method with other measurements of relaxation phenomena. The results in this paper show that side-chain internal motions in proteins have timescales and amplitudes which are sufficient to affect longitudinal relaxation rates. This implies that previous models[17] which consider that the only motions of significance for proton relaxation are overall tumbling and methyl group rotations are too simple. Preliminary results[16,44] have shown that data from inversion recovery and T_2 measurements at different frequencies can be interpreted in terms of a simple model of more general internal motion. Another approach is to use the σ_{ij} values in conjunction with calculations of protein dynamics. Recently, correlation functions have been calculated directly from the protein dynamics simulations.[45-47] From these correlation functions it has been predicted that ^{13}C spin-lattice relaxation times are simply changed by scaling factors from those expected for a rigid molecule. These scaling factors are directly analogous to the ratios of observed and calculated σ_{ij} values derived in the present studies.

Another important consequence of differences between observed σ_{ij} values and those calculated on the basis of a rigid model of the protein (i.e., of a variation in effective correlation times of different residues) relates to structural studies of proteins by using proton Overhauser effects. These studies have previously assumed[7] that a single correlation time describes the motion of all proton pairs. The nuclear Overhauser effect depends directly on the value of the correlation time as well as on the inverse sixth power of the distances between protons. If distances between the proton pairs were, however, to be calculated from Overhasuer effects, variations of even a factor of 3 in the scaling factor for σ_{ij} values would result in errors of at most 20% in relative distances. Because distances measured by this technique are generally 5 Å or less,[7] the errors resulting directly from neglect of motional effects of the type considered here are likely to be less than ±0.5 Å. These errors are not large when compared with those of even the highest resolution protein crystal structures and are unlikely to be the dominant errors in NMR distance measurements except at the very highest level of experimental accuracy. Distances measured from the cross-relaxation rates between protons whose separation is not motionally invariant will, however, be averaged over the various interconverting states. That the averaged distances obtained from nuclear Overhauser effects in proteins may not be greatly different from the distances between the average coordinates can be deduced[7] from the high level of correlation between Overhauser effects and distances derived from the crystallographic coordinates of lysozyme. The present results give confidence in the use of proton Overhauser effects for defining accurate structural and dynamical features of proteins in solution.

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Structure Revision of the Antibiotic Vancomycin. The Use of Nuclear Overhauser Effect Difference Spectroscopy

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Abstract: Through the use of nuclear Overhauser effect difference spectroscopy (nOeds), the negative nOe has a great potential for structure elucidation of relatively large organic molecules. This potential could be compromised by even limited spin diffusion. The antibiotic vancomycin has been examined by the nOeds technique to determine the amount of structural and stereochemical information which can be derived. Recognizing, and making allowances for, spin diffusion and "spin migration" (chemical exchange of perturbed NH and OH proton spins), the relative stereochemistry at the majority of the chiral centers of the aglycon portion can be derived. The power of the method is illustrated by the fact that the data demand a significant revision of the structure of vancomycin (previously based on an X-ray study of a crystalline degradation product, CDPI). Where spin diffusion is not involved, the interproton distances calculated from nOe's are normally in good agreement with those from X-ray data. Two exceptions are shown to indicate the oscillation of an aromatic ring in the antibiotic.

A nuclear Overhauser effect (nOe) is observed when irradiation of a proton causes a change in the integrated intensity of the resonance of a second proton. This second proton must be predominantly dipole-dipole coupled to the irradiated proton and relatively close in space to it. The rate of buildup of the nOe shows an r^{-6} dependence.[1] If the integrated intensity of the observed proton increases (positive nOe), information on relative interproton distances can normally be obtained; such is the case for molecules which have a relatively small rotational correlation time τ_c ($\omega\tau_c < 1$). However, when τ_c is relatively large ($\omega\tau_c > 1$), the nOe becomes negative. Under these circumstances, spin-lattice relaxation may become relatively inefficient (especially if $\omega\tau_c \gg 1$), and the perturbations of spin populations caused by the irradiating field pass from spin to spin; the nOe is said to spread by spin diffusion.[2-5] Spin diffusion is more noticeable if the

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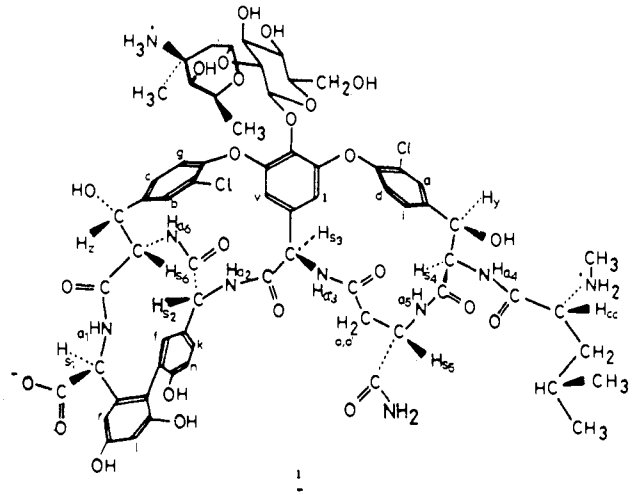
irradiating frequency is maintained for long periods, and data obtained in this manner may be misleading with regard to the inference of relative interproton distances. It is clearly important to establish the extent to which the negative nOe can be useful in structure elucidation. The effect is important since (i) it has the potential to yield structural information on macromolecules such as large antibiotics[6,7] and proteins ($\omega\tau_c > 1$),[8-10] (ii) a viscous solvent may be used[3,11] to change $\omega\tau_c$ from ca. 1 to >1.2 so that the nOe may be manipulated from approximately zero to a useful negative value, (iii) it is normally of greater magnitude than the positive nOe (maximum theoretical enhancements of −100% and +50%, respectively, but note that the maximum enhancement is seldom observed for molecules of molecular weight >200 daltons, because of their relatively slow tumbling rate), and therefore normally more readily observed, and (iv) controlled spin diffusion has the potential to increase the distance over which nOe's may be used to obtain structural information.

Under conditions where spin diffusion may occur, it is still true that the *initial* rate of buildup of the nOe is proportional to r^{-6}, and even when spin diffusion becomes significant at longer irradiation times, the rate of buildup of the nOe by *direct* interaction to an observed proton is proportional to r^{-6}. However, in the latter case there is also a contribution to the nOe from spin diffusion. Thus, rates of buildup of nOe's which are derived from data obtained over longer irradiation periods will lead to values of r which are too small if spin diffusion is involved. Further to the theoretical and experimental studies already cited,[2-11] we have undertaken a study of the rates of buildup of negative nOe's in the antibiotic vancomycin[6,7] (**1**). The nOe's observed in the ^1H



spectra of this molecule are negative because (i) it is studied in a relatively viscous solvent ($[^2H]_6$-Me_2SO) and (ii) it is of moderately high molecular weight (ca. 1400 daltons) and contains a relatively rigid, approximately globular, peptide portion. A more quantitative study of the nOe's than that undertaken earlier[6] is important since relative interproton distances in the fairly rigid portion of vancomycin can be taken from an X-ray study,[12] and compared with those obtained by the nOe. Thus, it is an ideal model to test the advantages and possible pitfalls associated with negative nOe's and spin diffusion.

Experimental Section

Spectra were run at 270 MHz on a Bruker WH-270 with a dedicated Nicolet computer, and at 400 MHz on a Bruker WH-400 with a dedicated Aspect-2000 computer. Typical spectra were obtained from 100 transients in 8K data points with spectral width of 3012 Hz and an acquisition time of 1.36 s, using quadrature detection. A line-broadening exponential factor of 0.5 Hz was used. nOe's were measured by subtraction of the irradiated spectrum from a "blank" spectrum (accumulated immediately before) irradiated at the same frequency for zero time. A typical pulse sequence was [decoupler on–τ_1–decoupler off–2 ms delay–observation pulse–8 s delay]$_n$. Thus spin populations were perturbed by means of a selective frequency decoupling, applied for a time τ_1, which in separate experiments was set at 0, 0.3, 0.6, 1.0, 1.8, and 6.4 s. The FID was then accumulated with the decoupler off, and an 8 s delay followed to allow the spins to revert to their equilibrium populations before the sequence was repeated.

The value τ_1 = 6.4 s was taken to give the steady state nOe value (η_∞). The time $t_{1/2}$, taken to reach half the steady state nOe, was used as a measure of the rate of buildup of the nOe.[2] Where possible, $t_{1/2}$ was measured graphically by the following method: if the nOe builds up to its steady state value in an exponential manner, i.e., $\eta = \eta_\infty(1 - e^{-kt})$, then a plot of ln ($h_\infty - h_t$) vs. t should give a straight line, gradient $-k$, where $k = \ln 2/t_{1/2}$ (h_t is the difference in heights between the peak in the blank and irradiated spectra at time t). When this plot gave marked deviation from linearity, straightforward interpolation was used. It is noteworthy that buildup of the nOe will not be a simple exponential when spin diffusion is involved,[3] but a perusal of some calculations[4] suggests that in many cases the quality of experimental data will be insufficient to show this deviation from exponentiality.

Solutions of vancomycin in $[^2H]_6$-Me_2SO were 0.02–0.04 M, and were dried and degassed by overnight pumping on an oil pump. Spectra were obtained at 35 °C. Samples in which exchangeable hydrogens were replaced by deuterium were prepared by repeatedly dissolving the samples in $[^2H]_2O$ and freeze–drying prior to dissolving in $[^2H]_6$-Me_2SO.

Results and Discussion

A. Data and General Considerations. The steady state nOe's (η_∞) and the times ($t_{1/2}$) taken to attain half the steady state nOe's are given in Table I. The data refer to irradiation of 8 protons (a_1, a_2, f, s_2, s_3, s_4, s_6, and z, see **1**), and have been limited by citing only the shorter $t_{1/2}$ values, and larger η_∞ values, in each case, in order to limit the discussion to that of reasonably close neighbors. In general it is found that, as anticipated, nOe's are transmitted most rapidly (and are larger) to nearest neighbors or to those protons connected by a strong spin diffusion pathway. However, there are two cases (included in Table I) where such a simple interpretation is not possible, and these can be identified and allowed for as shown below.

The first case can be summarized under the general heading of "spin migration". In several cases, relatively short $t_{1/2}$ values are observed which, in the light of the known structure of vancomycin, clearly do not reflect proximity of two protons. For example, upon irradiation of s_6, we observe $t_{1/2}$ = 0.64 s and η_∞ = 17% for l [data are subsequently quoted in the form {s_6}, l, 0.64, 17]. Such results generally arise when both the irradiated and observed protons have exchangeable protons as their nearest neighbors (e.g., s_6 and l). We believe that this effect is due to the chemical exchange of –OH and –NH protons to which the nOe has been transmitted. This hypothesis has been tested by exchanging such protons for deuterium. After such exchange, we observe {s_6}, l, 1.2, 5. Thus our hypothesis is supported and, indeed, such misleading effects can be identified and at least removed partially (if complete deuterium exchange is a problem for practical reasons) in this way. Although the majority of effects which clearly arise from this phenomenon have been omitted from Table I, a few are presented (in italics) to illustrate the care which should be taken in interpretation of data. Thus we have identified the nOe's from s_2, s_3, and z to cc as arising from spin migration. These nOe's must be due to migration of exchangeable protons from the vicinity of the irradiated nuclei to the positions a_4 and $^+NH_2Me$, all of which are adjacent to cc. On addition of D_2O, these nOe's are reduced so much as to be scarcely visible.

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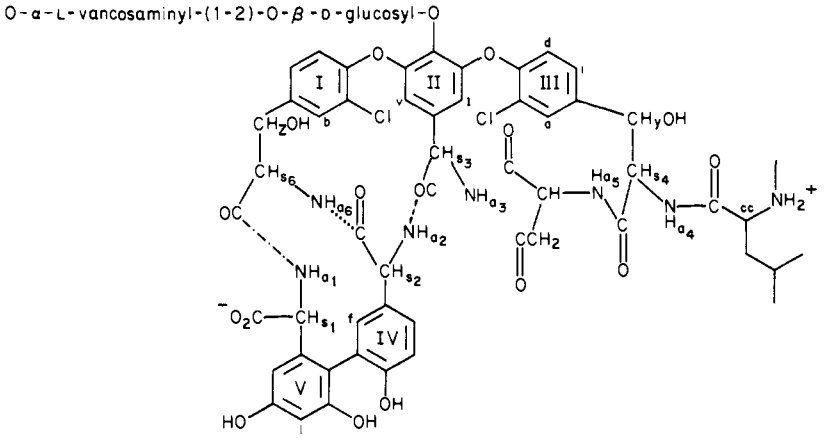
Table I. Values of $t_{1/2}$ (s) and η_∞ (%) for Some nOe's in Vancomycin[a]

obsd proton	irradiated proton								
	a_1	a_2	f[b]	s_2	s_3	s_4[b]	s_6	z	z[c]
a_1				0.29 (38)			0.25 (43)	0.35 (27)	0.67 (15)
a_2				0.41 (17)	0.24 (49)				
a						0.53 (20)			
a_5/a_6				0.34 (17)			0.35 (15)	0.50 (21)	
b	1.05 (8)		0.67 (28)	0.43 (33)			0.39 (40)	0.31 (49)	0.41 (32)
cc				*0.25 (19)*	*0.17 (22)*			*0.50 (36)*	
f	0.46 (6)			0.24 (56)			0.21 (48)	0.63 (23)	0.79 (12)
s_2	0.56 (2)[d]		0.27 (24)				0.24 (35)	0.45 (14)	
s_3		0.22 (53)	*1.2 (18)*	0.81 (25)					
s_4			*0.69 (8)*					0.35 (~42)	0.73 (7)
s_6	0.32 (11)	*0.55 (28)*	0.24 (47)	0.14 (56)				0.37 (30)	(18)
t			*0.75 (17)*		0.41 (39)				
v		(32)		0.55 (30)	0.62 (17)				
y						0.40 (27)			
z	0.39 (7)	*0.38 (24)*	0.86 (19)	*0.39 (30)*			0.29 (32)		

[a] $t_{1/2}$ values are followed by η_∞ values in parentheses. [b] Data recorded after addition of D_2O (40 μL). All other experiments were carried out on vancomycin in which ca. 80% of the exchangeable protons were exchanged for deuterium. [c] Irradiation carried out at 40 dB attenuation of 0.2 W level; all other irradiations at higher power (ca. 22 dB attenuation of 0.2 W level). [d] Part of this nOe is due to s_1, which has the same chemical shift as s_2 under these conditions.



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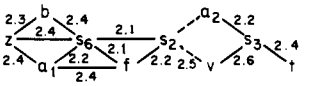
At this point it is worth mentioning that steady-state nOe's to isolated protons are often misleadingly large. This is especially true for those protons whose only near neighbors are exchangeable protons (e.g., l, r, s_1) as saturation of an exchangeable proton (or one whose nearest neighbor is exchangeable) gives large steady-state nOe's to protons whose major source of dipole–dipole relaxation is these exchangeable protons. These nOe's are also much reduced by addition of D_2O to the solution.

The second case where care must be taken is where the irradiating frequency excites more than one nucleus. The high power used to generate almost all the nOe's (see footnote to Table I) is sufficient to fully saturate the irradiated peak and corresponds to a bandwidth >10 Hz. Thus the result {z}, s_4, 0.35, 42 is in fact due to the spread of the irradiating frequency to y, which gives an nOe to s_4. The validity of this conclusion is demonstrated by the large increase in the $t_{1/2}$ value (0.73) upon reducing the irradiating power level (Table I).

B. Structure of Vancomycin. Bearing in mind the above two effects, and being aware of the potential complications due to spin diffusion, we now use the data to assemble the structure of vancomycin treating it as an "unknown". We start from the position existing after mass spectrometry and qualitative observation of a limited number of nOe's in the absence of the use of nOe difference spectroscopy (nOeds) had defined the unit **2** with protons assigned as indicated.[6,7] (The bonds indicated by broken lines in **2** are deduced by means which are detailed subsequently.) The problem is to complete a tricyclic structure (with stereochemical detail, so far as is possible) by making further interconnections in **2**, and adding the NH_2– group of a primary amide. This problem constitutes a realistic test of the current power of nOeds in the presence of a limited amount of spin diffusion (from the largest nOe observed, we calculated vancomycin has $\omega\tau_c \simeq 2.8$ at 35 °C).

We have used the tables of Bothner-By and Noggle[2] to deduce that, where nOe's arise directly and not through spin diffusion, $t_{1/2} = 0.14$ s represents a distance of 2.0 Å. From this, we have deduced the map of interproton distances shown in **3**. The



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proximity of s_2 to a_2 is independently established by their mutual coupling ($J = 6$ Hz). Note that the calculated distances are in fact $\langle r^{-6}\rangle^{-1/6}$ and in general (due to thermal motions) will tend to be less than the equilibrium or $\langle r\rangle$ value. We elaborate upon, and utilize, this point subsequently.

In the construction of such a proton map, direct nOe's are differentiated from spin diffusion as illustrated by the following examples. We observe {z}, s_6, 0.37 and {z}, s_2, 0.45 which could imply that the z–s_2 distance is 1.03× the z–s_6 distance (direct nOe's only) or that s_2 is very close to s_6, b, or a_1 (nOe from one of these protons to s_2 by spin diffusion). We show that the nOe is in fact being transferred by spin diffusion from s_6 to s_2 by {s_2}, s_6, 0.14, demonstrating almost van der Waals contact of s_2 and s_6. Similarly, {z}, f, 0.63 could arise either by a direct nOe (indicating a z–f distance of 2.6 Å) or by spin diffusion from s_6, b, or a_1. Spin diffusion from s_6 is established by {f}, s_6, 0.24 and {s_6}, f, 0.21. It is also clear from the irradiation of s_4 that s_4, a, and y form a

Table II. Some Vicinal Coupling Constants in Vancomycin, and Derived Dihedral Angles and Interproton Distances[a]

nuclei	J, Hz	θ, deg	r_{ij}, Å
s_1–a_1	7	140 ± 10	2.7
s_2–a_2	6	130 ± 10	2.7
s_3–a_3	8	145 ± 10	2.8
s_4–a_4	<2	45–110 (limits ±10)	2.2–2.5
s_5–a_5	7	140 ± 10	2.7
s_6–a_6	12	180 ± 10	2.8
z–s_6	<2	45–110 (limits ±10)	2.2–2.5
y–s_4	4	30 or 120 (±10)	2.1 or 2.6

[a] Dihedral angles (θ) are based on ref 13.

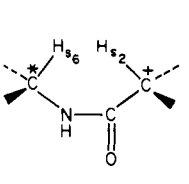
separate group of proximate protons.

The map **3** supports the prior proton assignments given in **2**. The short a_2–s_3 distance indicates the amide connection of ring II to ring IV (broken line in **2**).

At this stage in the argument, we apply the Karplus equation to the measured vicinal coupling constants[6] (Table II). From Table II it can be seen that z is expected to be about 2.3 Å distant from the α-CH proton adjacent to it, consistent with the assignments in both **2** and **3**. Given these assignments, s_2 and s_6 can only be brought into the necessary almost van der Waals contact (s_2–s_6 distance of 2.1 Å) by connecting the CO of ring IV to the NH of ring I via a cis amide bond (see dotted line in **2**). The connection of the ring V NH to the CO of ring I is then mandatory (dotted and dashed line in **2**). We can find no NMR evidence to determine which of the aspartic acid carbonyl groups is attached to the ring II NH, although the structure has been sufficiently defined at this stage to demand that one of them must be connected in this way. The carbonyl group not connected in this way must carry an NH_2 residue, giving the primary amide known to be present in vancomycin.[6,7]

From the data in Tables I and II we now consider the stereochemistry of vancomycin. The stereochemical details available from purely chemical experiments are that *N*-methylleucine and aspartic acid have the *R* and *S* absolute configurations, respectively. The stereochemical details which may be deduced from the present nOe study do not impinge upon these *R* and *S* centers, and therefore it is necessary to assume the absolute stereochemistry at one of the centers bearing protons s_3, s_2, s_1, s_6, and z, in order that the others may be deduced. We have chosen to define the chiral carbon atom bearing z as having the *R* absolute configuration (as is indeed found in the X-ray structure[12]).

The z–b distance of 2.3 Å shown in **3** demands that z and b be eclipsed or nearly so. It has already been deduced that s_2 and s_6 are extremely close (**3**), and connected via a cis amide bond (see above); this necessitates a roughly hexagonal arrangement of the six atoms (**4**). Since the unit **4** forms part of the peptide link between rings I and II, then both the H and N atoms extending from C* in **4** must be oriented to the right in **5** and both the CO and H atoms extending from C^+ must be oriented to the left in **5**. Thus the stereochemistries at C* and C^+ are *S* and *R*, respectively. Since the vicinal coupling constants z/s_6 and s_6/a_6 establish dihedral angles near 80° and 175°, the geometry from H_b to C^+ is quite precisely defined.



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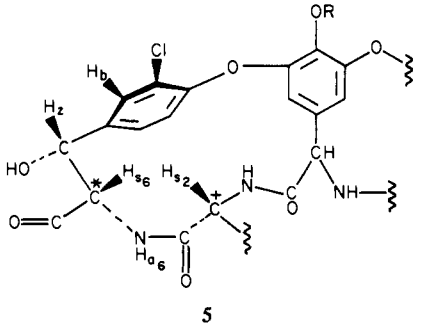
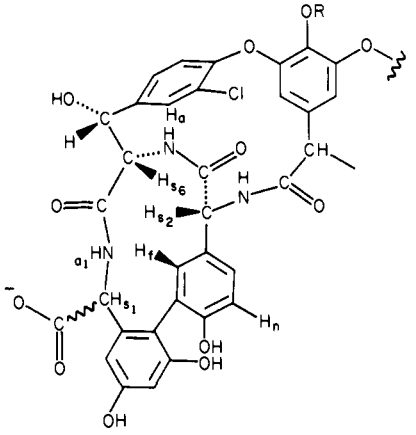


5

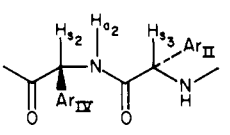
We now turn to the a_1, s_1, f region. The chirality of the biphenyl unit is clear from the fact that f is very close to both s_2 and s_6 (and hence must be on the "top" side of the molecule as represented in **5**), and the requirement that the NH of ring V must be oriented to the left in order to make the amide bond to the CO of ring I. Additionally, the proton map **3** establishes that a_1 is close to both z and s_6 and therefore is also on the "top" side of the molecule. Since the precisely defined geometry of **5** requires that the CO group attached to C* is oriented downwards and to the left from C*, the amide bond between this CO group and NH_{a_1} must be trans. These conclusions are summarized in **6**, and leave



6

only the stereochemistry at the carbon bearing s_1 undetermined in this region. Neither coupling constants (Table II) nor nOe's allow us to distinguish between *R* and *S* configurations at the carbon bearing s_1. However, the *R* configuration would require first-order broadening of H_n (see **6**) in the ^1H spectrum of the europium salt of vancomycin; this is not observed,[6] but the data are in good accord with the *S* configuration.

The s_2, a_2, s_3 region is now considered. The short a_2–s_3 distance (see **3**) demands that the amide bond connecting rings II and IV be trans as in **7** or **8**.



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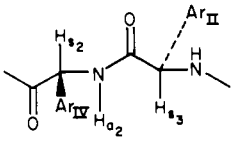


8

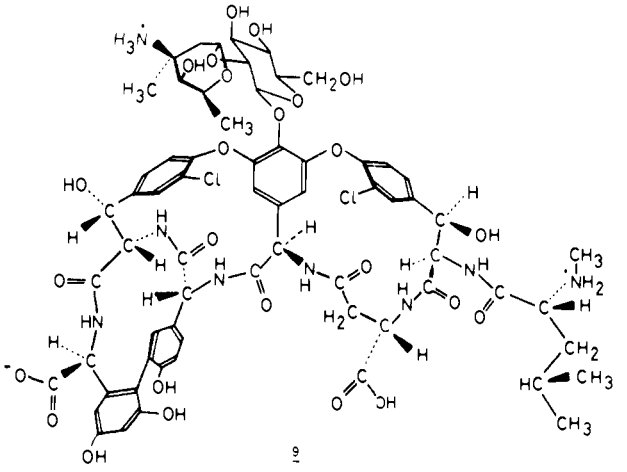
The dihedral angles between the vicinal s_2 and a_2 protons are consistent with either **7** or **8**, and the $t_{1/2}$ value does not securely distinguish them. However, a CPK model of **7** shows a severe steric interaction between the two carbonyl groups of **7**, which seems likely to preclude it. Structure **8** is relatively strain free, and is chosen on these grounds. Thus, the stereochemistry at the center carrying s_3 is determined to be *R*.

The preceding arguments show that it is possible to determine, principally by the use of nOeds and recognition of limited spin diffusion, the complete structure of the left-hand portion of vancomycin as represented in **1**. In the absence of the X-ray data, the mirror image of this portion of the structure would have been equally feasible (but in principle differentiable by means of the interaction with the cell wall analogue Ac-D-Ala-D-Ala[12]).

The stereochemistry at two asymmetric centers (those carrying s_4 and y), and numerous conformational details in the right-hand portion, remain to be considered on the basis of NMR evidence. The data {s}$_4$, y, 0.40 (27) and {y}, s_4, 0.35 show that the s_4, y dihedral angle (Table II) is 30° rather than 120°. Therefore, both protons are on the same side of the molecule. In addition, the nOe's {a_5}, s_4 and {s_4}, a_5 observed previously[6] establish that a_5 is on the same side of the molecule as s_4 and y. Since irradiation of s_4 gives an nOe to a, the most reasonable conclusion would be that a_5, s_4, and y are on the same side of the molecule as the chlorine atom of ring III (since a is next to Cl; see **2**). However,

(13) Cung, M. T.; Marraud, M.; Neel, J. *Macromolecules* **1974**, *7*, 606.

this is inconsistent with the X-ray structure (**9**), in which a is at



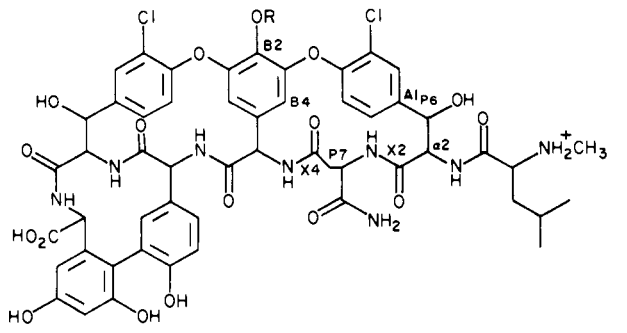
9

the front, but y and s_4 are at the back. **9** is not the structure of vancomycin itself, but of a derivative, CDP-I, formed by heating an aqueous solution of vancomycin at pH 4.2 and 80 °C for 3 days.[14] It lacks the primary amide group of vancomycin.

We first attempted to explain this anomalous nOe by noting that upon irradiation of s_4, spin diffusion could occur via y to the proton of the OH group attached to the same carbon as proton y, and thence to a. This rationalization is in accord with the fact that a is an isolated proton which should derive almost all its dipole–dipole relaxation from the OH proton. Thus, once the spins of the OH group are excited, and the energy diffuses to the "terminal" proton a, there may be no efficient mechanism by which this energy can be lost. However, experimental tests of this hypothesis do not support it. Thus, irradiation of the OH resonance at δ 9.35 does not produce a particularly fast or marked nOe to a. Also, exchange of OH for OD does not remove the {s_4}a nOe (Table I). Conformers of the right-hand portion of the molecule other than **9** do not lead to a more satisfying explanation of the anomaly. A further possibility is that vancomycin does in fact have the chlorine at the back (as in **1**) and that during the conversion of vancomycin to CDP-I, the ring is turned through ca. 180°, giving the conformation **9**. We believe this to be the true explanation, and further evidence for this is provided in the next section.

C. The Conversion of Vancomycin to CDP-I. In this section, we present further evidence for the inversion of ring III in the conversion of vancomycin to CDP-I, and suggest a mechanism for the conversion.

Synthesis of CDP-I usually gives a microcrystalline precipitate of a mixture of two isomers, in a ratio of 2:1 (by NMR). These two isomers will be referred to as M and m, respectively. ^1H and ^{13}C NMR both indicate that the difference lies in the right-hand side (i.e., near ring III), and for both nuclei the m isomer has chemical shifts closely similar to those of vancomycin, while the M isomer is somewhat different (Table III).



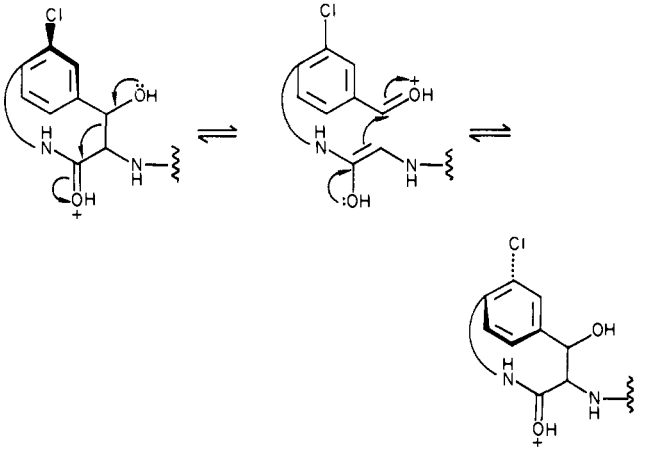
10

Table III. Chemical Shift Data for the CDP-I Isomers and Vancomycin[a]

	CDP-I(M)	CDP-I(m)	vancomycin
	(a) ^1H		
a_3	8.18	7.90	8.13
a	7.60	7.64	7.42
i	7.43	7.47	7.57
d	7.34	7.12	7.20
y	4.64	5.16	5.15
a_5	6.71	6.87	6.59
t	5.79	6.00	5.63
s_3	5.69	5.72	5.71
o	2.92	2.40	2.40
	(b) ^{13}C		
X2	170.8	170.3	170.1
X4	166.2	166.5	166.8
A1	140.8	140.5	139.6
B2	131.4	131.7	131.8
B4	106.8	105.4	107.4
P6	73.1	71.3	71.3
P7	36.0	36.9	36.9

[a] Spectra recorded at 70 °C. Vancomycin ^1H chemical shifts and assignments are taken from ref 6; ^{13}C nomenclature, chemical shifts, and assignments are from ref 15. A partial ^{13}C nomenclature is reproduced in 10. Chemical shifts are given in ppm downfield from Me_4Si.

Scheme I



A more careful crystallization produces only the M isomer, and this is presumed to be the isomer studied by X-ray analysis. We therefore conclude that the X-ray study was carried out on a compound whose stereochemistry was different from that of vancomycin, and this is supported by further nOe experiments, as detailed below.

In both vancomycin and CDP-I (M and m), nOe experiments show that protons s_4 and y are very close together; the three-bond coupling between them indicates a dihedral angle of $\lesssim$30°. These observations are only consistent with the stereochemistry at these centers being that in the crystal structure **9**, or the mirror image. Only the former allows a strong binding of vancomycin to bacterial cell wall analogues.[16]

Results of nOe experiments done on the M isomer of CDP-I are fully consistent with its having the same stereochemistry as the crystal; thus {i}, s_4, 0.60, {i}, y, 0.44, and {s_4}, i, 0.31. Irradiation of y gives no significant nOe to a. On the other hand, vancomycin and CDP-I(m) are similar to each other but different from CDP-I(M). Thus vancomycin {y}, a, 0.29, {s_4}, a, 0.53, {a}, y, 0.52, {a}, s_4, 0.47; {i}, d only and no significant nOe {s_4}, i or {y}, i, while

(14) Johnson, C. R. Thesis, University of Illinois, 1962.

(15) Bongini, A.; Feeney, J.; Williamson, M. P.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 2*, 1981, 201.

(16) Williams, D. H.; Butcher, D. W., unpublished work.

Table IV. Comparison of X-ray and nOe Derived Interproton Distances (Å)

proton (*i*) irrad.	proton (*j*) obsd	r_{ij}^{nOe}	$r_{ij}^{X\text{-}ray}$
a_1	f	2.4	2.2
a_1	z	2.4	2.5
a_1	s_2	2.5	4.0
a_1	s_6	2.3	2.3
s_4	a	2.5	3.9
s_4	y	2.4	2.4
f	z	2.7	~4.3
f	s_2	2.2	2.1
f	s_6	2.2	2.2
s_6	b	2.3	2.5
s_6	s_2	2.1	2.0
s_6	f	2.1	2.2
s_6	z	2.4	2.3
s_3	a_2	2.2	2.2
s_3	t	2.4	2.8
s_3	v	2.6	3.3

CDP-I(m) {s_4}, a, 0.65, {a}, s_4, 0.21; {i}, d only and no significant nOe {s_4}, i.

These data lead us to conclude that ring III undergoes a rotation of ca. 180° between vancomycin and the CDP-I crystal studied by X-rays, leaving the other chiral centers unchanged. Since in the intact molecule there would be very severe steric hindrance to such a rotation, this must involve a bond-breaking step, followed by the ring rotation and a bond-reforming step. The most reasonable candidate for this is the P6-α2 bond (see **10**), which is readily broken by a retro-aldol reaction (Scheme I). This converts both P6 and α2 into sp^2 centers, and therefore these centers can, in principle, be racemized. The fact that they are not significantly racemized in the isomers M and m detected by NMR is understandable in terms of stereochemical restraints imposed by the rest of the molecule.

This structural revision of vancomycin has interesting implications for binding of members of the vancomycin group of antibiotics to the bacterial cell wall and its analogues. Several members of the group[7] (actinoidin, avoparcin, and A-4696[17]) have only one aryl chlorine atom, which, by analogy with vancomycin, we can place at the front of ring I. By contrast, ristocetin has no chlorine substituents, and this leads to its having a greater tolerance for bulky side chains in the carboxyl terminus of cell wall analogues (e.g., Ac_2-L-Lys-D-Ala-D-Leu rather than Ac_2-L-Lys-D-Ala-D-Ala). Compounds with both chlorine atoms at the front (as **9**) may be expected to bind cell wall analogues less strongly, because of greater steric restraints on the C-terminal side chain. This was tested by adding Ac-D-Ala-D-Ala stepwise to a mixture of the two CDP-I isomers, under conditions in which fast exchange between free and bound forms would occur. This led to greater chemical shift changes for the m isomer, indicating that, as expected, the isomer with both chlorines at the front binds less strongly.

D. **Comparison of X-ray and nOe Distances.** A few representative comparisons are made in Table IV.

All values agree within 0.2 Å except those for {a_1}s_2, {s_4}a, {f}z, {s_3}t, and {s_3}v. The lack of agreement for {a_1}s_2 and {f}z is clearly due to spin diffusion, and recognizable as such by the method outlined previously in this paper. As discussed previously, the {s_4}a nOe arises from an inversion of ring III; the crystal distance s_4–i is 2.7 Å. In the cases of {s_3}t and {s_3}v, the nOe method gives distances, from the proton of the α-carbon of ring II to the isolated protons v and t, which are apparently too small. Spin diffusion cannot be involved. However, in these cases, the differences between nOe and X-ray data are readily understood, and are measuring a useful parameter. Since the rate of buildup of the nOe is proportional to r^{-6}, if one proton oscillates (thus periodically altering the internuclear distance), the rate of buildup will markedly increase, and the apparent internuclear distance will decrease. We conclude that the apparent r values obtained from the nOe's {s_3}t and {s_3}v, reflecting $\langle r^{-6}\rangle^{-1/6}$, are less than the X-ray values because ring II is oscillating. This oscillation periodically brings both v and t much closer to s_3 than the average distance. A calculation of the degree of oscillation of ring II from these data is not justified, since a simple pattern of oscillation by ring II (e.g., harmonic) is not followed.

Conclusion

We have shown that the use of nOeds, in the presence of limited and recognized spin diffusion, is capable of giving detailed structural information on complex organic molecules. In the present model study, the main conclusions are in accord with a prior X-ray structure determination; one result has necessitated a significant structural revision of vancomycin. If "spin migration" is recognized, and minimized when necessary through the use of deuterium exchange, then the method is an extremely powerful one for structure elucidation. Subsequent studies will be aimed at using the methods developed in this paper toward the structure elucidation of unknowns.

Acknowledgment. We thank the Science Research Council, Churchill College, Cambridge, and the Smith-Kline Foundation (U.K.) for financial support and Dr. J. Feeney and colleagues (National Institute for Medical Research, Mill Hill, London) for access to a Bruker WH-270 spectrometer.

(17) Hamill, R. L.; Stark, W. M.; DeLong, D. C. U.S. Patent 4 115 552.