C^{α}-Methyl, C^{α}-*n*-Propylglycine Homo-oligomers

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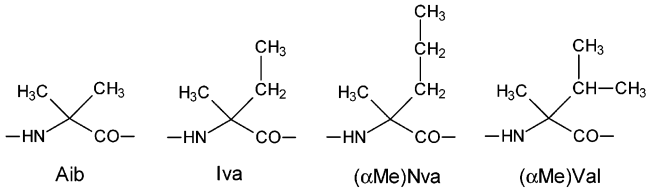
Received June 13, 2003; Revised Manuscript Received August 4, 2003

ABSTRACT: A series of N^{α}-protected, monodispersed homo-oligopeptide esters to the octamer level from L-C^{α}-methyl, C^{α}-*n*-propylglycine [or C^{α}-methylnorvaline, (αMe)Nva] has been synthesized by solution methods and fully characterized. The preferred conformation of these homo-oligomers in solution has been assessed by FT-IR absorption and ^{1}H NMR techniques. Moreover, the molecular structures of the homotrimer and homotetramer have been determined in the crystal state by X-ray diffraction. The obtained results strongly support the view that right-handed, single or multiple, and consecutive β bends are preferentially adopted by the conformationally restricted L-(αMe)Nva homo-oligomers. In particular, 3_{10} helices are formed by the longest homo-oligomers. It is our contention that the [(αMe)Nva]$_n$ peptides represent the best available choice among C^{α}-tetrasubstituted α-amino acid-based homo-oligomers for the construction of relatively easy to make, rigid foldamers with a well-defined screw-sense bias.

Introduction

A proper understanding of the nature of electron- and energy-transfer processes depends heavily upon our ability to design and synthesize conformationally constrained structures, the intercomponent geometry (rigid but precisely tunable) of which would be well defined. To this end, we have recently reported a few studies on the exploitation of short 3_{10}-helical peptides as spacers.[1−4] The 3_{10} helix, first predicted to be a reasonably stable peptide secondary structure more than 60 years ago, has only recently attracted the attention of structural biochemists.[5−7] It represents the third principal long-range 3D structure occurring in globular proteins and has been described at atomic resolution in model peptides and in peptaibol antibiotics. Its promising role as a spacer for spectroscopic and electrochemical applications is just emerging.

In this connection, the conformational preferences of homo-oligomers based on C^{α}-methylated α-amino acids with a linear, saturated aliphatic side chain of increasing length have particularly attracted our attention.[8−12]



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The experimental results obtained convincingly support our view that the Aib (α-amino isobutyric acid) and Iva (isovaline) residues are strong β-turn[13−15] and 3_{10}-helix formers (depending on the peptide main-chain length) and are much more efficient than their C^{α}-unmethylated parent amino acids. However, in terms of the screw-sense bias, Aib is achiral, thereby forming isoenergetic, equally probable, enantiomeric, right- and left-handed homo-oligomeric 3_{10}-helices. Peptides based on Aib and protein (L-) amino acids can indeed provide helical structures with a preferred (right-handed) screw sense; however, they tend to fold into *mixed* 3_{10}-/α-helical structures, which makes it difficult to determine their end-to-end distance precisely. Iva is chiral, but the small difference (one carbon atom only) between the two aliphatic substituents on its C^{α} atom does allow the formation of only a relatively limited, although sizable, excess of one 3_{10}-helix screw sense over the other in its homo-oligomers. (Likewise, with protein amino acids, the L residue gives a predominantly right-handed helix.[16,17]) However, the homopeptides from the C^{β}-branched, C^{α}-methylated α-amino acid (αMe)Val (C^{α}-methyl valine) are folded in rigid 3_{10}-helical structures (at least if very polar and protic solvents are avoided)[18,19] with a strong screw-sense bias.[20] However, with the coupling methods available to date, the high steric hindrance of the (αMe)Val residue, particularly at its nucleophilic amino function, prevents one from preparing a homo-oligomeric series in good yield and in a relatively short time.[20]

The work reported in this article was aimed at searching for an acceptable compromise between the 3D structural and reactivity properties required by a homo-

10.1021/ma030327v CCC: $25.00
Published on Web 09/18/2003

oligopeptide series from a C^{α}-methylated α-amino acid to be an attractive spacer system. On the basis of the results obtained, it is our contention that the (αMe)-Nva (C^{α}-methyl norvaline) homo-oligomeric series does represent the best choice currently available for a set of relatively easy to prepare, rigid, strongly 3_{10}-helix screw-sense-biased peptide spacers. Here we describe the synthesis, full chemical characterization, and conformational analysis in the crystal state (by X-ray diffraction) and in solution (by FT-IR absorption and ^{1}H NMR techniques) of the Z-[L-(αMe)Nva]$_n$-O*t*Bu (Z, benzyloxycarbonyl; O*t*Bu, *tert*-butoxy; n = 2−6, 8) homopeptide series.

Experimental Section

Synthesis of Peptides. The synthesis and characterization of the amino acid derivative Z-L-(αMe)Nva-OH[21] have already been described. Newly synthesized homopeptides are as follows.

Z-L-(αMe)Nva-O*t*Bu. Isobutylene (55 mL) was slowly bubbled into a solution of Z-L-(αMe)Nva-OH (10 g, 37.7 mmol) in anhydrous CH_2Cl_2 (110 mL). The solution was cooled to −60 °C. Concentrated H_2SO_4 (0.37 mL) was added, and the pressure-resistant reaction flask was hermetically sealed. After keeping the solution at room temperature for 7 days, it was poured into a 5% $NaHCO_3$ solution (200 mL). The organic solvent was removed under reduced pressure, and the aqueous phase was extracted with ethyl acetate (EtOAc). The organic layer was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness: yield 87%, oil. TLC (silica gel plates 60F-254 Merck): R_fI ($CHCl_3$−ethanol 9:1) 0.95, R_fII (1-butanol−acetic acid−water 3:1:1) 0.95, R_fIII (toluene−ethanol 7:1) 0.85; $[\alpha]^{20}_D$ −4.1° (*c* 0.3, methanol). IR absorption (film): ν_{max} 3418, 3361, 1719 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.34 (m, 5H, Z phenyl CH), 5.69 (br, 1H, NH), 5.07 (s, 2H, Z CH_2), 1.67 (dt, 1H, β-CH_2), 1.54 (s, 3H, β-CH_3), 1.45 (s, 9H, O*t*Bu 3 CH_3), 1.40−1.10 (m, 3H, 1 β-CH_2 and γ-CH_2), 0.89 (dd, 3H, δ-CH_3).

Z-[L-(αMe)Nva]$_2$-O*t*Bu. To a solution of Z-L-(αMe)Nva-OH (8.65 g, 32.6 mmol) in anhydrous CH_2Cl_2 cooled to 0 °C, 7-aza-1-hydroxy-1,2,3-benzotriazole (HOAt) (4.44 g, 32.6 mmol) and *N*-ethyl,*N*′-(3-dimethylamino)propylcarbodiimide (EDC) hydrochloride (6.26 g, 32.6 mmol) were added. When a clear solution formed, H-L-(αMe)Nva-O*t*Bu [obtained by catalytic hydrogenation of the corresponding Z derivative (9.52 g, 29.6 mmol) in anhydrous CH_2Cl_2] and 1 equiv of *N*-methylmorpholine (NMM) were added, and the reaction was stirred at room temperature for 7 days. Then, the solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was isolated by flash chromatography [eluant EtOAc−PE (petroleum ether) 3:7]: yield 71%, mp 71−73 °C (from EtOAc/PE). TLC R_fI 0.95, R_fII 0.95, R_fIII 0.40; $[\alpha]_D^{20}$ −0.9° (*c* 0.4, methanol). IR absorption (KBr): ν_{max} 3391, 3332, 1726, 1669, 1499 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.36 (m, 5H, Z phenyl CH), 6.93 (br, 1H, NH), 5.84 (br, 1H, NH), 5.08 (s, 2H, Z CH_2), 2.32 (dt, 1H, β-CH_2), 2.15 (m, 1H, β-CH_2), 1.68 (dt, 2H, β-CH_2), 1.56 (s, 3H, β-CH_3), 1.52 (s, 3H, β-CH_3), 1.47 (s, 9H, O*t*Bu 3 CH_3), 1.28−1.14 (m, 4H, 2 γ-CH_2), 0.87 (dd, 6H, 2 δ-CH_3).

Z-[L-(αMe)Nva]$_3$-O*t*Bu. To a solution of Z-L-(αMe)Nva-OH (5.86 g, 22.1 mmol) in anhydrous CH_2Cl_2 cooled to 0 °C, HOAt (3 g, 22.1 mmol) and EDC·HCl (4.24 g, 22.1 mmol) were added. When a clear solution formed, H-[L-(αMe)Nva]$_2$-O*t*Bu [obtained by catalytic hydrogenation of the corresponding Z derivative (9.60 g, 22.1 mmol) in anhydrous CH_2Cl_2] and 1 equiv of NMM were added, and the reaction was stirred at room temperature for 6 days. Then, the solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was isolated by flash chromatography (eluant EtOAc−PE 4:6): yield 57%, mp 114−115 °C (from EtOAc/PE). TLC R_fI 0.95, R_fII 0.95, R_fIII 0.30; $[\alpha]_D^{20}$ −7.2° (*c* 0.4, methanol). IR absorption (KBr): ν_{max} 3439, 3345, 3303, 1720, 1671, 1531 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.35 (m, 5H, Z phenyl CH), 7.10 (br, 1H, NH), 6.91 (br, 1H, NH), 5.72 (br, 1H, NH), 5.08 (2d, 2H, Z CH_2), 2.32−2.05 (m, 2H, β-CH_2), 1.78−1.63 (m, 4H, 2 β-CH_2), 1.58 (s, 3H, β-CH_3), 1.54 (s, 3H, β-CH_3), 1.51 (s, 3H, β-CH_3), 1.47 (s, 9H, O*t*Bu 3 CH_3), 1.34−1.03 (m, 6H, 3 γ-CH_2), 0.89 (m, 9H, 3 δ-CH_3).

Z-[L-(αMe)Nva]$_4$-O*t*Bu. To a solution of Z-L-(αMe)Nva-OH (3.48 g, 13.1 mmol) in anhydrous CH_2Cl_2 cooled to 0 °C, HOAt (1.79 g, 13.1 mmol) and EDC·HCl (2.52 g, 13.1 mmol) were added. When a clear solution formed, H-[L-(αMe)Nva]$_3$-O*t*Bu [obtained by catalytic hydrogenation of the corresponding Z derivative (5.97 g, 10.9 mmol) in anhydrous CH_2Cl_2] and 1 equiv of NMM were added. The reaction was stirred at room temperature for 6 days. Then, the solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was isolated by flash chromatography (eluant EtOAc−PE 3:7): yield 73%, mp 148−150 °C (from EtOAc/PE). TLC R_fI 0.95, R_fII 0.95, R_fIII 0.35; $[\alpha]_D^{20}$ 8.4° (*c* 0.5, methanol). IR absorption (KBr): ν_{max} 3428, 3334, 1732, 1705, 1672, 1527 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.36 (m, 5H, Z phenyl CH), 7.12 (br, 1H, NH), 7.09 (br, 1H, NH), 6.62 (br, 1H, NH), 5.33 (br, 1H, NH), 5.10 (2d, 2H, Z CH_2), 1.97−0.64 (m, 8H, 4 β-CH_2), 1.59 (s, 6H, 2 β-CH_3), 1.53 (s, 3H, β-CH_3), 1.51 (s, 3H, β-CH_3) 1.46 (s, 9H, O*t*Bu 3 CH_3), 1.60−1.12 (m, 8H, 4 γ-CH_2), 0.92 (m, 12H, 4 δ-CH_3).

Z-[L-(αMe)Nva]$_5$-O*t*Bu. To a solution of Z-L-(αMe)Nva-OH (2.18 g, 8.2 mmol) in anhydrous CH_2Cl_2 cooled to 0 °C, HOAt (1.11 g, 8.2 mmol) and EDC·HCl (1.57 g, 8.2 mmol) were added. When a clear solution formed, H-[L-(αMe)Nva]$_4$-O*t*Bu [obtained by catalytic hydrogenation of the corresponding Z derivative (4.50 g, 6.8 mmol) in MeOH] and 1 equiv of NMM were added. The reaction was stirred at room temperature for 8 days. Then, the solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was isolated by flash chromatography (eluant CH_2Cl_2−EtOH (ethanol) 95:5): yield 38%, mp 207−209 °C (from CH_2Cl_2/EtOH). TLC R_fI 0.95, R_fII 0.95, R_fIII 0.35; $[\alpha]_D^{20}$ −4.0° (*c* 0.3, methanol). IR absorption (KBr): ν_{max} 3424, 3329, 1728, 1698, 1673, 1665, 1533 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.37 (m, 5H, Z phenyl CH), 7.31 (br, 1H, NH), 7.22 (br, 1H, NH), 7.12 (br, 1H, NH), 6.33 (br, 1H, NH), 5.11 (br, 1H, NH), 5.10 (2d, 2H, Z CH_2), 1.46 (s, 9H, O*t*Bu 3 CH_3), 1.90−1.10 (m, 35H, 5 β-CH_3, 5 β-CH_2 and 5 γ-CH_2), 0.91 (m, 15H, 5 δ-CH_3).

Z-[L-(αMe)Nva]$_6$-O*t*Bu. Z-L-(αMe)Nva-OH (0.103 g, 0.39 mmol) and pyridine (73 μL, 0.74 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL). The solution was cooled to 0 °C, and cyanuric fluoride (63 μL, 0.74 mmol) was added. After stirring the reaction mixture for 3 h ice water was added and the organic layer was separated, washed with cool water, and concentrated to dryness to give Z-L-(αMe)Nva-F as an oil. To a solution of crude Z-L-(αMe)Nva-F in anhydrous CH_2Cl_2, H-[L-(αMe)Nva]$_5$-O*t*Bu [obtained by catalytic hydrogenation of the corresponding Z derivative (0.1 g, 0.13 mmol) in MeOH] and 1 equiv of NMM were added. The reaction was stirred at room temperature for 14 days. Then, the solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was isolated by flash chromatography (eluant CH_2Cl_2−EtOH 95:5): yield 33%, mp 81−82 °C (from CH_2Cl_2/EtOH). TLC R_fI 0.85, R_fII 0.95, R_fIII 0.30; $[\alpha]_D^{20}$ −4.4° (*c* 0.3, methanol). IR absorption (KBr): ν_{max} 3426, 3320, 1724, 1704, 1662, 1532 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.47 (br, 1H, NH), 7.40 (br, 1H, NH), 7.32 (m, 5H, Z phenyl CH), 7.25 (br, 1H, NH), 7.19 (br, 1H, NH), 6.28 (br, 1H, NH), 5.32 (br, 1H, NH), 5.05 (s, 2H, Z CH_2), 1.41 (s, 9H, O*t*Bu 3 CH_3), 1.90−1.10 (m, 42H, 6 β-CH_3, 6 β-CH_2 and 6 γ-CH_2), 0.87 (m, 18H, 6 δ-CH_3).

Table 1. Crystal Data and Diffraction Parameters for the Z-[L-(αMe)Nva]$_n$-O*t*Bu (n = 3, 4) Homo-oligomers

parameter	Z-[L-(αMe)Nva]$_3$-O*t*Bu	Z-[L-(αMe)Nva]$_4$-O*t*Bu dihydrate
mol formula	$C_{30}H_{49}N_3O_6$	$C_{36}H_{60}N_4O_7 \cdot 2H_2O$
mol wt, amu	547.7	696.9
crystal system	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1$
Z, unit cell	4	2
a (Å)	11.927(2)	10.671(2)
b (Å)	18.192(2)	15.804(5)
c (Å)	15.201(2)	13.787(2)
β (deg)	90	112.39(1)
V (Å^3)	3298.3(8)	2149.8(8)
d (calcd), g/cm^{-3}	1.103	1.077
radiation	CuK$_\alpha$ (1.54178 Å)	Cu K$_\alpha$ (1.54178 Å)
data collection method	$\theta/2\theta$	$\theta/2\theta$
θ range, deg	1−70	1−70
indep refls	3513	4056
obs refls	3076[$I > 2\sigma(I)$]	3591[$I > 2\sigma(I)$]
goodness of fit	0.939	1.675
solved by	SIR97[22]	SIR97[22]
refined by	SHELXL97[23]	SHELXL97[23]
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0572$, $wR_2 = 0.1912$	$R_1 = 0.0696$, $wR_2 = 0.1989$
R indices all data	$R_1 = 0.0637$, $wR_2 = 0.2082$	$R_1 = 0.0749$, $wR_2 = 0.2086$
$\Delta\rho$, e/Å^3	0.532/−0.316	0.542/−0.345
temp, K	293	293
crystallization solvent	EtOAc/PE[a]	CH_3CN/H_2O

[a] EtOAc, ethyl acetate; PE, petroleum ether.

5(4*H*)-Oxazolone from Z-[L-(αMe)Nva]$_4$-OH. Z-[L-(αMe)-Nva]$_4$−OH (0.09 g, 0.15 mmol), obtained by treating Z-[L-(αMe)Nva]$_4$-O*t*Bu with CH_2Cl_2-TFA (trifluoroacetic acid), 1:1 was dissolved in anhydrous CH_2Cl_2. Then, EDC·HCl (0.021 g, 0.17 mmol) was added. The reaction was stirred at room temperature for 3 h. Then, the solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness: yield 95%, oil. TLC R_f I 0.85, R_f II 0.95, R_f III 0.30; $[\alpha]_D^{20}$ −5.0° (*c* 0.5, methanol). IR absorption (film): ν_{max} 3330, 1816, 1707, 1671, 1526 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.37 (s, 5H, Z phenyl CH), 7.21 (br, 1H, NH), 6.58 (br, 1H, NH), 5.40 (br, 1H, NH), 5.11 (2d, 2H, Z CH_2), 1.85−0.63 (m, 8H, 4 β-CH_2), 1.50 (s, 3H, β-CH_3), 1.48 (s, 3H, β-CH_3), 1.39 (s, 6H, 2 β-CH_3), 1.33−1.05 (m, 8H, 4 γ-CH_2), 0.96−0.83 (m, 12H, 4 δ-CH_3).

Z-[L-(αMe)Nva]$_8$-O*t*Bu. The 5(4*H*)-oxazolone from Z-[L-(αMe)Nva]$_4$-OH (0.084 g, 0.14 mmol) was dissolved in CH_3-CN, and H-[L-(αMe)Nva]$_4$−O*t*Bu [obtained by catalytic hydrogenation of the corresponding Z derivative (0.10 g, 0.15 mmol) in MeOH] was added. The reaction was stirred under reflux for 12 days. The solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The product was isolated by flash chromatography (eluant EtOAc−PE 3:7): yield 18%, mp 225−226 °C (from EtOAc/PE). TLC R_f I 0.25, R_f II 0.95, R_f III 0.25; $[\alpha]_D^{20}$ 3.4° (*c* 0.3, methanol). IR absorption (KBr): ν_{max} 3425, 3314, 1703, 1659, 1534 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$, 10 mM): δ 7.68 (br, 1H, NH), 7.56 (br, 1H, NH), 7.52 (br, 1H, NH), 7.44 (br, 1H, NH), 7.38 (s, 5H, Z phenyl CH), 7.33 (br, 1H, NH), 7.23 (br, 1H, NH), 6.32 (br, 1H, NH), 5.18 (br, 1H, NH), 5.14 (2d, 2H, Z CH_2), 1.79−0.64 (m, 16H, 8 β-CH_2), 1.59−1.41 (m, 24H, 8 β-CH_3), 1.45 (s, 9H, O*t*Bu 3 CH_3), 1.40−1.28 (m, 16H, 8 γ-CH_2), 1.02−0.81 (m, 24H, 8 δ-CH_3).

Infrared Absorption. The solid-state infrared absorption spectra (KBr disk technique) were recorded with a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station and a model 660 printer. The solution spectra were recorded using a Perkin-Elmer model 1720 X FT-IR spectrophotometer, nitrogen-flushed and equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF_2 windows) were used. Spectrograde deuterochloroform (99.8% *d*) was purchased from Sigma. Solvent (baseline) spectra were obtained under the same conditions.

^{1}H Nuclear Magnetic Resonance. The ^{1}H NMR spectra for conformational analysis were recorded with a Bruker model AM 400 spectrometer. Measurements were carried out in deuterochloroform (99.96% *d*; Acros Organics) and dimethyl-d_6 sulfoxide (Me_2SO) (99.96% d_6; Acros Organics) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) was purchased from Sigma.

X-ray Diffraction. Colorless single crystals of the tripeptide Z-[L-(αMe)Nva]$_3$-O*t*Bu and the tetrapeptide Z-[L-(αMe)-Nva]$_4$-O*t*Bu dihydrate were grown by slow evaporation at room temperature from the solvents reported in Table 1. Data collection was carried out on a CAD4 Enraf-Nonius X-ray diffractometer at the Institute of Biostructure and Bioimaging, CNR, Naples. Unit-cell determinations were carried out by least-squares refinement of the setting angles of 25 high-angle reflections that were accurately centered. No significant variation was observed in the intensities of the standard reflections monitored at regular intervals during data collection, thus implying electronic and crystal stability. Lorentz and polarization corrections were applied to the intensities, but no absorption correction was made. Crystallographic data for the two compounds are listed in Table 1.

The two structures were solved by direct methods using the SIR 97 program.[22] The solution with the best figure of merit revealed the coordinates of all non-H atoms. Refinements were performed by full-matrix least-squares procedures with the SHELXL 97 program.[23] All non-H atoms were refined anisotropically. H atoms were calculated, and during the refinement they were allowed to ride on their carrying atoms, with U_{iso} set equal to 1.2 times the U_{eq} of the attached atom. The scattering factors for all atomic species were calculated from Cromer and Waber.[24] CCDC-216284 and CCDC-216283 contain the supplementary crystallographic data for Z-[L-(αMe)-Nva]$_3$-O*t*Bu and Z-[L-(αMe)Nva]$_4$-O*t*Bu dihydrate, respectively. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Results and Discussion

Peptide Synthesis. For the large-scale preparation of enantiomerically pure H-L-(αMe)Nva-OH, an eco-

nomically attractive and generally applicable chemoenzymatic synthesis was developed by DSM Research.[25−29] Racemic H-(αMe)Nva-NH_2 was prepared in 61% total yield by a partial Strecker synthesis using 2-pentanone and mild acid hydrolysis of the α-amino nitrile intermediate. H-D,L-(αMe)Nva-NH_2 was resolved using the α-amino amidase from *M. neoaurum* ATCC 25795. At 51% conversion, H-L-(αMe)Nva-OH was obtained with an ee >95%, the remaining H−D-(αMe)Nva-NH_2 having an ee >99.5%. This procedure resulted in a remarkably high enantiomeric ratio of the specificity constants ($E \cong 232$) for this enzymatic resolution.[30] The same resolution was also performed using the recently developed α-amino amidase from *Ochrobactrum anthropi* NCIMB 40321.[31] However, this enzyme proved to be less selective, resulting in $E \cong 97$.

Then, the preparation and full characterization of the terminally protected, monodispersed homo-oligomeric series Z-[L-(αMe)Nva]$_n$-O*t*Bu (n = 1−6, 8) were performed. Z-L-(αMe)Nva-O*t*Bu was obtained by esterification of the corresponding Z-protected amino acid with isobutylene in anhydrous methylene chloride in the presence of a catalytic amount of sulfuric acid. The synthesis from dimer to hexamer was carried out step-by-step in solution, beginning from the C-terminal α-amino *tert*-butyl ester. L-(αMe)Nva-L-(αMe)Nva peptide bond formation was achieved either by the EDC/HOAt[32] or by the acyl fluoride[33] C-activation procedure in the presence of a tertiary amine (NMM). Z-L-(αMe)-Nva-F was prepared in situ from the N-protected amino acid and cyanuric fluoride in pyridine. The synthesis of the homo-octamer was performed by the 4 + 4 segment condensation strategy using the 5(4*H*)-oxazolone from Z-[L-(αMe)Nva]$_4$-OH as the electrophilic C-activated component.[34−36] The latter derivative was synthesized from the N-protected tetrapeptide free acid, prepared in turn by mild acidolysis of Z-[L-(αMe)Nva]$_4$-O*t*Bu with a diluted TFA solution, using EDC in methylene chloride. Removal of the Z N-protecting group was achieved by catalytic hydrogenation. Using these methodologies, the L-(αMe)Nva-L-(αMe)Nva peptide bonds were formed in variable yields, generally decreasing with increasing peptide size.

Solution Conformation. The preferred conformations of the Z/O*t*Bu-protected L-(αMe)Nva homo-oligomers were investigated in a structure-supporting solvent ($CDCl_3$) by FT-IR absorption and ^{1}H NMR over the peptide concentration range from 10−0.1 mM.

The FT-IR absorption spectra in the N−H stretching (amide A) region (peptide concentration, 1 mM) are illustrated in Figure 1. The curves are characterized by bands at 3436−3425 cm^{-1} (free, solvated NH groups), 3398−3395 cm^{-1} (weakly H-bonded NH groups of fully extended conformations),[37] and 3368−3328 cm^{-1} (strongly H-bonded NH groups of folded conformations).[38−40] The band at 3398−3395 cm^{-1} is the only one observed below 3400 cm^{-1} for the dimer; it is still one of the dominant features in the spectrum of the trimer, but it is almost completely absent from the spectrum of the tetramer. The intensity of the lowest-frequency band relative to those of the higher-frequency bands increases significantly as the main-chain length increases. We have also been able to demonstrate that even at 10 mM self-association via intermolecular N−H···O=C H bonding is negligible or modest for all oligomers (results not shown). Therefore, the observed H bonding should be interpreted as arising almost exclusively from intramolecular N−H···O=C interactions.

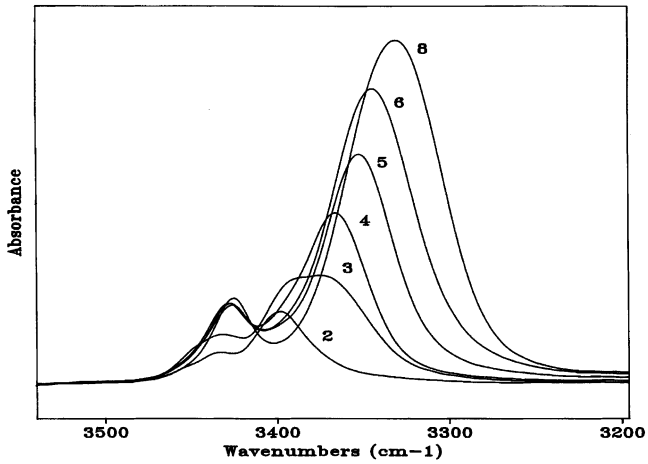

Figure 1. FT-IR absorption spectra in the N−H stretching region of the Z-[L-(αMe)Nva]$_n$-O*t*Bu (n = 2−6, 8) homo-oligomers in $CDCl_3$ solution. Peptide concentration, 1 mM.

The present FT-IR absorption analysis has provided evidence that intramolecular H bonding typical of folded conformations is the predominant feature of the terminally protected, longer L-(αMe)Nva homo-oligomers in $CDCl_3$ solution.

To get more detailed information on the preferred conformation in $CDCl_3$ of Z-[L-(αMe)Nva]$_8$-O*t*Bu, the longest and most significant homopeptide of this series, we carried out a 400-MHz ^{1}H NMR investigation.

The NH proton resonances were assigned by means of a 2D ROESY experiment beginning from the urethane N(1)H proton at higher field and by analogy with the results obtained with homo-octamers from other C$^\alpha$-tetrasubstituted α-amino acids. An analysis of the spectra as a function of peptide concentration (not shown) indicates that a 6-fold dilution (from 6 to 1 mM) produces a variation, albeit small (Δppm = 0.023, to higher fields), of the chemical shift of the N(1)H proton. For the N(2)H−N(8)H protons, the concentration effect is even less significant or negligible. In agreement with the FT-IR absorption results discussed above, we conclude that at 6 mM the modest self-association phenomenon involves the urethane N(1)H group as the main H-bonding donor.[39,41]

In the absence of self-association (peptide concentration, 1 mM), the delineation of inaccessible (or intramolecular H-bonded) NH protons was carried out with the use of the solvent (Me_2SO)[42] dependence of NH proton chemical shifts and free-radical (TEMPO)[43] -induced line broadening of NH proton resonances. Figure 2 (parts a and b) graphically describes the results obtained. Two classes of NH protons were clearly observed. The first class [N(1)H and N(2)H protons] includes protons whose chemical shifts are sensitive to the addition of the strong H-bonding-acceptor solvent Me_2SO[44] and whose resonances significantly broaden upon the addition of TEMPO. Interestingly, the sensitivity of the N(1)H proton is significantly higher than that of the N(2)H proton. The second class [N(3)H−N(8)H protons] includes those displaying a behavior that is characteristic of shielded protons (relative insensitivity of chemical shifts to solvent composition and of line widths to the presence of paramagnetic agent TEMPO).

The present ^{1}H NMR data support the view that at low concentration (1 mM) in $CDCl_3$ solution the N(3)H−

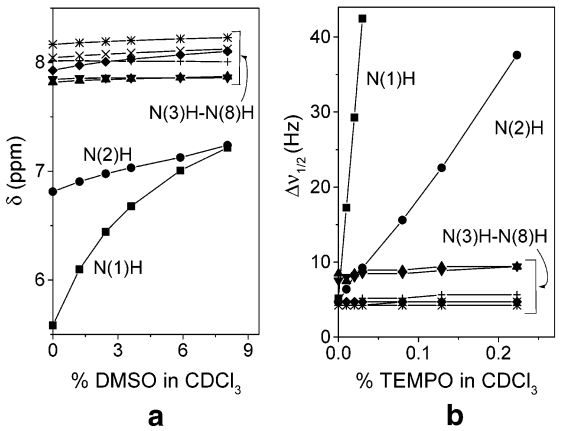

Figure 2. (a) Plot of NH proton chemical shifts in the ^{1}H NMR spectrum of the Z-[L-(αMe)Nva]$_8$-O*t*Bu homo-oligomer as a function of increasing percentages of Me_2SO with respect to the $CDCl_3$ solution (v/v). (b) Plot of the bandwidth of the NH protons of the same homo-oligomer as a function of increasing percentages of TEMPO with respect to $CDCl_3$ (w/v).

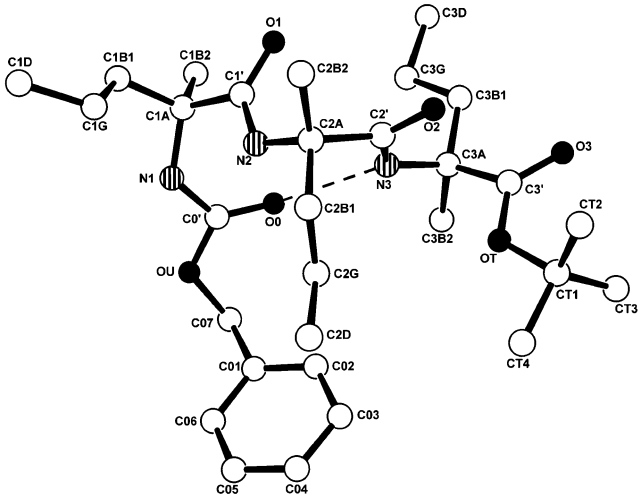

Figure 3. X-ray diffraction structure of homotrimer Z-[L-(αMe)Nva]$_3$-O*t*Bu with atom numbering. The intramolecular H bond is represented by a dashed line.

N(8)H protons of the octamer are inaccessible to solvent and perturbing agents and are therefore most probably intramolecularly H-bonded. The intramolecular H-bonding scheme of the octamer does not appear to change upon self-association [involving the N(1)H proton as the donor of the intermolecular H bond]. Because all NH protons, beginning with the N(3)H proton of Z-[L-(αMe)-Nva]$_8$-O*t*Bu, form intramolecular H bonds, we are inclined to conclude that the structure predominantly adopted in $CDCl_3$ by these peptides is the 3_{10} helix (a series of consecutive type-III β bends) 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.[5] These more detailed conclusions are in full agreement with the preliminary indications extracted from the FT-IR absorption study discussed above.

Crystal-State Conformation. We determined by X-ray diffraction the molecular and crystal structures of two Z/O*t*Bu-protected L-(αMe)Nva homo-oligomers, namely, the trimer and the tetramer (the latter in its dihydrated form). The molecular structures with the atomic numbering schemes are shown in Figures 3 and 4, respectively. Protecting groups, backbone, and side-

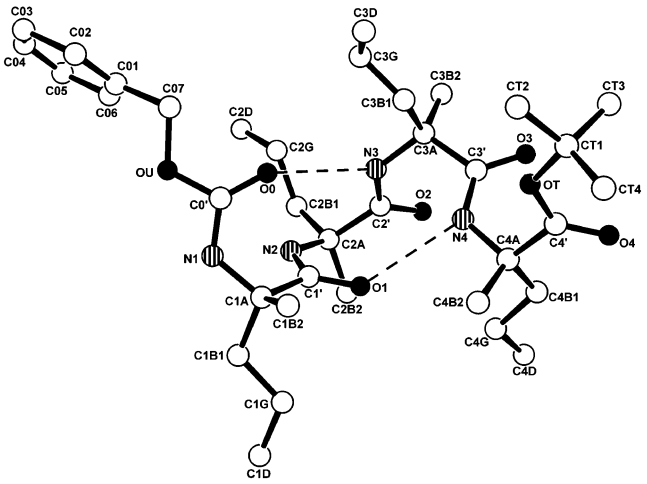

Figure 4. X-ray diffraction structure of homotetramer Z-[L-(αMe)Nva]$_4$-O*t*Bu with atom numbering. The two intramolecular H bonds are represented by dashed lines.

Table 2. Selected Torsion Angles (deg) for the Z-[L-(αMe)Nva]$_n$-O*t*Bu (n = 3, 4) Homo-oligomers

torsion angle	Z-[L-(αMe)Nva]$_3$-O*t*Bu	Z-[L-(αMe)Nva]$_4$-O*t*Bu
$\theta^{3,1}$	−101.6(5)	112.7(10)
$\theta^{3,2}$	79.6(4)	−66.6(10)
θ^{2}	94.3(4)	158.4(7)
θ^{1}	−177.4(3)	−177.0(7)
ω_o	−166.5(3)	−178.5(4)
ϕ_1	−62.0(4)	−50.9(6)
ψ_1	−27.6(4)	−35.9(5)
ω_1	−175.8(3)	−169.6(3)
ϕ_2	−64.2(4)	−54.3(6)
ψ_2	−29.5(4)	−30.0(6)
ω_2	−178.6(3)	−179.4(4)
ϕ_3	44.5(4)	−60.0(5)
ψ_3	51.3(3)[a]	−36.9(5)
ω_3	174.7(3)[b]	−175.7(3)
ϕ_4		49.7(5)
ψ_4		43.9 (4)[c]
ω_4		174.1(4)[d]
χ_1^1	60.9(4)	178.6(6)
χ_1^2	−169.0(3)	179.6(11)
χ_2^1	60.1(5)	63.7(6)
χ_2^2	−178.4(5)	−171.9(6)
χ_3^1	−60.5(5)	−68.0(8)
χ_3^2	−174.7(5)	−172.6(15)
χ_4^1		−61.6(5)
χ_4^2		176.6(6)

[a] $N_3-C^{\alpha}_3-C'_3-O_T$. [b] $C^{\alpha}_3-C'_3-O_T-C_{T1}$. [c] $N_4-C^{\alpha}_4-C'_4-O_T$. [d] $C^{\alpha}_4-C'_4-O_T-C_{T1}$.

chain torsion angles[45] are given in Table 2. In Table 3, the intra- and intermolecular H-bond parameters are listed. Figure 5 illustrates the packing mode of the tetramer in the crystal.

Bond lengths and bond angles (deposited) are in general agreement with previously reported values for the geometry of the benzyloxycarbonylamino urethane[46] and *tert*-butyl ester[47] moieties and the peptide unit.[48,49]

All seven L-(αMe)Nva residues populate the helical region (A or A*)[50] of the conformational (ϕ, ψ) space. The average values for the (ϕ, ψ) backbone torsion angles of the (αMe)Nva residue are ±55.1 and ±36.4°, close to those expected for a 3_{10} helix.[5] In both the trimer and tetramer, the signs of the (ϕ, ψ) values of the C-terminal residue are opposite with respect to those of the preceding ones. This observation is quite common in the X-ray diffraction structures of peptide esters that are heavily based on C^{α}-tetrasubstituted α-amino acids.[10,51] The 1−2 sequence of the trimer is folded into a

Table 3. Intra- and Intermolecular H-Bond Parameters for the Z-[L-(αMe)Nva]$_n$-O*t*Bu (n = 3, 4) Homo-oligomers

peptide	donor	acceptor	symmetry operation	N···O distance, Å	C′=O···N angle, deg
Z-[L-(αMe)Nva]$_3$-O*t*Bu	N_3H	O_0	x, y, z	3.083(3)	131.0(2)
	N_1H	O_3	$-x + 2, y + 1/2, -z + 1/2$	2.929(3)	143.6(2)
Z-[L-(αMe)Nva]$_4$-O*t*Bu dihydrate	N_3H	O_0	x, y, z	3.240(5)	131.8(3)
	N_4H	O_1	x, y, z	2.855(4)	136.8(3)
	N_1H	O_3	$1 + x, y, z$	2.958(8)	177.1(3)
	N_1H	O_{w1}	x, y, z	3.159(9)	
	N_2H	O_{w1}	x, y, z	3.086(6)	
	$O_{w2}H$	O_2	x, y, z	2.796(6)	168.8(4)
	$O_{w2}H$	O_4	$-x, y + 1/2, -z + 2$	2.917(7)	119.8(3)
	$O_{w1}H$	O_{w2}	$1 + x, y, z$	2.722(8)	

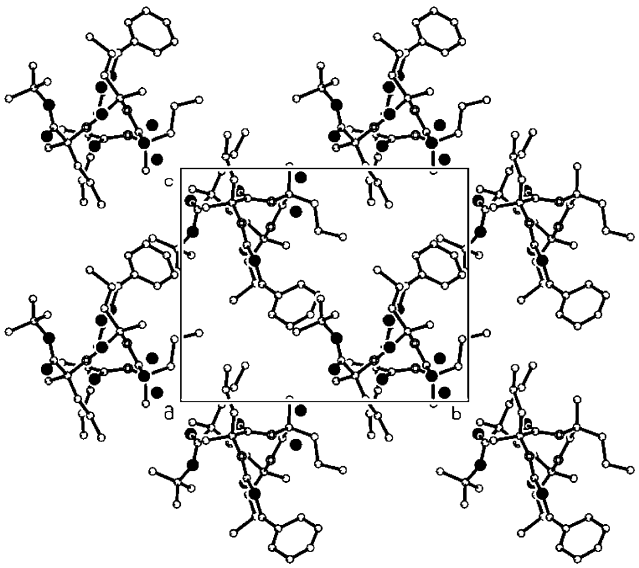

Figure 5. Packing mode of the molecules of Z-[L-(αMe)Nva]$_4$-O*t*Bu dihydrate in the crystal state as viewed along the *a* direction (black circles, oxygen atoms; patterned circles, nitrogen atoms).

1 ← 4 C′=O···H−N intramolecularly H-bonded β bend conformation of the helical (III) type.[13−15] The $C'_0{=}O_0{\cdots}H{-}N_3$ intramolecular separation is within the limits expected for such H bonds.[52−54] The 1−3 sequence of the tetramer adopts a regular, right-handed, incipient 3_{10}-helical structure, stabilized by two consecutive 1 ← 4 C′=O···H−N intramolecular H bonds. One of the two N···O distances, $N_3{\cdots}O_0$ [3.240(5) Å], is near the upper acceptable limit.

In each of the two molecules, only one significant deviation in the ω backbone torsion angles ($|\Delta\omega| > 10°$) from the ideal value of the trans-planar urethane, peptide, and ester units (180°) is observed: the urethane ω_0 value of the trimer and the peptide ω_1 value of the tetramer, which differ by 13.5 and 10.4°, respectively. The trans, trans arrangement of the θ^1, ω_0 torsion angles of the Z-NH-urethane moiety (type-b conformation) found for both homo-oligomeric molecules is that commonly reported for Z-protected peptides.[46] The *tert*-butyl ester conformation of both the trimer and tetramer with respect to the preceding $C^\alpha{-}N$ bond is intermediate between the anticlinal and antiperiplanar conformations.[55] In the seven L-(αMe)Nva residues of the two compounds examined, the $N{-}C^\alpha{-}C^\beta{-}C^\gamma$ (χ^1) torsion angle is either in the g^+ conformation (three times) or in the g^- (three times) and t (once) conformations (i.e., no clear side-chain conformation bias is observed for this parameter). Conversely, the $C^\alpha{-}C^\beta{-}C^\gamma{-}C^\delta$ (χ^2) torsion angles are all trans.

The crystal packing mode of the tripeptide is characterized by an intermolecular H bond ($N_1{-}H{\cdots}O_3{=}C'_3$), giving rise to rows of molecules H-bonded in a head-to-tail fashion along the *b* direction. van der Waals interactions link together rows of peptide molecules in the *a* and *c* directions.

In the crystals of the tetrapeptide, the molecules are connected through an intermolecular H bond ($N_1{-}H{\cdots}O_3{=}C'_3$), which generates rows in a head-to-tail arrangement along the *c* direction (Figure 5). These rows are held together by four intermolecular H bonds ($N_1{-}H{\cdots}O_{w1}$, $N_2{-}H{\cdots}O_{w1}$, $O_{w2}{-}H{\cdots}O_2{=}C'_2$, and $O_{w2}{-}H{\cdots}O_4{=}C'_4$) involving the peptide and the two water molecules. An additional intermolecular H bond is observed between the two water molecules. The crystal structure is further stabilized by van der Waals interactions between the hydrophobic groups.

Conclusions

In this paper, we have described the successful solution-phase synthesis of the sterically hindered L-(αMe)Nva homo-oligomers to the octamer level using either the step-by-step or the segment condensation approach. Furthermore, the results of the solution conformational analysis, combined with those extracted from the crystal-state X-ray diffraction study, also reported here, definitely confirm our earlier, preliminary findings[21] that L-(αMe)Nva has a remarkable propensity for β-bend and 3_{10}-helix formation. This conclusion strictly parallels those already reported for other C^α-methylated α-amino acids.[11,12] As for the relationship between (αMe)Nva α-carbon chirality and the screw sense of the bend/helix that is adopted by its peptides, the available X-ray diffraction data support the contention that this structural property is analogous to that exhibited by protein amino acids (i.e., L-amino acids fold into right-handed bends/helices).

In our view, the L-(αMe)Nva homo-oligomeric series is the best among those derived from C^α-tetrasubstituted α-amino acids for application as a set of rigid, foldameric spacers in physicochemical investigations for the following reasons: (i) It exhibits a stronger bias, compared to the L-Iva series,[16,17] toward right-handed bends and helical structures because the two alkyl substituents on the C^α atom differ by two carbons. (ii) It is much easier to synthesize than the series based on the more sterically demanding β-branched (αMe)-Val residue.[20]

Supporting Information Available: Tables of positional parameters, bond distances, bond angles, and torsion angles for the X-ray diffraction structures of Z-[L-(αMe)Nva]$_3$-O*t*Bu and Z-[L-(αMe)Nva]$_4$-O*t*Bu. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA030327V