minations and to Richard S. Ware for mass spectra analyses. We thank Dr. U. Hacksell for a preprint of ref 5.

Registry No. (±)-**3**, 97000-20-9; **4**·HCl, 20260-53-1; **5**·HCl, 59105-51-0; **5**·HBr, 97000-21-0; **6**·HCl, 22620-36-6; **7**, 97000-22-1; **8**, 97000-23-2; **9**, 97000-24-3; **9** (acid), 97000-30-1; (±)-**10**, 97000-25-4; (±)-**11**, 97000-26-5; (±)-**12** (isomer 1), 97000-27-6; (±)-**12** (ketone) (isomer 1), 97000-31-2; (±)-**12** (mesylate) (isomer 1), 97000-33-4; (±)-**12** (isomer 2), 97000-28-7; (±)-**12** (ketone) (isomer 2), 97000-32-3; (±)-**12** (mesylate) (isomer 2), 97000-34-5; (±)-**13**, 97000-29-8; 3-(3-methoxyphenyl)-5-(3-methoxybenzyl)pyridine, 97000-35-6.

Preparation of Biologically Active Ristocetin Derivatives: Replacements of the 1′-Amino Group

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A series of ristocetin analogues with modifications (OH, C=O, C=NOH, $NCOCH_3$) at the C-1′ amino group was synthesized and found to possess antibacterial activity against gram-positive bacteria and to bind to Ac_2-Lys-D-Ala-D-Ala, a model for the antibiotic's site of action. Due to the lack of a positively charged amino group, the active analogues could not form a salt bridge, indicating that an electrostatic interaction between the positively charged 1′-amino group of ristocetin and the carboxylate anion of the peptide is not required for complex formation. The only compound that did not exhibit good antibacterial activity was epiristocetin aglycone (an analogue with the 1′-amino group in the opposite configuration (*S*) as ristocetin). On the basis of NMR studies of epiristocetin aglycone in solution, the 1′-amino group is located in the proposed carboxylate binding pocket and may sterically block complex formation.

The glycopeptide antibiotics (ristocetin, vancomycin, avoparcin) have been the subject of intense investigation in recent years, especially with respect to structure and mode of action. It is now well established that the glycopeptide antibiotics exert their antibiotic activity by binding specifically to cell wall precursors terminating with the peptide D-alanyl-D-alanine.[1] The remarkable specificity of this complexation was demonstrated by Perkins using synthetic analogues of the cell wall peptide.[2] The binding of the glycopeptide antibiotics was found to be dependent on the chirality of either D-alanine residue of the synthetic analogue, the length of the amino acid side chains, and the presence of a free carboxyl group.

On the basis of extensive NMR studies of the complex formed between Ac-D-Ala-D-Ala and ristocetin, Williams and co-workers[3] have proposed a structure for the complex. The interactions postulated to stabilize the complex include three hydrogen bonds between three amide NH groups (2′, 3′, 4′) and the carboxylate ion of the peptide, two additional hydrogen bonds, hydrophobic interactions, and an electrostatic interaction between the protonated 1′-amine of ristocetin and the peptide carboxylate anion. For the vancomycin/Ac-D-Ala-D-Ala complex, a similar structure was proposed.[4] It has been shown that vancomycin undergoes a large conformational change on binding that orients the NH groups for hydrogen bonding and positions the amino group to allow the formation of a bent salt bridge with the carboxylate anion. Unlike vancomycin, however, ristocetin is more rigid due to the diphenyl ether linkage between the F and G rings and cannot orient the protonated amino group for an ideal electrostatic interaction. In fact, in the proposed structure of the complex the amino group is about 5 Å from the carboxylate and pointing in the wrong direction for salt bridge formation.[3] Nevertheless, an interaction between the peptide carboxylate and protonated amine of ristocetin has been postulated[5] to be important for the initial formation of the complex, resulting in fast on-rates and in the final structure of the complex. Although the peptide carboxylate anion and antibiotic cation are separated by a considerable distance (5 Å) in the final structure of the complex, the ristocetin salt bridge is proposed to be more important than anticipated due to its location in a hydrophobic environment formed by the C, F, and G rings of ristocetin.[5]

In order to assess the relative importance of an electrostatic interaction for complex formation, we have synthesized a series of ristocetin analogues (Figure 1) that cannot form a salt bridge. These compounds were tested for their antibacterial activity and ability for binding to Ac_2-L-Lys-D-Ala-D-Ala. Since ristocetin ψ-aglycone (II) has greater antimicrobial potency than the parent molecule and should be more amenable to selective modification, II was chosen as the starting point for our synthetic modifications. Due to the structural complexity of the target compounds, high magnetic fields and two-dimensional NMR methods were required to interpret their complicated ^1H NMR spectra. The NMR data were useful for the structural elucidation of the compounds as well as for providing information on the molecular conformations that might be related to the biological activity of these molecules.

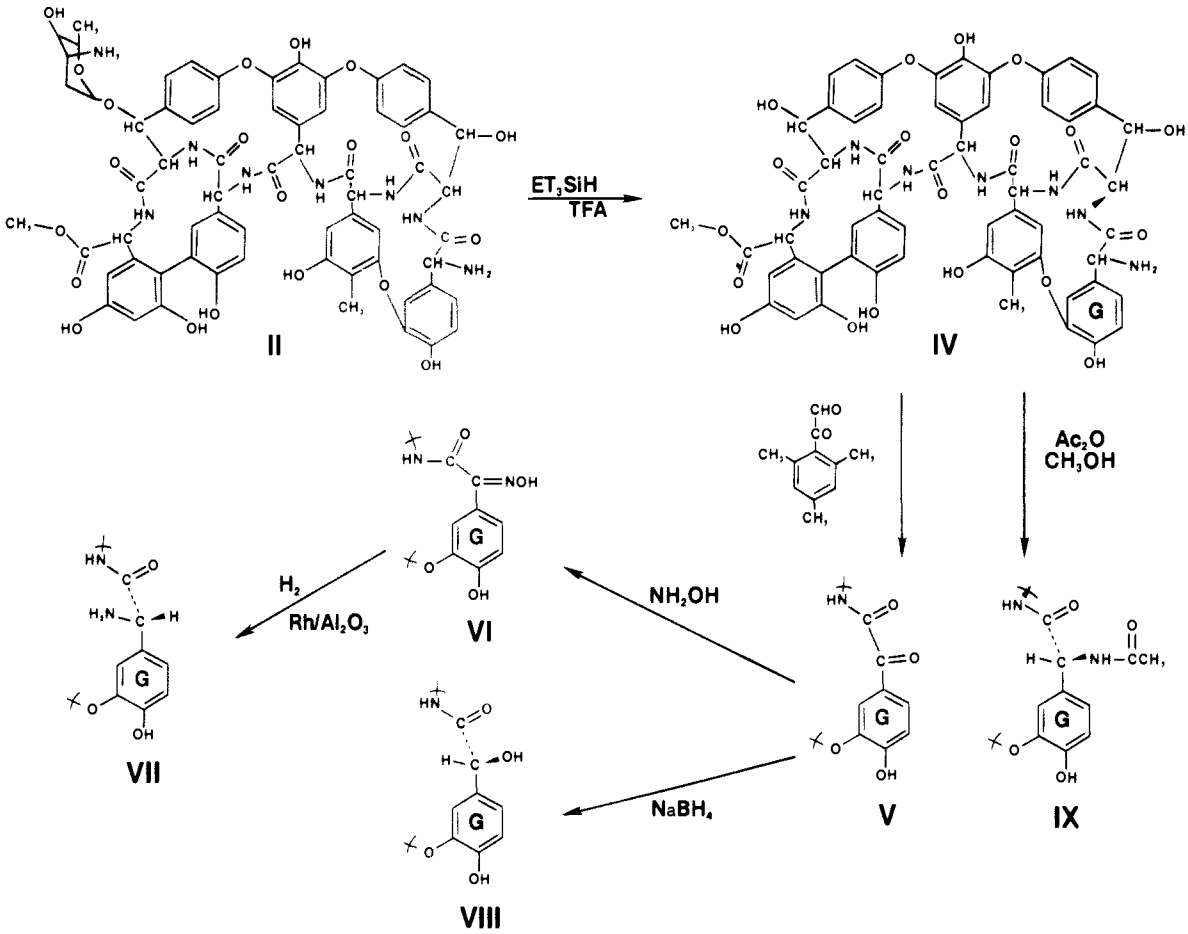
Results and Discussion

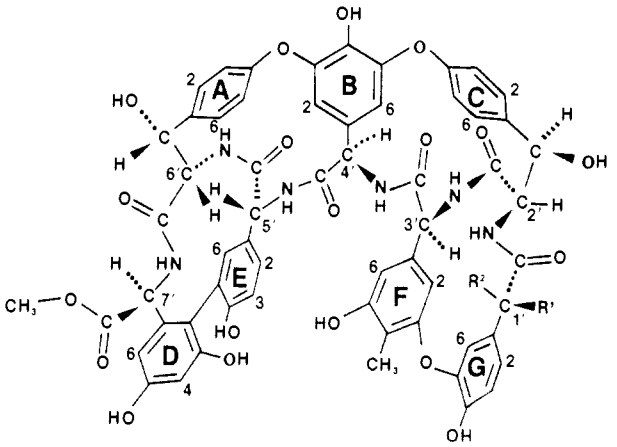
Chemistry. Ristocetin ψ-aglycone (II, Scheme I) was obtained by mild acid hydrolysis of ristocetin.[6] Attempts to prepare aglycone IV by acidolysis, as reported by Bognar,[7] resulted in concomitant ester cleavage to give the aglycone acid III (same structure as IV with the ester hydrolyzed). The aglycone was prepared cleanly by re-

(1) (a) Best, G. K.; Durham, N. N. *Arch. Biochem. Biophys.* **1965**, *11*, 685. (b) Perkins, H. R. *Biochem. J.* **1969**, *111*, 195. (c) Hammes, W. P.; Neuhaus, F. C. *Antimicrob. Agents Chemother.* **1974**, *6*, 722.
(2) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *124*, 845.
(3) Williams, D. H.; Williamson, M. P.; Butcher, D. W.; Hammond, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1332.
(4) Williams, D. H.; Butcher, D. W. *J. Am. Chem. Soc.* **1981**, *103*, 5697.
(5) Williamson, M. P.; Williams, D. H.; Hammond, S. J. *Tetrahedron* **1984**, *40*, 569.
(6) Rajananda, V.; Norris, A. F.; Williams, D. H. *J. Chem. Soc. Pak.* **1979**, *1*, 29.
(7) Bognar, R.; Sztaricakai, F.; Munk, M. E.; James, J. *J. Org. Chem.* **1974**, *39*, 297.

0022-2623/85/1828-1371$01.50/0

Scheme I





Number	Compound	R'	R²
IV	Ristocetin aglycone	NH_2	H
V	Ristocetin aglycone ketone		= O
VI	Ristocetin aglycone oxime		= NOH
VII	epi-Ristocetin aglycone	H	NH_2
VIII	Ristocetin aglycone alcohol	OH	H
IX	N-Acetyl ristocetin aglycone	$NHCCH_3$ (O)	H

Figure 1. Structures of new ristocetin derivatives, labeled to show the numbering system used in the text and in Table I.

ductive cleavage using triethylsilane in trifluoroacetic acid, which had been shown to cleave neutral glycosides.[8] Acetylation of IV with acetic anhydride in methanol provided the *N*-acetyl derivative IX. In order to oxidize the amino group of IV, Schiff base formation with mesitylglyoxal followed by rearrangement and hydrolysis to the ketone[9] was attempted. Surprisingly, treatment of IV with mesitylglyoxal in DMF followed by silica gel chromatography led directly to ketone V. In retrospect, this result might be explained by the highly cross-conjugated α-keto amide aromatic system of the resultant product. Conversion of V to the oxime VI followed by catalytic hydrogenation gave a single amino compound, VII, as the only isolatable product. We had anticipated from studies of space-filling models that oxime VI could only approach a catalyst surface in such a way that the *S* amino acid isomer would be produced. The structure of peptide VII is consistent with this prediction and is clearly different from that of aglycone IV. Support for this configurational assignment will be presented in the discussion of structural elucidation.

Sodium borohydride reduction of α-keto amide V gave rise to a product mixture from which a single isomeric alcohol was isolated. This product was shown, as discussed later, to have the *R* configuration at the reduced center. The other major component of the product mixture could not be identified by NMR and mass spectral analysis. The alcohol with the *S* configuration may have been lost upon chromatography; however, TLC analysis of the crude reaction product did not reveal a component with an R_f expected for the isomeric alcohol. NMR and mass spectral data suggest structure VII for the product of this reduction.

Structure Elucidation. The structures of the compounds shown in Figure 1 were analyzed on the basis of ^1H and ^{13}C NMR methods. The ^{13}C chemical shifts were

(8) Rolf, D.; Gray, G. R. *J. Am. Chem. Soc.* **1982**, *104*, 3539.

(9) Corey, E. J.; Achiwa, K. *J. Am. Chem. Soc.* **1969**, *91*, 1429.

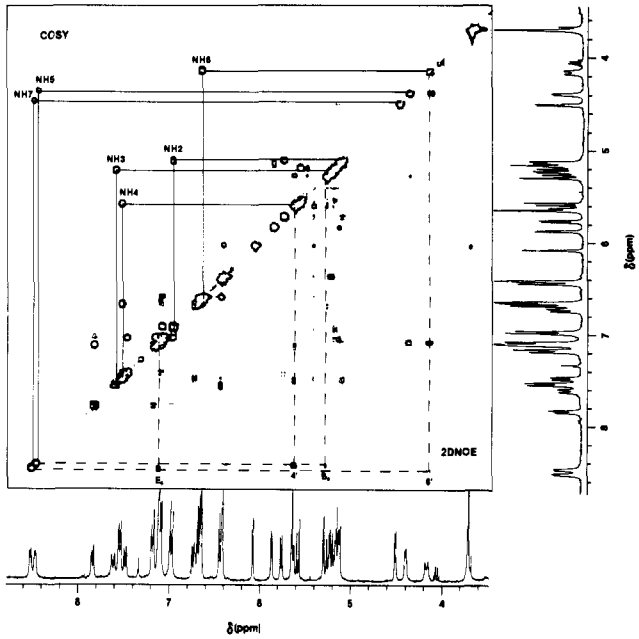


Figure 2. Contour plot of a COSY (upper half) and 2D NOE experiment (lower half) of ristocetin aglycone alcohol (VIII) in Me_2SO.

indicative of the particular functional groups (C=O, 190.8; C=NOH, 163.8; $NCOCH_3$, 22.3 ppm); however, from the ^{13}C data alone, the structures could not be unambiguously determined. For a complete structural analysis, we relied on ^1H NMR studies. The ^1H resonances were assigned by one- and two-dimensional NMR methods. For example, Figure 2 (top) depicts a contour map of a correlation spectroscopy (COSY)[10] experiment that allowed the identification of the protons that are scalar (through bond) coupled in ristocetin aglycone alcohol (VIII). The solid lines connect the cross peaks to the diagonal and identify the frequencies of the coupled NH and α-CH protons. A contour map of a 2D NOE[11] experiment is shown in Figure 2 (bottom). The dashed lines connecting the cross peaks indicate some of the protons that are dipolar (through space) coupled and, therefore, in close proximity. For example, NH7 is near E6 and 6′, and NH5 is in close proximity to 4′ and B2. From a detailed analysis of the COSY and 2D NOE data, many of the ^1H resonances were assigned. The remaining ^1H assignments were made from two-dimensional double-quantum NMR[12] and relayed correlation spectroscopy.[13] The double-quantum NMR experiments were especially useful in assigning the complicated aromatic region of the spectra by identifying the coupled protons with small differences in chemical shift and simplifying the contour maps by selectively enhancing the cross peaks corresponding to either the ortho ($J \sim 8$ Hz) or meta ($J \sim 2$ Hz) coupled protons. Connectivities between remote nuclei belonging to the same spin system were identified by double-quantum NMR and relayed correlation spectroscopy. The utility of these two-dimensional techniques for assigning the proton resonances of glycopeptide antibiotics is described elsewhere.[14]

(10) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
(11) Kumar, A.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1.
(12) Braunschweiler, L.; Bodenhausen, G.; Ernst, R. R. *Mol. Phys.* **1983**, *48*, 535.
(13) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731.

Table I. ^1H NMR Chemical Shifts (δ) of Ristocetin Aglycone (IV) and Its Analogues in Me_2SO at 25 °C (Reported Relative to Internal Tetramethylsilane (Me_4Si))

	δ				
assgnt	IV	V	VII	VIII	IX
NH7	8.51	8.48	8.49	8.50	8.50
NH5	8.45	8.53	8.41	8.44	8.44
C6	7.84	7.59	7.73	7.82	7.84
NH3	7.62	7.46	7.77	7.58	7.58
NH4	7.56	7.22	8.23	7.52	7.57
A6	7.52	7.48	7.52	7.52	7.52
A2	7.46	7.47	7.46	7.46	7.46
C5	7.17	7.23	7.21	7.16	7.20
C3	7.15	7.24	7.12	7.16	7.15
G2	7.10	7.68	7.12	7.06	6.99
E6	7.09	7.11	7.10	7.10	7.10
A3	7.09	7.09	7.11	7.08	7.08
C2	7.08	7.12	7.18	7.06	7.08
NH2	7.07	7.40		6.96	7.31
G3	6.96	7.14	6.91	6.96	6.92
A5	6.71	6.72	6.68	6.72	6.72
E2	6.68	6.67	6.68	6.68	6.68
E3	6.65	6.64	6.64	6.64	6.64
NH6	6.66	6.64	6.66	6.64	6.64
G6	6.62	7.22	6.73	6.63	6.67
F2	6.43	6.52	6.71	6.43	6.45
D4	6.41	6.42	6.40	6.40	6.39
F6	6.39	6.48	6.42	6.39	6.39
D6	6.05	6.07	6.06	6.06	6.05
B6	5.62	5.72	5.67	5.63	5.62
4′	5.62	5.64	5.64	5.61	5.61
B2	5.28	5.29	5.30	5.28	5.28
3′	5.23	5.21	5.28	5.22	5.25
Cbz	5.14	5.23	5.16	5.14	5.14
Abz	5.10	5.10	5.11	5.11	5.11
2′	5.09	5.05	4.94	5.11	5.04
1′	4.72		4.58	5.19	5.85
7′	4.48	4.50	4.48	4.48	4.48
5′	4.37	4.41	4.37	4.37	4.37
6′	4.14	4.14	4.15	4.14	4.14
$COOCH_3$	3.69	3.71	3.69	3.69	3.69
FCH_3	1.95	1.96	1.94	1.96	1.96
$NCOCH_3$					1.88

The ^1H chemical shifts for this series of glycopeptide antibiotics appear in Table I. The chemical shifts, scalar coupling constants ($^3J_{NH-\alpha-CH}$), and nuclear Overhauser effects for ristocetin aglycone (IV), ristocetin aglycone alcohol (VIII), and *N*-acetylristocetin aglycone (IX) were found to be very similar. These results suggest a similarity in the conformations of these compounds and indicate that the stereochemistry at the 1′-position of ristocetin aglycone alcohol (VIII) and ristocetin aglycone (IV) is the same. Although the ^1H chemical shifts differed at the N-terminus for epiristocetin aglycone (VII), the NOEs and scalar coupling constants were found to be similar to those of ristocetin aglycone, suggesting a similarity in their conformations. The upfield shifts observed for the NH3, NH4, G6, and F2 protons of epiristocetin aglycone can be explained by the presence of the protonated amine in a position above this part of the molecule with the opposite configuration as that of ristocetin aglycone (IV). For ristocetin aglycone ketone, some of the chemical shift differences suggest at least a slight difference in conformation of the 14-membered ring containing the ketone, which might be expected by a change from an sp^3 to an sp^2 center. However, the conformation of ristocetin aglycone ketone (V) cannot be drastically different from that of ristocetin aglcone since the same NOEs were observed between F6 and 3′, F2 and G6, and C2 and C_{BZ} and, in

(14) Fesik, S. W.; Perun, T. J.; Thomas, A. M. *Magn. Reson. Chem.*, in press.

Table II. Antibacterial Activities (MIC)[a] and Association Constants[b] for Ristocetin and Derivatives

	MIC, μg/mL				
compd	*Staphylococcus aureus* Smith	*Staphylococcus epidermis* 3519	*Streptococcus faecalis* 10541	*Streptococcus pyogenes* C203	K_A, M^{-1}
I	12.5	25	6.2	1.56	6.0×10^5
II	0.78	0.78	1.56	0.39	6.4×10^5
III	3.1	6.2	6.2	1.56	1.7×10^5
IV	3.1	3.1	3.1	3.1	6.7×10^5
V	6.2	12.5	12.5	3.1	5.6×10^5
VI	3.1	3.1	3.1	1.56	9.6×10^5
VII	25	50	50	25	2.0×10^4
VIII	12.5	12.5	6.2	6.2	1.5×10^5
IX	6.2	6.2	6.2	3.1	1.4×10^5

[a] Measured with a twofold agar dilution of the test compound in 10 mL of brain heart infusion agar. Test plates were incubated for 24 h at 35 °C. [b] K_A for association with Ac_2Lys-D-Ala-D-Ala, as determined by differential UV spectrophotometry in 10% methanol/sodium citrate buffer (pH 5.1).

addition, the three-bond coupling constants ($^3J_{NH-\alpha-CH}$) were found to be similar.

Antibacterial Activity. Each of the compounds II–IX were assayed for antibacterial activity against several gram-positive organisms by a standard agar dilution assay using ristocetin as a standard. As shown in Table II, only compound VII, the epiaglycone, showed markedly reduced activity. The nearly equivalent activity of the remaining compounds III–VI and IX suggested that the amino group of II was not critical for activity, providing that all of the compounds were acting by the same mechanism. To check this, we measured the binding of these compounds to Ac_2-Lys-D-Ala-D-Ala as described below.

Binding Studies. The binding of ristocetin (I) and analogues (II–IX) to the synthetic peptidoglycan analogue Ac_2-Lys-D-Ala-D-Ala was measured by the differential-UV assay described by Nieto and Perkins.[2] As shown in Table II, all the analogues, except epiristocetin aglycone (VII), had similar association constants. The association constant of VII was 10-fold less than those of the others.

Careful consideration of the binding models proposed by Williams et al.[3] and others[15] suggests that several interactions may be stabilizing the glycopeptide/Ac_2Lys-D-Ala-D-Ala interaction. These interactions include three hydrogen bonds between the NH groups (2′, 3′, 4′) of the antibiotic and the carboxylate of the peptide, two additional hydrogen bonds, and hydrophobic interactions. In addition to these interactions, for vancomycin, avoparcin, and ristocetin an electrostatic interaction between the protonated amine of the antibiotics and the carboxylate anion has been proposed to be important for binding. Since several of the ristocetin derivations that were synthesized (V, VI, VIII, IX) cannot form an electrostatic interaction and yet retain antibacterial activity and the ability to bind Ac_2Lys-D-Ala-D-Ala, we conclude that this salt bridge is not necessary for complex formation. However, the overall shape of the molecules may be important for biological activity as all of the active analogues displayed very similar conformations in solution. In particular, the overall size and shape of the "carboxylate binding pocket" formed by the three NH groups (2′, 3′, 4′) may be very important for antibacterial activity. The 10-fold reduction in activity of VII cannot be explained by changes in size or conformation of the metacyclophane ring (as judged by coupling constants and NOEs). Thus, it appears that the 1′-amino group is sterically blocking the binding site. Unlike other glycopeptides of this class,[3,16] ristocetin is relatively rigid at this end of the molecule due to the diphenyl ether linkage between the F and G rings. Therefore, epiristocetin is not expected to undergo large conformational changes to alleviate these unfavorable steric interactions.

Conclusions

Modification of the C-1′ amino group of aglycoristocetin does not abolish antibacterial activity or the ability to complex with Ac_2-Lys-D-Ala-D-Ala, clearly demonstrating that an ionic interaction with the peptide carboxylate group is not required for antibacterial activity. However, epiristocetin aglycone, which has the *S* configuration at C-1′, does not bind very well, suggesting that the size and shape of the carboxylate binding pocket is important for complexation. Although the present series of ristocetin derivatives demonstrates that an ionic interaction is not critical for binding, these analogues do not rule out the possibility of a dipole–dipole or hydrogen-bonding interaction stabilizing the complex. Replacement of the amino group of IV with a proton or alkyl group would test this possibility and shed more information on the molecular basis of peptide–antibiotic binding. Efforts to synthesize such compounds are under way.

Experimental Section

Purity of all the compounds was determined by TLC on silica gel using the solvent systems (A) 1-BuOH/EtOAc/H_2O/HOAc (4/3/2/1) and/or (B) PhMe/MeOH/HOAc (60/30/10) and by HPLC on a μ-Bondapak column using a Waters M-6000A pump and a Schoeffel GM770 UV detector. All new compounds gave an appropriate molecular ion from high-resolution peak-matched mass spectra. Mass spectra were obtained on a Kratos MS-50 mass spectrometer.

NMR spectra were recorded on a Nicolet NT-360 wide-bore spectrometer. Chemical shifts for both ^1H and ^{13}C NMR spectra are reported relative to internal tetramethylsilane (Me_4Si). NOE difference spectra were obtained as previously described.[16] A presaturation pulse of lower power was applied to a particular resonance and the FID stored. Similarly, a control FID was recorded after presaturating at a frequency where no resonance appeared in the spectrum. To process the data, the control FID was subtracted from the other FIDs that were collected, and the difference FID was exponentially multiplied (line broadening 2 Hz), Fourier-transformed, and phase corrected. Presaturation times of 0.2–0.4 s were employed. COSY experiments were performed with a (90°-t_1-90°-acquire)*n* pulse sequence. Typically, 512 × 1*K* FIDs were recorded with a sweep width of 3333 Hz and t_1 values of 0.3–153.6 ms. For the 2D NOE experiments, a (90°-t_1-90°-Υ_m-90°-acquire)*n* pulse sequence was employed with coherent magnetization transfer suppressed by a random variation of the mixing time, Υ_m. In these experiments 512 × 1*K* FIDs were collected for t_1 values incremented in 0.3-ms steps. The 2D data sets were processed in the absolute value mode, applying a sine bell window function before Fourier transformation.

Chemistry. Ristocetin A (I) was purchased from H. Lundbeck and Co. A/S, Ottiliavej 7-9, 2500 Copenhagen, Denmark.

ψ-Aglycoristocetin (II) was prepared as described by Rajananda et al.[6]

Aglycoristocetin Acid (III). Ristocetin sulfate, 3.0 g, was refluxed in 60 mL of 1 N hydrochloric acid for 40 min. The mixture was cooled to room temperature, adjusted to pH 6.5 with 0.5 N sodium hydroxide, concentrated at reduced pressure, and filtered to give 1.39 g of solid. This material was chromatographed on a 3.0 × 54 cm silica gel column, eluted with 1-BuOH/EtOAc/H_2O/HOAc (4/3/2/1) to give 0.5 g of partially purified material. This material was rechromatographed on a second silica

(15) Fesik, S. W.; Armitage, I. M.; Ellestad, G. A.; McGahren, W. J. *Mol. Pharmacol.* **1984**, *25*, 281.

(16) Fesik, S. W.; Armitage, I. M.; Ellestad, G. A.; McGahren, W. J. *Mol. Pharmacol.* **1984**, *25*, 275.

gel column to give 250 mg of product. Further purification was accomplished by chromatography on a 2.4 × 31 cm C_{18} silica column eluted with 20% MeOH/0.1% H_3PO_4 (adjusted to pH 3 with Et_3N). The appropriate fractions were combined, adjusted to pH 6.5 with 0.5 N sodium hydroxide, and concentrated to remove the methanol. The precipitate was collected to give 115 mg of product: UV (CH_3OH) λ_{max} 278 nm (ϵ 8600); $[\alpha]_D$ -66.7° (*c* 1.08, DMF).

Aglycoristocetin (IV). To a solution of 11.6 g of triethylsilane in 200 mL of trifluoroacetic acid was added 13.0 g of ristocetin ψ-aglycone. The solution was allowed to stand at room temperature for 48 h. The solvent was evaporated, and the residue was slurried with water and adjusted to pH 6.5 with 0.5 N NaOH. The precipitated solid was collected by filtration and dried to give 13.34 g of product. An analytical sample was prepared by chromatography of the crude product on a silica gel column eluted with 1-BuOH/EtOAc/H_2O/HOAc (4/3/2/1). The best fractions were combined and concentrated, and the residue was chromatographed on a C_{18} silica column. The product was eluted with 25% CH_3OH/0.1% H_3PO_4. The appropriate fractions were combined, concentrated to remove the CH_3OH, and adjusted to pH 6.5 with 0.5 N NaOH. The precipitate was collected to give 2.30 g of a white powder: UV (CH_3OH) λ_{max} 278 nm (ϵ 9100); $[\alpha]_D$ -85° (c 1.01,DMF); exact mass for $C_{60}H_{52}N_7O_{19}$ (*m/e*) calcd 1174.3317, found 1174.3299 (M + H).

Desaminoaglycoristocetin Ketone (V). To a solution of 4.988 g (4.25 mmol) of aglycoristocetin dissolved in 30 mL of dimethylformamide, chilled in an ice bath, was added 935 mg (5.31 mmole) of freshly distilled mesitylglyoxal in 5 mL of DMF. After the mixture was stirred at 5 °C for 1.5 h, the solvent was evaporated and the residue chromatographed on a 3.2 × 77 cm silica gel column and eluted with toluene/methanol/acetic acid (70/25/5). The appropriate fractions were combined to give 1.61 g of product: $[\alpha]_D$ -53° (*c* 1.02, DMF); exact mass for $C_{60}H_{49}N_6O_{20}$ (*m/e*) calcd 1173.3006, found 1173.2975 (M + H).

Desaminoaglycoristocetin Oxime (VI). To a solution of 1.490 g (1.27 mmol) of desaminoaglycoristocetin ketone in 25 mL of pyridine was added 890 mg (13 mmol) of hydroxylamine hydrochloride. The mixture was stirred overnight at room temperature. The solvent was evaporated and the last of the pyridine removed with a toluene azeotrope. The residue was kept in vacuo at 50 °C for 2 h to give 950 mg of the oximino derivative. HPLC and TLC analysis indicated this material was sufficiently pure (>90%) to be used without additional purification: $[\alpha]_D$ -94° (*c* 1.37, DMF).

Epiaminoaglycoristocetin (VII). The crude oxime derivative (950 mg) prepared as described above was treated with 5 g of 5% rhodium/alumina in 250 mL of MeOH at 3 atm H_2 pressure for 40 h. The catalyst was removed by filtration, and the solvent was evaporated. The residue was chromatographed on a 3.0 × 50 cm silica gel column and eluted with PhMe/MeOH/HOAc (60/30/10), to give 173 mg of epiaminoaglycoristocetin. An analytical sample was prepared by chromatography of this material on a C_{18} silica column eluted with 25% MeOH/0.1% H_3PO_4: exact mass for $C_{60}H_{52}N_7O_{19}$ (*m/e*) calcd 1174.3317, found 1174.3288 (M + H).

Desaminoaglycoristocetin Alcohol (VIII). A solution of 208 mg of desaminoaglycoristocetin ketone in 4 mL of methanol was treated with an excess of sodium borohydride, at room temperature for 2.5 h. The mixture was concentrated, chromatographed on a 3.0 × 54 cm silica gel column, and eluted with the solvent mixture PhMe/MeOH/HOAc (60/30/10). The second compound eluted from the column was the desired product. HPLC analysis on a 4 mm × 30 cm C_{18} silica column with acetonitrile/0.1% phosphoric acid showed a peak at 4.5 mL: exact mass for $C_{60}H_{50}N_6O_{20}$ (*m/e*) calcd 1174.3, found 1197 (M + Na).

***N*-Acetylaglycoristocetin (IX).** To a solution of 0.50 g of aglycoristocetin in 15 mL of CH_3OH, cooled in an ice bath, was added 1 mL of acetic anhydride. After 2 h the solvent was evaporated, and the residue was chromatographed on a silica gel column and eluted with BuOH/EtOAc/H_2O/HOAc (4/3/2/1). The partially purified product was rechromatographed on a C_{18} silica column eluted with 35% MeOH/0.1% H_3PO_4. The pure fractions were combined, the MeOH was evaporated, and the mixture was adjusted to pH 6.5 with NaOH. The precipitate was collected and dried to give 220 mg of product.

Microbiological Assay. Antibacterial activity expressed as MIC (minimal inhibitory concentration in μg/mL) was determined on a twofold agar dilution of the compounds in 10 mL of brain heart infusion agar. The organisms were grown for 24 h and diluted 1/100. The inoculum was delivered to the plates with a Steers replicator. The test plates were incubated at 35 °C for 25 h.

Binding Assays. The interaction of Ac_2-Lys-D-Ala-D-Ala with ristocetin and its derivatives was examined by UV difference spectroscopy as described by Perkins.[17] Experiments were carried out on a Cary 219 double-beam UV spectrophotometer using a tandem-cell arrangement with a total light path of 2 cm. The initial volume of antibiotic solution was 1.4 mL at a concentration of 50 μg/mL in 20 mM sodium citrate buffer (pH 5.1). Some of the ristocetin derivatives were not soluble in water and, therefore, had to be dissolved in methanol (10%) to complete the binding studies. The difference in absorbancy developed on addition of peptide was monitored at the wavelength that showed the maximal change. Association constants for complex formation were calculated by an nonlinear curve-fitting program[18] based upon the equations developed by Hunt and Vernon.[19]

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(17) Perkins, H. R. *Biochem. J.* **1969**, *111*, 195.
(18) Program was written by J. A. Wakat of Abbott Laboratories Application Systems Development.
(19) Hunt, A. H.; Vernon, P. D. *J. Antibiot.* **1981**, *34*, 469.