Nitrogen-15 Magnetic Resonance Spectroscopy. Natural-Abundance Spectra of Isomeric Homooligopeptides of L-Norvaline and L-Valine to the Tetramers[1]

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The ^{15}N NMR shifts of chemically and optically pure N- and C-protected homooligopeptides derived from L-norvaline and L-valine to the tetramers have been determined at the natural-abundance level of ^{15}N by using the Fourier-transform technique. The shift effects produced by increasing the chain length and temperature, as well as changing the solvent from a hydrogen-bonding donor to a hydrogen-bonding acceptor and replacing the linear side chain (as in the norvaline peptides) by a β-branched side chain (as in the valine peptides), are discussed.

A rapidly growing number of investigations have indicated that nitrogen-15 nuclear magnetic resonance (^{15}N NMR) can be a useful tool for sequence and conformational analyses of synthetic and naturally occurring polypeptides.[2-40] However, despite all that has been done, we feel that, at the present stage of our knowledge, more studies are needed of closely related peptide model com-

Table I. ^{15}N Chemical Shifts of *tert*-Boc-(L-Nva-)$_n$OCH$_3$ in Trifluoroethanol at Various Temperatures

n	temp, °C[a]	^{15}N chemical shifts[b]			
1	55	285.8			
2	25	285.8	256.5		
	63	285.5	257.5		
3	18	286.0	256.5	255.5	
	36	285.8	256.7	255.7	
	59	285.7	256.8	256.0	
4	25	285.7	257.7	256.3	255.5
	38	285.7	257.7		255.6
	62	285.6	257.7		255.7

[a] Temperatures are within ±0.5 °C. [b] In ppm upfield from external $D^{15}NO_3$; values corrected for shift of $D^{15}NO_3/D_2O$ with temperature. Where there are more than two Nva residues, no definite assignments of the ^{15}N resonances can be made other than for the residue at the N terminus, and the shifts are arranged not by residue number but in decreasing value.

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pounds to elucidate the potentialities and limitations of the technique. For this reason, we report here a ^{15}N NMR study at the natural-abundance level of ^{15}N, using Fourier-transform methods of monodispersed, chemically and optically pure N- and C-protected homooligopeptides derived from the isomeric α-amino acid residues L-norvaline (Nva) and L-valine (Val) to the tetramers, having the general formula *tert*-Boc-(L-X-)$_n$OCH$_3$ (X = Nva, Val; n = 1–4) (*tert*-Boc = *tert*-butoxycarbonyl). The shift effects resulting from changes in the chain length, the temperature, and the solvent from the hydrogen-bonding donor 2,2,2-trifluoroethanol to the hydrogen-bonding acceptor dimethyl sulfoxide and the replacement of the linear side chains of the Nva peptides by the β-branched side chains of the Val peptides are discussed. The conformational preferences of L-Nva and L-Val homopeptides in solvents of divergent polarity and at different temperatures determined by ultraviolet and infrared absorptions, circular dichroism, and ^1H NMR already have been reported.[41-48]

Experimental Section

Synthesis of Peptides. The chemically and optically pure *tert*-Boc-(L-Nva-)$_{2-4}$OCH$_3$[49] and *tert*-Boc-(L-Val-)$_{2-4}$OCH$_3$[41] were prepared according to the previously described procedures. Details of the synthesis of *tert*-Boc-L-Nva-OCH$_3$[46] have also been previously reported. The *tert*-Boc-L-Val-OH compound was prepared in 73% yield by the reaction of L-valine with *tert*-butyl azidoformate according to the procedure described by Schnabel;[50] mp 77–78 °C; $[\alpha]_D^{20}$ −6.5° (*c* 1, CH_3CO_2H). A sample of Ac-L-Val-OCH$_3$[51] was prepared from acetyl chloride and HCl·H-L-Val-OCH$_3$[41] in anhydrous chloroform by using *N*-methylmorpholine to deprotonate the ammonium group. The yield was 75%, mp 68–69 °C, after crystallization from ethyl acetate–petroleum ether; $[\alpha]_D^{20}$ −30.2° (c 1, CH_3OH); $[\alpha]_D^{20}$ −47.3 (*c* 1, H_2O). *tert*-Boc-L-Val-NHCH$_3$ was prepared from *tert*-Boc-L-Val-OH[50] and methylamine hydrochloride in anhydrous methylene chloride by using *N*-methylmorpholine to deprotonate the ammonium group and *N,N'*-dicyclohexylcarbodiimide as the condensing agent. The yield was 65% of material with a melting point of 132–133 °C after crystallization from ethyl ether–petroleum ether; $[\alpha]_D^{20}$ −11.7° (*c* 1, CH_3OH).

Anal. Calcd for $C_{11}H_{22}N_2O_3$: C, 57.3; H, 9.6; N, 12.2. Found: C, 58.2; H, 9.8; N, 12.2.

^{15}N NMR spectra were taken with a Bruker WH-180 spectrometer at a frequency of 18.25 MHz, using 25-mm sample tubes. All samples were run at a concentration of 1–2 g in 17 mL of solvent. Normal operating conditions employed a pulse width of 55 μs (70° flip angle), a repetition rate of 6 s, and broad-band proton decoupling. Under these conditions, spectra were obtained in 2–5 h. Chemical shifts were referenced to a 1.0 M D^{15}NO$_3$/D$_2$O capillary and corrected for the D^{15}NO$_3$/D$_2$O temperature dependence. Temperatures were obtained by direct measurement of the sample temperature after thermal equilibrium was reached.

Table II. ^{15}N Chemical Shifts of *tert*-Boc-(L-Nva-)$_n$OCH$_3$ in Dimethyl Sulfoxide at Various Temperatures

n	temp, °C[a]	^{15}N chemical shifts[b]			
2	52	285.6	259.9		
3	24	285.2	258.9	257.8	
	36	285.5	259.3	258.4	
	60	285.8	259.9	259.1	
4	24	285.3	258.7	258.4	257.6
	40	285.5	259.1	258.9	258.0
	65	285.8	259.4	259.4	258.5

[a] Temperatures are within ±0.5 °C. [b] In ppm upfield from external D^{15}NO$_3$; values corrected for shift of D^{15}NO$_3$/D$_2$O with temperature. Where there are more than two Nva residues, no definite assignments of the ^{15}N resonances can be made other than for the residue at the N terminus, and the shifts are arranged not by residue number but in decreasing value.

Results and Discussion

The ^{15}N chemical shifts for *tert*-Boc-(L-Nva-)$_n$OCH$_3$ (n = 1–4) in the hydrogen-bonding donor solvent trifluoroethanol and in the hydrogen-bonding acceptor solvent dimethyl sulfoxide at various temperatures are given in Tables I and II, respectively. The results in Table I should be compared to the corresponding ones for the isomeric homopeptides from L-Val listed in Table III. This last table also gives the ^{15}N resonance positions of some L-Val derivatives examined as models for the carbamate and amide functions. The upfield resonance at 285–290 ppm in each spectrum is readily assigned to the nitrogen atom of the single OCONH group. There is a significant dependence of this signal on the nature of the amino acid component. Thus, in trifluoroethanol solutions, the shifts of *tert*-Boc-Val nitrogens are about 3 ppm upfield of those of *tert*-Boc-Nva nitrogens. Small downfield changes (<0.8 ppm) of this resonance are apparent in the Nva series when the solvent is changed from trifluoroethanol to dimethyl sulfoxide. The resonance of the *tert*-Boc-Gly nitrogen in dimethyl sulfoxide solution has been reported at near 56.5 ppm downfield from the NH_4^+ resonance[9] (i.e., near 298.5 ppm upfield from D^{15}NO$_3$).[16] This result is in accord with the aforementioned substituent effect. Raising the temperature at which the ^{15}N NMR spectra of the carbamate nitrogens are taken causes small downfield shifts (<0.008 ppm/°C) with trifluoroethanol as solvent and somewhat larger upfield shifts (≃0.015 ppm/°C) with dimethyl sulfoxide as solvent. In general, the temperature effects are smaller with peptides with longer chain lengths. In trifluoroethanol, the amide resonances in the Val series move to lower fields on increasing the number of residues in the peptide chain. At the tripeptide level, the signal of the C-terminal nitrogen is not distinguishable from the internal nitrogen signals. However, the resonance of one nitrogen of the tetrapeptide is seen to be somewhat downfield from the others, and this may be the nitrogen of the C terminus. Short of ^{15}N labeling, there seems no easy way to assign the nitrogen resonances to specific Val residues, except for the nitrogen of the N-terminal peptide residue. Identical ^{15}N shifts have also been reported for

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Table III. ^{15}N Chemical Shifts in *tert*-Boc-Val Derivatives and Peptides in Trifluoroethanol

compd	temp, °C[a]	^{15}N chemical shifts[b]			
tert-Boc-L-Val-OH	28	290.6			
tert-Boc-(L-Val-)$_2$OCH$_3$	28	288.7	257.1		
tert-Boc-(L-Val-)$_3$OCH$_3$	28	288.9	256.1	256.1	
tert-Boc-(L-Val-)$_4$OCH$_3$	28	288.9	255.5	255.5	253.6
	56	288.7	256.5	255.9	254.2
CH$_3$CO-L-Val-OCH$_3$	34		255.3		
tert-Boc-L-Val-NHCH$_3$	35	289.1	268.7		

[a] Temperatures are within ±0.5 °C. [b] In ppm upfield from external D^{15}NO$_3$; values corrected for shift of D^{15}NO$_3$/D$_2$O with temperature. Where there are more than two Val residues, no definite assignments of the ^{15}N resonances can be made other than for the residue at the N terminus, and the shifts are arranged not by residue number but in decreasing value.

the second and third residues of CH$_3$CO-(Gly-)$_3$OH (in dimethyl sulfoxide and trifluoroacetic acid).[16,18] The Nva series appears to be deficient in that in neither trifluoroethanol nor dimethyl sulfoxide solutions is there a clear influence of peptide chain length on the amide nitrogen shifts.

The effect of changing the amino acid from Nva to Val is consistent for the carbamate nitrogen but rather ambiguous for the other nitrogens. Thus, there is an upfield shift difference of 0.6 ppm between the amide nitrogen of the Nva and Val dipeptides, but there are downfield shift differences for at least two amide nitrogens between the Nva and Val tetrapeptides. This seems to be the first instance where side-chain effects on the ^{15}N shifts of homopeptides are masked, or even reversed, by chain-length effects.

In contrast to the small downfield shift (<0.8 ppm) of the carbamate nitrogens on changing solvent from trifluoroethanol to dimethyl sulfoxide for the Nva series, rather larger upfield shifts of 1–3.5 ppm are found for the other peptide nitrogens. The differences become smaller with increasing chain length and decreasing temperature. An upfield shift of 0.4 ppm has recently been reported for the nitrogen resonance of the central residue of the homopeptide CH$_3$C$_6$H$_4$SO$_2$-(Gly-)$_3$OC$_2$H$_5$ under the same experimental conditions.[28] The observed solvent effects are rather small, considering the striking differences in properties of dimethyl sulfoxide and trifluoroethanol, the first being a very strong hydrogen-bond accepting solvent while the last is a strong hydrogen-bond donor solvent.[9,18,22,25,28] Matters are further complicated by the possibility for intramolecular >NH···O=C< hydrogen bonding, and both trifluoroethanol and dimethyl sulfoxide will tend to diminish such interactions by offering alternative hydrogen-bonding possibilities. Further difficulties in interpretation of the shifts arise because the shift of a –C(O)-NH– nitrogen is sensitive to not only whether its proton is forming a hydrogen bond but also whether its carbonyl group and/or nitrogen unshared pair is acting as a hydrogen-bond acceptor. At high concentrations (≥0.1 M), solvent-dependent formation of intermolecular >NH··· O=C< hydrogen bonds can also become a factor.[46,48]

We hoped that the effects of temperature on the ^{15}N resonances of these peptides would help sort out some of the conflicting influences that we can envision as complicating the interpretation of the conformational states. Unfortunately, the effects are small, but at least consistent, in that for both the Nva and Val series, there are upfield shifts of the amide nitrogen resonances with increasing temperature in either dimethyl sulfoxide or trifluoroethanol solutions. The size of the effect is somewhat larger with decreasing chain length and on going from Nva to Val peptides and from trifluoroethanol to dimethyl sulfoxide solutions. With *tert*-Boc-(L-Val-)$_4$OCH$_3$, the coincidence of two ^{15}N resonances is removed at 56 °C. The smallest change in ^{15}N shifts with temperature was observed for the Nva homotetrapeptide in trifluoroethanol solution. This lack of sensitivity of shifts to temperature could be ascribed to the stability of an aggregated structure for *tert*-Boc-(L-Nva-)$_4$OCH$_3$ in trifluoroethanol. The overall smallness of the temperature effects which have been observed in this study hardly permits any more definitive conclusion.

Registry No. *t*-Boc-L-Nva-OCH$_3$, 64896-37-3; *t*-Boc-L-Nva-OCH$_3$, 56558-25-9; *t*-Boc-L-Nva-L-Nva-L-Nva-OCH$_3$, 56613-62-8; *t*-Boc-L-Nva-L-Nva-L-Nva-L-Nva-OCH$_3$, 56558-26-0; *t*-Boc-L-Val-OH, 58561-04-9; *t*-Boc-L-Val-L-Val-OCH$_3$, 33857-88-4; *t*-Boc-L-Val-L-Val-L-Val-OCH$_3$, 53197-49-2; *t*-Boc-L-Val-L-Val-L-Val-L-Val-OCH$_3$, 19794-14-0; Ac-L-Val-OCH$_3$, 1492-15-5; *t*-Boc-L-Val-NHCH$_3$, 71988-70-0; acetyl chloride, 75-36-5; H-L-Val-OCH$_3$·HCl, 6306-52-1; methylamine hydrochloride, 593-51-1.