Deaminoteicoplanin and Its Derivatives. Synthesis, Antibacterial Activity, and Binding Strength to Ac-D-Ala-D-Ala

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Teicoplanin and its acid hydrolysis products were deaminated with hydroxylamine-*O*-sulfonic acid (HOS). A few amides of these deaminoteicoplanins were also prepared. The loss of the terminal amino group reduces in vitro activity against staphylococcal and streptococcal bacteria to one-half to one-third, while binding strength to Ac-D-Ala-D-Ala, measured by differential UV spectroscopy, is reduced to one-tenth that of teicoplanin. The in vitro activity is further reduced by the presence of serum, and this is attributable to the increased lipophilicity and total negative charge of the deamino compounds. Comparison of UV spectra of deaminoteicoplanins with those of parent compounds made it possible to single out the most acid phenol group of teicoplanin aglycon (OH-4; pK = 8.2).

The glycopeptide antibiotics vancomycin, avoparcin, ristocetin, and teicoplanin exert their activity by binding specifically to the cell-wall precursor that terminates in D-alanyl-D-alanine.[1] The specificity of this binding was initially studied with vancomycin, with synthetic analogues of the cell-wall peptide.[2] The structure of the complex between Ac-D-Ala-D-Ala and the glycopeptide antibiotics has been elucidated by NMR studies.[3] Barna et al.[4] reported on the role of the terminal amino group of ristocetin in binding strength and antibacterial activity. By comparing the association and dissociation rates of protonated (pH 5) and deprotonated (pH 10) ristocetin with those of its *N*-acetyl derivative, these investigators demonstrated the importance of the charged N-terminus in the binding strength of ristocetin to Ac-D-Ala-D-Ala.

Herrin et al.,[5] on the other hand, in order to assess the relative importance of an electrostatic interaction in complex formation, synthesized a series of ristocetin analogues that cannot form an ionic bond with the carboxyl group of Ac-D-Ala-D-Ala and found that antibacterial activity and binding were not abolished. They noticed, however, that in this series there might be dipole–dipole or hydrogen-bonding interaction stabilizing the complex.

Teicoplanin (I, Figure 1), a glycopeptide structurally related to ristocetin,[6-8] is a complex of five closely related factors having the same skeleton and sugars but different acyl aliphatic chains.[9] It can be transformed into two pseudoaglycons (II and III) and into one aglycon (IV) by sequentially removing *N*-acylglucosamine moieties, mannose, or *N*-acetylglucosamine, respectively, by acid hydrolysis.[10] During the course of chemical modification of I we considered it of interest to replace the amino group (R = NH_2, Figure 1) with a proton (R = H) to obtain additional information about the relative importance of the electrostatic interaction in complex formation between this antibiotic and the cell-wall precursor that terminates in D-alanyl-D-alanine. Deamination of all these compounds gave the corresponding deamino compounds (Iw, IIw, IIIw, and IVw).

Another observation of Barna[4] was that the amino sugars in ristocetin and vancomycin to some extent favor the binding of these antibiotics when the N-terminal amino group is deactivated, e.g., by acylation. To see whether or not the introduction of a protonable function in glycopeptides, such as an amine instead of the original N-terminal amino group, can facilitate the binding, we also synthesized amide derivatives of IVw (VIw and VIIw, Figure 1) with an amino group. For comparison purposes, one neutral amide Vw was also prepared, starting from V.

NMR, UV, and IR data, pK values, HPLC retention times (t_R), biological activities, and binding association constants for Ac-D-Ala-D-Ala are reported.

Results and Discussion

Chemistry. Deamination of teicoplanins with sodium nitrite resulted in several unknown products, as shown by the HPLC profile of this reaction. Selective deamination was achieved with hydroxylamine-*O*-sulfonic acid (HOS) by operating at controlled pH 8 and 50 °C instead of the strong alkaline conditions at 0 °C as reported by Doldouras et al.[11] These last conditions remove proton X_3 (Figure 1), causing the C-3 epimerization with the consequent destruction of the binding pocket of molecules.[12a] Teicoplanins with *R* configuration at C-3 do not show significant antibacterial activities.[12b] The "reductive deamination" with HOS proved to be specific for primary amino group. Side reactions with secondary amides or tertiary amines, present in teicoplanin derivatives, were not observed. The reaction yield was generally higher than 60%.

Compounds IIw–IVw can be obtained from I through two pathways: first sugar hydrolysis followed by reductive deamination or vice versa, the final yield being independent of the route of synthesis. The derivatives *N*,*N*-dimethylamide (Vw) and *N*-quinuclid-3-ylamide (VIIw) were synthesized by reductive deamination of the corresponding amides V and VII, which were obtained from I and IV, respectively, with diphenyl phosphorazidate (DPPA)[13] as the condensing agent. *N*-[3-(Dimethyl-

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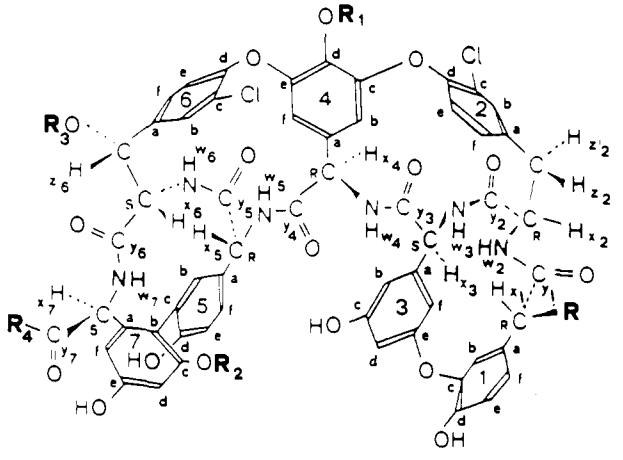
Table I. Teicoplanin Derivatives (Figure 1)

compd	HPLC,[a] t_R, min	pK_{MCS}[a] COOH	pK_{MCS}[a] NH_2	formula[a]	MW
I	26.51[b]	5.0	7.1	$C_{88}H_{97}O_{33}N_9Cl_2$[b]	1879.7
Iw	34.24[b]	5.2	absent	$C_{88}H_{96}O_{33}N_8Cl_2$[b]	1864.7
II	9.60	4.7	6.9	$C_{72}H_{68}O_{28}N_8Cl_2$	1564.3
IIw	22.25	5.1	absent	$C_{72}H_{67}O_{28}N_7Cl_2$	1549.3
III	11.58	4.7	6.8	$C_{66}H_{58}O_{23}N_8Cl_2$	1402.2
IIIw	25.77	4.9	absent	$C_{66}H_{57}O_{23}N_7Cl_2$	1387.2
IV	14.78	4.6	6.8	$C_{58}H_{45}O_{18}N_7Cl_2$	1199.0
IVw	28.02	4.6 (3.7)[d]	absent	$C_{58}H_{44}O_{18}N_6Cl_2$	1184.0
V[c]	28.85[b]	nd[f]	nd	$C_{90}H_{102}O_{32}N_{10}Cl_2$[b]	1906.7
Vw	36.05[b]	absent	absent	$C_{90}H_{101}O_{32}N_9Cl_2$[b]	1891.7
VI[c]	18.25	nd	nd	$C_{63}H_{57}O_{17}N_9Cl_2$[b]	1299.2
VIw	34.19	absent	nd[e]	$C_{63}H_{56}O_{17}N_8Cl_2$·HCl	1320.6
VII[c]	20.23	nd	nd	$C_{65}H_{56}O_{17}N_9Cl_2$	1306.2
VIIw	35.26	absent	nd[e]	$C_{65}H_{55}O_{17}N_8Cl_2$·HCl	1327.6

[a] See the Experimental Section. [b] Value of the highest peak of the "complex". [c] Reference 17. [d] When the sample was dissolved in the minimum amount of MCS and then diluted 10 times with water, the pK value decreased. Under these conditions the pK_a of OH-4 was also observed; it was 8.2. [e] Amino groups with a pK value of 8 are not detectable because they are masked, in these titration conditions, by the phenolic groups of the molecule. [f] Not determined.



	I	Iw	II	IIw	III	IIIw	IV
R	NH_2	H	NH_2	H	NH_2	H	NH_2
R_1	X	X	H	H	H	H	H
R_2	Y	Y	Y	Y	H	H	H
R_3	Z	Z	Z	Z	Z	Z	H
R_4	OH	OH	OH	OH	OH	OH	OH

	IVw	V	Vw	VI	VIw	VII	VIIw
R	H	NH_2	H	NH_2	H	NH_2	H
R_1	H	X	X	H	H	H	H
R_2	H	Y	H	H	H	H	H
R_3	H	Z	Z	H	H	H	H
R_4	OH	S	S	T	T	U	U

X = N-acyl-β-D-glucosamine, Y = α-D-mannose, Z = *N*-acetyl-β-D-glucosamine, S = $N(CH_3)_2$, T = $NHCH_2CH_2CH_2N(CH_3)_2$, U =

Figure 1. Structures of teicoplanin derivatives. The numbering system used is that proposed by Williams et al.[21]

amino)propyl]amide VIw was prepared by the same method, starting from IVw.

UV Spectra. The UV spectra of deamino compounds in water solution show a peak at about 280 nm at pH 7.4 that is shifted to 300 nm by increasing the pH from 8 to 12 (Figures 2 and 3) as does that of teicoplanin. In teicoplanins the phenol groups play the major role in UV absorption, while the ionization of the carboxyl group has no influence and the amino group influences it only to a minor extent. The changes in the 300-nm absorption seen in the teicoplanin UV curves, between pH 6 and 8 (Figure

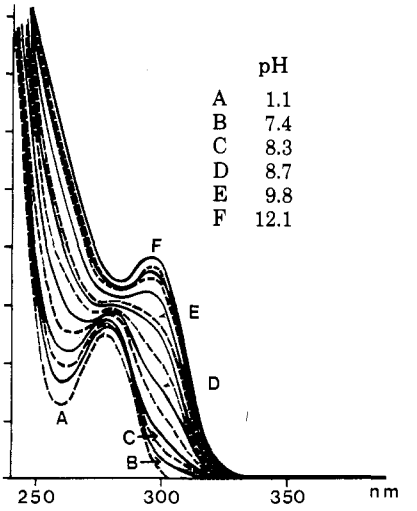


Figure 2. UV spectra of teicoplanin (I) in water solution at different pH values.

2, curves B and C), when phenols are not yet ionized, can be attributed to the deprotonation of NH_2 in the pH range of 6–8. Furthermore, comparison of the UV curves of Iw and IIw at pH 7.4 (Figure 3) indicates that the removal of the sugar at position OH-4 (Figure 1) induces absorption at about 300 nm attributable to the ionization of the more acidic phenol (OH-4). Therefore the differences in UV absorption at about 300 nm around pH 7.4 are due to the differences in ionization of both the amino and 4-phenol groups in compounds II–IV and of only the 4-phenol group in compounds IIw–IVw.

Potentiometric Titration. The potentiometric titrations (Table I) clearly demonstrate that there is no NH_2 group in the w compounds. When IIw–IVw are titrated in water solution, the pK of COOH is lower than in MCS/water 4/1, as expected, and the curves show an inflection corresponding to a pK_a value of 8.2, attributable to the OH-4 phenol, as discussed above for the UV spectrum changes around pH 8.

IR Spectra. The new class of compounds (Iw–IVw) cannot form internal salts. The carboxyl group of the derivatives is in the non-ionized form, as shown by its $\nu_{C=O}$ stretching at 1725 cm^{-1} (in the internal salts this band is at 1610 cm^{-1}, COO^-). In addition to the general picture for the aromatic glycopeptide molecule (peptidic ν_{NH}, glucosidic ν_{OH}, and phenolic ν_{OH}, 3700–3100 cm^{-1}; amide I, 1655–1645 cm^{-1}; amide II, 1515–1510 cm^{-1}; glycosidic and

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Table II. Proton NMR Assignments of Selected Signals (δ, ppm)[a]

	I[a]	Iw	II	IIw	III[b]	IIIw	IV[c]	IVw	Vw	VIw	VIIw
CH_3 groups	0.84	0.82									
CH_2 groups	1.13–1.24	1.13–1.24									
CH_2 β to C=O and $CH(CH_3)_2$	1.44	1.42									
acetyl G	1.88	1.86	1.87	1.84	1.87	1.85			1.86		
CH_2 α to C=O	2.03	2.01							2.01		
anomeric H of acetyl-G	4.30–4.50	4.30–4.50	4.30–4.50	4.30–4.50	4.38	4.32			4.30–4.50		
anomeric H of mannose	5.22	5.25	5.25	5.25					5.26		
anomeric H of acyls-G	5.40	5.40							5.35		
X_1	4.73		4.56		4.60		4.66				
X_2	4.99	4.80	4.90	4.83	4.97	4.91	4.97	4.92	4.82	4.94	4.91
X_3	5.34	5.34	5.31	5.35	5.34	5.32	5.33	5.35	5.34	5.34	5.34
X_4	5.64	5.68	5.62	5.63	5.60	5.64	5.67	5.64	5.69	5.65	5.65
X_5	4.30–4.50	4.30–4.50	4.30–4.50	4.30–4.50	4.30	4.28	4.33	4.36	4.30–4.50	4.35–4.50	4.36
X_6	4.10	4.12	4.12	4.14	4.13	4.13	4.11	4.13	4.10	4.18	4.17
X_7	4.30–4.50	4.30–4.50	4.30–4.50	4.30–4.50	4.46	4.44	4.39	4.44	4.30–4.50	4.30–4.50	4.47
Z^2	nd	2.85	2.82	2.84	2.84	2.84	2.84	2.83	nd	2.84	2.85
Z_2'	nd	nd	nd	nd	3.31	nd	3.31	3.27	nd	nd	3.28
Z_6	5.27	5.30	5.32	5.30	5.30	5.24	5.08	5.10	5.30	5.36	5.29
4_b	5.56	5.60	5.62	5.61	5.57	5.54	5.50	5.54	5.61	5.52	5.52
4_f	5.11	5.11	5.08	5.08	5.10	5.08	5.10	5.13	5.11	5.13	5.12
7_f	6.30	6.33	6.32	6.32	6.29	6.22	6.26	6.25	6.35	6.20	6.22
H-C_1-H		3.78		nd		3.50		3.83	nd	3.80	nd
		3.60				3.49		3.52		3.54	
$CON(CH_3)_2$									2.89		
									2.74		
$NH^+(CH_3)_2$										1.90	
NH-quinuclid-3-yl										2.5–2.7	
										1.7–2.3	
										3.4–3.8	

[a] G = glucosamine; nd = not determined. [b] Reference 21. [c] reference 22. IV corresponds to A41060B.

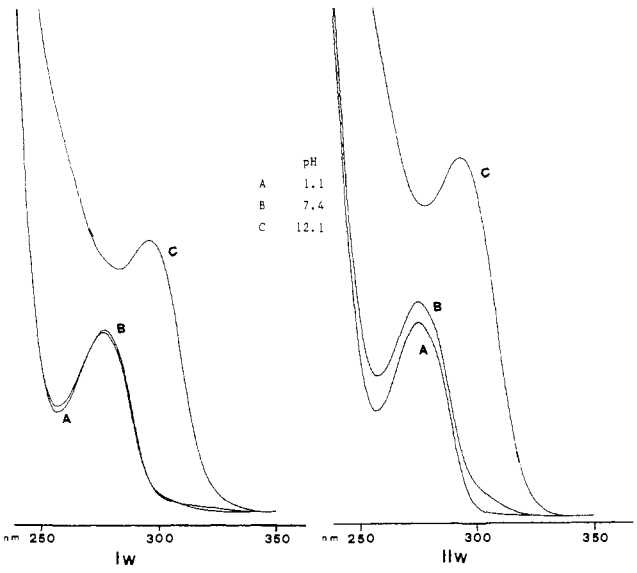

Figure 3. UV spectra of Iw and IIw at different pH values in water solution.

phenolic δ_{OH} and ν_{C-O} mixed vibrations, 1235–1225, 1160–1140, 1060, 1025–1005 cm^{-1}), the IR spectrum of structures containing mannose clearly indicates the presence of this sugar on the basis of the characteristic OH vibration at 970 cm^{-1}.

NMR Analysis. The selected ^1H NMR spectral attributions, reported in Table II, were based on comparison with data for teicoplanin and other glycopeptides,[14] on selective decoupling, and on two-dimensional COSY experiments. The absence of X_1 signal (Figure 1) in the w compounds is counterbalanced by the appearance of a CH_2 group which gives rise to an AB system (two doublets at about 3.50 and 3.80 ppm with J_{gem} = 15.4 Hz). The low field shift from 5.10 to about 5.30 ppm of Z_6 proton signal is noteworthy; it must be caused by the terminal amide or by the acetylglucosamine moiety. In the latter case this shift is due to both the inductive and anisotropic effects of the sugar attached to the carbon bearing Z_6-H. In the former case, there could be an anisotropic effect on Z_6 caused by a change of conformation in the surroundings induced by a hydrogen bond between the NH of the terminal amide and the carbonyl Y_6. When both sugar and amide moieties are present (as in Vw), only one of these has its deshielding effect on Z_6, and it is logical to assume that the amide moiety effect is excluded, since this group is prevented from assuming the conformation suitable to affect the chemical shift by the steric hindrance of the sugar moeity.

Antibacterial Activity. Each of the w compounds was assayed for antibacterial activity against several Gram-positive organisms, and their MICs, compared with those of parent compounds, are reported in Table III. There are moderate decreases of activity for all w compounds, more evident for the acidic ones (Iw–IVw). All the deamino compounds (w) show a remarkable reduction of the antibacterial activity in the presence of serum. This result is consistent with previous findings concerning the correlation of physical–chemical properties of a drug and its pharmacokinetics.

It is known that increases in drug lipophilicity, generally, are associated with increases in serum binding.[15a] It is also reported that positively charged drugs display low

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Table III. Antibacterial Activities (MIC)[a] of Teicoplanin and Derivatives

	MIC, μg/mL						
compd	*Staph. aureus* Tour	*S. aureus* Tour[b]	*S. aureus* Tour[c]	*S. epidermidis* ATCC12228	*Strep. pyrogenes* C 203	*Strep. pneumoniae* UC 41	*Strep. faecalis* AACC7080
I	0.12	0.5	0.5	0.25	0.06	0.06	0.12
Iw	0.5	2	8	0.12	0.12	0.25	0.5
II	0.25	0.5	0.5	0.25	0.5	0.5	2
IIw	2	8	16	1	4	4	4
III	0.5	0.25	0.5	0.12	0.5	1	1
IIIw	2	2	16	1	8	2	8
IV	0.06	0.12	0.25	0.016	0.12	0.12	0.12
IVw	0.12	0.25	1	0.25	0.06	0.06	0.12
V[d]	0.12	0.12	0.5	0.06	0.06	0.06	0.12
Vw	0.12	1	4	0.12	0.12	0.12	0.25
VI[d]	0.06	0.12	0.25	0.016	0.06	0.06	0.12
VIw	0.12	0.12	4	0.12	0.12	0.2	0.12
VII[d]	0.12	0.12	0.5	0.06	0.06	0.06	0.12
VIIw	0.12	0.25	4	0.12	0.12	0.12	0.25

[a] Determined by the 2-fold dilution method in microtiter system. The media used were Iso-sensitest broth (Oxoid) for staphylococci, and *S. faecalis* and Todd-Hewitt broth (Difco) for streptococci. The final inoculum was about 10^4 cfu/mL. MIC was read as the lowest concentration that permitted no visible growth after 18–24 h of incubation at 37 °C. [b] Inoculum 10^6 cfu/mL. [c] Determined in Iso-sensitest broth supplemented with 30% bovine serum and an inoculum of about 10^4 cfu/mL. [d] Reference 17.

Table IV. Association Constants[a] for Teicoplanins and Ac-D-Ala-D-Ala at 31 °C

compd	K_A, M^{-1}
I	1.1×10^6
Iw	1.3×10^5
Vw	2.0×10^5
IV	1.8×10^5
IVw	1.1×10^4
VIIw	2.2×10^4

[a] Measured by differential UV spectrophotometry 10% methanol/0.02 M sodium citrate buffer (pH 5).

binding to serum and negatively charged drugs exhibit high binding to serum. These statements have been confirmed on glycopeptide antibiotics by pharmacokinetic studies.[15b] The lack of N-terminal amino groups in teicoplanin derivatives causes an increased lipophilicity (Iw–VIIw) and the formation of a whole negative charge (Iw–IVw). These molecular features cause an increased serum binding observed as reduction of the in vitro activity. Lipophilicity of the reported compounds, was measured by reverse-phase HPLC method and the values, expressed as retention time (t_R), are reported in Table I (see Figure 4). The comparison of each w compound with the parent compounds shows a remarkable lipophilic difference.

Binding to Acetyl-D-Ala-D-Ala. To determine the importance of the amino group in binding, we measured the association constants (K_A, M^{-1}) of I and IV and of deamino Iw and IVw with Ac-D-Ala-D-Ala. As shown in Table IV, compounds Iw and IVw retain affinity for Ac-D-Ala-D-Ala but the binding parameter is only one-tenth that of parent compounds. The K_As of the amides Vw and VIIw are intermediate between those of I and Iw, IV and IVw, respectively.

Conclusion. Elimination of the terminal amino group from teicoplanin and its hydrolysis products II–IV reduces the in vitro antibacterial activities to one-half to one-third those of the corresponding analogues. The reduction of the association constants of Iw and IVw to Ac-D-Ala-D-Ala to one-tenth confirms the importance of the Coulomb attraction between the protonated N-terminal amino group of the antibiotic and the carboxyl anion of Ac-D-Ala-D-Ala.

Aglycons, which are the most active molecular species in vitro, have a lower binding strength to Ac-D-Ala-D-Ala than teicoplanins (Table IV), as also previously observed[4] and justified with the removal of the D-mannose (R_2, Figure 1). We now consider, in a homogeneous class, the role of the chemical modification on the binding strength. The replacement of the N-terminal amino group with a proton reduces the K_A 8-fold in teicoplanins (compare I and Iw) and 16-fold in aglycons (compare IV and IVw). On the contrary, substituting the carboxyl group of glycopeptide antibiotics, the negative charge of which can repulse the carboxylate of the Ac-D-Ala-D-Ala, with a neutral amide group increases K_A 1.5-fold (Vw versus Iw). This increase is 2-fold if the amide carries a basic group (VIIw versus IVw). This result demonstrates that a positive charge, such as a protonated amino group, in glycopeptide antibiotics aids the diffusion-controlled reaction[16] that affects the binding strength. However, the N-terminal amino group produces the best effect because of its strategic position near the binding pocket.

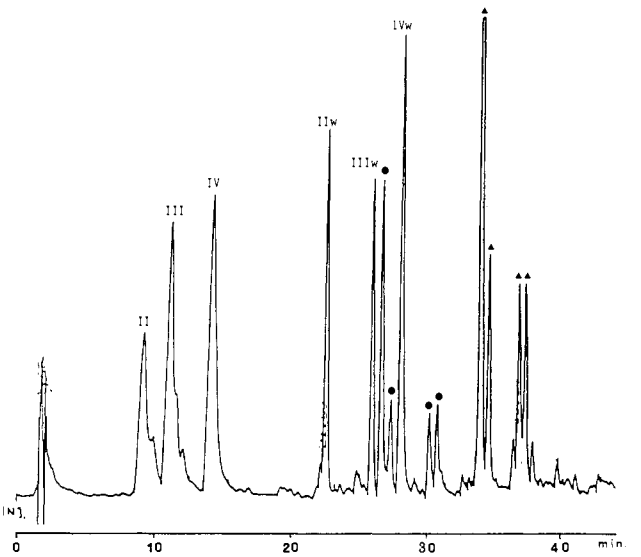

Figure 4. HPLC chromatogram of a prepared mixture of "natural" and 15-deamino teicoplanins: (●) I peaks, (▲) Iw peaks.

Experimental Section

General Procedures. Teicoplanin is manufactured by Gruppo Lepetit Spa; hydrolysis products II–IV were prepared as described by Malabarba et al.[17] Amides V–VII were synthesized by Ma-

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labarba.[17] Ac-D-Ala-D-Ala was synthesized according to the method of Nieto and Perkins[18] and the purity was verified by TLC and ^1H NMR. Thin-layer chromatography was performed on Merck silica gel 60 plates with the following solvent systems: (1) 1-butanol/acetic acid/water 6:2:2, (2) CH_3CN/acetic acid/water 8:1:1. Peptides were located by ninhydrin or by the method of Reindel.[19] Evaporations were carried out in a rotary evaporator at 45–50 °C (bath temperature) under reduced pressure, with 1-butanol added to prevent foaming. Column chromatographies were performed on silanized silica gel 60 (0.06–0.2 mm, Merck). IR spectra were recorded on a Perkin-Elmer 580 spectrophotometer (in Nujol mull). UV spectra were run on a Perkin-Elmer Model 320 spectrophotometer. The ^1H NMR spectra were recorded with a 250-MHz Bruker instrument equipped with an Aspect 3000 computer. ^1H NMR spectra (δ, ppm) were obtained in DMSO-d_6, with TMS as the internal standard. HPLC assays were run with a Hewlett-Packard 1084A chromatograph equipped with a UV detector at 254 nm and a Hibar-LiCrosorb RP-8 (250 mm, 7 μm) column (injection volume 20 μL; flow rate 1.5 mL/min; mobile phases (A) CH_3CN–0.02 M aqueous NaH_2PO_4, 5:95, (B) CH_3CN–0.02 M aqueous NaH_2PO_4, 75:25; linear step gradient [min (% B)] as follows: 0 (8), 10 (15), 20 (25), 40 (40). All compounds were analyzed for C, H, N, and Cl on samples previously dried at 140 °C under an N_2 atmosphere. Weight loss was determined by thermogravimetric analysis (TGA) at 140 °C; inorganic residue was determined after heating of the sample at 900 °C in an O_2 atmosphere. The analytical results were in accordance with the theoretical vaues. The pK_{MCS} values were determined potentiometrically in methyl Cellosolve (MCS)/water 4/1 (v/v) solution. An excess of 0.01 N HCl in the same mixture was added and the resulting solution was titrated with 0.01 N NaOH in the same solvent mixture.

UV Difference Spectroscopy. Binding constants between peptide and teicoplanin or its derivatives were measured essentially as described by Nieto and Perkins[18] except that tandem arrangement of cells was not required, since the peptide used in this work has no significant absorption in the range 250–340 nm. Experiments were carried out with the UV instrument mentioned above. Cells with 4-cm light path were used. Solutions (10 mL) containing antibiotic (25 μM in 10% methanol/0.02 M sodium citrate buffer, pH 5) were placed in the sample and reference cells, and the difference in absorbance that developed upon addition of Ac-D-Ala-D-Ala (10–50 μL of a 4 mM solution) to the sample cell and the same volume of buffer to the reference cell was measured at 282–285 nm. The temperature was 31 °C. Association constants were determined by Scatchard plots. The antibiotic concentrations were determined by UV, with the specific absorption of teicoplanin at 279 nm ($E^{1\%}_{1cm}$ = 59.4) as reference.[20] The peptide titer in distilled water was first determined by potentiometric titration, and then the suitable concentration was prepared by dilution.

Microbiological Assay. Antibacterial activity, expressed as MIC (minimal inhibitory concentration in μg/mL), was determined by the 2-fold dilution method in a microtiter system. The media used were Todd-Hewitt (Difco) for streptococci and Isosensitest broth (Oxoid) for staphylococci. The final inoculum was about 10^4 cfu/mL. MICs were read as the lowest concentrations that permitted no visible growth after 18–24 h of incubation at 37 °C.

General Procedure for Preparation of Deamino Compounds. Deaminoteicoplanin Pseudoaglycon (IIIw). As a representative example, antibiotic III (500 mg, 0.37 mequiv) was dissolved in water (15 mL) and acetonitrile (10 mL). The pH of the solution was maintained in the range of 8–9 with 1 N NaOH while a total of 165 mg of HOS (1.46 mequiv) was added over 6 h and the reaction mixture was kept at 60 °C. The end of reaction was checked by HPLC analysis. When the transformation was completed, the reaction mixture was loaded on the top of a flash column containing 150 g of silica gel RP-8 (LiChroprep, Merck, 40–60 μm) prepared in water/acetonitrile 90/10 (v/v). The column was developed with linear gradient, from 10 to 50% of acetonitrile, collecting 20-mL fractions. Fractions containing pure product were pooled and evaporated. The residue was treated with 5 mL of acetone and the product precipitated by adding 100 mL of ethyl ether. After standing 1 h, the precipitate was collected by filtration, washed with ether, and dried in vacuo overnight at 50 °C; yield 0.2 g of IIIw.

Deamino-*N*-quinuclid-3-ylteicoplanin Aglycon Amide Hydrochloride (VIIw·HCl). Compound VII[17] (600 mg, 0.46 mequiv) was suspended in water (20 mL) and acetonitrile (10 mL), and then 103.9 mg of HOS was added and the pH of the suspension was corrected to 8 by adding 1 N NaOH. The reaction mixture was kept at 50 °C for 5 h, with 50 mg of HOS added at 2-h intervals. After the mixture was allowed to stand overnight at room temperature, the reaction was complete. The acetonitrile was evaporated and the water extracted with 1-butanol (2 × 40 mL) after acidification to pH 2 with 1 N HCl. The organic layer was washed with water and evaporated in vacuo. The residue was suspended in 20 mL of acetone and 100 mL of ethyl ether. The product was filtered, washed with ether, and dried in vacuo at 50 °C overnight, yielding 440 mg of VIIw·HCl.

Deamino-*N*,*N*-dimethylteicoplanin Amide (Vw). Compound V[17] (1 g, 0.5 mequiv) was dissolved in water (40 mL) and acetonitrile (20 mL), and then HOS (147 mg, 1.30 mequiv) was added. The solution was heated at 40 °C maintained at pH 8.5 by adding 2.5 N NaOH. Four additions of HOS (50 mg × 4) were made during 10 h of reaction. The reaction mixture was adjusted to pH 5 with 1 N HCl and the organic solvent evaporated. The residual water solution was extracted twice with 1-butanol (30 mL), the organic phase evaporated to 10 mL, and the product precipitated by adding 150 mL of ether. The solid was filtered, washed, and dried in vacuo overnight at 50 °C, yielding 770 mg of Vw.

Deamino-*N*-[3-(dimethylamino)propyl]teicoplanin Aglycon Amide Hydrochloride (VIw·HCl). Diphenyl phosphorazidate (DPPA) (0.160 mL) was added at 0 °C to a stirred mixture of IVw (1 g, 0.8 mequiv) and 3-(dimethylamino)-1-propylamine (0.160 mL) in DMF (30 mL), followed by the addition of triethylamine (0.1 mL). The mixture was stirred at 0–5 °C for 5 h and then at room temperature overnight. A second portion of DPPA (0.160 mL) and dimethylpropylamine (0.160 mL) was added and after 24 h the reaction was complete. The product was precipitated by adding ethyl ether and collected by filtration. The crude material was purified on a chromatographic column prepared with 150 g of silanized silica gel and eluted with a gradient from 20% CH_3CN/0.1% HCl to 50%. The pure fractions were pooled and evaporated, yielding 100 mg of pure VIw.

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Registry No. II, 93616-27-4; IIw, 117226-73-0; III, 91032-39-2; IIIw, 117226-74-1; IV, 89139-42-4; IVw, 117226-75-2; VI, 117226-72-9; VIw, 117226-76-3; VIw·HCl, 117306-59-9; VII, 117251-07-7; VIIw, 117251-10-2; VIIw·HCl, 117307-53-6; HOS, 2950-43-8; Ac-D-Ala-D-Ala, 19993-26-1; teicoplanin, 61036-62-2.

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