Use of a Guest Amino Acid for the Complete Proton Nuclear Magnetic Resonance Assignments of Blocked Linear Methionine Homooligopeptides

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Abstract: Proton magnetic resonance spectra at 220 MHz are reported for $Boc(L-Met)_n$-OMe ($n = 2-7$) at dilute concentrations in $CDCl_3$. A new approach, the inclusion of a guest amino acid residue in a linear homooligopeptide, is shown to allow unequivocal ^{1}H NMR assignments for the NH and α-CH resonances of homooligopeptides. Assignments for the homologous series $Boc-Met_n$-OMe are obtained by comparison with co-oligopeptides of methionine with glycine.

Protected linear homooligopeptides exhibit various secondary structures in solution such as the α helix or β sheet. Of particular importance are the findings that the critical chain length for folding often occurs at about the hexamer or heptamer stage and that the secondary structure varies widely with the solvent, concentration, temperature, and other conditions.[2]

High resolution ^{1}H NMR[3] is a valuable technique to study the possible conformational equilibria and the local structure at each residue of these linear peptides. Past ^{1}H NMR studies on homooligopeptides[4-6] have revealed that the amide (NH) and α-methine (α-CH) proton resonances, which are useful as probes of peptide structure, are usually well separated from other proton resonances arising from the side chains and/or blocking groups. Moreover, while in some cases overlapping NH or α-CH signals occur, individual NH and α-CH resonances with different chemical shift values can often be observed. For example, the NMR spectra of the γ-ethyl glutamates in trifluoroethanol show individual NH resonances for all oligopeptides from dimer to hexamer.[6a] To date, however, unambiguous ^{1}H NMR assignments have only been obtained for homodipeptides of norvaline,[4] alanine,[5] isoleucine,[5d] glutamic acid,[6] and a homotripeptide of norvaline.[4]

Unequivocal assignments for a single protected homooligopeptide[7] would allow a detailed analysis of the peptide structure in solution. Assignments for an entire homologous series would further aid exploration of the chain length dependence of peptide structure. Such assignments might also have important implications for the establishment of some better additivity rules for peptide ^{1}H NMR spectra.

In this paper, we introduce a new strategy, i.e., the inclusion of a guest amino acid into the sequence of a linear homooligopeptide chain, as a means to obtain unequivocal assignments of protected homooligopeptides. Specifically we have compared a series of protected methionine homooligopeptides, $Boc-L-Met_n$-OMe ($n = 2-7$), with co-oligopeptide derivatives where a "host" amino acid, methionine, is replaced by the "guest" amino acid, glycine, at each position of a homooligopeptide chain. We discuss here our NMR observations of these peptides in $CDCl_3$ or 99% $CDCl_3$/1% Me_2SO-d_6 media. We emphasize at the outset that these peptides, particularly the hexamers and heptamers, are almost insoluble in $CDCl_3$ media, and we have worked at relatively dilute concentrations for NMR spectroscopy, typically 10^{-3} M peptide.

Experimental Section

Details of the synthesis and chemical and optical purity of $Boc-L-Met_n$-OMe ($n = 2-7$)[8] and the co-oligopeptides with glycine[9] are reported elsewhere. The solvents, $CDCl_3$ (99.8%) and Me_2SO-d_6

(99.8%), were purchased from Aldrich. All peptides except $Boc-Met-Gly-Met$-OMe (which was an oil) are crystalline compounds. Weighed amounts of peptides could be placed in NMR sample tubes and a known volume of solvent added.

^{1}H NMR spectra were obtained at 0.2% (w/v) peptide in the Fourier transform (FT) mode on a HR-220 spectrometer fitted with a Nicolet TT-100 System. Spectra were accumulated into 8K points using a 45° pulse of 28 μs to cover a spectral width of 2500 Hz and were exponentially line broadened by 0.25 Hz. In the absence of line broadening the line width of singlet resonances was 2.0 Hz. Under these conditions, the six $-SCH_3$ singlets of $Boc-Met_6$-OMe are completely resolved.

Homonuclear spin-decoupling experiments were carried out in the FT mode with a home-built double-irradiation apparatus assembled from a 73-MHz crystal oscillator mixed with a 10-MHz oscillator in a Syntest synthesizer and tripled up to 220-MHz frequencies. With this apparatus, relationships between α-CH and NH resonances were established by first irradiating the α-CH region, observing change in the NH resonances, and then irradiating individual NH peaks and observing change in the α-CH region.

Results

In Figure 1 we present 220-MHz ^{1}H NMR spectra over the NH and α-CH regions for dilute solutions of the homologous series of peptides, $Boc-Met_n$-OMe ($n = 2-7$) in $CDCl_3$. All of the NH resonances and most of the α-CH resonances in these homooligopeptides have different chemical shifts. The spectrum of the dipeptide, $Boc-Met_2$-OMe (Figure 1A) exhibits two resolved NH doublets (5.16, 6.80 ppm) and two resolved α-CH multiplets (4.29, 4.73 ppm). The tripeptide, $Boc-Met_3$-OMe, shows a spectrum with three NH doublets (5.15, 6.89, 6.95 ppm), one resolved α-CH resonance (4.25 ppm), and two overlapping α-CH resonances (4.62, 4.67 ppm). In the tetrapeptide ($Boc-Met_4$-OMe) spectrum four NH doublets (7.36, 7.20, 7.02, 5.32 ppm), *two* resolved α-CH signals (4.19, 4.53 ppm), and two overlapping α-CH resonances (4.65, 4.67 ppm) are observed. In the spectrum of $Boc-Met_5$-OMe (Figure 1B) five NH doublets may be identified. Four NH signals (7.74, 7.46, 7.06, 5.46 ppm) are individually resolved in Figure 1B, while a fifth NH signal ($\sim$7.30 ppm) is located under the solvent $CHCl_3$ signal. Two resolved α-CH resonances (4.09, 4.34 ppm) and three partially overlapping α-CH resonances (4.63, 4.59, 4.53 ppm) are observed for this pentapeptide. For the hexapeptide, $Boc-Met_6$-OMe, all six NH doublets (7.86, 7.66, 7.39, 7.16, 7.08, 5.51 ppm) are remarkably resolved from each other and the solvent $CHCl_3$ peak. Four envelopes are observed in the methine region. Two envelopes integrate to single, resolved α-CH resonances (4.08, 4.44 ppm), and the other two envelopes each integrate to two partially overlapping α-CH resonances (4.64, 4.57 ppm and 4.30, 4.26 ppm). Finally, in the spectrum of the heptapeptide,
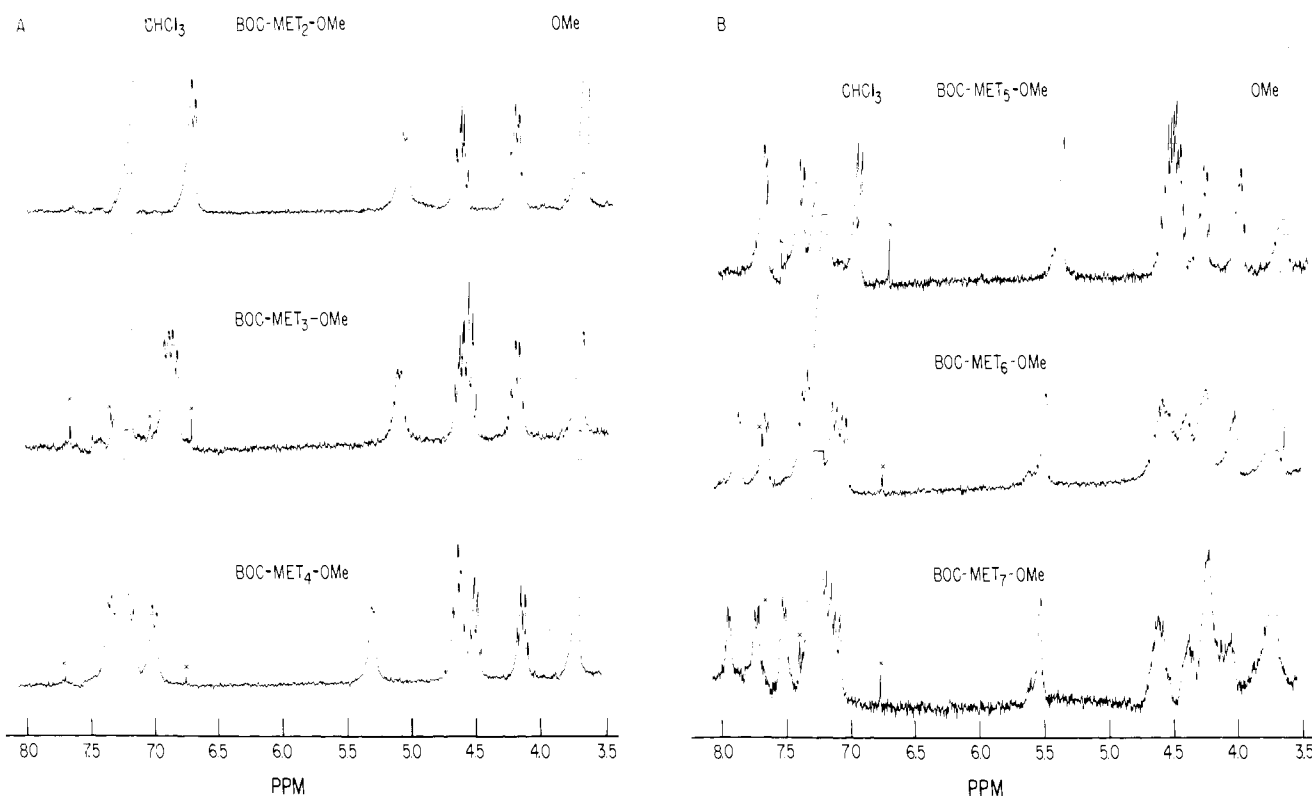
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Figure 1. Partial 220-MHz ^{1}H NMR spectra over a 1000-Hz range showing the NH and α-CH resonances of (A) Boc-Met$_2$-OMe (0.0050 M, 600 scans), Boc-Met$_3$-OMe (0.0038 M, 800 scans), and Boc-Met$_4$-OMe (0.0031 M, 1024 scans); and (B) Boc-Met$_5$-OMe (0.0025 M, 1026 scans), Boc-Met$_6$-OMe (0.0022 M, 1600 scans), and Boc-Met$_7$-OMe (0.0019 M, 2000 scans), in CDCl$_3$ at 23 °C. X denotes spinning side bands of the solvent CHCl$_3$ peak. The methionine side chain β-CH$_2$ and γ-CH$_2$ groups each show overlapping proton signals at ~2.1 and 2.6 ppm, respectively. Two, three, four, five, and six –SCH$_3$ singlet resonances at ~2.1 ppm were observed for Boc-Met$_2$-OMe, Boc-Met$_3$-OMe, Boc-Met$_4$-OMe, Boc-Met$_5$-OMe, and Boc-Met$_6$-OMe. In Boc-Met$_7$-OMe three –SCH$_3$ singlets are resolved and four overlap.

Boc-Met$_7$-OMe, seven NH resonances may be identified. Six individual NH resonances (7.99, 7.76, 7.52, 7.18, 7.11, 5.53 ppm) are resolved while the seventh (7.24 ppm) is buried under the solvent CHCl$_3$ peak. Four envelopes are again seen in the methine region. Two envelopes are single proton α-CH signals (4.08, 4.44 ppm), a third envelope integrates to two partially overlapping α-CH resonances (4.63, 4.58 ppm), and the fourth envelope involves three overlapping α-CH resonances (~4.27 ppm).

Homonuclear double-resonance experiments were carried out to establish relationships of scalar spin–spin coupling between the α-CH and NH resonances of each of these homo-oligopeptides. The full results of those experiments are summarized later in the paper. As an example, we list here the relationships obtained in the case of the homohexamer, Boc-Met$_6$-OMe (numbers in parts per million): α-CH, 4.08, 4.26, 4.29, 4.41, 4.57, 4.64; coupled NH, 5.51, 7.86, 7.66, 7.39, 7.16, 7.08.

The decoupling experiments in general establish that the upfield NH and α-CH resonances of each homooligopeptide are coupled to each other. For the other NH and α-CH signals, it should be noted that the most deshielded NH proton is coupled to the most shielded α-CH proton. The second most deshielded NH proton corresponds with the second most shielded α-CH proton, etc. These trends are observed for each oligopeptide—from Boc-Met$_2$-OMe to Boc-Met$_7$-OMe.

In considering assignments for each methionine NH and α-CH resonance to a specific residue of an oligopeptide chain, we number the amino acid residues from the amino terminus, so that the nitrogen and α-carbon protons at this end of the

peptide chain are labeled Met^1 NH and Met^1 α-CH and so on.

The high-field NH resonance (5.15–5.53 ppm) of each homooligopeptide, Boc-Met$_n$-OMe, can be assigned to the N-terminal methionine residue (Met^1) as a urethane group ROCONHR is well known to shift the NH proton to considerably higher fields as compared to RCONHR.[4-6] Our spin-decoupling experiments and previously reported assignments for the N-terminal α-CH resonances of alanine[5b] and norvaline peptides[4] in CDCl$_3$ show that the corresponding high-field α-CH resonance (4.29–4.06 ppm) can also be assigned to the N-terminal residue. The assignment of the N-terminal residue trivally allows the assignment of the 6.80-ppm NH and 4.73-ppm α-CH resonances of the dipeptide, Boc-Met$_2$-OMe, to the C-terminal residue. The remaining methionine resonances of all the other homooligopeptides, however, cannot be assigned on the basis of the above results.

To identify the NH and α-CH resonances of these other methionine residues, we examined co-oligopeptides of methionine singly substituted with glycine. To obtain the assignments for Boc-Met$_6$-OMe, the following co-oligopeptides were synthesized:[9]

> Boc-Gly-Met-Met-Met-Met-Met-OMe
> Boc-Met-Gly-Met-Met-Met-Met-OMe
> Boc-Met-Met-Gly-Met-Met-Met-OMe
> Boc-Met-Met-Met-Gly-Met-Met-OMe
> Boc-Met-Met-Met-Met-Gly-Met-OMe
> Boc-Met-Met-Met-Met-Met-Gly-OMe

These co-oligopeptides proved insoluble in CDCl$_3$. Addition

of 1% Me$_2$SO-d_6 improved their solubility. The NMR spectra for dilute solutions of Boc-Met$_6$-OMe and the six co-oligopeptides in 99% CDCl$_3$/1% Me$_2$SO-d_6 (v/v) are compared in Figure 2A-G.

The following interesting features are observed. (a) The glycyl NH appears as a distinct triplet and the α-CH$_2$ usually appears in an upfield position(3.8-4.0 ppm), clearly distinguishing these resonances from methionine resonances.[10] (b) The methionine resonances of the co-oligopeptides and Boc-Met$_6$-OMe are remarkably similar in spectral appearance. The seven hexapeptides show methionine resonances which are virtually identical in chemical shift, with the largest variation in chemical shift ~0.1 ppm. These data are summarized in Table I. (c) Each co-oligopeptide derivative leads to a different simplification of the methionine α-CH and NH regions vis-á-vis the same regions in Boc-Met$_6$-OMe. Arrows are placed in Figure 2 to indicate the methionine NH and α-CH resonance missing in each co-oligopeptide. Each pair of missing resonances allow a separate assignment to be made for one NH and one α-CH resonance of Boc-Met$_6$-OMe. For example, the spectrum of Boc-Gly-Met$_5$-OMe confirms the assignment of the upfield NH and α-CH resonances to the N-terminal residue (Met^1). The six co-oligopeptides together give complete assignments (Table I) for Boc-Met$_6$-OMe in 99% CDCl$_3$/1% Me$_2$SO-d_6. In addition, homonuclear spin-decoupling experiments were carried out for all seven hexamers in 99% CDCl$_3$/1% Me$_2$SO-d_6. These experiments confirm the assignments listed in Table I by showing the correct pairing of α-CH and NH resonances. Me$_2$SO-d_6 induces shift changes of peptide peaks from their positions in CDCl$_3$.[4,5a] The small amounts of Me$_2$SO-d_6 present here, however, do not greatly change the spectral appearance of Boc-Met$_6$-OMe in pure CDCl$_3$. (See Figures 1B and 2A.) Thus, extrapolation of chemical shift data at various amounts of Me$_2$SO-d_6 to CDCl$_3$ yields chemical shift assignments for Boc-Met$_6$-OMe in CDCl$_3$.

The chemical shift assignments and established coupling relationships of the NH and α-CH resonances of the Boc-Met$_n$-OMe (n = 2-7) homooligopeptides in CDCl$_3$ are now given in Table II.

The assignments for Boc-Met$_2$-OMe, Boc-Met$_3$-OMe, and Boc-Met$_6$-OMe are unequivocal as the necessary co-oligopeptides—Boc-Gly-Met-OMe, Boc-Met-Gly-OMe, Boc-Gly-Met$_2$-OMe, Boc-Met-Gly-Met-OMe, Boc-Met$_2$-Gly-OMe, Boc-Gly-Met$_5$-OMe, Boc-Met-Gly-Met$_4$-OMe, Boc-Met$_2$-Gly-Met$_3$-OMe, Boc-Met$_3$-Gly-Met$_2$-OMe, Boc-Met$_4$-Gly-Met-OMe, and Boc-Met$_5$-Gly-OMe[9]—were available for comparisons. These assignments are also confirmed by the double-resonance experiments. For Boc-Met$_4$-OMe, Boc-Met$_5$-OMe and Boc-Met$_7$-OMe, the peptides Boc-Gly-Met$_3$-OMe, Boc-Gly-Met$_4$-OMe, and Boc-Gly-Met$_6$-OMe were available. Thus the Met^1 NH and α-CH are directly confirmed in these peptides. The remaining resonances of these three homooligopeptides are assigned from those for Boc-Met$_6$-OMe and the results of the spin-decoupling experiments. The latter reveal that each NH and α-CH resonance of a given oligopeptide from the dimer to the heptamer can be described by the one, unique pattern of coupling relationships given in Table II.

Discussion

Our results suggest that, if the introduction of a "guest" amino acid into a homooligopeptide does not grossly affect the peptide conformation, NMR assignments for a homooligopeptide may be deduced by comparison with various co-oligopeptide derivatives where a single "guest" amino acid replaces a "host" amino acid at different positions. The approach is useful in that, combined with standard decoupling experi-
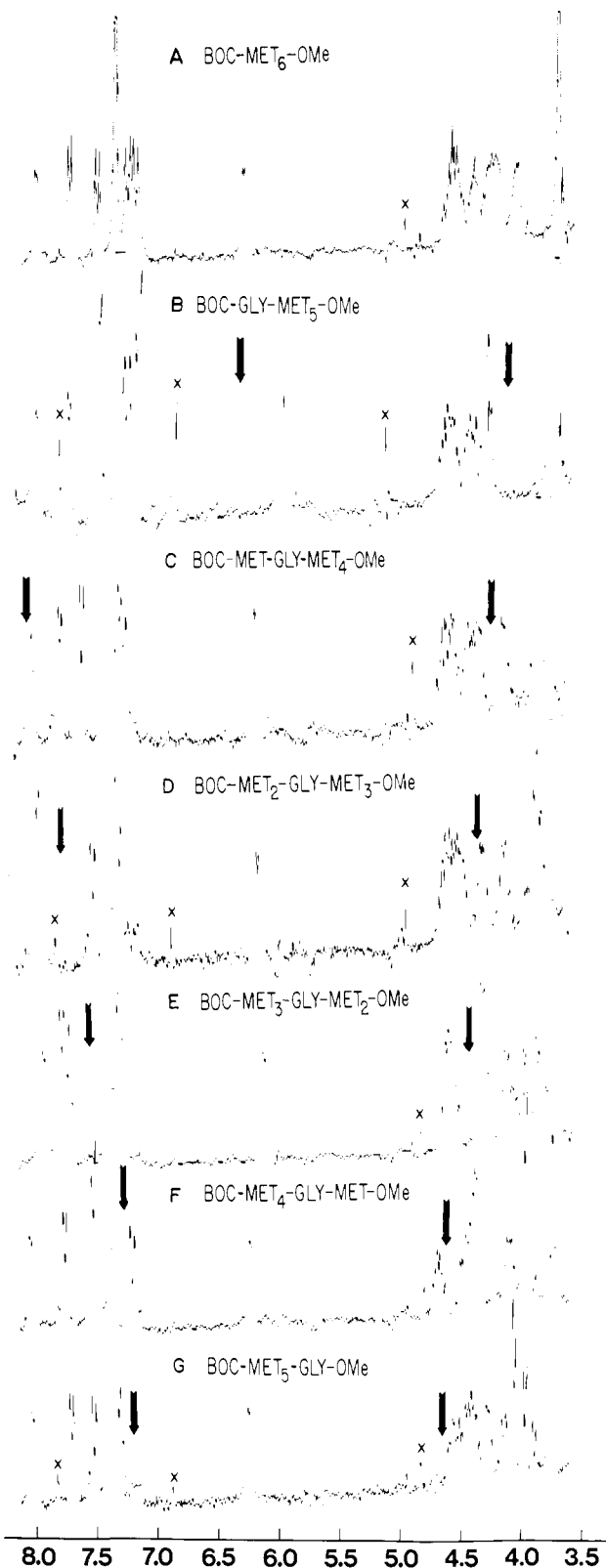

Figure 2. Partial ^{1}H NMR spectra over a 1000-Hz range showing the amide and α-methine regions of methionine hexapeptides in 99% CDCl$_3$/1% Me$_2$SO-d_6 at 220 MHz: (A) Boc-Met$_6$-OMe, (B) Boc-Gly-Met$_5$-OMe, (C) Boc-Met-Gly-Met$_4$-OMe, (D) Boc-Met$_2$-Gly-Met$_3$-OMe, (E) Boc-Met$_3$-Gly-Met$_2$-OMe, (F) Boc-Met$_4$-Gly-Met-OMe, (G) Boc-Met$_5$-Gly-OMe. A was at 1.09 × 10^{-3} and B-G were at 1.18 × 10^{-3} M. Each NMR spectrum was obtained by accumulating 2000 scans over a sweep width of 2500 Hz. Arrows indicate the methionine NH and α-CH resonances missing in each co-oligopeptide spectrum (B-G) compared with the spectrum of the homooligopeptide (A). X denotes spinning side bands of the solvent CHCl$_3$ peak or a coherent side band from the spin-decoupling apparatus. The methionine side-chain β-CH$_2$ and γ-CH$_2$ groups each show overlapping proton signal at ~2.1 and 2.6 ppm, respectively. The six -SCH$_3$ groups of Boc-Met$_6$-OMe appear as singlet resonances at ~2.1 ppm.

Table I. Chemical Shift Assignments for Methionine Oligopeptides in 99% $CDCl_3$/1% $Me_2SO\text{-}d_6$[a]

Protected oligopeptides	Amide region Met NH						Gly NH	α-Methine region Met α-CH						Gly α-CH$_2$
Boc-Gly-Met$_5$-OMe	8.04	7.75	7.50	7.27	7.20		6.12	4.61	4.56	4.41	4.29	4.24		4.27
Boc-Met-Gly-Met$_4$-OMe		7.76	7.58	7.27	7.24	6.16	7.99	4.62	4.56	4.40	4.33		4.12	3.84
Boc-Met$_2$-Gly-Met$_3$-OMe	7.94		7.50	7.3[b]	7.28	6.15	7.96	4.62	4.55	4.49		4.30	4.12	4.0, 3.8[c]
Boc-Met$_3$-Gly-Met$_2$-OMe	7.93	7.78		7.3[b]	7.27	6.12	7.72	4.62	4.57		4.32	4.29	4.12	4.0, 3.8[c]
Boc-Met$_4$-Gly-Met-OMe	8.03	7.75	7.51		7.19	6.22	7.50	4.68		4.46	4.40	4.40	4.09	3.95
Boc-Met$_5$-Gly-OMe	8.02	7.70	7.51	7.3[b]		6.24	7.28		4.55	4.50	4.40	4.26	4.06	4.0, 3.8[c]
Boc-Met$_6$—OMe	8.01	7.75	7.50	7.26	7.19	6.30		4.61	4.57	4.42	4.29	4.23	4.06	
Residue assignment	Met^2	Met^3	Met^4	Met^5	Met^6	Met^1		Met^6	Met^5	Met^4	Met^3	Met^2	Met^1	

[a] Data for peptide at 1 mg/mL (1.09 × 10⁻³ M for Boc-Met$_6$-OMe, 1.18 × 10⁻³ M for Boc-(5Met,Gly)-OMe compounds) in 99% $CDCl_3$/1% $Me_2SO\text{-}d_6$ at 22 °C. Chemical shifts in parts per million downfield from internal Me$_4$Si. [b] Chemical shift estimated as amide peak is buried under the solvent CHCl$_3$ peak. [c] Nonequivalent glycyl α-methylene protons.

Table II. Chemical Shift Assignments,[a] Coupling Pattern,[b] and Relative Intensities[c] for NH and α-CH Resonance of Boc-Met$_n$-OMe Oligomers

Oligomer	Methionine NH Region							Methionine α-CH Region						
Boc-Met$_2$-OMe	5.16	6.80											4.73	4.29
Boc-Met$_3$-OMe	5.15	6.95	6.89									4.67[d]	4.62[d]	4.25
Boc-Met$_4$-OMe	5.32	7.36	7.20	7.02							4.67[d]	4.65[d]	4.53	4.19
Boc-Met$_5$-OMe	5.46	7.74	7.46	7.30	7.06					4.63[e]	4.59[e]	4.53[e]	4.34	4.09
Boc-Met$_6$-OMe	5.51	7.86	7.66	7.39	7.16	7.08			4.64[d]	4.57[d]	4.41	4.30[d]	4.26[d]	4.08
Boc-Met$_7$-OMe	5.53	7.95	7.76	7.52	7.24	7.18	7.11	4.63[d]	4.58[d]	4.42	4.27[e]	4.27[e]	4.27[e]	4.08
Observed coupling	◊	□	△	▽	○	+	●	●	+	○	▽	△	□	◊
Residue assignment	Met^1	Met^2	Met^3	Met^4	Met^5	Met^6	Met^7	Met^7	Met^6	Met^5	Met^4	Met^3	Met^2	Met^1

[a] Data for peptides in CDCl$_3$ at 23 °C. Exact concentrations of peptides are given in the legend to Figure 1. [b] Coupling pattern is verified by irradiating independently in the NH and α-CH regions. See Experimental Section for more details. [c] The resolved NH and α-CH proton signals integrate to one proton each unless indicated. [d] Two partially overlapping proton resonances. [e] Three overlapping proton resonances.

ments, complete, unambiguous assignments of all NH and α-CH resonances of a homooligopeptide may be possible.

A more obvious method for assignment of the NH and α-CH resonances of a protected peptide would be the use of homooligopeptides isotopically enriched at a specific nitrogen with ¹⁵N or a specific α-carbon with ¹³C or ²D. Nakamura and Jardetzky have elegantly employed the deuterium label in obtaining unequivocal assignments for the methylene proton resonances of water-soluble di-, tri-, tetra-, and pentaglycines.[7] This latter procedure is generally applicable. However, a considerable number of labeled peptides will be necessary to obtain assignments for a given homologous series of protected homooligopeptides, and would require large amounts of enriched amino acids. In our procedure, the label no longer need be an isotopically enriched amino acid but instead merely another amino acid with different side chain.

Our method may not be useful in every case. An underlying assumption is that the homo- and co-oligopeptides have similar shifts reflective of similar residual backbone structure. Thus, our method appears most likely applicable in situations where only the nearest neighbor amino acids on the average have significant effect on the chemical shifts of the α-CH and NH resonances, e.g., homooligopeptides in random coil, β structures, extended forms. The method may not be useful when residual peptide structure brings nonadjacent residues into close proximity in the homooligopeptide, e.g., the presence of a β or γ turn or the formation of α helix. The introduction of a "guest" amino acid then may possibly induce a large conformational change.

We have elsewhere reported the ¹H NMR assignments for the Boc-Met$_n$-OMe homooligopeptides in $Me_2SO\text{-}d_6$.[12] This strongly hydrogen-bonding solvent supports random coil forms for linear oligopeptides.[6a,13] In $Me_2SO\text{-}d_6$, the interior Met NH resonances appear upfield from the C-terminal Met NH. Thus the assignments in CDCl$_3$ differ from those in $Me_2SO\text{-}d_6$ and we may exclude random coil conformations for the Boc-Met$_n$-OMe homooligopeptides (except the dimer) in CDCl$_3$.

Our assignments suggest that in each homooligopeptide the NH proton of Met^2 participates in the strongest hydrogen bond while the NH protons of Met^3, Met^4, etc., are progressively involved in weaker hydrogen bonds on proceeding to the C-terminal residue. Two pieces of evidence suggest that the hydrogen bonding may likely be intramolecular. First, IR spectroscopy indicates that Boc-Met$_3$-OMe and Boc-Met$_4$-OMe have, respectively, ~30 and 55% intramolecular, hydrogen-bonded folded forms.[14] Secondly, in the chemical shift data for Boc-Met$_6$-OMe (Tables I and II), the presence of small quantities of $Me_2SO\text{-}d_6$ preferentially shifts the N-terminal Met^1 NH proton by >0.80 ppm while other methionine NH resonances shift by <0.1 ppm. These points obviously need further investigation and, in a future paper, we hope to comment further on the conformations of these Boc-Met$_n$-OMe oligopeptides in CDCl$_3$ with more extensive concentration, temperature, and solvent dependence studies.[15,16]

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(8) The chemical syntheses of complete series of Boc-Met$_n$-OMe oligopeptides has been reported: (a) the mixed anhydride method to the nonamer by F. Naider and J. M. Becker (*Biopolymers*, **13**, 1011 (1974)) and (b) the acylazide method to the heptamer by F. M. Bonora and C. Toniolo (*ibid.*, **13**, 2179 (1974)). Toniolo and co-workers report that their Boc-Met$_n$-OMe oligopeptides are insoluble in CHCl$_3$ at the hexapeptide level.[14] We find limited solubility for the hexamer and heptamer in this solvent, and increased solubility with addition of Me$_2$SO-d_6.
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(11) Our assignment of the lowest field α-CH resonance (4.61 ppm) to the C-terminal residue of the Boc-Met$_n$-OMe homooligopeptides (n = 2–7) agrees favorably with similar assignments made by Pysh and Toniolo for *dilute* norvaline dimers, trimers, and tetramers in CDCl$_3$.[4] Pysh and Toniolo make their assignment by noting an unusual downfield shift for the lowest field α-CH with temperature.[4]
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Role of the Pyrimidine Base in Ribonuclease A Hydrolysis of RNA. Determination of the Conformation of Cyclic β-Cytidine 2',3'-Phosphate and Cyclic β-Uridine 2',3'-Phosphate in Solution

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Abstract: Ribonuclease A (RNase) cleaves ribonucleic acid at the 3'-pyrimidine nucleotide sites, producing 2',3' cyclic nucleotides which subsequently react with RNase to produce 3' nucleotides. The relative orientation of the pyrimidine base relative to the ribose moiety is an important aspect of the mechanism of cleavage and subsequent hydrolysis of the cyclic intermediates. Using NMR chemical-shift changes induced by the complexation of Pr^{3+} and Dy^{3+} to the phosphate group of cyclic β-cytidine 2',3'-phosphate (2',3'-cCMP) and cyclic β-uridine 2',3'-phosphate (2',3'-cUMP), the glycosyl angle, as defined by Donahue and Trueblood, is $120 \pm 16°$ for 2',3'-cCMP and $116 \pm 16°$ for 2', 3'-cUMP, both syn conformations. The broadening of ^{1}H resonances caused by Gd^{3+} is consistent with the angles deduced from shift data. Circular dichroism (CD) spectra and ^{1}H-^{1}H spin–spin coupling constants are the same for uncomplexed and complexed cyclic nucleotides, showing that complexation does not change their conformation. The constancy of the CD spectra at elevated temperatures (to 85 °C) shows a minimum rotation barrier for base rotation of 25 kcal/mol. Previously proposed mechanisms for the RNase-catalyzed hydrolysis of RNA have invoked the interaction of the C(2) carbonyl oxygen atom with the enzyme as a possible feature of the selection of 3'-pyrimidine nucleotides as cleavage sites as well as interaction of this atom with the phosphate group in the second step of the hydrolysis. The syn conformation of the pyrimidine bases and the high rotation barrier, however, strongly suggest that the role of the C(2) oxygen atom is not a decisive factor in the mechanism of RNA hydrolysis by RNase.

In the hydrolysis of RNA catalyzed by bovine pancreatic ribonuclease A (RNase), the cyclic pyrimidine nucleotides, cyclic β-cytidine 2',3'-phosphate (2',3'-cCMP) and cyclic β-uridine 2',3'-phosphate (2',3'-cUMP), are formed. Subsequently, RNase catalyzes the hydrolysis of these cyclic nucleotides specifically to the 3'-monophosphates.[1,2] Acidic or

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basic hydrolysis in the absence of enzyme leads to mixtures of the 2' and 3' isomers. The enzyme RNase has been particularly well studied,[3,4] including full sequencing[5] and structural determination via x-ray diffraction.[6] In view of the known structural detail of the enzyme, several proposals for the intimate mechanism of hydrolysis of the cyclic intermediates to the 3'-monophosphates have been suggested.[7-15] In these mechanisms, consideration is given to the role of the pyrimidine