and warmed to room temperature. The aqueous phase was extracted with $Et_2O$ (4 × 20 mL) and the combined organics were dried ($MgSO_4$) and concentrated in vacuo. Silica gel chromatography gave 35.3 mg (96%) of silyl ether **17**: mp 62–63 °C; IR ($CHCl_3$) 2950, 2925, 2880, 2850, 1460, 1360, 1250, 1130, 1090 cm$^{-1}$; $^1$H NMR (490 MHz, $CDCl_3$) δ 7.36–7.28 (m, 5 H), 6.07 (s, 1 H), 5.00 (d, *J* = 2.6 Hz, 1 H), 4.85 (s, 1 H), 4.72 (d, *J* = 12.1 Hz, 1 H), 4.48, (d, *J* = 12.1 Hz, 1 H), 4.28 (t, *J* = 5.6 Hz, 1 H), 2.20 (s, 3 H), 2.13–2.07 (m, 1 H), 2.07–2.01 (m, 1 H), 1.94 (s, 3 H), 1.07 (d, *J* = 7.6 Hz, 3 H), 0.96 (d, *J* = 7.2 Hz, 3 H), 0.92 (s, 9 H), 0.08 (s, 6 H); MS, *m/e* (relative intensity) 444 (M$^+$, 45), 387 (26), 336 (29), 279 (45), 240 (31), 239 (73), 221 (23), 209 (27), 201 (20), 187 (25), 173 (39), 172 (72), 149 (30), 143 (65), 145 (21), 143 (38), 136 (66), 135 (24), 131 (65), 125 (42), 124 (21), 123 (37), 119 (36), 116 (44), 115 (77), 109 (32), 92 (52), 91 (100), 85 (21), 79 (20), 77 (40), 75 (65), 73 (67), 65 (26). Exact mass Calcd: 444.2696. Found: 444.2689.

(3*R**,4*R**,5*S**,6*R**)-Tetrahydro-3,5-dimethyl-4-((*tert*-butyldimethylsilyl)oxy)-6-(4,5-dimethyl-2-furyl)-2*H*-pyran-2-ol (18). To a cold (−78 °C) solution of pyran **17** (94.8 mg, 0.213 mmol) in $Et_2O$ (5.0 mL) and $NH_3$ (ca. 10 mL) was added sodium metal (ca. 150 mg, ca. 15 equiv). After 25 min at −78 °C, $NH_4Cl$(s) was added and the $NH_3$ was evaporated. After addition of $H_2O$ (20 mL) and extraction with $Et_2O$ (4 × 20 mL) the combined organics were dried ($MgSO_4$) and concentrated in vacuo. Silica gel chromatography gave lactol **18** (49.5 mg, 66%) as a 2.7:1 mixture of anomers. IR ($CHCl_3$) (of mixture) 3600, 3400 (b), 2960, 2940, 2900, 2860, 1460, 1390, 1255, 1130, 1110, 1090, 1070, 1010, 975, 900, 880, 840 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$), major isomer, δ 6.04 (s, 1 H), 5.22 (br s, 1 H), 5.18 (d, *J* = 3.0 Hz, 1 H), 4.28 (t, *J* = 5.5 Hz, 1 H), 2.50 (d, *J* = 3.0 Hz, 1 H), 2.19 (s, 3 H), 2.15–2.05 (m, 1 H), 2.03–1.97 (m, 1H), 1.92 (s, 3 H), 1.08 (d, *J* = 7.6 Hz, 3 H), 0.94 (d, *J* = 7.2 Hz, 3 H), 0.92 (s, 9 H), 0.09 (s, 3 H), 0.08 (s, 3 H); $^1$H NMR (500 MHz, $CDCl_3$), minor isomer, δ 6.09 (s, 1 H), 4.89 (dd, *J* = 7.2, 2.8 Hz, 1 H), 4.53 (dd, *J* = 2.9 Hz, 1 H), 3.97 (t, *J* = 5.2 Hz, 1 H), 3.17 (d, *J* = 7.2 Hz, 1 H), 1.04 (d, *J* = 7.2 Hz, 3 H), other signals hidden by major isomer.

(1*R**,2*S**,3*S**,4*S**)-1-(4,5-Dimethyl-2-furyl)-2,4-dimethylpentan-1,3,5-triol (5-*tert*-Butyldimethylsilyl Ether) (19b). To a cold (−78 °C) solution of pyran **17** (204.5 mg, 0.460 mmol) in $Et_2O$ (10.0 mL) and $NH_3$ (ca. 15 mL) was added sodium metal (ca. 300 m, (25 to 30 equiv)). After 5 min at −78 °C (solution goes dark blue), the reaction mixture

was warmed to reflux (−33 °C) for 1 h. The excess sodium was then quenched with $NH_4Cl$(s) and the $NH_3$ was evaporated. After addition of $H_2O$ (20 mL) and extraction with $Et_2O$ (4 × 20 mL), the combined organics were dried ($MgSO_4$) and concentrated in vacuo. Silica gel chromatography gave diol **19** (101.6 mg, 62%): IR ($CHCl_3$) 3425 (b), 3015, 2965, 2940, 2860, 1470, 1260, 1100, 1075, 840 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 6.04 (s, 1 H), 5.05 (br s, 1 H), 4.78 (d, *J* = 2.7 Hz, 1 H), 4.25 (d, *J* = 2.9 Hz, 1 H), 3.91 (dd, *J* = 10.0, 3.7 Hz, 1 H), 3.66 (br dt, *J* = 7.4, 3.9 Hz, 1 H), 3.62 (dd, *J* = 10.0, 7.9 Hz, 1 H), 2.18 (s, 3 H), 2.14–2.08 (m, 1 H), 2.06–1.99 (m, 1 H), 1.92 (s, 3 H), 1.03 (d, *J* = 7.1 Hz, 3 H), 0.92 (s, 9 H), 0.91 (d, *J* = 7 Hz, 3 H), 0.12 (s, 3 H), 0.11 (s, 3 H); MS, *m/e* (relative intensity) 356 (M$^+$, 2.0), 338 (2), 297 (6), 281 (6), 239 (2), 203 (4). Exact mass Calcd: 356.2383. Found: 356.2372.

# The Role of the Chlorine Substituents in the Antibiotic Vancomycin: Preparation and Characterization of Mono- and Didechlorovancomycin

**Constance M. Harris,\* Rajamoorthi Kannan, Hana Kopecka, and Thomas M. Harris**

*Contribution from the Chemistry Department, Vanderbilt University, Nashville, Tennessee 37235. Received April 5, 1985*

**Abstract:** Mono- and didechlorinated derivatives of the antibiotic vancomycin (**1**) have been prepared by catalytic dehydrogenation with Pd/C (10%) catalyst. Initial dehalogenation occurs on residue 2 to give monochloro derivative **3** (MDCV), followed by a slower removal of the second chlorine to give didechloro derivative **4** (DDCV). Heating of **3** and **4** (pH 4.2, 80 °C) leads to CDP-I type rearrangement products **6** and **7** which were also obtained by catalytic dehalogenation of CDP-I. Rearrangement product **7** failed to show detectable antibiotic activity; the inward-facing chlorine on residue 2 is not, therefore, the sole reason for the lack of activity of CDP-I itself. Binding studies with di- and tripeptides, **8–14**, which are analogues of the natural peptide binding site in bacterial peptidoglycan, indicate that both **3** and **4** bind peptides less effectively than **1**, although **4** is somewhat more tolerant of larger side chains on the C-terminal residue of tripeptides. The role of the chlorines in stabilizing and defining the shape of the peptide binding site is discussed.

Vancomycin, a glycopeptide antibiotic elaborated by *Streptomyces orientalis*,[1a] has been the subject of numerous investigations

in recent years.[1b,c,2] Several revisions in its structure have been made, but **1** is now generally accepted to be the correct one.[3] Prior

(1) (a) McCormick, M. H.; Stark, W. M.; Pittenger, G. E.; Pittenger, R. C.; McGuire, J. M. *Antibiot. Annu.* **1955/1956**, 606. For reviews covering the chemistry and mode of action of the vancomycin-group antibiotics see: (b) Williams, D. H.; Rajananda, V.; Williamson, M. P.; Bojesen, G. *Top. Antibiot. Chem.* **1980**, *5*, 119. (c) Perkins, H. R. *Pharmacol. Ther.* **1982**, *16*, 181. (d) Williams, D. H. *Acc. Chem. Res.* **1984**, *17*, 364.

(2) Recent papers on clinical uses of vancomycin include the following: (a) Fekety, R. *Med. Clin. North Am.* **1982**, *66*, 175. (b) McHenry, M. C.; Gavan, T. L. *Pediatr. Clin. North Am.* **1983**, *30*, 31. (c) Geraci, J. E.; Herman, P. E. *Mayo Clin. Proc.* **1983**, *58*, 88. (d) Symposium on Vancomycin Therapy: Wise, R., Reeves, D., Eds. *J. Antimicrob. Chemother.* **1984**, *14*, Suppl. D.

to the elucidation of the structure of **1**, it was shown that vancomycin exerts its primary antibacterial action by interfering with the cross-linking of peptidoglycan in susceptible bacteria.[4] Vancomycin does this by binding preferentially to biosynthetic intermediates terminating in acyl-D-ala-D-ala. In the early 1970s



**1**
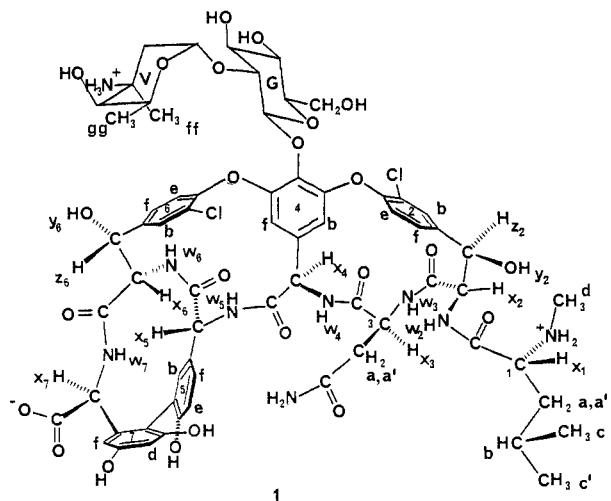
Nieto and Perkins prepared an extensive series of aliphatic peptides and studied their binding to vancomycin and to the closely related antibiotic, ristocetin.[5] From these studies they concluded that (1) a free C-terminal carboxyl group on the peptide was an absolute requirement, (2) the amino acids at positions 1 and 2 (numbering from the C-terminus) of the peptide had to be either glycine or possess the D configuration, and (3) ristocetin could tolerate larger side chains at residue **1** than could vancomycin whereas the reverse was true with residue **2**.



2a R = R' = Cl
b R = H; R' = Cl
c R = R' = H
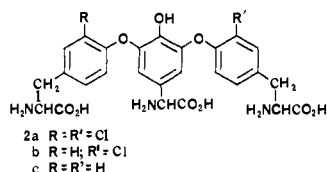
Subsequent to the work of Nieto and Perkins the interaction between the antibiotic and small aliphatic peptides has been examined by high-field ¹H NMR by Williams' group at Cambridge[6] and also by Feeney and co-workers[7]. In the model for complexation proposed by Williams (Figure 1), the peptide is bound to the antibiotic by several hydrogen bonds between the terminal carboxylate anion and the amides in the peptide and the amide backbone of the antibiotic; the side chains of the peptide fit into hydrophobic pockets formed by the aromatic rings. The cluster of hydrogen bonds near the N-terminus of the antibiotic is stabilized by the folding in of the *N*-methylleucine side chain to form a hydrophobic wall. In recent papers Williamson et al.[8] have
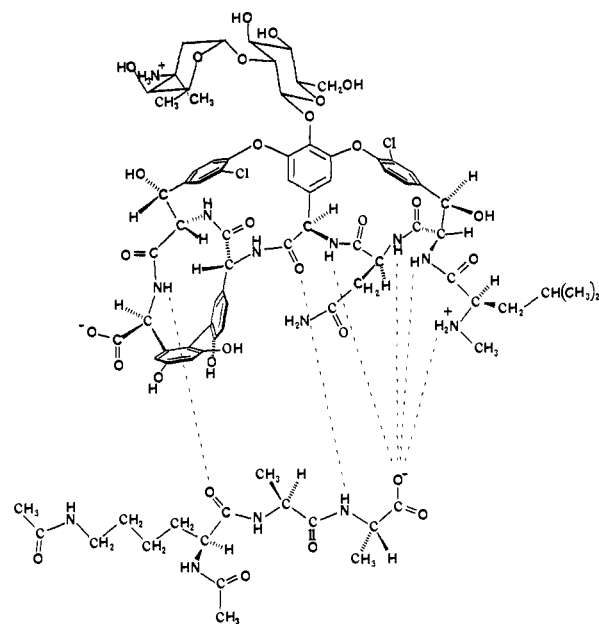


**Figure 1.** Proposed model for binding of peptidoglycan analogues to vancomycins.[1d,6] Dashed lines indicate hydrogen bonds and a salt bridge.

stressed that a proper fit of the side chains into the hydrophobic areas is necessary to give the complex sufficient rigidity for strong complementary hydrogen bonds to form between peptide and antibiotic. Optimum binding clearly requires a very delicate interplay of steric and electronic factors. The structures of a number of antibiotics in this class are now known, including those of the teicoplanins which appeared very recently,[9] and the following question can be asked: what features in these compounds are essential for effective binding? The answers are important not only for this group of antibiotics but also because of the light they can shed on other peptide or protein binding phenomena. One of the structural features found in many of the vancomycin group antibiotics is chlorination of one or more aromatic rings. In vancomycin itself the chlorine on residue **6** has been proposed to be involved in formation of a tight binding site for the methyl of the C-terminal *D*-ala in the peptide.[8a] In this paper we report the preparation and characterization of mono- and dichlorovancomycin.

## Experimental Section

**General.** Vancomycin was obtained as a gift from Eli Lilly. ¹H and ¹³C NMR spectra were obtained on a JEOL FX-90Q or a Bruker AM-400 spectrometer. Optical rotations were measured on an Autopol III spectropolarimeter in 1-dm cells. UV spectra were obtained on a Varian 210 spectrophotometer. FAB spectra were obtained by using a glycerol matrix. Vancomycin, MDCV, and DDCV were submitted for FAB spectra as hydrochloride salts. FAB results are reported in terms of the largest peak in the isotope cluster. The calculated value represents species containing ¹²C and ³⁵Cl.

**Chromatography.** Thin-layer chromatography was performed on Merck silica gel 60 or Whatman silica gel K6F plates with the following solvent systems: (1) 1-butanol/acetic acid/$H_2O$, 6:2:2; (2) 1-butanol/acetic acid/$H_2O$/pyridine, 45:9:36:30; (3) $CHCl_3$/MeOH, 95:5; (4) $CH_3CN$/acetic acid/$H_2O$, 8:1:1; (5) $CH_3CN$/acetic acid/$H_2O$, 34:3:3. Final purification of peptides was carried out by employing silica gel plates and solvent no. 5 in a Chromatotron apparatus (Harrison Research, Palo Alto, CA). Flash chromatography[10] of intermediates was performed on silica gel (Baker RP-7024) with elution by $CHCl_3$–MeOH mixtures. Peptides were located by means of ninhydrin (0.1% in 2-propanol) or by the method of Reindel.[11] HPLC was performed with

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a Spectra-Physics SP7000 system or an IBM 9533 Ternary Liquid Chromatograph. A C-18 column (Altex or IBM, 4.0 × 250 mm) was used with 10% acetonitrile/0.1% trifluoroacetic acid as eluent. Components were eluted in the following order: **7**, **5a**, **6**, **3**, **4**, **1**, and **5b**.

**Preparation of Monodechlorovancomycin (MDCV, 3).** To a solution of vancomycin (0.5 g) in $H_2O$ (25 mL) was added Pd/C (1.0 g, 10%). The mixture was hydrogenated at 4 atm in a Parr apparatus for 4 days at which time HPLC analysis showed one major product. The reaction mixture was filtered through Celite and the filtrate lyophilized. A portion of the lyophilizate (0.150 g) was purified by low-pressure chromatography on a 15 × 500 mm column of Whatman Partisil 40 ODS-3 with elution by 10% $CH_3CN$ in 0.1 M ammonium formate. Fractions were assayed by HPLC and 73 mg of purified material was obtained. FABMS: 1415 (calcd $MH^+$ 1414.6).

**Preparation of Didechlorovancomycin (DDCV, 4).** Didechlorovancomycin was prepared as for the monodechloro compound except that additional catalyst (~0.5 g) was added periodically as shaking was continued over a period of 3 to 4 weeks. The second reduction appeared to occur much more slowly than the first. The product was isolated as described for the mono derivative. FABMS: 1381 (calcd $MH^+$ 1380.6).

**Rearrangement Products of MDCV and DDCV (6 and 7).** Rearrangement products from MDCV and DDCV were prepared essentially by the procedure of Marshall.[12] Samples (5 mg/mL, 0.05 M potassium phosphate, pH 6.0) were heated at 80 °C, and aliquots were removed at intervals for analysis by HPLC. The rearrangement of **3** and **4** was complete within 24 h. Upon cooling **6** precipitated as does CDP-I (**5**), but **7** did not. Compounds **6** and **7** were also formed by dehydrogenation of CDP-I (10% Pd/C) in $H_2O$–2-propanol (8:2) containing 0.1% TFA. FABMS (**7**): 1381 (calcd $MH^+$ 1381.5).

**Degradation of Vancomycin, MDCV, and DDCV with HI.** The reductive HI hydrolyses were carried out essentially as described previously.[13,14]

Aglycovancomycin (0.10 g) was hydrolyzed (24 h, 106 °C) in 10 mL of freshly distilled HI containing 80 mg of red P in a Pierce hydrolysis tube. After removal of the HI in vacuo, the residue was suspended in $H_2O$ (2 mL), filtered, and lyophilized. The lyophilizate was analyzed by ion-exchange chromatography as previously described.[3c] Amino acid **2a** (16.3 mg) was obtained as well as the expected *N*-methylleucine, aspartic acid, and actinoidinic acid. Amino acid **2a**: $^1H$ NMR ($D_2O$/DCl) δ 7.47 (d, 2 H, $J$ = 2 Hz), 7.23 (dd, 2 H, $J$ = 8 + 2 Hz), 7.01 (d, 2 H, $J$ = 8.5 Hz), 6.78 (br s, 2 H), 4.35 (t, 2 H, $J$ = 6.7 Hz), 3.30–3.27 (m, 4 H); the α-proton on the phenylglycine ring was obscured by the HOD peak; $^{13}C$ NMR δ 171.98, 171.33, 151.82, 146.19, 140.23, 132.32, 130.26, 125.22, 124.90, 120.78, 115.09, 56.96, 54.85, 35.20 (2×).

The aglycon of MDCV was hydrolyzed similarly to give 13.5 mg of amino acid **2b**, previously isolated from actinoidin by Berdnikova et al.[13b] Amino acid **2b**: $^1H$ NMR ($D_2O$/DCl) δ 7.47 (d, 1 H, $J$ = 2.0 Hz), 7.29 (d, 2 H, $J$ = 8.8 Hz), 7.17 (d, 1 H, $J$ = 8.5 + 2.2 Hz), 7.00 (d, 3 H, $J$ = 8.5 Hz), 6.92 (d, 1 H, $J$ = 2.2 Hz), 6.78 (d, 1 H, $J$ = 1.9 Hz), 4.81 (s, 1 H), 4.26 (t, 2 H, $J$ = 8 Hz), 3.27–3.10 (m, 4 H); $^{13}C$ NMR δ 172.30, 172.15, 171.65, 157.19, 151.93, 146.35, 146.08, 141.20, 132.43, 131.89, 130.37, 130.04, 125.22, 120.83, 118.61, 117.15, 115.52, 57.07, 55.00 (2×), 35.67 (2×).

The aglycon of DDCV (50 mg) yielded 5.9 mg of didechlorovancomycinic acid (**2c**), identical by TLC and $^1H$ NMR to the compound previously isolated from ristocetin.[13a,14] Amino acid **2c**: $^1H$ NMR ($D_2O$/DCl) δ 7.50 (d, 4 H, $J$ = 8.5 Hz), 7.22 (d, 4 *H*, $J$ = 8.5 Hz), 7.14 (br s, 2 H), 4.38 (t, 2 H, $J$ = 6.7 Hz), 3.42 (m, 4 H); the α-proton of the phenylglycine ring was obscured by the HOD signal; $^{13}C$ NMR δ 173.82, 158.00, 146.83, 142.65, 132.64, 131.04, 127.02, 119.34, 118.25, 118.18, 58.52, 56.42 (2×), 36.67 (2×).

**Synthesis of Peptides.** *t*-BOC-D-alanine was purchased from Peninsula Laboratories. α,ε-Bis(*t*-BOC)-L-lysine was prepared by the procedure of Itoh et al.[15] All *t*-BOC derivatives were purified by flash chromatography with $CHCl_3$ as eluent. D-Alanine benzyl ester was prepared as described by Erlanger and Hall.[16] The benzyl esters of D-2-aminobutyric acid and D-leucine were prepared by the method of Miller et al.[17] Synthesis of peptides was carried out by conventional solution methods with dicyclohexylcarbodiimide as the coupling agent.[18] Protected tri-

peptides were purified by flash chromatography or with the Chromatotron (solvent no. 3). Following removal of the *t*-BOC group with HCl in ethyl acetate the amino groups were acetylated with excess acetic anhydride in pyridine. Acetylated peptides were purified on the Chromatotron with solvent no. 4. Benzyl esters were removed either by hydrogenation in $CH_3OH$ with 10% Pd/C or by hydrogenolysis with ammonium formate as the hydrogen source.[19] Purity of peptides was established by TLC (solvent no. 1, 2, and 4), NMR, and amino acid analysis. Peptides **8–11** and **14** had optical rotations in agreement with those reported previously by Nieto and Perkins.[5c]

**Di-NAc-L-lys-D-ala-2-aminobutyric acid (12):** $[\alpha]^{27}_D$ +36 (*c*, 0.47, 0.01 M HCl); $^1H$ NMR ($Me_2SO$-$d_6$) δ 12.50 (1 H, br s, COOH), 8.13 (1 H, d, $J$ = 7.5 Hz, ala NH), 8.0 (1 H, d, $J$ = 8.0 Hz, lys αNH), 7.96 (1 H, d, $J$ = 7.5 Hz, 2-ab NH), 7.76 (1 H, t, $J$ = 6.0 Hz, lys ε-NH), 4.31 (1 H, m, ala α-H), 4.20 (1 H, m, lys α-H), 4.06 (1 H, m, 2-ab α-H), 2.97 (2 H, m, lys 6-$CH_2$), 1.82 (3 H, s, $COCH_3$), 1.77 (3 H, s, $COCH_3$), 1.65 (2 H, m, 2-ab $CH_2$), 1.47 (2 H, m, lys 3-$CH_2$), 1.30 (2 H, m, lys 5-$CH_2$), 1.18 (2 H, m, lys 4-$CH_2$), 1.15 (3 H, d, $J$ = 7.5 Hz, ala $CH_3$), 0.86 (3 H, t, $J$ = 7.5 Hz, 2-ab $CH_3$). FABMS: 424 (calcd for $M^-K^+$ 424.3).

**Di-NAc-L-lys-D-ala-aminoisobutyric acid (13):** $[\alpha]^{27}_D$ +29.1 (*c*, 0.62, 0.01 M HCl); $^1H$ NMR ($D_2O$) δ 4.04 (1 H, q, ala α-H), 3.97 (1 H, t, lys α-H), 2.94 (2 H, t, lys 6-$CH_2$), 1.80 (3 H, s, $COCH_3$), 1.75 (3 H, s, $COCH_3$), 1.50 (2 H, m, lys 3-$CH_2$), 1.28 (2 H, m, lys 5-$CH_2$), 1.24 (3 H, s, aib $CH_3$), 1.22 (3 H, s, aib $CH_3$), 1.18 (2 H, m, lys 4-$CH_2$), 1.14 (3 H, d, ala $CH_3$). FABMS: 387 (calcd for $MH^+$ 387.2).

**UV Difference Spectroscopy.** Binding constants between peptides and vancomycin and its derivatives were measured essentially as described by Nieto and Perkins[5b,c] except that the tandem arrangement of cells was not required, since the peptides used in this work had no significant absorption in the range 250–320 nm. Cells with a 5-cm light path were used. Solutions (15 mL) containing antibiotic (0.08-0.1 mg/mL in 0.02 M sodium citrate buffer, pH 5.1) were placed in the sample and reference cells and the difference in absorbance which developed upon addition of peptide (5–100 μL of a 0.015 M solution) to the sample cell and buffer to the reference cell was measured at 282–285 or 292–294 nm. The difference in extinction was measured with an accuracy of ±0.0003 absorbance units. Additions in the sample cell were carefully compensated for in the reference cell. The temperature was 25 ± 2 °C. Association constants were determined by means of a nonlinear least-squares program or by Scatchard plots,[5b] based on the assumption of a simple $V + P \rightleftharpoons VP$ equilibrium. Binding constants of $10^4$ or less were obtained with a standard deviation of ±5–10%: because of the steepness of the curve for peptides with association constants of $10^5$ or greater, the standard deviation in these cases was greater, ranging from 10 to 20%. Antibiotic concentrations were determined by UV, using a value of $\epsilon^{1\%}$ 45.0 at 280 nm.[5b] Peptide concentrations were determined by amino acid analysis.

**Antibiotic NMR Studies.** These were carried out at 400-MHz with 10-mg/mL solutions in $Me_2SO$-$d_6$. Simple spectra were obtained employing spectral widths of 4000 Hz and 16K of data points. NOE difference spectra were obtained by subtraction of two FIDs where one was accumulated with the decoupler set on a peak of interest and the second with the decoupler upfield of 0 ppm. The decoupler was gated off during data acquisition, and the minimum feasible irradiation times and decoupler power were employed to avoid spin diffusion. Sufficient transients were accumulated to assure good signal to noise after subtraction and transformation. In a typical experiment, COSY spectra were obtained with a 90–τ–45 pulse sequence, using 1K of data points in F2 and 256 points in F1. F1 data were zero-filled once before transformation. Sine-bell multiplication was employed in both domains, and the transformed data were symmetrized.

**Antibiotic Assays.** Antibiotic assays were carried out by disc diffusion on agar with *B. subtilis* (Difco) as the test organism.[20,21] Assays were run in triplicate with vancomycin standards on each plate.

## Results and Discussion

**Monodechlorovancomycin (MDCV, 3).** The monodechloro derivative was prepared by hydrogenolysis of vancomycin in aqueous solution (pH 4–7, 4 atm $H_2$, 10% Pd/C). The reaction was carried out at room temperature and required several days for complete disappearance of starting material. At the end of this time, HPLC analysis showed mainly one peak, faster moving than vancomycin itself. Purification was carried out by re-

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Table I. $^1$H NMR Chemical Shifts in Mono- and Didechlorovancomycin[a,b]

| proton | vancomycin (1) | | MDCV (3) | | DDCV (4) | |
|---|---|---|---|---|---|---|
| | 295 K | 315 K | 295 K | 315 K | 295 K | 315 K |
| $\underline{w_5}$ | 8.59 | 8.52 | 8.63 | 8.55 | 8.73 | 8.63 |
| $\underline{w_7}$ | 8.32 | 8.35 | 8.34 | 8.32 | ~8.40 | 8.41 |
| $w_4$ | 8.22 | 8.19 | 8.28 | d | 8.24 | 8.17 |
| $\underline{w_2}$ | 7.80 | 7.80 | 7.87 | d | ~8.10–7.90 | ~7.97 |
| $\underline{6b}$ | 7.89 | 7.88 | 7.87 | 7.83 | 7.60 | 7.61 |
| $\underline{2f}$ | 7.50 | 7.52 | 7.56 | 7.58 | 7.60 | 7.58 |
| 6f | 7.44 | 7.48 | 7.44 | 7.44 | 7.42 | 7.44 |
| $\underline{2b}$ | 7.32 | 7.37 | 7.26 | 7.28 | 7.26 | 7.28 |
| $\underline{6c}$ | | | | | 7.19 | 7.17 |
| $\underline{5b}$ | 7.16 | 7.18 | 7.16 | 7.15 | 7.11 | 7.11 |
| $\underline{6e}$ | 7.32 | 7.32 | 7.34 | 7.29 | 7.12 | 7.10 |
| $\underline{2c}$ | | | 7.14 | 7.12 | 7.01 | 7.03 |
| $\underline{2e}$ | 7.23 | 7.23 | 7.06 | 7.04 | 6.83 | 6.86 |
| 5f | 6.73 | 6.77 | 6.73 | 6.76 | 6.71 | 6.73 |
| 5e | 6.68 | 6.70 | 6.67 | 6.69 | 6.66 | 6.67 |
| $\underline{w_6}$ | 6.60 | 6.54 | 6.62 | 6.53 | 6.50 | 6.43 |
| 7d | 6.34 | 6.36 | 6.34 | 6.34 | 6.33 | 6.34 |
| 7f | 6.32 | 6.36 | 6.32 | 6.34 | 6.33 | 6.34 |
| $\underline{w_3}$ | 6.87 | 6.64 | 6.95 | 6.27 | ~6.32 | 6.26 |
| $x_4$ | 5.73 | 5.72 | 5.72 | 5.67 | 5.70 | 5.68 |
| $\underline{4b}$ | 5.50 | 5.57 | 5.56 | 5.60 | 5.48 | 5.54 |
| $\underline{4f}$ | 5.22 | 5.23 | 5.20 | 5.20 | 5.54 | 5.52 |
| $\overline{V_A}$ | 5.22 | 5.25 | 5.20 | 5.24 | 5.28 | 5.23 |
| $G_A$ | 5.24 | 5.31 | 5.25 | 5.24 | 5.23 | 5.23 |
| $z_2$ | 5.14 | 5.16 | 5.15 | 5.15 | 5.16 | 5.16 |
| $z_6$ | 5.11 | 5.13 | 5.10 | 5.13 | 5.13 | 5.14 |
| $\underline{x_2}$ | 4.88 | 4.85 | 4.76 | 4.74 | 4.70 | 4.66 |
| $\underline{V_5}$ | 4.67 | 4.67 | 4.65 | 4.65 | 4.59 | 4.57 |
| $x_5$ | 4.40 | 4.45 | 4.40 | 4.43 | 4.38 | 4.40 |
| $x_7$ | 4.35 | 4.41 | 4.35 | 4.40 | 4.35 | 4.38 |
| $x_3$ | 4.40 | 4.38 | 4.36 | 4.34 | 4.40 | 4.33 |
| $x_6$ | 4.16 | 4.18 | 4.16 | 4.16 | 4.14 | 4.15 |
| Glu | 3.70–3.30 | 3.70–3.28 | 3.70–3.20 | 3.72–3.20 | 3.70–3.10 | 3.73–3.15 |
| $\underline{V_4}$ | 3.22 | 3.17 | 3.16 | 3.13 | c | 3.10 |
| $x_1$ | 3.06 | 3.06 | 3.02 | 0.03 | 3.01 | 3.01 |
| $\underline{3a'}$ | 2.35 | 2.42 | 2.49 | 2.50 | 2.50 | 2.57 |
| 1d | 2.31 | 2.31 | 2.30 | 2.30 | 2.34 | 2.31 |
| 3a | 2.13 | 2.15 | 2.10 | 2.13 | 2.13 | 2.14 |
| $V_2'$ | 1.91 | 1.89 | 1.90 | 1.87 | 1.91 | 1.88 |
| 1b | 1.70 | 1.73 | 1.70 | 1.73 | 1.74 | 1.76 |
| $\underline{V_2}$ | 1.70 | 1.69 | 1.70 | 1.63 | 1.65 | 1.65 |
| 1a,a' | 1.44 | 1.45 | 1.42 | 1.44 | 1.45 | 1.44 |
| ff | 1.26 | 1.29 | 1.26 | 1.29 | 1.29 | 1.30 |
| $\underline{gg}$ | 1.06 | 1.07 | 1.07 | 1.08 | 1.12 | 1.10 |
| 1c' | 0.89 | 0.91 | 0.90 | 0.90 | 0.90 | 0.91 |
| 1c | 0.84 | 0.87 | 0.86 | 0.87 | 0.86 | 0.88 |

[a] Solvent: Me$_2$SO-$d_6$; concentration of antibiotic = 10 mM. [b] Chemical shifts in $\delta$ (ppm). [c] Obscured by solvent peak. [d] Could not be assigned unambiguously.

verse-phase chromatography. The material obtained showed an ion in the fast-atom bombardment mass spectra at 1415, consistent with the loss of one chlorine. Reductive hydrolysis of the aglycon of the monodechloro derivative by HI gave monodechlorinated triphenylamino acid, **2b**, previously isolated from the related antibiotic actinoidin.[13b] Similar hydrolysis of vancomycin itself gives acid **2a**. The site of dechlorination (residue **2** or **6**) was determined by high-field $^1$H NMR studies, described below, on the intact antibiotic.

**Didechlorovancomycin (DDCV, 4).** The didechloro derivative was prepared by allowing the hydrogenation to proceed with occasional additions of fresh catalyst until HPLC indicated that the monochloro derivative had been converted to a new slightly slower moving component. The reaction was very slow and required more than 14 days. The purified didechlorovancomycin gave M + H$^+$ at 1381 in the FAB mass spectrum and yielded didechlorinated acid, **2c**, upon reductive hydrolysis with HI; **2c** had previously been obtained from ristocetin.[14]

**High-Field $^1$H NMR Studies.** Further information on the structures of the dechloro derivatives was obtained by $^1$H NMR studies at 400 MHz. The studies were carried out at 295 and 315 K in Me$_2$SO-$d_6$; assignments were made by analogy to vancomycin and by specific decoupling, NOE difference, and COSY experiments. The spectra at 315 K were obtained to help

in the assignment of protons which overlap at room temperature, such as $x_3$ with $x_5$ and $V_A$ and $G_A$ with **4f**. The assignments are shown in Table I; the designation of protons is analogous to the system used most recently by Williams.[9b]

The initial studies were carried out on the monodechloro derivative to determine (1) if the material isolated as a single chromatographic fraction contained both or only one of the possible monodechlorinated species and (2), if the latter were the case, the site of dechlorination. The spectrum that was obtained showed only one isomer; the other, if formed, must be present in very low yield. The initial evidence for the site of dechlorination in the monodehalogenated derivative was that proton 6b, the furthest downfield carbon-bound proton, appeared to be unchanged. This suggested that ring 6 was unaltered and, therefore, that the chlorine on ring 2 had been removed. With proton 6b as the starting point, 6f and 6e was assigned from the COSY spectrum; no significant change in their chemical shifts was seen. In the COSY spectrum the meta coupling of 6b and 6f can be seen using a 90–τ–45 sequence. When the chlorine is removed from the benzene ring of residue **2**, it is replaced by a proton designated as 2c which is ortho to the 2b proton. In the monodechloro derivative 2b becomes a doublet. Protons 2b, 2c, 2e, and 2f were assigned with the help of decoupling and 2-D NMR experiments. When the 2f proton, which is slightly upfield from 6b, is irradiated,

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the peak at 7.06 ppm becomes a singlet. It therefore must be 2e. Proton 2e shows meta coupling to a downfield neighboring peak—the latter must be the new proton, 2c. The remaining peak at 7.26 ppm must be 2b; when it is irradiated, 2c becomes a singlet. A singlet which overlaps 2c shows coupling to 5f so it must be 5b (7.16 ppm).

Table I shows that 2b, 2c, and 2e occur at relatively high field. Once the chlorine has been removed, proton 2e is shifted from 7.23 to 7.06 ppm; 2b is also shifted to slightly higher field. Other protons occur in the same place as in vancomycin except $w_3$, $w_2$, 4b, and 3a which occur slightly downfield and $x_2$ and $V_4$ which move slightly upfield. These results show the location of the remaining chlorine to be in ring 6 in monodechlorovancomycin, and the structure can now be designated as 3.

The spectrum of didechlorovancomycin was examined next. When both of the chlorines are removed, the spectrum of the aromatic region becomes complex. For example, in the spectrum at 295 K the doublets for the 6b and 2f protons exactly overlap to give what appears to be a singlet doublet at 7.60 ppm. At 315 K the peaks are better separated and 6b and 2f appear as a triplet resulting from partial overlap of the doublets. The overlap was confirmed by an NOE experiment in which 2e (6.86 ppm) was irradiated, giving rise to a singlet at 7.58 ppm (2f) with 6b remaining as a doublet. Proton 2e was assigned by its meta coupling to a proton (2c) at 7.03 ppm which is coupled to a peak at 7.28 ppm which must be 2b. In ring 6 the assignment of 6b was supported by NOE difference experiments in which $x_6$ and 6c were irradiated, both giving rise to a difference peak at 7.61 ppm (6b). Decoupling at 7.61 ppm caused the peak at 7.17 to become a singlet, supporting its assignment as 6c. In the COSY plot 6b also showed coupling to a signal at 7.44 ppm which must be 6f. Proton 6f showed ortho coupling to a peak at 7.10 ppm which, therefore, is 6e. A second signal at 7.10 ppm shows coupling to a peak at 6.73 ppm and can be assigned as 5b which is meta to 5f.

All other protons in didechlorovancomycin have the same chemical shift as in the monodechloro derivative except $w_5$, 6b, 6e, 2c, $w_6$, and 4f (Table I). Amide proton $w_3$ could not be assigned unambiguously at 295 K, but at 315 K it came at 6.26 ppm (compared to $\delta$ 6.27 for MDCV and 6.64 for vancomycin itself at that temperature). Proton 4f is shifted far downfield in DDCV as compared to MDCV. At 315 K protons 4f and 4b occur at 5.52 ppm, overlapping each other. This was confirmed only by NOE difference spectra. When $w_5$ and $x_5$ were irradiated, there was a difference peak at 5.52 ppm (proton 4f). When 2e and $w_4$ were irradiated there was an NOE at 5.52 ppm (proton 4b). When $x_4$ was irradiated, two overlapping peaks at 5.52 ppm in the NOE difference spectrum were seen. This clearly shows that 4f is shifted far downfield in DDCV. At 295 K irradiation of $w_5$ gave an NOE at 5.54 ppm, which must be 4f, and irradiation at $w_4$ produced an NOE at 5.48 ppm which therefore is 4b.

The protons which show significant change in chemical shift in MDCV and DDCV as compared to vancomycin itself are underlined in Table I. When the first chlorine is removed there are changes only in residues 2 and 3. When the second chlorine is removed to give DDCV, changes occur not only in the dechlorinated rings but also in the ring of residue 4, where 4f moves downfield, and along the peptide backbone, where $w_5$ and $w_6$ are affected. It is also important to note that protons 2c and 2e in the ring of residue 2 move upfield (by ~0.2 ppm) when the chlorine is removed from residue 6. The observed changes in chemical shift must be interpreted with caution. In addition to the expected changes in the protons on the dechlorinated rings numerous other shifts are seen, which are probably the result of changes in the conformations of the aromatic rings of residues 2, 4, and 6. For example, in MDCV the ring of residue 2 may be tilted upward compared to its position in vancomycin, leading to increased shielding of $V_4$ and decreased shielding of 4b and 3a'. Likewise, the deshielding of 4f in DDCV may result from a change in the relative orientation of the rings of residues 4 and 6. There appears to be little change in the amide coupling constants in the dechlorinated species which implies that the peptide

backbone exists in essentially the same average conformation as in the parent antibiotic.

**Rearrangement Products of MDCV and DDCV.** Vancomycin, upon heating or prolonged standing in solution at ambient temperature, undergoes rearrangement to give a crystalline product, CDP-I (5),[12] in which residue 3, the asparagine, has become an isoaspartic acid residue.[3c] Both MDCV and DDCV were found to undergo a similar rearrangement upon heating, yielding 6 and 7, respectively (Scheme I). CDP-I (5) exists as atropisomers, which differ in the orientation of the chlorine in residue 2. The major isomer (5b) has the chlorine on the inner, or concave, side of the molecule and the minor one (5a) has the chlorine on the backside as in vancomycin itself. This conclusion, reached by Williams on the basis of NMR evidence,[3b] is reinforced by our finding that the dechlorinated rearrangement products exist in only one form. It is noteworthy that MDCV and DDCV both rearranged more rapidly than vancomycin itself. When solutions of 1, 3, and 4 were heated (pH 6.0, 80 °C), rearrangement of 3 and 4 to 6 and 7 was essentially complete after 24 h whereas 50% of the vancomycin was unchanged. A possible explanation is that the chlorine of residue 2 in vancomycin hinders the rearrangement process. In its absence MDCV and DDCV can more readily achieve the requisite conformation for rearrangement to occur.

Compounds 6 and 7 were independently synthesized by catalytic hydrogenation of CDP-I (Scheme I). Interestingly, the dehalogenation of CDP-I appeared to go much more quickly to the didechloro stage than in the case of vancomycin; within 2 days, no CDP-I (major or minor) remained and 7 was the predominant product. The more open structure of CDP-I may make it more accessible to the catalyst than is the parent antibiotic. The rearrangement product 7 had no detectable antibiotic activity;[22] this provides evidence that it is not the inward-facing chlorine on the major form of CDP-I which is solely responsible for its lack of activity. More likely, the increased distance between the hydrogen-bonding sites at the C-terminal and N-terminal ends produced by the additional methylene group present in the backbone of CDP-I makes it impossible for peptides to bind simultaneously at both sites.

**Peptide binding Studies.** Peptide binding studies were carried out by UV difference spectroscopy, essentially as described by Nieto and Perkins.[5c] Inasmuch as the steepness of the binding curve, especially for $K_A$ values greater than $10^4$, renders the determination of accurate binding constants difficult, caution must be exercised in interpreting the results in Table II. However, certain trends are discernible. The initial studies were done with three dipeptides, 8–10 (Table II). These data show that loss of the chlorine on residue 2 leads to decreased binding and that loss of both chlorines leads to still further decrease in binding effectiveness. The finding that the monodechloro derivative showed decreased binding was somewhat surprising. The location of the lost chlorine, on the backside of residue 2, would not seem to be of any importance for direct interaction with the peptide. One explanation may be that the loss of the chlorine on the aromatic ring of residue 2 leads to increased mobility of the ring and of the amide bonds in the macrocyclic ring in which it is embedded, which leads, consequently, to weaker intermolecular hydrogen bonding to the peptide; the facile rearrangement of 3 as compared to 1 supports this idea. The loss of the second chlorine should not lead to much loss of rigidity in the C-terminal region of the antibiotic which is severely constrained by the biphenyl linkage of residues 5 and 7, but it may make the hydrophobic pocket too large for the methyl on the C-terminal D-ala to make a tight fit; in the case where the first residue is glycine (10) the fit is apparently so poor that no binding could be measured for the didechloro derivative.

To extend this study further, four tripeptides (11–14) with D-ala and three more bulky groups on the C-terminal residue were prepared and their binding to vancomycin and the dechlorinated

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(22) When 5 was assayed, a mixture of 5a and 5b was actually used; 5a and 5b equilibrate in a matter of hours to an ~2:1 mixture. No inhibition was seen at concentrations up to 168 $\mu$g/mL (vancomycin is inhibitory at <6 $\mu$g/mL).

Scheme I



5a  R=Cl; R'=H
 b  R=H; R'=Cl

a: $H_2$, Pd/C (10%)
b: pH 6.0, 80 °C
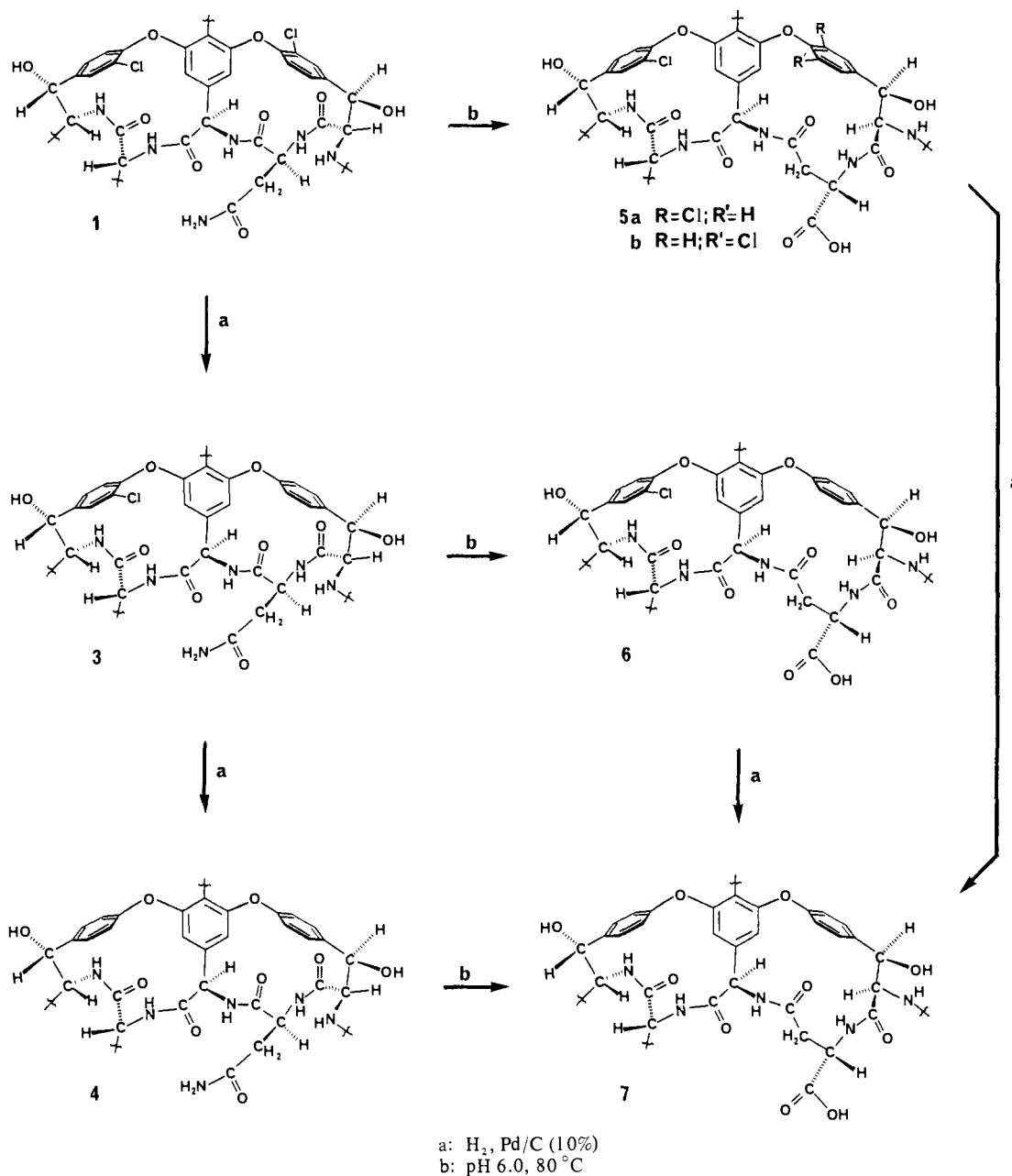
derivatives was measured (Table II). The binding of DiAc-L-lys-D-ala-D-ala is similar to the trend seen in the dipeptides—the loss of chlorine leads to decreasing binding with the $K_A$ for the didechloro derivative being only 10% of that for vancomycin. When the side chain is increased to ethyl (12), binding affinity is markedly decreased for vancomycin and the monodechloro derivative but remains relatively unchanged for the didechloro compound. This result suggests that in didechlorovancomycin there is enough room for the ethyl side chain to fit without disturbing the hydrogen bond geometry; the ethyl group, on the other hand, does not appear to fit sufficiently better than methyl to increase the binding affinity. In the case of peptide 13, which has aminoisobutyric acid at its C-terminus, decreased binding (as compared to 11 and 12) is shown by vancomycin and MDCV and no complex formation with DDCV could be detected. The second methyl group on the α-carbon of 13 may interfere with the folding around of the N-methylleucyl side chain which is proposed to occur upon complex formation.[6b,7c] This would lead to destabilization of the complex at the carboxylate end of the peptide. It is also possible that steric constraints of the aminoisobutyric acid residue in peptide 13 destabilize the conformation required for complex formation.[23]  We had previously observed that the dipeptide

Ac-D-ala-aib did not show any detectable binding to vancomycin[21]—the addition of the lysyl residue apparently contributes enough stability for measurable complexation to occur. However, in the case of DDCV, the increased conformational mobility of the antibiotic coupled with the poor affinity of the aib residue for the N-terminal binding site leads to complete loss of detectable binding. When the side chain is enlarged to isobutyl (14) a decrease in binding is seen for vancomycin and the monodechloro compound as compared to 12. The didechloro derivative also shows a marked decrease compared to 12 which was rather unexpected. We had hypothesized that didechlorovancomycin would bind Ac-L-lys-D-ala-D-leu almost as well as ristocetin ($K_A$ = 6.1 × 10⁵).[5d] The decreased binding by DDCV may be due

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**Table II.** Association Constants[a] for the Combination of Vancomycin and Mono- and Didechlorovancomycin with Aliphatic Peptides

| peptide | vancomycin (1) | MDCV (3) | DDCV (4) |
|---|---|---|---|
| Ac-D-ala-D-ala (8) | $6.4 \times 10^4$ | $2.4 \times 10^4$ | $1.0 \times 10^4$ |
| Ac-gly-D-ala (9) | $2.1 \times 10^4$ | $1.2 \times 10^4$ | $4.6 \times 10^3$ |
| Ac-D-ala-gly (10) | $5.9 \times 10^3$ | $7.8 \times 10^3$ | very weak |
| DiAc-L-lys-D-ala-D-ala (11) | $1.5 \times 10^6$ | $5.9 \times 10^5$ | $1.6 \times 10^5$ |
| DiAc-L-lys-D-ala-D-ab[b] (12) | $9.1 \times 10^4$ | $1.4 \times 10^5$ | $1.3 \times 10^5$ |
| DiAc-L-lys-D-ala-aib[c] (13) | $2.4 \times 10^4$ | $1.1 \times 10^4$ | undetectable |
| DiAc-L-lys-D-ala-D-leu (14) | $2.3 \times 10^4$ | $1.9 \times 10^4$ | $1.8 \times 10^4$ |

[a] Association constants were determined by UV difference spectroscopy (see Experimental Section). [b] Ab = 2-aminobutyric acid. [c] Aib = aminoisobutyric acid.

to the increased flexibility of its N-terminal region as compared to that of ristocetin. The isobutyl side chain in **14** may also interfere with the effective positioning of the *N*-methylleucine in the complex.

The decreased peptide binding shown by both the mono- and didechloro derivatives is reflected in their decreased antibiotic activity as measured by disc diffusion on agar with *B. subtilis* as the test organism. The monodechlorinated derivative, **3**, has approximately 70% of the activity of vancomycin itself while didechloro dervative **4** has only about 50% of the activity of the parent antibiotic.

### Conclusions

The antibiotic vancomycin can be selectively dehalogenated on residue **2** to give a monodechloro derivative, **3**; further reduction gives didechloro derivative **4**. Neither **3** nor **4** binds di- and tripeptides as effectively as the parent antibiotic. Compounds **3** and **4** rearrange to CDP-I-type products, **6** and **7**, respectively. The rearrangements are more facile than the rearrangement of vancomycin. The picture which is emerging is that both chlorines decrease the mobility of the rings to which they are attached and the mobility of the antibiotic as a whole. In addition the chlorine on residue **6** helps to define the shape of the pocket into which the side chain of residue **1** of the binding peptide fits. The overall effect of the chlorines is to contribute to both the stability and specificity of the binding site.

A survey of other members of this class of antibiotics reveals that the aromatic rings of residues **2** and **6** of the triphenylamino acid are frequently chlorinated.[24] Position 6c is the most common

site of substitution. Thus far, chlorination at position 2c has only been observed for antibiotics simultaneously bearing a chlorine at 6c. In one recently reported case, aridicin,[25e] chlorine is found on position 6e, as well as 2c and 6c. No examples have been described in which a substituent is present at 2e, and an examination of molecular models reveals that a chlorine substituent at that position would block peptide complexation. Chlorines have also been found, but less frequently, on the other aromatic residues at sites that would not interfere with complexation. It is quite possible that in the other known antibiotics in this class chlorines on residues **2** and **6** may not play as important a role as they do in vancomycin, because the compounds have aromatic residues at positions 1 and 3, often joined together via a diphenyl ether linkage. The aromatic residues contribute conformational stability to the binding pocket, particularly when they are linked together.

Further studies are now in progress to evaluate the role of other structural features of these antibiotics in determining their biological activity.

(24) Sites of chlorination in vancomycin-type antibiotics include the following: ristocetin (none), avoparcin (6c, 3c),[25a] actinoidin (6c, 1c),[13] A35512B (3c),[25b] A41030 and the related A47934 (2c, 6c, 5e),[25c] teicoplanin (2c, 6c),[9] actaplanin (6c),[25d] and aridicin (2c, 6c, and 6e).[25e]

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