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Proton Magnetic Resonance Assignments of the Polypeptide Antibiotic Telomycin†

N. G. Kumar and D. W. Urry*

ABSTRACT: The 220-MHz proton magnetic resonance spectrum of telomycin, a cyclic undecapeptide antibiotic, has been analyzed, and the resonances have been assigned to specific hydrogens of the constituent amino acids. The majority of resonance assignments were made possible by proton homonuclear spin-decoupling experiments in different solvents at different temperatures. Temperature dependence of the peptide NH chemical shifts in different solvents was also utilized in confirming the identification of amide resonances and in achieving complete assignment for a given solvent and temperature.

Telomycin, an undecapeptide antibiotic, was isolated in 1958 by Misiek *et al.* from the culture broth of an unidentified streptomyces. Sheehan *et al.* (1963, 1968) determined the primary structure of telomycin as given in Figure 1. Hle is *erythro*-3-hydroxyleucine[1] and Δ-Trp is α,β-didehydrotryptophan. *trans*-3-Hyp and *cis*-3-Hyp are *trans*- and *cis*-3-hydroxyproline, respectively. All amino acids in telomycin are reported to be of the L configuration (Sheehan *et al.*, 1963, 1968).

One prerequisite for a successful conformational analysis of a polypeptide by nmr is the identification of the resonance pattern of individual protons of the constituent amino acids and assignment of individual resonances to specific protons. Therefore, in this paper we wish to report the analysis of the nmr spectra of telomycin in dimethyl-d_6 sulfoxide and the assignment of proton resonances to specific hydrogen atoms. In dimethyl-d_6 sulfoxide, telomycin exhibited features indicative of defined secondary structure. These conformational aspects will be discussed in a subsequent paper.

Experimental Section

Spectra were recorded on a Varian Associates HR-220 spectrometer. Chemical shifts were measured relative to tetramethylsilane as an internal reference. The chemical-shift

† From the Laboratory of Molecular Biophysics, the University of Alabama Medical Center, Birmingham, Alabama 35294. *Received March 9, 1973.*

[1] Abbreviations used are: Hle, *erythro*-3-hydroxyleucine; Δ-Trp, α,β-didehydrotryptophan; 3-Hyp, 3-hydroxyproline; nmr, nuclear magnetic resonance; BAWP, butanol–glacial acetic acid–water–propanol (30:6:24:20); α-L-Thr, *allo*-L-threonine.

TELOMYCIN

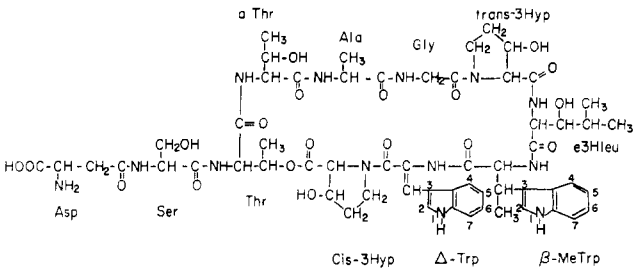

FIGURE 1: Primary structure of telomycin.

difference between resonances of ethylene glycol or methanol was used to determine the probe temperature. Proton spin-decoupling experiments were performed with a field-tracking decoupling accessory designed in this laboratory; by this accessory a resonance may be irradiated continuously as the remainder of the spectrum is scanned. Most of the decoupling experiments were performed at 46 and 69°, and then coupled resonances were followed as a function of temperature to determine their positions in the 40° spectrum.

Telomycin was a gift from Bristol Laboratories and was shown to be homogeneous by thin-layer chromatography on silica gel G in BAWP[1] (30:6:24:20) and propanol–H_2O (7:3) solvent systems.

Deuterium-exchanged samples were prepared by heating a solution of telomycin in 20% D_2O–Me_2SO-d_6 (v/v) overnight at 65°. The solution was evaporated under vacuum and the deuterated sample was redissolved in Me_2SO-d_6. A small amount of back-exchange of labile deuterons with traces of H_2O in the solvent had occurred, but the α-CH region was free of an intense H_2O resonance which could otherwise obscure some of the α-CH absorptions.

Results and Discussion

Assignment of Resonances

The proton magnetic resonance spectra of unexchanged and exchanged telomycin in Me_2SO-d_6 are presented in Figure 2, together with an assignment of resonances. The lower spectrum was recorded after exchanging all the peptide protons for deuterium, as indicated in the Experimental Section. The peptide proton region of the spectrum is shown in Figure 3. Amide resonances are numbered in the order of increasing field as they appear in Me_2SO-d_6.

Assignment of multiplets to specific protons or groups of protons in telomycin is complicated by several factors. One is that several amino acids appear more than once in the 11 amino acid sequence; there are two threonines (one *allo*-L-threonine and one L-threonine), two prolines (one *cis*-3-hydroxy-L-proline and one *trans*-3-hydroxy-L-proline), and two tryptophans (one β-methyl-L-tryptophan and one Δ-tryptophan). Six of the 11 amino acids have only one proton in the β position, thereby resulting in a large number of similar splitting patterns for the α-proton resonances. The second complicating factor is that there are two indole rings whose resonances overlap with some of the peptide proton resonances, thereby making the corresponding assignments difficult. The assignment of individual resonances to specific protons is indicated in Figure 2. Identification of proton magnetic resonances was achieved in the majority of cases by performing homonuclear spin decoupling experiments in different solvents at different temperatures. The publication

TABLE I: Proton Magnetic Resonance Parameters of the Peptide NH Resonances of Telomycin.

Amino Acid Residue	Coupling Constant[a] ($J_{\alpha\text{-CH-NH}}$, Hz)	Chemical Shift[b] (ν_{NH}, Hz) (at 220 MHz)
Gly	6.5 ± 0.3 ($J_{\alpha\text{-N}}$)	1925
	3.8 ± 0.3 ($J_{\alpha'\text{-N}}$)	
Hle	9.2 ± 0.2	1635
β-MeTrp	7.3 ± 0.2	1465
Δ-Trp	0.0	2205
Thr	8.1 ± 0.2	1706
*a*Thr	9.0	1904
Ala	8.0 ± 0.2	1693
Ser	~0 (v. small)	1890

[a] Coupling constants within experimental error remained constant between 30 and 68°. [b] Spectra were recorded in Me_2SO-d_6 at 40°.

by McDonald and Phillips (1969) locating resonance positions of amino acids in random coil polypeptides was helpful as an initial point of reference.

Glycine. The peptide NH proton centered at 1915 Hz (no. 1) was assigned to glycine, since it is the only amino acid whose peptide NH proton can exhibit a triplet pattern, resulting from coupling to two magnetically equivalent α-CH_2 protons or a quartet pattern (doublet of doublets) from coupling to two nonequivalent α-CH_2 protons. At 46°, the peptide NH proton of Gly resonates as a quartet (doublet of doublets) centered at 1915 Hz and was decoupled from the α-CH_2 resonances located at 818 and 838 Hz. In Me_2SO-d_6 the quartet pattern is not very well resolved and appears as a triplet, but in the Me_2SO-d_6–CH_3OH (3:7, v/v) mixture at 40°, the quartet structure is apparent. The resonance at 838 Hz was labeled the α'-CH of Gly. Irradiation at this frequency collapsed the small splittings (~3.8 Hz, Table I) of the amide resonance of Gly. Irradiation of a resonance at 818 Hz resulted in collapse of the large splittings (~6.5 Hz, Table I) of amide resonance (no. 1). The resonance at 818 Hz is then assigned to the α-CH of Gly with a large $J_{\alpha\text{-CH-NH}}$ coupling constant (Table I). The chemical-shift difference between the α'-CH and α-CH is ~20 Hz. The α-CH of Gly resonates at an upfield position ($\nu_{\alpha\text{-CH}}$ = 818 Hz) relative to the α'-CH proton. This suggests that the α-CH is slightly shielded either by ring currents, *e.g.*, due to an indole moiety, or the magnetic anisotropy of a nearby peptide moiety.

In the spectrum of the deuterium-exchanged sample, the α-CH and the α'-CH protons resonate as doublets, an AB part of an ABX spin system, centered at 818 and 838 Hz, respectively. The geminal coupling constant (absolute value) between the α-CH and α'-CH protons ($J_{\alpha\alpha'}$ = |15.5 ± 0.5 Hz|, Table II) was obtained by analysis of the AB part.

allo-L-*Threonine Residue.*[2] The methyl resonance of α-L-Thr resonates on the high-field side of the L-Ala methyl resonance and is located at 240 Hz. The methyl CH_3 doublets of *a*-L-Thr and L-Ala overlap at 46°, but are resolved at 69°. The coupling constant between the CH_3 and β-CH protons is 6.0

[2] Pmr parameters are listed in Table II. *a*-L-Thr is distinguishable from L-Thr as the β-CH proton of L-Thr is at lower field due to participation in the lactone bond. The β oxygen of L-Thr is an alkyl oxygen.

Hz. The CH_3 doublet at 240 Hz was decoupled from the resonance located at 830 Hz.

In the deuterium exchanged sample at 69°, irradiation of the CH_3 resonance at 240 Hz collapsed the multiplet at 830 Hz into a doublet (doublet separation ~10 Hz). The resonance located at 976 Hz was in turn decoupled from the multiplet at 830 Hz.

At 46°, the one-proton peptide NH doublet (no. 2) at 1895 Hz was decoupled from the resonance at 976 Hz. At 69°, irradiation of the amide resonance (no. 2) at 1885 Hz collapsed the multiplet at 977 Hz into a doublet (separation of the doublet ~10 Hz). Therefore, the resonances at 1885, 976, 830, and 240 Hz were assigned to the peptide NH (no. 2), the α-CH, the β-CH, and the CH_3 protons of *a*-L-Thr, respectively.

The observed vicinal coupling constant ($J_{\alpha,\beta} \approx 10.0$ Hz) between the α-CH and β-CH protons defines stereochemistry around the C_α–C_β bond and indicates, as is common with subsequent residues, a conformer in which the relative orientation of these two protons is trans.

It might be pointed out that the β-CH proton of *a*-L-Thr resonates at an upfield position relative to its α-CH proton. In the spectrum of the free L-Thr amino acid in D_2O at 40°, the β-CH proton resonates at a lower field position than the corresponding α-CH proton (McDonald and Phillips, 1969). It is quite conceivable that the β-CH proton is shielded either by ring currents of one of the indole moieties or by the magnetic anisotropy of the neighboring carbonyl moieties.

L-Ser Residue. At 23°, in Me_2SO-d_6 the peptide NH protons of L-Ser (no. 3) and *a*-L-Thr (no. 2) overlap. The peptide NH proton (no. 3) resonates at 1917 Hz on the low-field side of the amide resonance (no. 2), and it exhibits a near-zero coupling constant. At 68°, the peptide NH proton (no. 3) appeared as a broad resonance at 1875 Hz and it is coupled to the resonance located at 961 Hz, which in turn is coupled to the resonance at 902 Hz. Therefore, the resonances at 961 and 902 Hz originate from the α-CH and β-CH protons of L-Ser, respectively.

At 23°, in trifluoroethanol–Me_2SO (1:1, v/v) and at 7° in CH_3OH–Me_2SO-d_6 (7:3, v/v) solvent mixtures, the peptide NH proton (no. 3) of L-Ser appears as a well-resolved resonance, appears at lower field than the amide resonance (no. 2) of *a*-L-Thr, and again exhibits a near-zero coupling constant.

L-Threonine.[3] The multiplet located at the low-field position (~1100 Hz) originated from the β-CH proton of the L-Thr involved in the lactone bond (Pitner and Urry, 1972). The β-CH proton resonates as a multiplet (two overlapping quartets) and its splitting pattern is identical in the exchanged and unexchanged samples. Irradiation at 1100 Hz collapsed the methyl doublet centered at 258 Hz into a singlet. The methyl resonance at 258 Hz, therefore, corresponds to the L-Thr involved in the lactone bond. The coupling constant between the methyl CH_3 and β-CH protons is 6.3 Hz.

At 46°, the one-proton peptide NH doublet at 1699 Hz (no. 4) was decoupled from the resonance located at 986 Hz. At 69°, irradiation of the peptide NH resonance (no. 4) at 1695 Hz collapsed a multiplet located at 990 Hz into a doublet. Upon irradiation of the β-CH multiplet at 1100 Hz, in the deuterium exchanged sample at 69°, the doublet of the resonance located at 990 Hz collapsed into a singlet. Therefore, the resonances at 1695, 1100, and 990 Hz were identified as the peptide NH (no. 4), the β-CH, and the α-CH protons of L-Thr, respectively.

[3] Pmr parameters are listed in Table II.

TABLE II: Proton Magnetic Resonance Parameters[a] of the Proton Resonances of Telomycin in Me_2SO-d_6.

Amino Acid Residue	$\nu_{\alpha\text{-CH}}$	$\nu_{\beta\text{-CH}}$	$\nu_{\gamma\text{-CH}_3}$	ν_{CH_3}	$J_{\alpha,\alpha'}$	$J_{\alpha,\beta'}$	$J_{\beta,\beta'}$	$J_{CH_2\text{-}\beta\text{-CH}}$	Other J
Gly	818 (α) 838 (α')				$\lvert 15.5 \pm 0.5 \rvert$				
Hle	888	438	433	179 192		9.5 ± 0.5			$J_{CH_3\text{-}\gamma\text{-CH}} = 6.5 \pm 0.2$
β-MeTrp	999	800		275		~10.5 ± 0.5		6.8 ± 0.2	
Thr[b]	989	1100		258		~10.8		6.3	
*a*Thr	976	830		240		~10.0		6.0	
Asp	858	600 (β') 526 (β)				11.0 ± 0.5 (α,β') 3.8 ± 0.2 (α,β)	12.5 ± 0.5		
Ala	973			243					$J_{CH_3\text{-}\alpha\text{-CH}} = 6.5$
Ser	961	902							

[a] Chemical shifts and coupling constants are reported in hertz. [b] Thr is involved in the lactone bond.

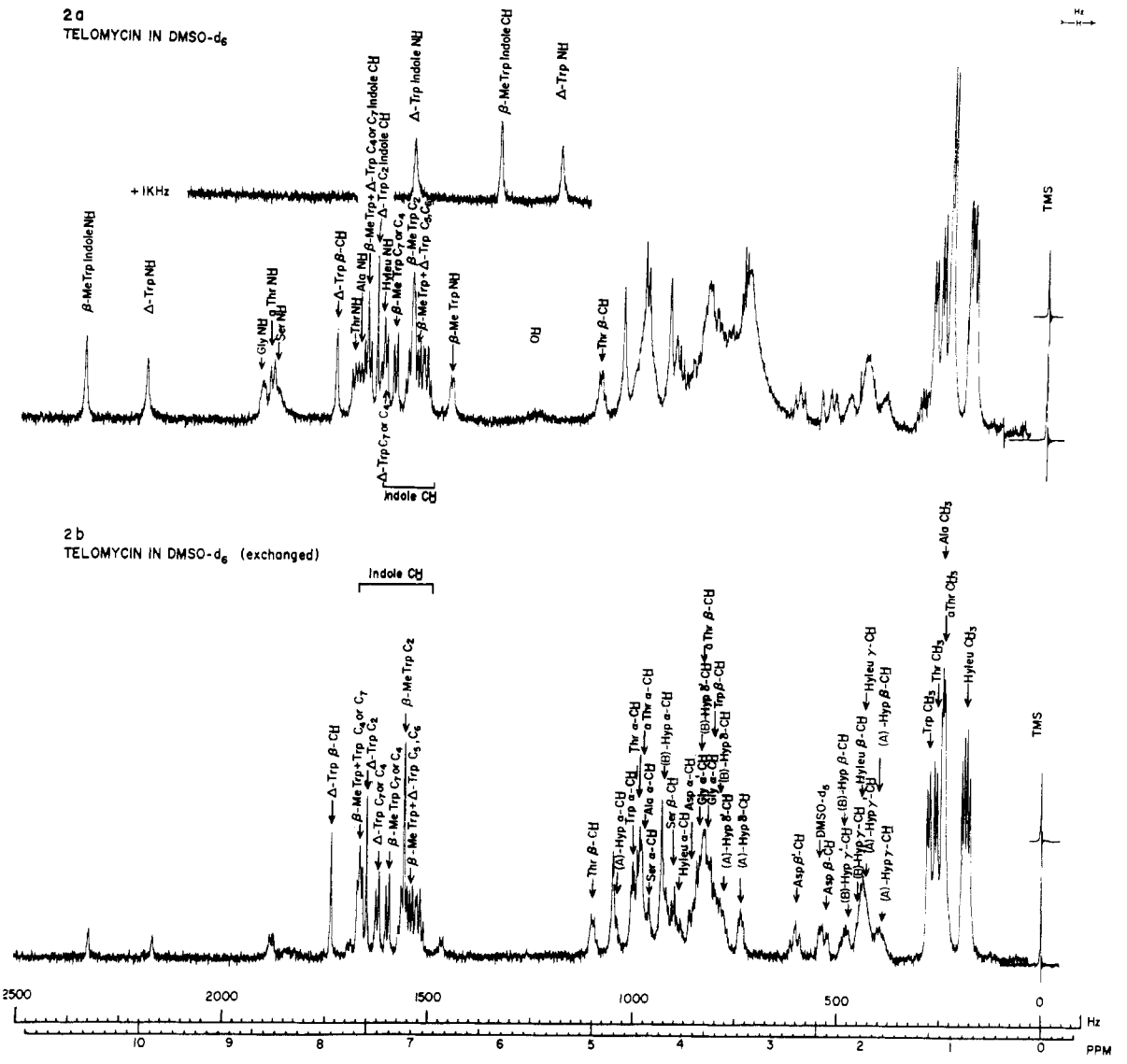

FIGURE 2: Proton magnetic resonance spectra (220 MHz) of telomycin (a) 8% in dimethyl-d_6 sulfoxide at 40° and (b) 10% in dimethyl-d_6 sulfoxide with peptide protons exchanged at 68°.

L-*Alanine Residue.*[3] The methyl protons of L-Ala resonate at 243 Hz and overlap with the methyl protons of L-Thr in Me_2SO-d_6 at 46°; both these CH_3 resonances are resolved at 69°. The coupling constant between the CH_3 and α-CH protons of L-Ala is 6.5 Hz.

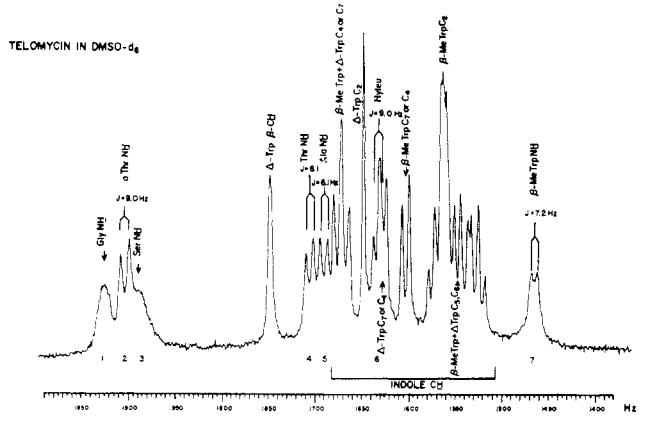

FIGURE 3: Proton magnetic resonance spectra (220 MHz) of the peptide proton resonances in dimethyl-d_6 sulfoxide at 40°.

At 46°, irradiation of the multiplet located at 973 Hz collapsed the doublet centered at 243 Hz into a singlet and also collapsed the doublet of the amide resonance (no. 5) at 1681 Hz. Since this is the only amino acid residue which has a β-methyl group, spin decoupling identifies the resonances at 973, 1681, and 243 Hz as the α-CH, the peptide NH, and CH_3 protons of L-Ala, respectively. These assignments were further supported by the following observations. In the deuterium exchanged sample, irradiation of the methyl CH_3 doublet at 243 Hz collapsed the quartet pattern of the α-CH resonance at 973 Hz. In the unexchanged sample, at 69°, the α-CH multiplet at 973 Hz was decoupled from the amide resonance (no. 5) at 1673 Hz.

erythro-3-Hydroxyleucine. The methyl protons of Hle resonate as a pair of doublets (each of three protons) centered at 179 and 192 Hz. The chemical-shift difference between the two CH_3 protons is ~13 Hz. The methyl protons were decoupled from a resonance located at 433 Hz. The resonance at 433 Hz was assigned to the γ-CH of Hle. The coupling constant between the CH_3 and γ-CH protons is ~6.5 Hz (Table II).

A peptide proton NH resonance signal at 1633 Hz (no. 6) at 40° was decoupled from the resonance at 890 Hz. In the

spectrum of the deuterium exchanged sample at 46°, the one-proton resonance at 888 Hz was simplified and became a clearly resolved doublet, which was decoupled from the resonance located at 438 Hz. This frequency, though near to, is downfield from the region where β and γ protons of leucine usually resonate (345–374 Hz; McDonald and Phillips, 1969). As would be expected, the inductive effect of the β-OH group shifts the β-CH and γ-CH protons downfield. Accordingly, the resonances at 1633, 888, and 438 Hz are assigned to the peptide NH, the α-CH, and the β-CH protons, respectively, of the Hle residue. These assignments were further supported by decoupling the α-CH resonance from the amide resonance (no. 6). In the spectrum of the unexchanged sample at 46°, the α-CH resonance of Hle appears as a triplet centered at 890 Hz. This triplet pattern results from approximately equal vicinal couplings to the β-CH and the peptide NH protons (Tables I and II). Irradiation of the amide resonance at 1630 Hz (no. 6) collapsed the triplet pattern into a doublet. In the decoupled spectrum, the doublet splitting is approximately equal to the vicinal coupling between the α-CH and the β-CH protons.

The observed value of vicinal coupling between the α-CH and the β-CH protons ($J_{\alpha,\beta} = 9.5 \pm 0.5$ Hz) was obtained from the splittings of the α-CH resonance in the spectrum of the deuterium exchange sample.

At 20° in Me_2SO-d_6, the amide proton (no. 6) of Hle overlaps with the C-2 indole CH resonance of Δ-Trp, but at 40° the amide proton (no. 6) resonates as a doublet on the low-field side of the C_7 or C_4 indole CH resonance of Δ-Trp. The identity of the peptide NH proton (no. 6) was confirmed from spin decoupling and chemical-shift temperature dependence in Me_2SO-d_6.

β-Methyl-L-tryptophan.[4] The methyl doublet at 275 Hz was assigned to the CH_3 protons of β-MeTrp, since it was decoupled from the multiplet at 800 Hz. This frequency is located very close to the region in which β-CH protons of tryptophan generally resonate (McDonald and Phillips, 1969). The resonance centered at 800 Hz is assigned to the β-CH proton of β-MeTrp. The coupling constant between the CH_3 and the β-CH protons is 6.8 Hz.

At 46°, the peptide proton NH resonance at 1462 Hz (no. 7) was decoupled from the resonance at 995 Hz. In the spectrum of the deuterium exchanged sample at 69°, irradiation of the β-CH resonance at 800 Hz collapsed the doublet of the resonance at 999 Hz into a singlet. At 69°, irradiation of the peptide NH proton at 1467 Hz (no. 7) collapsed the multiplet (quartet) of the resonance at 999 Hz into a doublet. Therefore, resonances at 1467, 800, and 999 Hz were assigned to the peptide NH, the β-CH, and the α-CH protons, respectively, of β-MeTrp. The vicinal coupling constant between the α-CH and the β-CH protons ($J_{\alpha,\beta} = 10.5 \pm 0.5$ Hz) was obtained from the observed splitting in the deuterium-exchanged sample and by spin decoupling. The amide proton (no. 7) of β-MeTrp resonates at the highest field position in the amide region of the spectrum. The α-CH and CH_3 protons resonate at the lowest field position in the α-CH and the methyl regions of the spectrum, respectively (Urry *et al.*, 1972).

Δ-Trp. The Δ-Trp residue has no α-CH proton; hence at 40° its peptide NH proton (no. 8) appears as a sharp singlet at 2205 Hz. It may also be noted that there is a large difference in acidity between the peptide NH proton of Δ-Trp and the other amide protons of telomycin. The resonance at 1750 Hz was assigned to the β-CH proton of Δ-Trp because its low-field position is characteristic of an olefinic proton geminal to an aromatic group and because it is not coupled to any resonances in the spectrum and does not exchange with deuterium.

[4] Pmr parameters are listed in Tables I and II.

TABLE III: Chemical Shifts[a] of the Proton Resonances of 3-Hydroxy-L-proline Residues of Telomycin in Dimethyl-d_6 Sulfoxide.

Amino Acid Residue	$\nu_{\alpha\text{-CH}}$	$\nu_{\beta\text{-CH}}$	$\nu_{\gamma\text{-CH}}$	$\nu_{\gamma'\text{-CH}}$	$\nu_{\delta\text{-CH}}$	$\nu_{\delta'\text{-CH}}$
A-3-Hyp	1040	400	390	430	738	778
B-3-Hyp	923	485	450	473	788	833

[a] Chemical shifts (ν) are reported in hertz.

L-Aspartic Acid Residue.[3] Since the dipeptide tail of telomycin is formed by a peptide bond between the α-carboxyl group of L-Asp and the α-amino group of L-Ser, the L-Asp residue has no peptide NH proton.

The resonance at 858 Hz was assigned to the α-CH proton of L-Asp, since it was decoupled from the resonances at 600 and 526 Hz. The resonance at 600 Hz is located in that region of the spectrum where β-CH_2 protons of L-Asp normally resonate (McDonald and Phillips, 1969). The α-CH proton of L-Asp resonates as a doublet of doublets at 858 Hz due to vicinal couplings to the β′- and β-CH protons with one large (~11.0 Hz) and one small (3.8 Hz) coupling constant. Irradiation of the resonance at 600 Hz removed the large splitting (between the α and β′ protons) of the α-CH resonance, and irradiation at 526 Hz collapsed the small splittings of the α-CH resonance. Accordingly, the resonances at 858, 600, and 526 Hz were assigned to the α-, β′, and β-CH protons of L-Asp, respectively. The β′-CH resonance appears as a triplet at 600 Hz, resulting from an approximately equal magnitude of geminal coupling to the β-CH proton ($J_{\beta,\beta'}$ = 13.0 Hz, absolute value) and vicinal coupling to the α-CH proton ($J_{\alpha,\beta'}$ = 11.0 Hz). The β-CH resonance pattern appears as a doublet of doublets at 526 Hz due to large geminal and small vicinal coupling constants (~3.8 Hz; Table II).

The resonance assignments were further confirmed by the nmr spectrum of neotelomycin. It is a cyclic decapeptide antibiotic, a telomycin-like molecule which has a proline residue instead of a *cis*-3-Hyp and it lacks aspartic acid. The nmr spectrum of neotelomycin lacks the resonance signals assigned to the α-CH, β′-CH, and β-CH protons of the L-aspartic acid residue of telomycin.

The 3-Hydroxy-L-proline Residues. In the present manuscript the two hydroxyproline residues will be labeled A and B and the assignments of the set of protons—α, β, γ, γ′, δ, and δ′—for each residue will be so indicated. The delineation of cis and trans is assisted by conformational arguments to explain both the $J_{\alpha,\beta}$ coupling constants and chemical shifts and as such will be treated in the subsequent manuscript.

A-3-Hydroxy-L-proline. A-3-Hyp lacks the amide proton; hence the α-CH proton is only coupled to protons appearing further upfield. The resonance at 1040 Hz is not coupled to any peptide NH resonances, but decouples from the resonance at 400 Hz, near the region of the spectrum in which the β-CH_2 protons of proline usually appear (456 and 510 Hz; McDonald and Phillips, 1969). The β-CH proton resonates at 400 Hz (Table III) as a doublet of triplets, resulting from large vicinal

TABLE IV: Coupling Constants[a] of the Proton Resonances of 3-Hydroxy-L-proline Residues of Telomycin.

Amino Acid Residue	$J_{\alpha,\beta}$	$J_{\beta,\gamma}$	$J_{\beta,\gamma'}$	$J_{\delta,\delta'}$	$J_{\delta,\gamma'} = J_{\delta',\gamma}$	$J_{\delta,\gamma} = J_{\delta',\gamma'}$
A-3-Hyp	8.7 ± 0.3	1.5 ± 0.5	3.5 ± 0.3	8.8 ± 0.3	6.3 ± 0.2	3.2 ± 0.3
B-3-Hyp	3.9 ± 0.3	3.8 ± 0.2	1.5 ± 0.5	8.8 ± 0.3	6.3 ± 0.2	3.2 ± 0.3

[a] Coupling constants (J) are reported in hertz.

coupling to the α-CH proton (~9 Hz, Table IV) and small splittings attributed to the interactions with the γ'-CH and γ-CH protons (~3.5 and 1.5 Hz, Table IV). Irradiation of the α-CH resonance at 1040 Hz collapses the large splittings of the β-CH resonance at 400 Hz. Therefore, resonances centered at 1040 and 400 Hz were assigned to the α- and β-CH protons of A-3-Hyp, respectively (Table III).

A single proton multiplet at 738 Hz was assigned to the δ-CH proton of A-3-Hyp. This assignment was confirmed by the following observations. The resonating frequency of the δ-CH proton occurs in the region of the spectrum in which δ-CH_2 protons of proline usually resonate (McDonald and Phillips, 1969). The splitting pattern of the δ-CH proton appears as a doublet of doublets, resulting from geminal coupling to the δ'-CH proton, and vicinal couplings to the γ'-CH and γ-CH protons with one large (~6.3 Hz) and one small (~3.2 Hz) coupling constant (Table IV). The quartet pattern of the δ-CH resonance resulting from geminal coupling to the δ'-CH and large vicinal coupling to the γ'-CH proton is well resolved in Me_2SO-d_6 at 46°, but the small splittings resulting from coupling to the γ-CH proton are only resolved at 69°. Irradiation of the multiplet at 390 Hz removed small splittings from the δ-CH resonance, thereby collapsing the δ-CH multiplet centered at 738 Hz into a quartet. In the decoupled spectrum, the quartet pattern resulted from coupling to the δ'- and γ'-CH protons. The δ-CH proton was also decoupled from a multiplet located at 430 Hz. Irradiation at this frequency collapsed the large splittings of the δ-CH resonance centered at 738 Hz. Hence, decoupling frequency of 430 Hz collapsed the quartet pattern (excluding small vicinal coupling to the γ-CH proton) of the δ-CH resonance into a doublet. The proton at 430 Hz resonates in the region of the spectrum in which γ protons of proline usually appear (McDonald and Phillips, 1969), but the resonance at 390 Hz is shifted upfield. Therefore, the resonances at 390 and 430 Hz were assigned to the γ- and γ'-CH protons of A-3-Hyp, respectively (Table III). The observed values of the geminal coupling between the δ and δ' protons and vicinal couplings between the δ and γ' protons were obtained from the observed splittings and spin decoupling (Table IV).

A multiplet at 778 Hz was assigned to the δ'-CH proton of A-3-Hyp because spin decoupling experiments indicate that this proton is coupled to the γ-CH and γ'-CH protons of A-3-Hyp. The frequency of the δ'-CH proton lies near the region of the spectrum in which δ-CH_2 protons of proline usually resonate (McDonald and Phillips, 1969). The δ'-CH proton appears as a doublet of doublets due to geminal coupling to the δ-CH proton and one relatively large vicinal coupling to the γ-CH proton and one small coupling to the γ'-CH proton. Irradiation of the γ'-CH resonance at 430 Hz removed small splittings from the δ-'CH resonance at 778 Hz, thereby collapsing the multiplet into a quartet. In the decoupled spectrum, the quartet resonance pattern is due to geminal coupling to the δ proton and one relatively large vicinal coupling to the γ-CH proton. The δ'-CH proton was also decoupled from the γ-CH resonance at 390 Hz. Irradiation at this frequency collapsed the large splittings of the δ'-CH resonance. Therefore, a decoupling frequency of 390 Hz collapsed the multiplet of the δ-CH resonance centered at 778 Hz into a doublet (excluding small vicinal coupling to the γ' proton). The geminal coupling constant between the δ' and δ protons and vicinal couplings between the δ' and γ and the δ' and γ' protons are listed in Table IV. It is noteworthy that the γ-CH proton of A-3-Hyp is ring current shifted (Johnson and Bovey, 1958) to high field by 40 Hz relative to the γ'-CH proton, whereas the δ'-CH proton is deshielded by 40 Hz relative to the δ-CH proton.

B-3-Hydroxy-L-proline. Decoupling experiments indicated that the resonance at 923 Hz was not coupled to any peptide NH protons in the downfield region of the spectrum, but was only coupled to the resonance at 485 Hz. This frequency is in the region of the spectrum where β-CH_2 protons of proline are expected to resonate (McDonald and Phillips, 1969). The splitting pattern of the resonance at 485 Hz appears as a triplet of doublets. This resonance pattern resulted from an approximately equal value of vicinal couplings of the β-CH proton to the α- and γ-CH protons, and one small coupling to the γ'-CH proton. Irradiation of the resonance at 923 Hz collapsed the triplet pattern (excluding small coupling to the γ'-CH proton) of the resonance at 485 Hz into a doublet. Therefore, the resonances at 923 and 485 Hz were assigned to the α- and β-CH of B-3-Hyp, respectively (Table III). The vicinal coupling constant between the α and β protons is small (see Table IV).

The multiplet at 788 Hz was assigned to the δ-CH proton of B-3-Hyp. This frequency is located near the region of the spectrum where δ-CH_2 protons of proline are expected to resonate (McDonald and Phillips, 1969). The resonance at 788 Hz was decoupled from the resonances located at 450 and 473 Hz. These latter frequencies are in the region of the spectrum in which β,γ proline protons appear (β-CH_2, 456 and 510 Hz; γ-CH_2, 443 Hz; McDonald and Phillips, 1969). The splitting pattern of the resonance at 788 Hz appears as a doublet of doublets, resulting from geminal coupling to the δ'-CH proton and vicinal couplings to the γ- and γ'-CH protons with one small (~3.1 Hz) and one large (~6.3 Hz) coupling constant. Irradiation of the resonance at 473 Hz removed large splittings from the δ-CH resonance centered at 788 Hz, thereby collapsing the multiplet into a doublet (excluding small coupling between the δ and γ protons). The resonance at 788 Hz was also decoupled from the resonance at 450 Hz; irradiation at this frequency collapsed the multiplet at 788 Hz into a quartet by removing small splittings between the δ- and γ-CH protons. The quartet pattern in the decoupled spectrum resulted from geminal coupling between the δ and δ' protons and one relatively large vicinal coupling between the δ and γ' protons. Therefore, the resonances at 788, 473, and 450 Hz originated from the

δ-, γ′, and γ-CH protons of the B-3-Hyp residue, respectively (Table III).

These assignments were further supported by spin decoupling of the γ′- and γ-CH protons from the δ-CH proton. The γ′-CH proton resonates as a doublet of doublets at 473 Hz. The splitting pattern of the γ′-CH proton is due to vicinal couplings to the δ-CH and the δ′-CH and the β-CH protons with one relatively large (~6.3 Hz) and one small (~1.5 Hz) coupling constant (Table IV). Irradiation of the δ-CH resonance at 788 Hz caused the γ′-CH quartet (excluding the small vicinal coupling between the β and γ′ protons) at 473 Hz to collapse to a doublet, thereby removing large coupling between the γ′- and δ-CH protons.

The γ-CH multiplet at 450 Hz was also decoupled from the δ-CH resonance at 788 Hz. The splitting pattern of the γ-CH proton appears as a doublet of triplets, resulting from approximately equal vicinal couplings to the δ- and β-CH protons, and one relatively large coupling to the δ′-CH proton (Table IV). Irradiation of the δ-CH resonance at 788 Hz collapsed the γ-CH multiplet into a quartet by removing the coupling between the γ- and δ-CH protons. The quartet pattern of the γ-CH resonance in the decoupled spectrum resulted from vicinal coupling to the δ′- and the β-CH protons. The observed values of the geminal coupling between the δ- and δ′-CH protons and vicinal couplings were obtained by analysis of the splitting patterns and spin decoupling (Table IV).

The multiplet at 833 Hz was identified as originating from the δ′-CH proton of B-3-Hyp, because spin decoupling indicated that the proton at this frequency was coupled to the γ- and γ′-CH protons of B-3-Hyp. The δ′-CH resonance pattern appears as a doublet of doublets. This splitting pattern is due to geminal coupling to the δ-CH proton, two vicinal couplings to the γ- and γ′-CH protons with one large (~6.3 Hz) and one small (~3.2 Hz) coupling constant. Decoupling frequency of 473 Hz collapsed the 3.2-Hz splittings of the δ′-CH multiplet at 833 Hz. In the decoupled spectrum, the δ′-CH resonance appears as a quartet due to geminal coupling to the δ-CH proton and vicinal coupling to the γ-CH proton.

The δ′-CH multiplet at 833 Hz was also decoupled from the γ-CH resonance at 450 Hz. Irradiation at this frequency removed ~6.3 Hz splittings from the δ′-CH resonance, thereby collapsing the multiplet into a doublet (excluding small splitting between the δ′ and γ′ protons). As expected, in the decoupled spectrum the separation of the doublet (~8.8 Hz) is approximately equal to the geminal coupling constant between the δ- and δ′-CH protons (Table IV).

These assignments were further supported by spin decoupling of the γ-CH and the γ′-CH protons from the δ′-CH resonance. The γ′-CH proton resonates as a quartet (excluding small coupling between the β- and γ′-CH protons) at 473 Hz. Irradiation of the δ′-CH resonance at 833 Hz collapsed the quartet of the γ′-CH resonance into a doublet. The doublet separation (~6.3 Hz) in the decoupled spectrum is approximately equal to the coupling constant between the γ′- and δ-CH protons (Table IV).

The γ-CH proton was also decoupled from the δ′-CH resonance at 833 Hz. The γ-CH proton resonates as a double of triplets at 450 Hz. This splitting pattern resulted from a relatively large vicinal coupling to the δ′-CH proton and two approximately equal couplings to the β- and the δ-CH protons. Irradiation at 833 Hz collapsed the γ-CH multiplet at 450 Hz into a triplet by removing large couplings between the γ- and δ′-CH protons. In the decoupled spectrum, the triplet pattern of the γ-CH resonance resulted from approximately equal vicinal couplings to the β- and the δ-CH protons.

Assignments of the Tryptophan Indole NH and CH Resonances of Telomycin

The peptide NH and indole NH and CH resonances of telomycin in Me_2SO-d_6 are shown on an expanded scale in Figure 3. The peptide NH and indole NH protons were distinguished from indole CH resonances of β-MeTrp and Δ-Trp by exchanging labile NH protons with deuterium (Figure 2b). The indole NH proton of Δ-Trp is in conjugation with the α–β unsaturated carbon–carbon double bond and carbonyl moiety of Δ-Trp; hence it should resonate at the lowest field position in the spectrum. Therefore, the one-proton resonance at 2564 Hz was assigned to the indole NH proton of Δ-Trp and it exhibited no coupling. The one-proton resonance at 2355 Hz was assigned to the indole NH proton of β-MeTrp. This resonating frequency is in agreement with the chemical shifts of the indole NH resonances of a series of tryptophan analogs in Me_2SO-d_6 (Urry *et al.*, 1972). As anticipated, this sharp singlet at 2355 Hz is not coupled to any resonances in the spectrum.

Assignment of the tryptophan indole CH resonances was accomplished by comparison to the spectrum of the free amino acid (McDonald and Phillips, 1967, 1969). The sharp singlet at 1565 Hz originated from the C_2 indole CH resonance of β-MeTrp, and, as expected, the C_2 indole CH resonance of Δ-Trp is relatively deshielded and resonates as a sharp singlet at 1650 Hz. The multiplet centered at 1550 Hz was assigned to the C_5,C_6 indole CH protons of β-MeTrp and Δ-Trp. The two doublets (each of one-proton intensity) centered at 1603 (J = 8.1 Hz) and 1628 Hz (J = 8.1 Hz) were assigned to the C_7 or C_4 indole CH protons of β-MeTrp and Δ-Trp, respectively. The two doublets (each of one-proton intensity) at 1669 (J = 8.1 Hz) and 1677 Hz (J = 8.1 Hz), originating from the C_4 or C_7 indole CH protons of β-MeTrp and Δ-Trp, respectively, have an inner line in common (the inner lines of both doublets overlap), and therefore they appear as a triplet at 1673 Hz.

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