] The critical N-C^α-C' (τ) bond angle of the Iva residue, 111.1 (11)° in molecule A and 110.6 (10)° in molecule B, is remarkably different from that observed in molecule A, 103.2 (3)°, and in molecule B, 105.2 (3)°, of the *fully extended* mClAc-*R*-Iva-OH, a clear, although preliminary, indication of the different conformations adopted by this residue in the two compounds.

The succession of similar pairs of *positive* ϕ,ψ values[52] along the first four residues in the chain of molecule A gives rise to a helical structure, which can be described as a *left*-handed 3_{10}-helix, very close to the ideal case (54.0°,28.4°).[53] The critical ψ torsion angles range from 26.9 (24)° to 36.4 (24)°. The helical structure is formed by three consecutive type III' β-bends (4→1 intramolecularly hydrogen-bonded structures).[54-56] The three intramolecular hydrogen bonds occur between the N-H groups of the Iva[3], Aib[4], and Aib[5] residues and the C=O groups of the acetamido moiety and the Aib[1] and Aib[2] residues [the N···O distances are 3.015 (16) Å, 2.938 (15) Å, and 2.990 (17) Å, respectively[57,58]].

The α-helical structure, although having pairs of ϕ,ψ torsion angles (55°,45°) close to those of the 3_{10}-helix, is not compatible with the observed hydrogen-bonding scheme, being characterized

Table I. Torsion Angles (deg) for Molecules A and B of Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe (with ESD's in Parentheses)

	molecule A	molecule B
C(1)-O(1)-C(2)-C(3)	-179.7 (16)	174.8 (16)
C(1)-O(1)-C(2)-O(2)	-4.2 (29)	-3.1 (28)
C(3)-N(1)-C(6)-O(3)	8.2 (29)	-7.5 (31)
C(3)-N(1)-C(6)-C(7)	-177.9 (17)	179.6 (16)
C(6)-N(1)-C(3)-C(2)	-52.5 (23)	48.2 (24)
C(6)-N(1)-C(3)-C(5)	-163.5 (17)	166.6 (19)
C(6)-N(1)-C(3)-C(4)	78.7 (23)	-71.8 (22)
C(7)-N(2)-C(10)-O(4)	4.5 (31)	-1.3 (32)
C(7)-N(2)-C(10)-C(11)	-178.3 (16)	-179.1 (18)
C(10)-N(2)-C(7)-C(6)	52.0 (24)	-53.4 (25)
C(10)-N(2)-C(7)-C(9)	-70.7 (23)	66.7 (24)
C(10)-N(2)-C(7)-C(8)	168.9 (18)	-171.0 (18)
C(11)-N(3)-C(15)-O(5)	3.7 (31)	1.0 (32)
C(11)-N(3)-C(15)-C(16)	-179.9 (17)	-175.1 (17)
C(15)-N(3)-C(11)-C(10)	55.9 (24)	-55.3 (24)
C(15)-N(3)-C(11)-C(13)	-65.0 (24)	-169.8 (18)
C(15)-N(3)-C(11)-C(12)	169.9 (17)	66.9 (24)
C(16)-N(4)-C(19)-O(6)	0.4 (29)	-1.4 (35)
C(16)-N(4)-C(19)-C(20)	173.8 (17)	179.2 (17)
C(19)-N(4)-C(16)-C(15)	53.0 (24)	-48.7 (25)
C(19)-N(4)-C(16)-C(18)	167.0 (18)	-166.6 (19)
C(19)-N(4)-C(16)-C(17)	-71.2 (24)	71.4 (25)
C(20)-N(5)-C(23)-O(7)	-0.6 (32)	3.8 (33)
C(20)-N(5)-C(23)-C(24)	179.3 (18)	-174.7 (18)
C(23)-N(5)-C(20)-C(19)	50.2 (25)	-55.1 (25)
C(23)-N(5)-C(20)-C(22)	169.0 (19)	-175.3 (20)
C(23)-N(5)-C(20)-C(21)	-71.0 (25)	65.0 (25)
O(1)-C(2)-C(3)-N(1)	-44.4 (22)	47.2 (20)
O(2)-C(2)-C(3)-N(1)	140.0 (18)	-135.0 (20)
O(2)-C(2)-C(3)-C(4)	8.2 (25)	-15.8 (26)
O(1)-C(2)-C(3)-C(4)	-176.1 (16)	166.4 (15)
O(2)-C(2)-C(3)-C(5)	-110.4 (20)	104.8 (22)
O(1)-C(2)-C(3)-C(5)	65.3 (20)	-73.0 (19)
O(3)-C(6)-C(7)-N(2)	-151.4 (19)	147.7 (19)
N(1)-C(6)-C(7)-N(2)	34.6 (23)	-39.4 (24)
N(1)-C(6)-C(7)-C(8)	-80.4 (21)	82.4 (21)
O(3)-C(6)-C(7)-C(8)	93.6 (22)	-90.5 (22)
N(1)-C(6)-C(7)-C(9)	155.0 (18)	-161.8 (17)
O(3)-C(6)-C(7)-C(9)	-31.0 (26)	25.4 (25)
O(4)-C(10)-C(11)-N(3)	-154.6 (19)	153.8 (19)
N(2)-C(10)-C(11)-N(3)	28.2 (24)	-28.3 (24)
N(2)-C(10)-C(11)-C(12)	-88.4 (20)	-150.5 (18)
O(4)-C(10)-C(11)-C(12)	88.8 (22)	31.6 (26)
N(2)-C(10)-C(11)-C(13)	151.9 (18)	86.0 (20)
O(4)-C(10)-C(11)-C(13)	-30.8 (25)	-91.9 (22)
C(10)-C(11)-C(13)-C(14)	173.8 (17)	168.0 (14)
N(3)-C(11)-C(13)-C(14)	-63.4 (23)	-74.4 (17)
C(12)-C(11)-C(13)-C(14)	59.5 (22)	46.7 (19)
O(5)-C(15)-C(16)-N(4)	-156.7 (20)	148.3 (18)
N(3)-C(15)-C(16)-N(4)	26.9 (24)	-35.3 (24)
N(3)-C(15)-C(16)-C(17)	151.2 (18)	-157.4 (18)
O(5)-C(15)-C(16)-C(17)	-32.4 (28)	26.2 (24)
N(3)-C(15)-C(16)-C(18)	-86.5 (21)	83.8 (22)
O(5)-C(15)-C(16)-C(18)	89.9 (24)	-92.6 (22)
O(6)-C(19)-C(20)-N(5)	-150.4 (20)	150.6 (19)
N(4)-C(19)-C(20)-N(5)	36.4 (24)	-30.0 (25)
N(4)-C(19)-C(20)-C(21)	160.4 (18)	-148.2 (19)
O(6)-C(19)-C(20)-C(21)	-26.5 (28)	32.4 (26)
N(4)-C(19)-C(20)-C(22)	-81.4 (22)	90.4 (23)
O(6)-C(19)-C(20)-C(22)	91.7 (25)	-89.1 (24)

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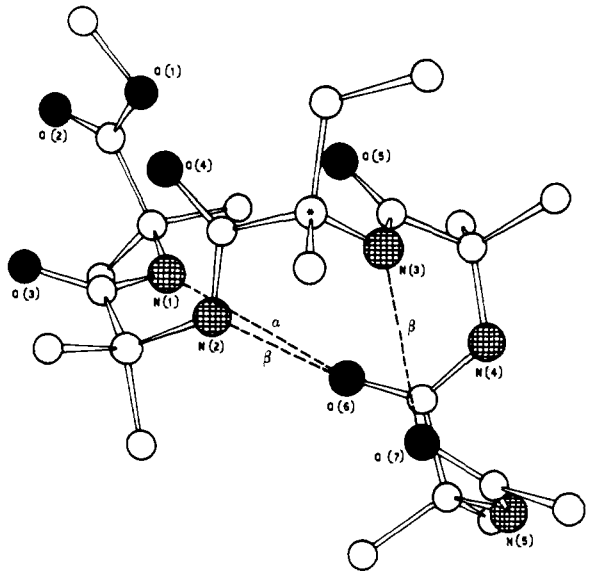

Figure 3. Conformation corresponding to the absolute energy minimum for Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe (conformation 1 in Table II). Intramolecular H-bonds are shown as dashed lines. The type of bend structure (whether α or β) is indicated, and the chiral α-carbon is starred.

by 5→1 intramolecularly hydrogen-bonded forms (α-bends[55]). The signs of the ϕ,ψ torsion angles of the C-terminal Aib residue are *opposite* with respect to those of the preceding residues, a general observation for Aib-rich 3_{10}-helices.[12-15] The $\chi^{1,1}$ torsion angle, defining the orientation of the Iva ethyl side chain, is −63.4 (23)° (g^-).

Interestingly, also molecule B is folded in a 3_{10}-helix, with ϕ,ψ angles very close to those of molecule A, but its *screw sense* is reversed (*right-handed*). Nevertheless, the $\chi^{1,1}$ angle is still g^- [−74.4 (17)°].

The methyl ester group of both molecules adopts a conformation with respect to the C–N bond intermediate between the *anticlinal* and *antiperiplanar* conformations,[59] the O(2)–C(2)–C(3)–N(1) torsion angle being 140.0 (18)° for molecule A and −135.0 (20)° for molecule B. The secondary acetamido[45,46] and peptide groups[50,51] in both molecules A and B are in the usual trans conformation with no significant deviations from planarity. The ester group is also trans planar,[48] the C(1)–O(1)–C(2)–C(3) angle being −179.7 (16)° for molecule A and 174.8 (16)° for molecule B.

In the crystal packing no short contacts (less than 3.50 Å) are seen between A and B molecules. Rather, rows of A molecules are held together through (acetamido) N—H···C=O (Aib^4) intermolecular hydrogen bonds along the *y* direction. The O(3)···N(5) ($x, y + 1, z$) separation is 2.777 (12) Å. A similar hydrogen-bonding motif, with an O(3)···N(5) ($x, y - 1, z$) separation of 2.840 (13) Å interconnects the B molecules along the same direction.

Conformational energy calculations of Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe were performed with a modified ECEPP/2 program. Both the tetrahedral symmetric geometry for the substituents on the α-carbon atom of the Aib residues and the asymmetric geometry, derived from well-defined X-ray diffraction structures, were used in the calculations.[15] The minimum energy conformations are listed in Table II, and the conformation corresponding to the absolute energy minimum 1 is shown in Figure 3.

Conformation 1 is a *left-handed* helical structure, intermediate between a 3_{10}- and an α-helix. In particular, the ψ values range from 36.2° to 50.5°, and three intramolecular hydrogen bonds are seen between the N–H groups of Iva^3, Aib^4, and Aib^5 residues and the C=O groups of the acetamido moiety and the Aib^1 residue (the latter acting as a double acceptor[60]). The N(3)···O(7), N(2)···O(6), and N(1)···O(6) distances are 2.87, 3.06, and 3.26 Å, while the N(3)–H···O(7), N(2)–H···O(6), and N(1)–H···O(6) distances are 2.02, 2.44, and 2.31 Å, respectively. Therefore, the C-terminal β-bend observed in the X-ray structure is missing, being replaced by a weak α-bend. In addition, the conformation of the Iva ethyl side chain is still g^- (−57.1°), as found in the crystal state, but the ϕ,ψ angles of the C-terminal Aib have the *same* signs as those of the preceding residues.

Table II. Minimum Energy Conformations[a] for Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe

no.		ϕ	ψ	$\chi^{1,1}$ [b]	ϕ	ψ	ΔE[c]
1[d]	Aib^1–Aib^2	53.4	36.2		52.8	42.3	
	S-Iva^3	59.8	50.5	−57.1			0.000
	Aib^4–Aib^5	61.4	43.1		55.5	40.4	
2[d]	Aib^1–Aib^2	−52.1	−38.8		−53.6	−43.1	
	S-Iva^3	−67.4	−47.2	−65.8			0.032
	Aib^4–Aib^5	−60.2	−44.3		−55.8	−39.6	
3[d]	Aib^1–Aib^2	54.7	34.4		53.5	37.5	
	S-Iva^3	58.3	47.5	−57.2			0.248
	Aib^4–Aib^5	60.3	35.0		−51.1	−38.3	
4[d]	Aib^1–Aib^2	−50.4	−46.3		−56.9	−44.8	
	S-Iva^3	−67.9	−44.5	−65.9			0.379
	Aib^4–Aib^5	−57.7	−38.2		51.2	38.1	
5[e]	Aib^1–Aib^2	49.6	51.4		55.4	47.3	
	S-Iva^3	60.1	48.9	−57.6			1.831
	Aib^4–Aib^5	57.0	48.0		53.8	45.8	
6[e]	Aib^1–Aib^2	−49.6	−50.5		−55.1	−45.9	
	S-Iva^3	−66.9	−45.6	−66.3			1.948
	Aib^4–Aib^5	−56.3	−48.8		−54.2	−44.9	
7[f]	Aib^1–Aib^2	50.2	36.4		53.0	26.9	
	S-Iva^3	55.9	28.2	−63.4			
	Aib^4–Aib^5	52.0	34.6		−52.5	−44.4	
8[f]	Aib^1–Aib^2	−55.1	−30.0		−48.7	−35.3	
	S-Iva^3	−55.3	−28.3	−74.4			
	Aib^4–Aib^5	−53.4	−39.4		48.2	47.2	

[a] The energies are given in units of kcal/mol. [b] N(3)–C(11)–C(14)–C(13) torsion angle. [c] E_o = 6.750 kcal/mol. [d] Using *asymmetric* geometry for the substituents on the C^d atom of the Aib residues. [e] Using *symmetric* geometry. [f] These values, given for comparison, are those obtained from the X-ray diffraction analysis (7 corresponds to molecule A, while 8 to molecule B).

The diastereomeric *right-handed* helical structure 2 is only slightly higher in energy (ΔE = 0.032 kcal/mol). This result is in agreement with our published data on Ac-*R*-Iva-NHMe.[9] The two diastereomeric conformations 3 and 4, closely resembling A and B observed in the crystal state (in these two helices the signs of the ϕ,ψ angles of the C-terminal residue are opposite with respect to those of the preceding residues), do not differ greatly in energy from conformation 1 (ΔE = 0.248 and 0.379 kcal/mol, respectively). In any case, we attribute the experimentally observed reversal of helicity of the C-terminal residue to packing forces operative in the crystal state. Conformations 1–4 have been calculated by use of Aib α-carbon asymmetric geometry, typically found in crystal structure analyses.[15,46] However, conformations 5 and 6, generated by use of symmetric geometry, are 1.831 and 1.948 kcal/mol less stable and exhibit torsion angles closely resembling those of the ideal α-helical structure (from 44.9° to 51.4°). Similar results on the sensitivity of peptide conformation to geometry have been reported by Paterson et al.[15] for Ac-$(Aib)_n$-NHMe (n = 1–3).

Conclusions

We have previously shown that in the crystal state an Iva residue can be accommodated in a *fully extended* conformation.[17] Recently, Marshall et al.[18] described the crystal structure of two N^α-protected tetrapeptides having an Iva residue in position 1. In both cases, two consecutive type III β-bends are observed. In

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this work the X-ray diffraction study of an Iva-containing 3_{10}-*helical* peptide is reported. These findings agree well with the results of our conformational energy computations of the Iva monopeptide.[9]

However, on the basis of the present crystallographic analysis we have been unable to correlate Iva configuration with helix handedness. Surprisingly, it appears that a single *chiral* Iva residue is *not* sufficient to induce a preferential handedness in a 3_{10}-helical, Aib-rich pentapeptide. As a result, both right- and left-handed helices concomitantly occur in the same crystal, as already observed for fully blocked, *achiral* $(Aib)_5$ homopeptides.[61-63] This result is in contrast with that obtained in an X-ray diffraction analysis of *p*BrBz-$(Aib)_n$-*S*-Leu-$(Aib)_2$-OMe (n = 4, 5), which have been shown to adopt the expected *right*-handed 3_{10}-helical structure in the crystal state,[64] and, to our knowledge, represents the *first observation* of a helical peptide containing a chiral residue *not* showing a discrimination between screw senses under these conditions. Work is in progress to determine (*i*) the minimum length of the R side chain in the chiral -NH-C(CH_3)R-CO- X residue of Ac-$(Aib)_2$-X-$(Aib)_2$-OMe and/or (ii) the minimum number and main-chain position of Iva residues in an N-acetylated, C-methoxylated Aib/Iva pentapeptide required to induce a preferential screw sense.

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Registry No. PChd-Aib-OH, 74763-76-1; H-Aib-Ome·HCl, 15028-41-8; PChd-Aib-Aib-OMe, 121141-82-0; H-Aib-Aib-OMe·TFA, 121141-83-1; Ac-Aib-OH, 5362-00-5; Ac-Aib-Aib-OMe, 121141-84-2; Ac-Aib-Aib-OH, 118724-99-5; H-*S*-Iva-OMe·HCl, 92760-72-0; Ac-Aib-Aib-*S*-Iva-OMe, 121141-85-3; Ac-Aib-Aib-*S*-Iva-OH, 121141-86-4; Ac-Aib-Aib-*S*-Iva-Aib-Aib-OMe, 121141-87-5.

Supplementary Material Available: Tables of angles and bond lengths for molecules A and B of Ac-$(Aib)_2$-(*S*)-Iva-$(Aib)_2$-OMe (2 pages). Ordering information is given on any current masthead page.

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Tetraalkylammonium Salts and Phospholipid Polymorphism

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Abstract: A series of tetraalkylammonium salts were tested for their ability to change the L_α-H_{II} phase transition temperature of dielaidoylphosphatidylethanolamine. Tetrapropylammonium bromide has little effect on this transition while tetrabutyl- and tetrapentylammonium bromides increase the transition temperature by 5 and 15 K/mol fraction, respectively. The longer chain tetrahexyl- and tetraoctylammonium bromides cause a rate of change of this transition temperature of -85 and -650 K/mol fraction of additive. This decrease in transition temperature was independent of the concentration or nature of the anion. It is concluded that long-chain tetraalkylammonium salts partition into the hydrophobic region of phospholipids as dissociated cations and they are potent promoters of the H_{II} phase. We estimate that approximately 23 kcal/mol of hydrophobic and van der Waals interactions is required to compensate for the unfavorable free energy change of bringing a charged group into the membrane.

Phosphatidylethanolamines readily interconvert between a planar bilayer (L_α) structure and an aggregate of water-filled cylinders, the inverted hexagonal phase (H_{II}).[1] The temperature at which this phase transition occurs is very sensitive to the presence of low concentrations of hydrophobic substances.[2] Only substances that partition mainly into the hydrophobic region of the membrane are good hexagonal-phase promoters. Inorganic salts, at high concentration, also modulate the L_α-H_{II} transition through their effects on lipid solvation.[3] In this work we wish to determine how a series of homologous tetraalkylammonium salts can modify the L_α-H_{II} phase transition temperature of dielaidoylphosphatidylethanolamine (DEPE). Tetraalkylammonium salts with short alkyl chain lengths will not penetrate deeply into the lipid structure. Hence, these salts will cause the membrane surface to expand, and they will introduce electrostatic repulsion, especially in the H_{II} phase. These factors would raise the L_α-H_{II} transition temperature. Increasing the alkyl chain length initially should favor increased partitioning from water into the membrane phase as well as cause a greater increase in headgroup area. Both factors would lead to an increase in the L_α-H_{II} transition temperature. We also wished to determine if the tetraalkylammonium salts would eventually become sufficiently hydrophobic to penetrate into the bilayer and promote H_{II}-phase formation. This would indicate the degree of hydrophobicity which is required to bring a charged group into a membrane.

Experimental Section

Materials. DEPE was purchased from Avanti Polar Lipids, Birmingham, AL. Its purity was ascertained by its phase transition characteristics as determined by differential scanning calorimetry (DSC). Tetraalkylammonium bromides were purchased from Aldrich Chemical Co., Milwaukee, WI.

Sample Preparation. DEPE was codissolved with varying amounts of a tetraalkylammonium bromide in $CHCl_3$/methanol (2/1 v/v). The

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