would experience even greater interaction of the interior bpy ligands. In earlier work we have observed similar diastereoselectivity in the coordination of 3,3'-tetramethylene-2,2'-bipyridine.[16] Since the crystal is centrosymmetric, the enantiomeric Λ,Λ form is present in equal amount. Recent studies on chiral ruthenium tris-diimine complexes have shown that the Λ form binds preferentially to the left-handed helical form of DNA and upon irradiation becomes an A-conformation-specific DNA cleaver.[17] The implications for complexes such as the one prepared in this study are under consideration.

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M43 Antibiotics: Methylated Vancomycins and Unrearranged CDP-I Analogues

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Vancomycin is a clinically important antibiotic discovered in 1956.[1] One of the three original vancomycin-producing strains of *Amycolatopsis orientalis*[2] (previously designated *Nocardia orientalis* and *Streptomyces orientalis*) numbered M43-05865 (NRRL 2450), produced a new antibiotic designated M43A as the major product. The ratio of M43A to vancomycin produced by strain M43A-05865 is about 2,5:1. Among the minor metabolites produced by this strain are several previously described compounds and include A51568A and traces of A51568B,[3] desvancosamine A51568A, agluco A51568A, desvancosamine vancomycin, aglucovancomycin, desvancosamine M43A (also named M43C), and agluco M43A.[4] This strain also produced small amounts of M43D and trace quantities of M43B. Antibiotic M43A is a tri-*N*-methylleucine analogue of vancomycin, while M43B is the desamido derivative of M43A. The minor metabolite, M43D, is the di-*N*-methylleucine analogue of vancomycin.

The filtered broth of culture *Amycolatopsis orientalis* M43-05865 was purified on the cation resin Dowex 50W-X4, and the M43 complex was obtained on lyophilization of the eluates. The individual M43 factors were obtained by chromatographic purification on RP-18 reversed phase and then desalted by using a Diaion HP20 column.

The HPLC retention times of M43 factors D and A are different from those of vancomycin, M43D is less polar than vancomycin, and M43A is less polar than both M43D and vancomycin.

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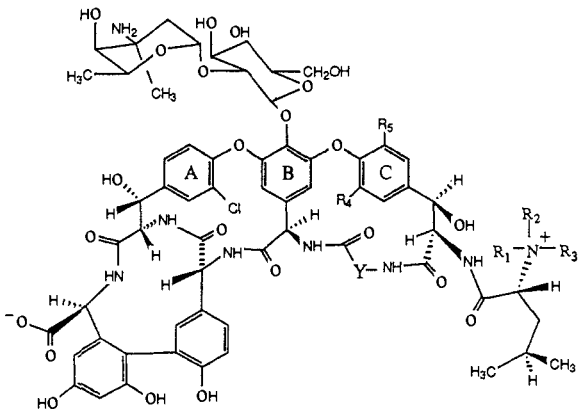
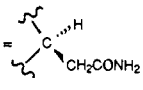
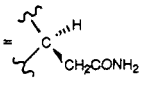
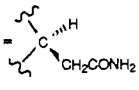
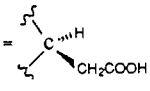
Chart I

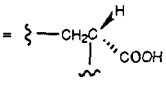


1. Vancomycin: R_1= R_2= R_4= H; R_3= CH_3; R_5= Cl; Y = 

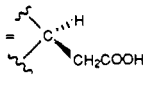
2. M43D: R_1= R_4= H; R_2= R_3= CH_3; R_5= Cl; Y = 

3. M43A: R_1= R_2= R_3= CH_3; R_4=H; R_5= Cl; Y = 

4. M43B: R_1= R_2= R_3= CH_3; R_4= H; R_5 = Cl; Y = 

5. CDP-1: R_1= R_2= R_5= H; R_3= CH_3; R_4= Cl; Y = 

6. R_1= R_2= R_3= CH_3; R_4= Cl; R_5= H; Y = 

8. M43F: R_1= R_2= R_4=H; R_3= CH_3; R_5= Cl; Y = 

The FABMS of M43D shows that it is 14 mass units higher than vancomycin and that M43A is 28 mass units higher than vancomycin. The FABMS also shows cleavages of vancosaminyl and vancosaminyl-*O*-glucosyl fragments from both M43D and M43A, thereby suggesting that the additional mass of 14 and 28 units in M43D and M43A, respectively, are present in the aglucone moieties of the two antibiotics and not in the sugar residues.

The ^{1}H NMR spectra of M43D and M43A are similar to those of vancomycin, except for the signals due to the N-methylated leucine portion of the metabolites (see Table I, Supplementary Material). The intensities of the *N*-methyl signals at 2.13 ppm for M43D and 3.20 ppm for M43A were higher than those due to vancomycin at 2.34 ppm. The above mass spectral and ^{1}H NMR data suggested that M43D contained *N,N*-dimethylleucine and M43A included *N,N,N*-trimethylleucine, and these compounds were assigned structures **2** and **3**, respectively.

This structural assignment of M43A was confirmed by X-ray analysis of a crystalline derivative **6** of M43A. A 100-mg sample of M43A hydrochloride was dissolved in 2 mL of water. The resulting solution at pH 4.2 was heated at 65 °C without stirring for 24 h, when crystals of the rearranged M43A derivative **6** were deposited. Under similar conditions, vancomycin yields CDP-I[5] **5** (crystalline degradation product I), and its X-ray structure determination represented a major step in the structure elucidation of vancomycin.[6] Later revisions of vancomycin structure[7,8] have

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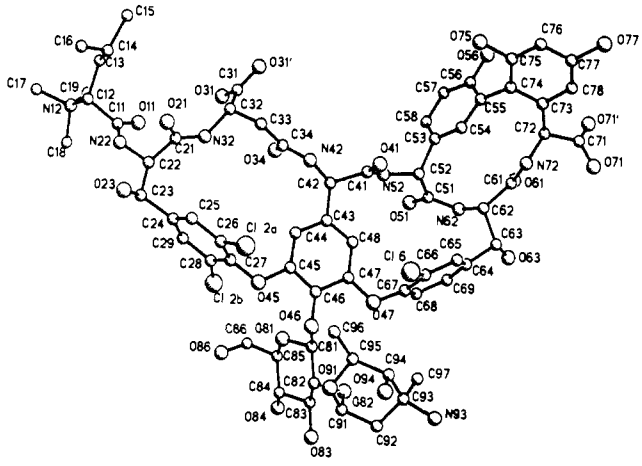
established the structural relationship of CDP-I to vancomycin.

M43A derivative **6** crystallizes as colorless prisms in the orthorhombic space group $P2_12_12_1$ with four molecules in a unit cell with the dimensions a = 14.018 (2), b = 21.460 (5), and c = 33.673 (8) Å. The calculated density is 1.33 g cm^{-3}. A total of 5809 unique reflections with 2θ less than 116.0° were measured on an automated four-circle diffractometer with monochromatic copper radiation. Crystals of vancomycin CDP-I[6] have the same space group and very similar unit cell dimensions. It was assumed that the two structures are nearly isomorphous and a difference electron density map was calculated by using positions reported for vancomycin CDP-I and structure factors for **6**. The difference map clearly showed two additional peaks in the correct positions for methyl groups (C18 and C19 in **7**).



The structure was refined by the least-squares method with anisotropic temperature parameters for all the non-hydrogen atoms of **6**. A difference electron density map showed 28 additional peaks which seemed to be at reasonable positions for water molecules. Eight of these were included in the refinement as oxygen atoms with full occupancy factors and isotropic temperature parameters. The other 20 putative water molecule oxygen atoms were given fixed isotropic temperature parameters, and their occupancy factors were allowed to refine. Of the 78 hydrogen atoms in **6**, 68 were included in the refinement at calculated positions with isotropic temperature parameters. Because of a persistent unexplained peak in the electron density map, we were able to show that chlorine atom 2 in **7** is disordered and exists in approximately 90% of the molecules in position 2a and the other 10% in position 2b. This supports earlier work[7,8] that suggested that in vancomycin the phenyl ring C to which this chlorine is attached flips approximately 180° when forming the CDP-I **5**. It would appear that, as in the case of vancomycin,[7] the chlorine atoms in M43A are on opposite sides of the molecule. The final R factor was 0.127 for 5297 observed reflections. Figure 2 shows an ORTEP plot of the molecule.

Amycolatopsis orientalis M43-05865 also produced trace amount of M43B. As in the case of vancomycin CDP-I **5**, the rearranged product **6** eluted in HPLC as two peaks with retention times 7.77 and 11.71 min, respectively. This is due to the fact that **6** exists as a mixture of two atropisomers as shown by the above X-ray data. In solution, both CDP-I[8] and **6** exist as an equilibrium mixture of atropisomers in which the chlorine-containing aromatic ring is slowly rotating with a half-life of several hours, resulting in separation of the atropisomers by HPLC. However, M43B elutes as a single peak with a retention time of 13.13 min. The new metabolite, M43B, had the same molecular weight and elemental composition as the rearranged M43A derivative **6** by FABMS peak match experiment.[9] Both compounds, M43B and **6** showed fragments with identical masses of 1334 and 1172 in the FABMS by cleavages of vancosaminyl and vancosaminyl-*O*-glucosyl moieties, respectively. These data suggest that M43B has the unrearranged desamido structure **4**.

The antibacterial activity of M43 factors A and D is similar, if not identical, to that of vancomycin suggesting that the state of methylation of the leucine residue does not affect their antibacterial activity. However, M43B is about 20–40 times less active than vancomycin. The corresponding unrearranged desamido analogue of vancomycin, designated M43F (**8**) is about ten times less active than vancomycin.[10] The rearranged M43A compound **6** and CDP-I **5** are completely devoid of antibacterial activity. *Clearly, the negative charge on the aspartate strongly depressses antibacterial activity in* **4**, **5**, **6**, and **8**. The presence of the negative carboxyl group in M43B, M43F, **5**, and **6**, near the binding site, and the change in the conformational geometry[8] in the rearranged **5** and **6** for the binding of D-Ala-D-Ala-carboxyl terminus of UDP-*N*-acetylmuramylpentapeptide to the N-terminal *N*-methylleucine contribute to the diminution in the biological activity in compounds M43B, M43F, **5**, and **6**, respectively. Details of the structure activity relationships of the M43 factors, the rearranged compound **6**, and other vancomycin derivatives will be discussed elsewhere.[11]

Supplementary Material Available: Tables of HPLC retention times and FABMS and ^{1}H NMR spectral data for vancomycin, M43A, and M43D and the atomic coordinates and temperature factors, bond lengths, bond angles, anisotropic temperature factors, and hydrogen coordinates and temperature factors for the M43A derivative **6** (11 pages). Ordering information is given on any current masthead page.

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(9) Fast atom bombardment peak match for **6**: MH^+ = 1477.4524. Calculated for $C_{68}H_{79}N_8O_{25}Cl_2$ = 1477.4534.

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^{1}H NMR Evidence for a Non-Chair Conformation for the Six-Membered Ring Attached Apical Equatorial to Pentacovalent Phosphorus. Potential Implications for Enzyme-Catalyzed Reactions of Cyclic Nucleotides

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Pentacovalent phosphorus derivatives, of long standing interest in their own right,[1] are likely key intermediates in the enzymatic reactions of biologically important phosphates including nucleoside cyclic 3′,5′-monophosphates.[2] We recently concluded from an NMR study of **1** ⇄ **2**[3] that enzyme–substrate binding energy would be sufficient to convert the normal chair form phosphate ring of a cAMP or cGMP into the twist conformation. Consideration of twist conformations for *pentacovalent* cyclic nucleotide adducts in enzymic systems also was suggested.

Little is known about the conformational properties of six-membered rings attached to *pentacovalent* phosphorus. The ^{1}H NMR techniques successfully applied to **1** ⇄ **2**,[3] the corresponding tricoordinate phosphite system,[4] and to P(IV) 2-oxo- and 2-

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