Structural Modifications of the Active Site in Teicoplanin and Related Glycopeptides. 1. Reductive Hydrolysis of the 1,2- and 2,3-Peptide Bonds

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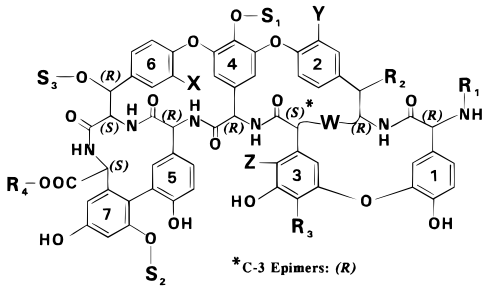
Received October 27, 1994 (Revised Manuscript Received January 4, 1996⊗)

Reaction of teicoplanin glycopeptides with sodium borohydride in aqueous ethanol solutions produced open pentapeptide derivatives in which the amide bond between amino acids 2 and 3 was hydrolyzed and the carboxyl group of amino acid 2 was reduced to a primary alcohol. Other glycopeptides of the dalbaheptide family, such as vancomycin, ristocetin, and A-40,926, underwent selective reductive hydrolysis (RH) of the heptapeptide backbone at the same position as in teicoplanins, while antibiotic A-42,867 and vancomycin hexapeptide were resistant. Also, teicoplanin and vancomycin were resistant to RH-treatment when the N-terminus was protected as carbamate. In contrast, open hexapeptides in which the 1,2-peptide bond was hydrolyzed and the carboxyl group of amino acid 1 was reduced to hydroxymethyl were obtained from carbamate derivatives of sugar-free compounds deglucoteicoplanin (**TD**) and vancomycin−aglycon (**VA**) under RH-conditions. Limited to **BOC** or **CBZ-TD**, the 3,4-amide bond was also affected. A possible RH-mechanism is proposed for natural glycopeptides and their derivatives. Teicoplanin-derived RH penta- and hexapeptides maintained residual antibacterial activity. As other analogous RH-glycopeptides, they are key intermediates for the synthesis of new members of this family of antibiotics. A synthetic approach to ring-closed derivatives of **TD** hexapeptide alcohol (**TDHPA**) and their activities are also reported.

A current challenge for glycopeptides of the dalbaheptide group,[1] to which teicoplanin (**CTA**, Figure 1)[2] belongs, is the emerging resistance in VanA enterococci[3] due to the replacement of antibiotic's target peptide D-Ala-D-Ala by a D-Ala-D-hydroxy acid depsipeptide (Chart 1)[4] which is not sufficiently bound by glycopeptides to prevent the bacterial growth. Considering that the binding properties of these antibiotics in part depend on the structure of amino acids 1 and 3,[5] activity against glycopeptide-resistant enterococci could be pursued by replacement of these amino acids with new amino acids or other moieties suitably selected to interact with the modified target.

Chemical[6] and biological[7] methods for the removal of amino acid 1 of vancomycin (**V**, Figure 2) have been reported, but these procedures could not be applied to **V**[8] or resulting hexapeptide (**VHP**) for the removal of amino acid 3. In teicoplanin, the N-terminal amino acid also cannot be removed by Edman degradation[9] since amino acids 1 and 3 are linked together through a diphenyl ether bridge. Therefore, a chemoselective process for the displacement of these amino acids following Edman degradation implies preliminary hydrolysis of the peptide bond between amino acids 2 and 3.[10] The reductive hydrolysis (RH) of the 2,3-amide in **CTA**, its



CTA (RH-CTA): S_1 = N-acylglucosamine, S_2 = mannose, S_3 = N-acetylglucosamine; X = Y = Cl, Z = R_1 = R_2 = R_3 = R_4 = H

TB (RH-TB): S_1 = H, S_2 = mannose, S_3 = N-acetylglucosamine; X = Y = Cl, Z = R_1 = R_2 = R_3 = R_4 = H

TC (RH-TC): S_1 = S_2 = H, S_3 = N-acetylglucosamine; X = Y = Cl, Z = R_1 = R_2 = R_3 = R_4 = H

TD (RH-TD): S_1 = S_2 = S_3 = H; X = Y = Cl, Z = R_1 = R_2 = R_3 = R_4 = H

A-40 (RH-A-40): S_1 = N-acylglucuronic acid, S_2 = mannose, S_3 = H; X = Cl, Y = H, Z = Cl, R_1 = Me, R_2 = R_3 = R_4 = H

R, HR (RH-HR):** S_1 = tetrasaccharide, S_2 = mannose, S_3 = rhamnosamine; X = Y = Z = R_1 = H, R_2 = OH, R_3 = R_4 = Me

**[HR (RH-HR): COO-R_4 = CH_2OH]

Figure 1. Structure of teicoplanin-like dalbaheptides (W = −NHCO−) and their 2,3-RH-derivatives (W = $-NH_2$, $HOCH_2$−).

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⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1996.

(1) Parenti, F.; Cavalleri, B. *Drugs Future* **1990**, *15*, 57.

(2) Coronelli, C.; Gallo, G. G.; Cavalleri, B. *Il Farmaco, Ed. Sci.* **1987**, *10*, 767.

(3) Johnson, A. P.; Uttley, A. H. C.; Woodford, N.; George, R. C. *Clin. Microbiol. Rev.* **1990**, *3*, 280.

(4) Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, *9*, 199.

(5) Naturally occurring glycopeptides are highly modified linear peptides made up of seven amino acids, five of which are aryl amino acids and are common to all members of the group. The differentiation of glycopeptides into four main classes is due to the remaining two amino acids in positions 1 and 3 which can be both aromatic (free or linked together through a diphenyl ether bridge) or one aliphatic and one aromatic.

(6) (a) Booth, P. M.; Stone, D. M.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* **1987**, 1694. (b) Nagarajan, R.; Schabel, A. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1306. (c) Booth, P.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2335.

(7) Zmijewski, M. J.; Logan, R. M.; Marconi, G.; Debono, M.; Molloy, R. M.; Chadwell, F.; Briggs, B. *J. Nat. Prod.* **1989**, *52*, 203.

(8) The probability to enzymatically remove amino acid 3 is actually negligible considering its positioning inside the binding pocket hardly approachable by enzymes.

(9) Edman, P. *Acta Chem. Scand.* **1950**, *4*, 277.

(10) Malabarba, A.; Ciabatti, R. *J. Med. Chem.* **1994**, *37*, 2988.

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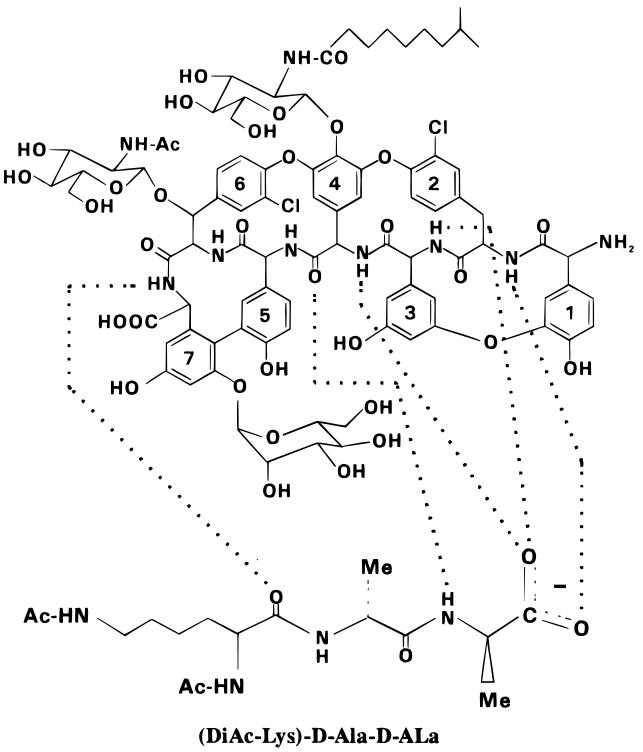
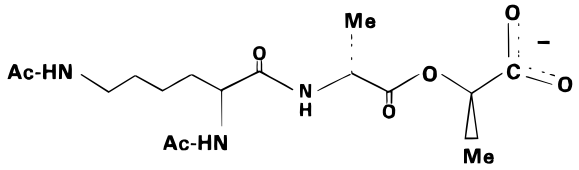
Chart 1. Teicoplanin − (DiAc-Lys)-D-Ala-D-Ala Complex





pseudoaglycons (**TB**, **TC**), and aglycon (**TD**) upon treatment of aqueous ethanol solutions of teicoplanin glycopeptides with $NaBH_4$ is described in this paper. Under these conditions, open pentapeptide derivatives (**RH-teicoplanins**, Figure 1) were obtained in which the carboxyl group of amino acid 2 was reduced to hydroxymethyl.

To assess whether this RH-method was generally applicable to natural dalbaheptides and their derivatives, six compounds were properly selected and treated with $NaBH_4$ under conditions similar to those used for the preparation of RH-teicoplanins from teicoplanins, namely: glycopeptides ristocetin (**R**)[11] and A-40,926 (**A-40**)[12] (Figure 1), which are structurally related to teicoplanin but significantly differ from **CTA** in the sugar content and distribution, **V** and its aglycon (**VA**),[6] in which amino acids 1 and 3 are aliphatic, and one vancomycin-like glycopeptide A-42,867 (**A-42**)[13] and vancomycin-derived hexapeptide (**VHP**)[6,7] (Figure 2), which show conformational changes[14] in their peptide backbone with

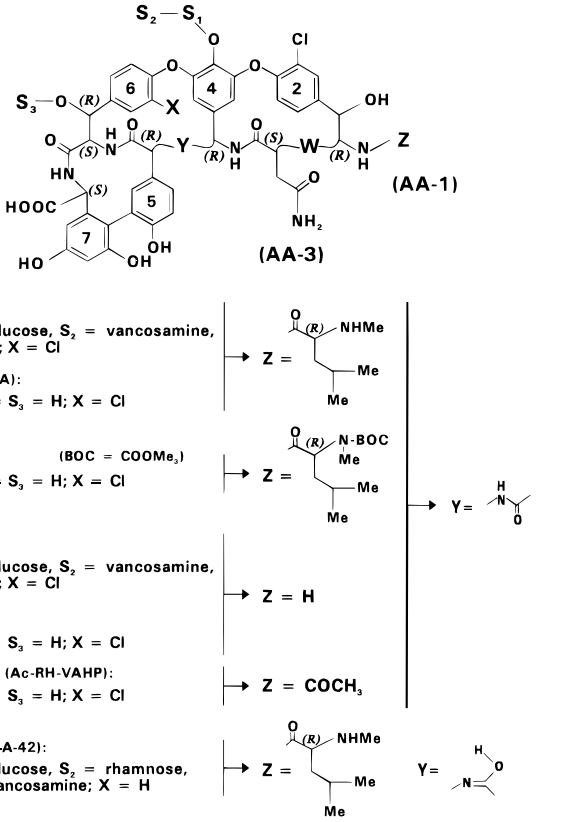


Figure 2. Structure of vancomycin-like dalbaheptides (W = −NHCO−), their hexapeptide derivatives (W = −NHCO−, Z = H) and the corresponding 2,3-RH-products (W = $-NH_2$, $HOCH_2$−).

respect to **V**. As reported below, only **A-42** and **VHP** were resistant.

On the basis of these findings, a possible RH-mechanism was thought (Scheme 1A) which is strictly related to the particular conformation of the heptapeptide chain of "natural" glycopeptides. In this case, the opening of the 2,3-amide would be favored by the formation, under weakly basic conditions, of a series of hydrogen-bonding systems whose direction is driven by the presence of the N-terminus as free base. Accordingly, amide bonds in enolic form are not susceptible to reductive hydrolysis. A similar mechanism was expected when the N-terminal amino group is protected as carbamate, but with different orientation of the hydrogen bonds (Scheme 1C,D) so as to allow the reduction of one or both of the 1,2- and 3,4-amides. Results achieved with carbamate derivatives of **TD** and **VA** upon treatment with $NaBH_4$ in different $EtOH/H_2O$ solutions are discussed. Most experiments have been done on **BOC** and **CBZ-TD** (Figure 3) and

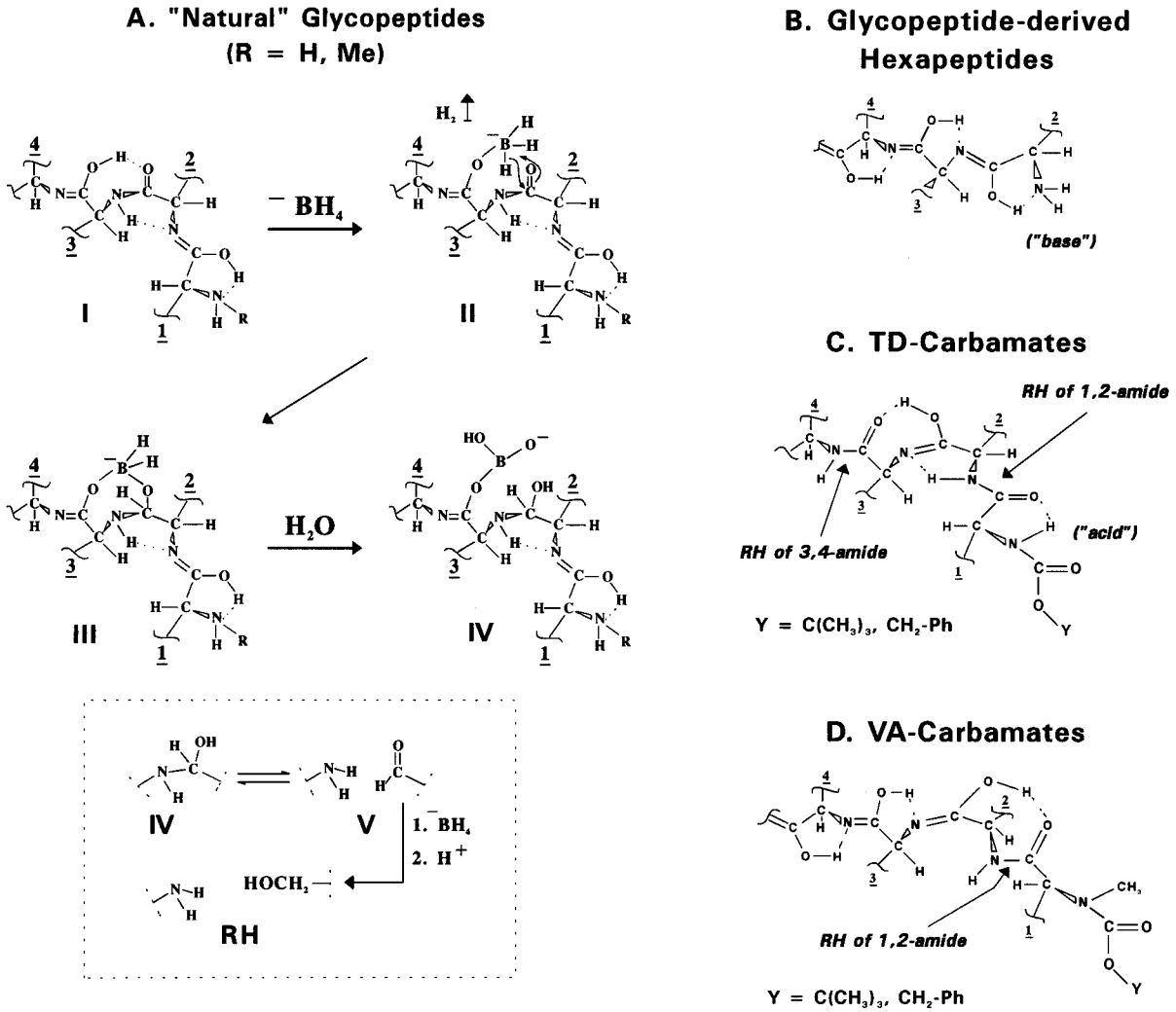
(11) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 897.

(12) (a) Goldstein, B. P.; Selva, E.; Gastaldo, L.; Berti, M.; Pallanza, R.; Ripamonti, F.; Ferrari, P.; Denaro, M.; Arioli, V.; Cassani, G. *Antimicrob. Agents Chemother.* **1987**, *31*, 1961. (b) Selva, E.; Gastaldo, L.; Beretta, G.; Borghi, A.; Goldstein, B. P.; Arioli, V.; Cassani, G.; Parenti, F. U.S. Patent No. 4,935,238 (June 19, 1990).

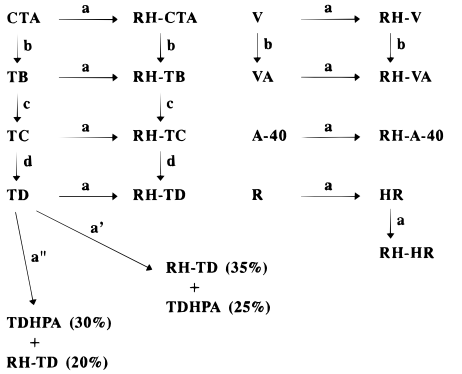
(13) Riva, E.; Gastaldo, L.; Beretta, M. G.; Ferrari, P.; Zerilli, L. F.; Cassani, G.; Selva, E.; Goldstein, B. P.; Berti, M.; Parenti, F.; Denaro, M. *J. Antibiot.* **1989**, *42*, 497.

(14) A significant difference between **A-42** and **V** is the opposite orientation of the 3,4 and 4,5-amide bonds. In **V**, as in the majority of the other dalbaheptides, the amide-COs of residues 2 and 3 are both pointing backward on the rear face of the molecule and are close to each other, while the amide-CO of residue 4 points forward on the front face. In **A-42**, the 4,5-amide is stabilized in the enolic form by a hydrogen bond with the amino group of the sugar vancosamine on amino acid 6. The OH resulting from this tautomerism is on the back face of the molecule. It follows a change in the orientation of the 3,4-amide whose CO is on the front face, while the amide-CO of residue 2 still lies on the back face and so its oxygen results far from that of the amide-CO of residue 3. In **VHP**, the absence of the N-terminal methylleucine causes (at basic pH) a change in conformation of the N-terminal region in which the 2,3-*trans* amide unit exhibits a 180° flipping motion, from having the NH-proton at the front of the molecule, as in **V**, to having it at the rear. This results in the opposite orientation of the amide-COs of residues 2 and 3.

Scheme 1. Proposed RH-Mechanism



Scheme 2. Synthesis of RH-Glycopeptide Derivatives



Method a: $NaBH_4$ in EtOH/H_2O 35/65, r.t.
Method a': $NaBH_4$ in EtOH/H_2O 60/40, r.t.
Method a": $NaBH_4$ in EtOH/H_2O 90/10, r.t.
Method b: 100% TFA, r.t.
Method c: HCl, r.t. (DME)
Method d: HCl, 70°C (TFE)

BOC-VA (Figure 2), since carbamate derivatives of parent sugar-containing glycopeptides **CTA** and **V** proved to be stable under all reaction conditions.[15] The aim of this work was to assess the actual possibility to selectively reduce the 1,2-amide in sugar-free glycopeptide carbamates so as to provide a suitable synthetic procedure to obtain **TD**-derived hexapeptide alcohol (**TDHPA**, Figure 3) and to propose a new method to produce **VA** hexapeptide (**VAHP**, Figure 2) with higher yields with respect to Edman degradation.[6] The *N*-acetyl derivative (**Ac-VAHP**) of **VAHP** was also treated with $NaBH_4$ under the above conditions to achieve additional information on the RH-mechanism.

Hexapeptides **VAHP** and **TDHPA** are useful intermediates for the preparation of new structurally different dalbaheptides. In particular, from **VAHP** there is the possibility to obtain a number of **VA**-derived glycopeptides by direct condensation of the terminal amino group with a series of different amino acids. Also, the availability of **BOC** or **CBZ-TDHPA**, more than the unprotected **TDHPA**, provides the opportunity for the direct synthesis of new families of related dalbaheptides with a common diphenyl ether−amino acid fragment 3[16] and different amino acid 1-residues. In fact, in **TDHPA** carbamates the only remaining free amino group is suitable to this goal. They are also useful intermediates to obtain **TD** derivatives with an enlarged 1,2,3-macrocyclic ring. To possibly establish further structure−activity relationships, two ring-closed compounds (**RC-1**

(15) The resistance of **CTA** and **V** carbamates to reductive hydrolysis might be ascribed to a possible steric effect of their sugar moieties on aromatic ring 4 on the formation and structure of hydrogen bonding/boron complex systems. This might favor the reduction of the 2,3-amide in **CTA** and **V**, while preventing the reduction of the 1,2-peptide bond in these compounds and their carbamate derivatives.

(16) In new semisynthetic dalbaheptides derived from **TDHPA**, amino acid 3 is a double amino acid residue formed by the diphenyl ether moiety originally belonging to amino acids 1 and 3 of **TD**.

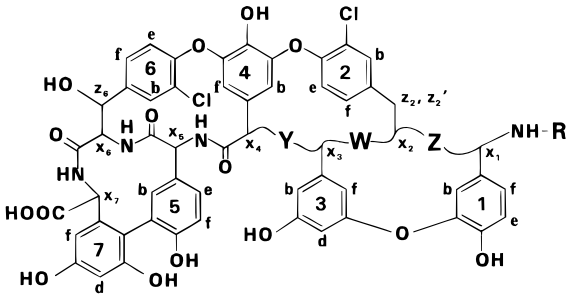


Figure 3. Structure of (R = H) **TD** (Y = W = Z = −NHCO−), **RH-TD** (W = $-NH_2$, $HOCH_2-$, Y = Z = −NHCO−), **TDHPA** (Y = W = −NHCO−, Z = $-NH_2$, $HOCH_2-$), and **TDTPDA** (Y = Z = $-NH_2$, $HOCH_2-$, W = −NHCO−) and their **BOC** (R = CO_2CMe_3) and **CBZ** (R = CO_2CH_2Ph) carbamate derivatives (with nomenclature of aromatic, benzylic, and HC-α protons.

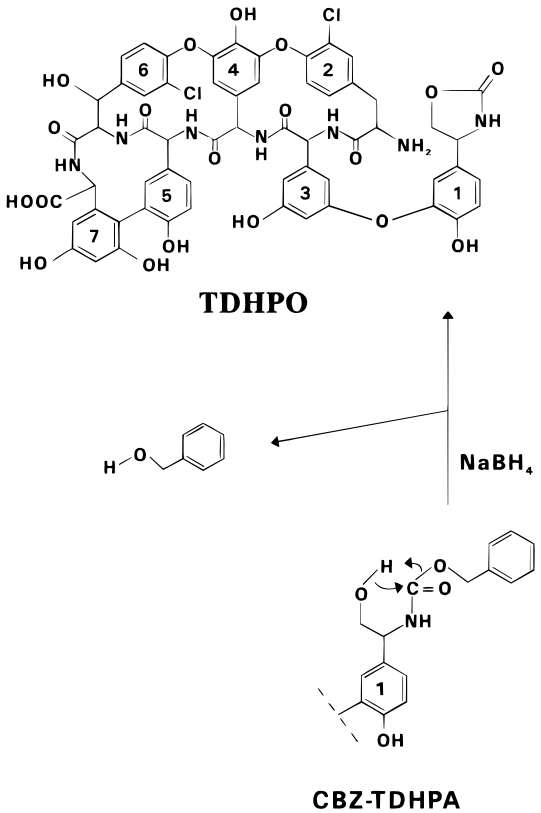


Figure 4. Structure and formation of **TDHPO**.

and **RC-2**, Figure 5) were synthesized in which the original 1,2,3-macrocyclic ring of **TD** was enlarged by a spacer of 1 and 2 methylene groups, respectively.

Results and Discussion

Reductive Hydrolysis of the 2,3-Peptide Bond. Treatment of teicoplanin antibiotics **CTA**,[17] **TB**, **TC**, and **TD** (Figure 1) at room temperature with a large excess of $NaBH_4$ in a EtOH/H_2O 35/65 solution (Scheme 2) afforded corresponding pentapeptide amino alcohol derivatives **RH-CTA**, **RH-TB**, **RH-TC**, and **RH-TD** with relatively good yields (method a, Table 1). Epimerization at C-3,[18] which generally occurs in teicoplanins under prolonged basic treatment, was only observed with **TD**. The different conversion rate (**CTA** > **TB** > **TC** ≫ **TD**) seems to play a critical role in the epimerization of teicoplanins. Epimers, once formed, were not susceptible to reductive hydrolysis upon treatment with $NaBH_4$. This is likely the reason why **RH-TD** was obtained with lower yields than the other **RH-teicoplanins**. Also, the presence of the sugar moieties seems to favor this transformation while preventing epimerization. In order to increase yields in **RH-TD** while decreasing epimerization, new reaction conditions were studied.[19] It was found that the formation of the C^3-epimer (*epi*-**TD**) increased with increasing reaction time which was inversely related to the concentration of $NaBH_4$, while the conversion rate of **TD** into **RH-TD** was somewhat proportional to the concentration of $NaBH_4$. It was also assessed that the concentration of water in the aqueous alcohol mixtures was critical, since the transformation yields of **TD** into **RH-TD** were lower when the EtOH/H_2O ratio was close to or higher than 1. In particular, when a large excess of $NaBH_4$ was used in a EtOH/H_2O 6/4 solution (method a′), deglucoteicoplanin hexapeptide alcohol (**TDHPA**) formed as byproduct. Yields in **TDHPA** were increased using a EtOH/H_2O 9/1 mixture (method a″). Under these conditions, a small (∼5%) amount of C^3-epimer (*epi*-**TDHPA**) was also obtained. Among teicoplanin dalbaheptides, only **TD** was susceptible to reduction of the 1,2-peptide bond. Pseudoaglycons **RH-TB**, **RH-TC**, and aglycon **RH-TD** were also prepared from **RH-CTA** by hydrolysis of the sugars under controlled acidic conditions (methods b, c, and d).

Following method a, analogous RH-derivatives (Table 2) were obtained from vancomycin (**V**) and its aglycon (**VA**), ristocetin (**R**), and A-40,926 (**A-40**). Under these conditions, the C-terminal methyl ester of **R** was previously reduced to hydroxymethyl, so that the final compound corresponded to the RH-derivative (**RH-HR**) of decarboxyhydroxymethylristocetin (**HR**).[20] Unexpectedly, A-42,867 (**A-42**) and **V**-derived hexapeptide (**VHP**) were resistant to reduction with $NaBH_4$. These results indicated that only glycopeptides which possess the "natural" dalbaheptide conformation[14] are susceptible to reductive hydrolysis.

The reduction of an amide with $NaBH_4$ to give an amino alcohol is a rather uncommon reaction which might occur in dalbaheptides according to a specific mechanism of activation of the 2,3-peptide bond due to the particular conformation of their heptapeptide chain. The following RH-mechanism is supported by a possible analogy with that proposed by Micovic and Mihailovic[21] for the reduction of amides to alcohols with lithium aluminum hydride.

Proposed RH-Mechanism for Glycopeptides and Their Derivatives. The conformation of the heptapeptide chain in most of glycopeptides is such as to allow the formation of a series of hydrogen bonding systems

(17) Teicoplanin (**CTA**, Figure 1) is a complex of five strictly related components (namely, factors A2-1, 2, 3, 4, 5) which only differ in the structure of the acyl chain of the *N*-acylglucosamine unit on aromatic residue 4. The acyl COR moieties are: R = $(CH_2)_2CH{=}CH(CH_2)_4CH_3$ (A2-1); R = $(CH_2)_6CH(CH_3)_2$ (A2-2); R = *n*-C_9H_{19} (A2-3); R = $(CH_2)_6$-$CH(CH_3)CH_2CH_3$ (A2-4); R = $(CH_2)_7CH(CH_3)_2$ (A2-5).

(18) Barna, J. C. J.; Williams, D. H.; Strazzolini, P.; Malabarba, A.; Leung, T.-W. C. *J. Antibiot.* **1984**, *37*, 1204.

(19) Different concentrations of $NaBH_4$ and H_2O in various aqueous alcohol mixtures were investigated. The detailed experimental results are not reported.

(20) The selective reduction of the C-terminal methyl ester of **R** to give **HR** occurred before starting the reductive hydrolysis of the 2,3-amide bond. Upon treatment of **R** with $NaBH_4$ under the same above RH conditions (method a) the conversion of **R** into **HR** was completed within 90 min.

(21) Micovic, V. M.; Mihailovic, M. L. *J. Org. Chem.* **1953**, *18*, 1190.

Table 1. RH-Teicoplanin Derivatives

compd	yield, % (method)	HPLC[a] t_R, min	titration pK_a-1	pK_a-2	pK_a-3	EW[b]	FAB MS $(M + H)^+$	formula	MW
RH-CTA[c]	82 (a)[d]	15.3	4.7	5.9	7.3	637	1882	$C_{88}H_{101}N_9Cl_2O_{33}$	1883.7
RH-TB	75 (a),[d] 75[e] (b)	10.2	4.7	5.9	7.3	549	1567	$C_{72}H_{72}N_8Cl_2O_{28}$	1568.3
RH-TC	62 (a),[d] 55[e] (c)	10.9	4.6	5.8	7.5	477	1405	$C_{66}H_{62}N_8Cl_2O_{23}$	1406.2
RH-TD	47 (a),[f] 20[e] (d)	11.6	4.5	5.5	7.4	425	1202	$C_{58}H_{49}N_7Cl_2O_{18}$	1203.0

[a] HPLC (method A). Relative (vs. parent unmodified teicoplanin) t_R's: 0.96 (**RH-CTA**), 0.98 (**RH-TB**), 0.93 (**RH-TC**), 0.92 (**RH-TD**). [b] Equivalent weight. The values given are corrected for solvent content (TG). [c] Data are referred to factor A2-2. [d] Molar ratio $NaBH_4$/substrate: 75; reaction time: 2 h (3 h for **RH-TC**). [e] From **RH-CTA**. [f] Molar ratio $NaBH_4$/substrate: 250; reaction time: 16 h.

Table 2. Other RH-Glycopeptide Derivatives

compd	molar ratio ($NaBH_4$ vs. substrate)	reaction[a] time, h	reaction[a] yield, %	HPLC[d] t_R, min	titration EW[e]	FAB MS[b] $(M + H)^+$ found	FAB MS[b] $(M + H)^+$ calcd	^{1}H NMR[c] (CH_2OH) δ, ppm	formula	MW
RH-A-40	80	24	68	20.2	470	1735.5	1735.5	3.27	$C_{83}H_{92}N_8Cl_2O_{29}$	1736.6
RH-V	200	48	61	7.6	430	1452.5	1452.5	3.28	$C_{66}H_{79}N_9Cl_2O_{24}$	1453.3
RH-VA	250	36	44	12.8	390	1146.2	1146.3	3.35	$C_{53}H_{56}N_8Cl_2O_{17}$	1148.0
RH-HR	120	16	59	8.5	750	2043.6	2043.7	3.38[f]	$C_{94}H_{114}N_8O_{43}$	2044.0
(**HR**	50	3	86	8.8	1090	2039.6	2039.7	*f*	$C_{94}H_{110}N_8O_{43}$	2040.0)

[a] Method a. [b] Mass numbers refer to the lowest mass isotope of a cluster. [c] Data referred to the new methylene protons of residue 2. [d] HPLC (method A). Relative (vs. parent unmodified glycopeptide) t_R's: 0.91 (**RH-A-40**), 0.79 (**RH-V**), 0.73 (**RH-VA**), 0.87 (**RH-HR**), 0.86 (**HR**). [e] Equivalent weight. The values given are corrected for solvent content (TG). [f] The new methylene protons of the C-terminal CH_2OH of **RH-HR** and **HR** resonate at δ 3.51 and 3.50 ppm, respectively.

in the right-hand side of these molecules that involve the OHs of the amides between residues 1,2, and 3,4, both postulated in the enolic form to be unsusceptible to reductive hydrolysis, and the N-terminus of residue 1 and the CO of the 2,3-amide, respectively (Scheme 1A). In these systems, which are favored by the weak basic pH of the reaction medium, the direction of hydrogen bonds is driven as depicted in structure **I** by the presence of the N-terminal amino group as free base. As a consequence, the formation of a hydrogen bond between the NH of the 2,3-amide and the adjacent −N═ atom of the vinylog form of the 1,2-amide is hypothesized to stabilize the −NHCO− structure of the 2,3-amide. The formation of a seven-membered hydrogen-bonding system involving the OH of the 3,4-amide, in the enolic form, and the amide-CO of residue 2, is allowed by the proximity of both of these carbonyl groups on the rear face of the dalbaheptide molecule. An initial interaction between the borohydride and the enolic-OH of residue 3 in structure **I** would give adduct **II**, followed by the formation of partially reduced cyclic boron complex **III**, as the result of an unusual intramolecular nucleophilic addition of an hydride ion to the CO-carbon atom of residue 2 and the formation of a boron−oxygen bond involving the carbonyl−oxygen of residue 2 and the boron atom of adduct **II**. A further nucleophilic attack by water at the C-atom of the carbinol−boron complex (**III**), with the simultaneous displacement of the borate anion, gives carbinolamine **IV**. This hemiaminal is in equilibrium with the amino aldehyde form **V** which is readily reduced to the final amino alcohol (**RH**) by the excess borohydride.

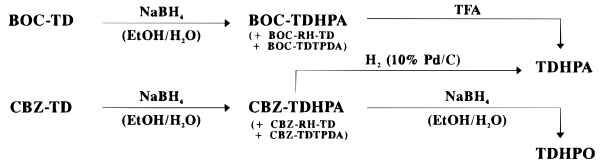
The resistance of **A-42** and **VHP** to reductive hydrolysis demonstrates the need for the "natural" conformation of the heptapeptide chain to promote the RH-mechanism in glycopeptides. In this sense, a critical role is played by the N-terminal amino acid 1. In fact, in the absence of this amino acid, as in **VHP**, a different hydrogen bonding system (Scheme 1B) is hypothesized due to a change in the orientation of the 2,3-amide-CO at basic pH. In glycopeptide-derived hexapeptides, the formation of a hydrogen bond between the N-atom of the terminal amino group and the enolic-OH of the adjacent amide would be favored by the presence of the unshared electron pair on the nitrogen of free NH_2. A further support is given by the resistance of teicoplanin epimers to reductive hydrolysis. With respect to the corresponding unmodified antibiotics, these compounds show opposite configuration at C-3.[18] As a consequence, the amide-CO of residue 3 points forward on the front face while the amide-CO of residue 2 is still pointing backward.

In glycopeptide antibiotics, the possible existance of the 1,2- and 3,4-peptide linkages in the enolic form (Scheme 1A) at basic pH could explain the different binding strength to synthetic models of the antibiotic's target peptide at pH 5 and 9.[22] In fact, the values of the association constants are generally about 10 times higher when determined at pH 5 with respect to those found at pH 9. Given that the NH-protons of residues 2 and 4, besides that of residue 3, are directly involved in the hydrogen bonding interaction with the carboxylate anion of the synthetic dipeptide model, the contribution of the NH-protons of amides 1,2 and 3,4 to the complex formation (Chart 1) would be lower at pH 9 for the presence of these amides mainly in the vinylog form.

As mentioned above, the driving force which possibly determines the direction of hydrogen bonds in "natural" dalbaheptides is the basic character of the N-terminal amino or methylamino group. It follows that different situations are expected when amino acid 1 is present but the N-terminus is acylated or protected as carbamate. Accordingly, in carbamate derivatives of glycopeptides possessing a terminal primary amino group, as **TD**-carbamates (Scheme 1C), the carbonyl oxygen of the 1,2-amide should act as the base and the carbamate-NH as the acid in the hydrogen bonding formation. In carbamate derivatives of vancomycin-like dalbaheptides, as **VA**-carbamates, because of the substitution of the carbamate NH-proton by a methyl group, a further different hydrogen bonding system (Scheme 1D) was hypothesized. This allowed us to predict the reductive hydrolysis of the 1,2- and 3,4-amides in **BOC** or **CBZ-TD** and of the 1,2-amide in **BOC-VA**.

(22) (a) Barna, J. C. J.; Williams, D. H.; Williamson, M. P. *J. Chem. Soc., Chem. Commun.* **1985**, 254. (b) Malabarba, A.; Trani, A.; Tarzia, G.; Ferrari, P.; Pallanza, R.; Berti, M. *J. Med. Chem.* **1989**, *32*, 783.

Scheme 3. Reductive Hydrolysis of TD-Carbamates[a] and Synthesis of TDHPA and TDHPO



[a] Reaction conditions and yields in Table 3.

Reductive Hydrolysis of the 1,2- and 3,4-Peptide Bonds. For **TD**-carbamates, the experiments were run using *N-tert*-butyl (**BOC-TD**) and benzyl (**CBZ-TD**) oxycarbonyl derivatives of **TD**, which were treated with $NaBH_4$ (Scheme 3) in various aqueous alcohol media containing decreasing percentages (from 80 to 10%) of water. The amount of the reductive agent was also increased to reduce the reaction time while minimizing epimerization at C-3. The results (Table 3) indicated that all of the 1,2-, the 2,3-, and the 3,4-amide bonds were susceptible to reduction, though to a different extent depending on the relative percentage of water in the solvent mixture. The opening of the 1,2-peptide linkage to give **BOC-** or **CBZ-TDHPA** (Figure 3) was favored with decreasing percentages of water, while **BOC-** or **CBZ-RH-TD** were the main products when the relative amount of water was higher than 40%. The highest RH-selectivity (60%) of the 1,2-amide was achieved (from **BOC-TD**) in a $EtOH/H_2O$ 9/1 mixture. The reduction of both the 1,2- and 3,4-amides was also observed (5−10%). Resulting carbamate (**BOC** or **CBZ**) derivatives of deglucoteicoplanin tetrapeptide dialcohol **TDTPDA** (Figure 3) were obtained as byproducts under all reaction conditions. In this case, the opening of the 1,2-peptide bond likely occurred simultaneously with the opening of the 3,4-amide, or at faster rate after the 3,4-cleavage, since there was no chromatographic (HPLC) evidence of the formation of an intermediate RH-product at the 3,4-position. On the other hand, the double RH-products cannot be derived from the **TDHPA** carbamates since these latter compounds were stable to RH-treatment under all described conditions. When *i*-PrOH instead of EtOH was used,[23] similar results were observed as above, but relatively longer (∼30%) times were necessary to complete the reactions. Water was always necessary since no transformation occurred in absolute MeOH, EtOH, or *i*-PrOH. Between the two carbamates selected for this investigation,[24] **BOC-TD** was more suitable for the preparation of **TDHPA** (*via* intermediate **BOC-TDHPA**).[25] In fact, **BOC-TDHPA** was stable once formed while **CBZ-TDHPA** underwent a further, though slow, transformation into oxazolidinone derivative **TDHPO** (Figure 4), as the result of an intramolecular nucleophilic cyclization involving the newly formed primary hydroxyl group of residue 1 and adjacent CBZ-carbonyl group.

In contrast to **BOC-TD**, **BOC-VA** was completely transformed into hexapeptide **VAHP** even under conditions ($EtOH/H_2O$ 2/8) favorable to the opening of the 2,3-peptide bond (Scheme 4, Table 4). As expected, the selectivity of reduction of the 1,2-amide was markedly higher in **BOC-VA** than in **BOC-TD**.

Acetylation of **VAHP** at the N-terminus with MeCOCl/TEA (DMF) yielded **Ac-VAHP** which was treated with $NaBH_4$ in $EtOH/H_2O$ 3/7 solution. A 6/4 mixture of **VAHP** and the *N*-acetyl derivative **Ac-RH-VAHP** was obtained (Scheme 4, Table 4). This result further supported the overall RH-mechanism proposed for glycopeptides and their derivatives. Accordingly, in the case of N-acylated glycopeptide-derived hexapeptides, both the 1,2-mimicking amide and the 2,3-peptide bonds are susceptible to reduction (Scheme 5).

Some physicochemical and analytical data of RH-products obtained from carbamate and acetyl derivatives are given in Table 5.

TDHPA-Derived Dalbaheptides. The enlargement of the 1,2,3-macrocyclic ring of **TD** by one and two methylene groups was achieved by acylation of the free-terminal amino group of the hexapeptide chain of **BOC-TDHPA** with bromoacetyl and 3-bromopropionyl chloride, respectively, in Me_2CO/H_2O 1/1 solution in the presence of $NaHCO_3$ (Scheme 6). The BOC protective group was then removed from the resulting bromoacetyl (**BOC-Ac-1**) and bromopropionyl (**BOC-Ac-2**) derivatives with TFA at room temperature. Final cyclization to give **RC-1** and **RC-2** (Figure 5) was carried out upon treatment of intermediates **Ac-1** and **Ac-2**, respectively, with K_2CO_3 at room temperature in DMF solution. Reaction yields are given in Table 6.

Structure Elucidation. Most of the NMR work was carried out on **RH-TD**, **TDHPA**, and *epi*-**TDHPA**. The structures of the other RH-derivatives were determined by comparison of their ^1H NMR spectra with those of the above compounds and corresponding unmodified glycopeptides. The MWs of all derivatives were confirmed by mass spectrometry. The FAB MS spectra of **RH-teicoplanins** were particulary studied.

NMR Structural Studies of RH-TD. A first inspection of its ^1H NMR spectrum compared to that of **TD**[26] shows two main differences: (i) there is a strong upfield shift of proton x_2,[27] and (ii) one of the two aromatic high-field protons does not belong to aromatic ring 4. In a resolution-enhanced spectrum, the signal at 5.83 ppm shows triplet multiplicity, indicating meta-coupling to two aromatic protons, whereas the aromatic protons of ring 4 appear as small doublets, due to the substitution pattern of this system. The through-bond connectivities for the heptapeptide core were easily determined in a DQF-COSY experiment,[28] giving the NH,HCα connectivities. The proton assigned as x_2 shows cross-peaks to two different aliphatic CH_2 groups, namely the protons z_2, z_2', and the newly introduced CH_2 moiety deriving from the reductive cleavage of the 2,3-amide bond. The ^{13}C chemical shift of the new CH_2 at 61 ppm, determined in an inverse H,C correlation, indicates the substitution by a hydroxyl group. The assignment for the aromatic

(23) The use of MeOH instead of EtOH was unsuitable for these studies due to the faster decomposition of the reducing agent, the lower selectivity, and relatively poor reaction yields.

(24) The BOC and CBZ protective groups were selected for these studies considering that the other functional groups of the aglycon structure of glycopeptides are generally stable to the reaction conditions (TFA, room temperature, for BOC; H_2, Pd/C, for CBZ) required for their removal from corresponding carbamate derivatives.

(25) The removal (TFA, room temperature) of the BOC protective group from **BOC-TDHPA** gave **TDHPA** with high (95%) yields.

(26) Malabarba, A.; Ferrari, P.; Gallo, G. G.; Kettenring, J.; Cavalleri, B. *J. Antibiot.* **1986**, *39*, 1430.

(27) By analogy with the proton nomenclature adopted for glycopeptides, HCα protons of amino acid fragments ($n = 1-7$) are indicated with the symbol x_n, C*H*(OH) or CH_2 benzyl protons are defined as z_n or z_n, z_n', while symbol w_n is used for NH or NH_2 protons. Aromatic, benzylic, and HCα protons are defined as shown in Figure 3.

(28) Marion, D.; Wuethrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967.

Table 3. Reductive Hydrolysis of TD-Carbamates

solvent mixture			reductive hydrolysis products[a]				
EtOH (%)	H_2O (%)	starting compd	**CBZ-TDHPA** (%)	**CBZ-RH-TD** (%)	**CBZ-TDTPDA** (%)	epimers (%)	**TDHPO** (%)
RH-POSITION			1,2	2,3	1,2 and 3,4		
90[b]	10	CBZ-TD	~50[c]	30	10	10	45[d]
60	40	CBZ-TD	~35[c]	35	10	20	27[d]
20[b]	80	CBZ-TD	~10[c]	45	5	40	9[d]

solvent mixture			reductive hydrolysis products[a]				
EtOH (%)	H_2O (%)	starting compd	**BOC-TDHPA** (%)	**BOC-RH-TD** (%)	**BOC-TDTPDA** (%)	epimers (%)	**TDHPO** (%)
90[b]	10	BOC-TD	60	25	7	~8	absent
60	40	BOC-TD	40	30	10	20	absent
20[b]	80	BOC-TD	15	40	5	40	absent

[a] The percent product composition of final reaction mixtures was determined by HPLC. [b] When *i*-PrOH was used instead of EtOH, a similar behavior was observed, but longer reaction times. [c] Highest percentage of **CBZ-TDHPA** observed (first step) before rearrangement into **TDHPO**. [d] Percentage of **TDHPO** present in the final reaction mixture (second step); transformation yield of **CBZ-TDHPA** into **TDHPO**: ~90%.

Scheme 4. Reductive Hydrolysis of VA-Carbamates and Ac-VAHP

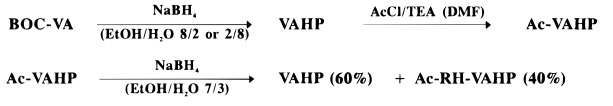


Table 4. Reductive Hydrolysis of BOC-VA and *N*-Ac-VAHP

solvent mixture		starting	reductive hydrolysis products	
EtOH (%)	H_2O (%)	compd	**VAHP**	**Ac-RH-VAHP**
80	20	**BOC-VA**	100	
20	80	**BOC-VA**	100	
30	70	**Ac-VAHP**	60	40

protons of **RH-TD** was done on the basis of their typical spin systems. The following spin systems are as expected: four ABX (rings 1, 2, 5, 6), two AB (rings 4, 7), and one AMN (ring 3). They are identified through their typical coupling pattern in the DQF-COSY spectra. So, for example, the four ABX-spin systems each show a strong cross-peak due to the ortho-coupled protons and a weak cross-peak for the meta-coupled protons. The connection between the individual aromatic rings and their benzylic protons was achieved by a carefully tuned COSY experiment with enhancement of long-range couplings,[29] showing weak cross-peaks between the HCα protons and the aromatic ortho protons of the attached aromatic moieties. These results were confirmed by NOE measurements that gave the through space connectivities between these protons.

^{1}H NMR Conformational Studies of RH-TD. A first indication of the conformational changes in **RH-TD** with respect to **TD** results from the two aromatic highfield protons. In **TD**, aromatic protons 4b and 4f are influenced by the anisotropic effect of aromatic moieties 6 and 2, documented by their highfield shift. In **RH-TD**, only 4f is shifted to a considerably high field (5.49 ppm), while 4b resonates at 6.45 ppm. On the other hand, aromatic proton 3b experiences the anisotropy of aromatic ring 1, thus shifted by 0.5 ppm to higher field than its counterpart in **TD**. The NOESY[30] spectrum reveals further details of the conformation of **RH-TD**. The protons on the left-hand part of the molecule up to the x_4 position show the same NOE pattern and therefore the same conformation as **TD**. At the center x_3 epimerization seems to have occurred, likely due to the basic conditions. This is the best way to explain the strong NOE between proton x_3 and aromatic protons 3f and 3b and amidic w_4. Furthermore, the NOE's show that the right-hand part of the molecule is folded backward. Instead of having the typical pocket-like conformation of teicoplanin,[31] the molecule forms in **RH-TD** two half-pockets pointing in opposite directions (Figure 6).

NMR Spectra of the Other 2,3-RH-Derivatives. All **RH-teicoplanins** show the same NOE pattern in their NOESY spectra like **RH-TD**, thus confirming their structural and conformational similarity. The ^{1}H NMR spectra of the other **RH-glycopeptides** are similar to those of **RH-teicoplanins**. In their DQF-COSY spectra, the proton assigned as x_2 exhibits cross-peaks to two different aliphatic CH-groups, to the proton (s) assigned as z_2 and the newly introduced CH_2 moiety deriving from the reductive cleavage of the amide bond between amino acids 2 and 3. Furthermore, the NOESY spectra indicate the same conformational changes as in **RH-teicoplanins**.

NMR Spectra of TDHPA and *epi*-TDHPA. A first inspection the ^{1}H NMR spectra of the two compounds compared to that of **TD** shows that for both the left-hand part of the molecule is unchanged. The DQF-COSY spectra allowed the assignment of most of the NH,HCα connectivities. In both spectra only crosspeaks from the HCα proton to the newly introduced alcoholic function are present, so that the reductive hydrolysis has occurred between amino acids 1 and 2. Only for **TDHPA** was the proton spectrum completely assigned by homonuclear experiments since the chemical shifts, particularly those of protons of the aromatic region, are quite similar to those of **TD**. For *epi*-**TDHPA** further studies were necessary due to a drastic change in the chemical shifts of the aromatic protons which leads to severe overlapping in the ^{1}H NMR spectra. However, the COSY spectra show that the two compounds have the same primary structure and that **TDHPA** has a conformation similar to that of **TD**. For a detailed conformational study of *epi*-**TDHPA** with the help of NOESY[32] experiments the whole proton spectrum had to be assigned, which was possible only by heteronuclear experiments. In the

(29) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
(30) Macura, S.; Wuethrich, K.; Ernst, R. R. *J. Magn. Reson.* **1982**, *47*, 351.
(31) (a) Barna, J. C. J.; Williams, D. H.; Stone, D. J. M.; Leung, T.-W. C.; Doddrell, D. M. *J. Am. Chem. Soc.* **1984**, *106*, 4895. (b) Hunt, A. H.; Molloy, R. M.; Occlowitz, J. L.; Marconi, G. C.; Debono, M. *J. Am. Chem. Soc.* **1984**, *106*, 4891.
(32) (a) Bodenhausen, G.; Kogler, H.; Ernst, R. R. *J. Magn. Reson.* **1984**, *58*, 370. (b) Macura, S.; Wuethrich, K.; Ernst, R. R. *J. Magn. Reson.* **1982**, *47*, 351.

Scheme 5. Proposed RH-Mechanism in Ac-VAHP

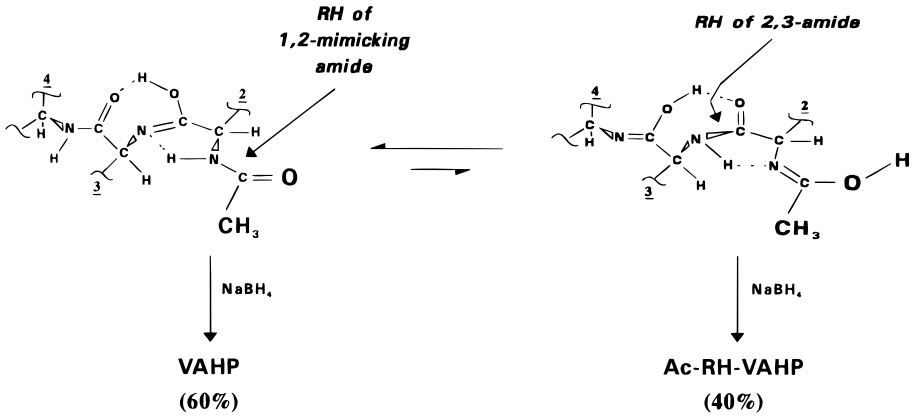


Table 5. Hexapeptide and Carbamate Derivatives

compd	HPLC[a] t_R, min (method)	FAB MS $(M + H)^+$	formula	MW
BOC-TDHPA	16.1 (A), 6.8 (B)	1302	$C_{63}H_{57}N_7Cl_2O_{20}$	1303.0
CBZ-TDHPA	18.4 (A)	N.D.[b]	$C_{66}H_{55}N_7Cl_2O_{20}$	1337.1
TDHPA	12.1 (A)[c]	1202	$C_{58}H_{49}N_7Cl_2O_{18}$	1203.0
BOC-RH-TD	17.7 (A), 7.3 (B)	N.D.	$C_{63}H_{57}N_7Cl_2O_{20}$	1303.0
CBZ-RH-TD	21.2 (A)	1336	$C_{66}H_{55}N_7Cl_2O_{20}$	1337.1
TDHPO	12.6 (A)	1228	$C_{59}H_{47}N_7Cl_2O_{19}$	1229.0
BOC-TDTPDA	13.9 (A)	1306	$C_{63}H_{61}N_7Cl_2O_{20}$	1307.1
VAHP	11.3 (A)	1016	$C_{46}H_{39}N_7Cl_2O_{16}$	1016.8
Ac-RH-VAHP	10.7 (A)	1062	$C_{48}H_{45}N_7Cl_2O_{17}$	1062.8

[a] HPLC (method A), t_R: **BOC-TD**, 24.0 min; **CBZ-TD**, 29.0 min. [b] N.D. = not determined [c] For *epi*-**TDHPA**, t_R 12.3 min.

HMQC[33] spectrum of *epi*-**TDHPA**, the crosspeaks of the protonated carbon atoms appear as doublets since no decoupling was done during acquisition. As expected, in the aromatic region 19 correlations are detected and only few can be immediately assigned, viz., 4f, 4b and 3b, 3d, and 3f. The HMBC[34] experiment show correlations *via* ^2J, ^3J, and ^4J couplings which allow, with the help of the aforementioned homonuclear experiments, the assignment of all ^{1}H and ^{13}C resonances of **TDHPA** and *epi*-**TDHPA**. The assignment of the aromatic protons of ring 2 of *epi*-**TDHPA** is given here as an example. The z_2 proton shows long-range couplings to one quaternary carbon at 134.94 ppm and to one protonated carbon atom at 131.43 ppm, which is correlated in the HMQC spectrum to a small doublet (^3J = 2 Hz) at 7.84 ppm in the proton spectrum and can be assigned to the aromatic proton 2b. In the DQF-COSY spectrum, this signal is correlated *via* a small crosspeak (*meta* coupling) to 2f, 6.94 ppm, and this proton shows a strong *ortho* coupling to a doublet at 7.12 ppm, which has to be assigned to 2e. In the HMBC spectrum, 2b shows long-range correlations to quaternary carbon atoms at 134.94, 128.47, and 150.10 ppm, and 2e to the signals at 134.94 and 128.47 ppm. The resonance at 134.94 ppm has to be assigned to 2a, as this signal is also correlated to z_2. The carbon atom at 150.10 ppm due to the chemical shift value has to be 2d, and one remaining resonance at 128.47 ppm has to be assigned to 2c. It is worth mentioning that in both cases long range crosspeaks are detected between 5b and 7b, thus confirming the presence of the diphenyl linkage between these two aromatic rings. The HMQC experiment allowed the assignment of all nitrogen resonances of *epi*-**TDHPA** and most of those of **TDHPA**. For *epi*-**TDHPA**, five amidic nitrogens are detected and readily identified by their common crosspeaks with the already assigned NH proton resonances. The two NH_2-groups are shifted in comparison with the amide resonances about 80 ppm to higher field, again confirming the primary structure. The NOE's of **TDHPA** correspond to the NOE's found in **TD**, except for the hydrolyzed part. In *epi*-**TDHPA**, additional NOE interactions are detected between proton x_3 and the amide proton w_4 and between w_3 and proton x_2. The same NOE's were detected in *epi*-**TD**[18] where epimerization occurred at the chiral center x_3. Accordingly, the molecule of *epi*-**TDHPA**, instead of having the typical pocket-like conformation of **TD** and **TDHPA**, is formed by two half-pockets pointing in opposite directions as **RH-TD**.

Main ^{1}H NMR Assignments for BOC- and CBZ-RH-TD, BOC-TDHPA, TDHPO, and BOC-TDTPDA. The assignments have been derived from the two-dimensional experiments COSY phase-sensitive double quantum filter (COSYPHDQ) and COSY, relayed coherence transfer (COSYRCT),[35] and NOE phase-sensitive (NOESYPH) experiments, and were based on the well-established spectra−structure correlations in the teicoplanin field.[26,31,36]

As expected, the spectra of **BOC** and **CBZ-RH-TD** show a downfield shift ($\Delta\delta \sim +0.8$ ppm) of proton x_1 due to the involvement of w_1 into the carbamate formation. A similar trend ($\Delta\delta$ +0.3 ppm) is found for **BOC-TDHPA**. The ^{1}H NMR spectrum of **TDHPO** is very similar to that of **TDHPA** except for the presence of signals due to a CH_2CH system in an oxazolidinone moiety[37] instead of a $HOCH_2CH$ system as in **TDHPA**.

The structure of **BOC-TDTPDA** was clearly deduced from the above ^{1}H NMR experiments carried out in DMSO-d_6 solution added with traces of CF_3COOH. The reduction of the 1,2- and 3,4-amide bonds could be defined by the coupling of the two newly formed NH_3^+ groups with their adjacent CH's (x_2 and x_4) which, in turn, were identified by NOE or long-range coupling interactions with the protons of the surrounding part of the molecule. Actually, the ^{1}H NMR data allow the buildup of the whole molecule of **BOC-TDTPDA**. Starting from the left-hand side of the molecule, the chemical shift values and NOE interactions concerning amino acid fragments 7−5 do not show any difference as compared to those of **TD·HCl**. Considering amino acid residue 4, the x_4 signal is

(33) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.
(34) (a) Bax, A.; Summers, M. F. *Chem. Soc.* **1986**, *108*, 2093. (b) Bax, A.; Marion, D. *J. Magn. reson.* **1988**, *78*, 186.
(35) Wagner, G. *J. Magn. Reson.* **1983**, *55*, 151.
(36) Malabarba, A.; Trani, A.; Ferrari, P.; Pallanza, R.; Cavalleri, B. *J. Antibiot.* **1987**, *40*, 1572.
(37) The presence of an oxazolidinone moiety was confirmed by the $\nu_{C=O}$ band at 1755 cm^{-1} in the IR spectrum of **TDHPO**.

Scheme 6. Synthesis of RC-1 and RC-2 (Figure 5)

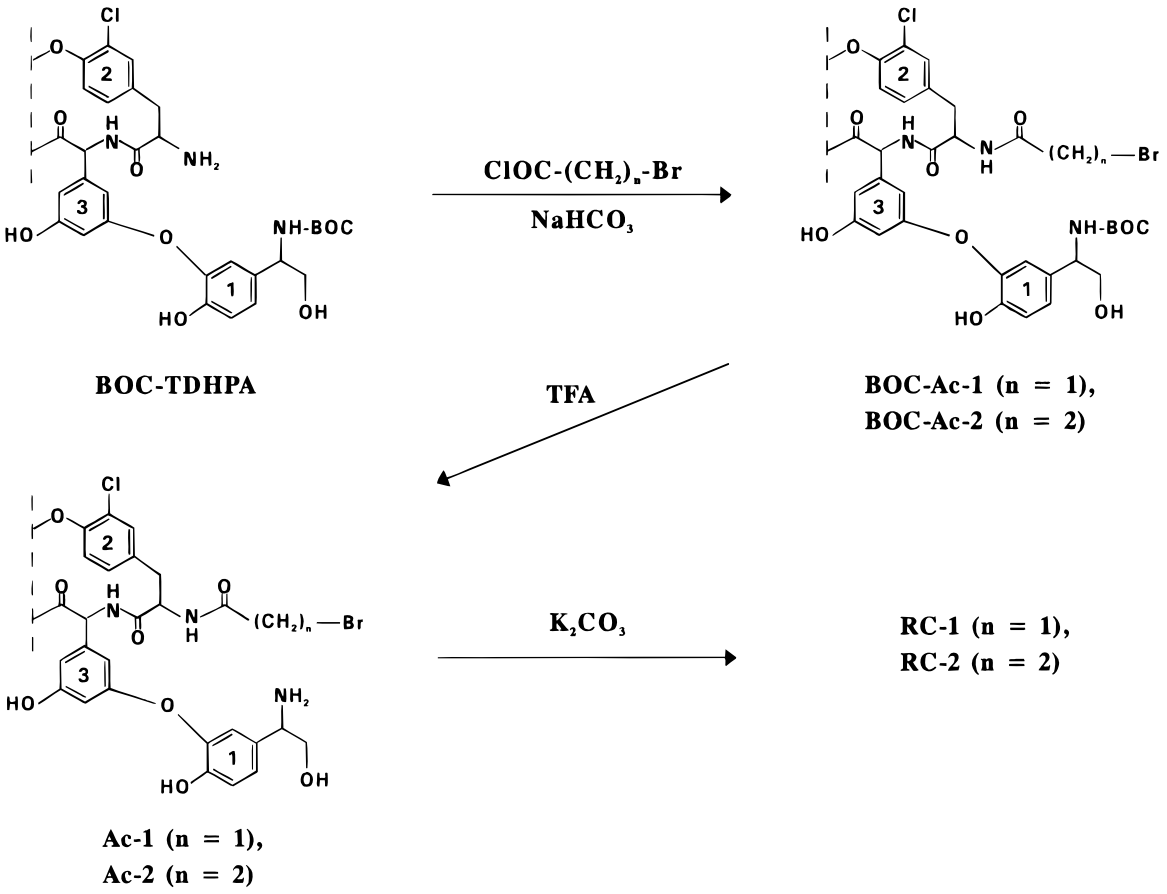


Table 6. TDHPA-Derived Dalbaheptides and Their Intermediates

compd	yield[a] (%)	HPLC t_R, min (method)	FAB MS $(M + H)^+$	formula	MW
BOC-Ac-1	70	10.8 (B)	1423	$C_{65}H_{58}N_7BrCl_2O_{21}$	1424.0
BOC-Ac-2	95	11.9 (B)	1437	$C_{66}H_{60}N_7BrCl_2O_{21}$	1438.0
Ac-1	70	14.75 (A), 5.9 (B)	1323	$C_{60}H_{50}N_7BrCl_2O_{19}$	1323.9
Ac-2	95	15.8 (A), 6.3 (B)	1337	$C_{61}H_{52}N_7BrCl_2O_{19}$	1337.9
RC-1	60	12.7 (A)	1242	$C_{60}H_{49}N_7Cl_2O_{19}$	1243.0
RC-2	85	13.1 (A)	1256	$C_{61}H_{51}N_7Cl_2O_{19}$	1257.0

[a] From **BOC-TDHPA**.

identified on the basis of its spatial proximity, revealed by NOE interactions, to w_5 and 4f, which in turn is identified by its signal generally at very high field in the teicoplanin family with respect to the other aromatic protons. Compared with that of **TD·HCl**, the chemical shift of the x_4 signal indicates some changes in the x_4 region. Its coupling with an NH_3^+ group, whose signal resonates at 8.50 ppm, shows that the 3,4-peptide linkage has been hydrolyzed. In addition, the presence of the CH_2OH group in the structure of residue 3 confirms that the 3,4-amide underwent a reductive hydrolysis. Residue 3 is characterized by the mutual *meta* coupling of three aromatic protons, one of which shows NOE interactions with the aliphatic CH, CH_2, and NH groups. The signals of the CH_2 protons of residue 2 show a NOE effect with the 2b proton and coupling with the $CHNH_3^+$ system.

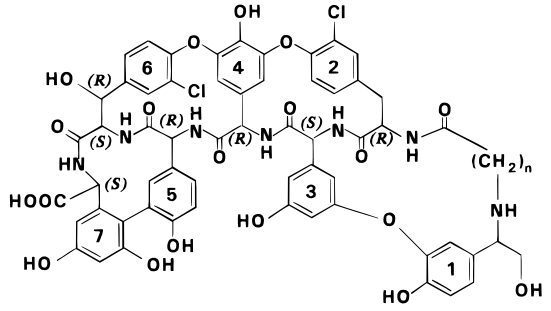


Figure 5. Structure of **RC-1** ($n = 1$) and **RC-2** ($n = 2$).

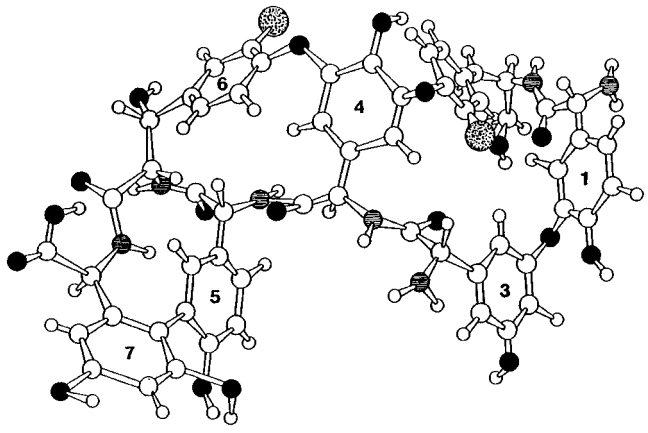


Figure 6. Stereostructure model of **RH-TD**.

Table 7. Assignments of Characteristic Signals of the ^{1}H NMR Spectra of RC-1 and RC-2 in Comparison with TD (in DMSO-d_6, TMS Internal Reference, δ in ppm)

assignment	RC-1[a]	RC-2[a]	TD[a]
CH_2OH	3.72	3.64	
$CHCH_2OH$	5.55	5.46	
CH_2CH_2-C=O		3.30	
H_2N^+CH	7.65	8.19	
$CH_2NH_2^+$	4.40, 4.26	4.13, 3.60	
x_2	4.85	4.71	4.92
z_2	2.75	2.76	2.87
x_3	5.34	5.66	5.35
x_4	5.57	5.72	5.60
x_5	4.33	4.34	4.33
x_6	4.12	4.12	4.10
x_7	4.45	4.42	4.42
4b	5.63	5.47	5.50
4f	5.10	5.33	5.08

[a] ·HCl.

This finding identifies the amide bond linking amino acid fragments 1 and 2 as the second hydrolysis site. The reduction of the 1,2-peptide linkage is confirmed by the structure of residue 1, which was identified by decouplings, obtained from the COSY spectrum in the system CH_2CHNH, where the NH group is part of a carbamate moiety, as shown by its ^{1}H NMR signal which does not change on adding CF_3COOH.

NMR Spectra of RC-1 and RC-2. The structures of **RC-1** and **RC-2** were defined on the basis of their COSY spectra and by comparing their ^{1}H NMR spectra with that of **TD·HCl** (Table 7). The two ring-closed compounds are in the form of hydrochlorides as shown by the NH_2^+ signal in their ^{1}H NMR spectrum. As expected, the main changes are limited to the right-hand side of the molecule while the overall conformation of the core structure appears to be almost unmodified in **RC-1**. Some minor changes are observed in **RC-2**.

FAB MS Spectra. The FAB MS spectra of **RH-teicoplanins** show abundant molecular ion clusters. The lowest mass isotopes of the protonated molecular ions were found to be 1882.7 (**RH-CTA**, factor A2-2),[17] 1567.4 (**RH-TB**), 1405.4 (**RH-TC**), and 1202.3 (**RH-TD**), in agreement within 0.1 Da with the values calculated for the structures assigned. The molecular masses calculated for average isotope content are about 1.0 Da higher. The isotope pattern of the molecular ion clusters is also in agreement with the proposed structures. Due to traces of Na in the samples, all spectra show various amounts of molecular ions $(M + Na)^+$. Losses of sugar units can also be observed, giving further structural information. All these ions are accompained by peaks formally derived by losses of 16 and 34 mass units, and these are common to all teicoplanin derivatives. The latter process, which occurs in the liquid matrix under FAB conditions, was also observed in the FAB study of chlorinated nucleosides and is due to a displacement of a Cl by an H.[38] The isotope distribution of the $(MH - 34)^+$ peak suggests the loss of one Cl atom. By analogy, a 16 Da loss indicates displacement of an OH by an H, which may proceed with a similar mechanism. To our knowledge, this is the first observation of an OH/H exchange occurring in FAB conditions. This adds to the growing evidence that bombardment of a liquid matrix with fast neutrals produces reactions not unlike to those observed in photodecompositions. It might be interesting to add that, in addition to the $(MH - 16)^+$ peak, there is a much smaller one formed by H_2O loss which is a "real" gas phase process, as indicated by a corresponding metastable peak. A very interesting correlation was observed between the facility to lose a sugar unit in FAB and the reactivity of the same sugar unit in solution chemistry. Hydrolysis of these compounds proceeds as follows:[39] **RH-CTA** easily loses the acylglucosamine, and then the mannose is lost, but this reaction requires much stronger conditions. The acetylglucosamine is lost only in the last step. The fragment ion abundances, as observed in FAB (reactivity of gas phase ions), are parallel with this: the loss of the acylglucosamine unit gives a very abundant fragment, while the loss of either mannose or acetylglucosamine gives small peaks, but mannose loss seems to be slightly favored. This correlation is interesting for fundamental studies on the reactivity of ions and neutrals but also has practical importance in estimating the reactivity of certain groups based on FAB spectra.

The FAB spectra of the other **RH-derivatives** are analogous to those of **RH-teicoplanins**. All compounds give abundant molecular ion cluster four mass units higher than the starting material. These derivatives, similar to those of teicoplanin, give nucleophilic substitution products at 16 (OH → H) and, except for **RH-HR**, 34 (Cl → H) Da lower masses under FAB conditions. It is noteworthy that sensitivity for **RH-HR** is much higher than that for the other compounds. This might indicate that the chlorine substitution in glycopeptides reduces FAB sensitivity. The spectrum of **BOC-TDTPDA** gives a molecular ion 8 mass units higher than that of **BOC-TD**, in accordance with the reduction of two amide bonds, while the MW of **TDHPO** was found to be 26 mass units higher than that of **TDHPA**, which is in agreement with the introduction of one CO and loss of two H and consequently with the oxazolidinone structure of this compound. The MWs found for **RC-1** and **RC-2** are in accordance with their structure of ring closed compounds.

Peptide Binding Studies and Antibacterial Activity. The ability of **RH-teicoplanins** and related **2,3-RH-glycopeptide derivatives**, as well as that of **TDHPA**, its C^3-epimer *epi*-**TDHPA**, and ring-closed compounds **RC-1** and **RC-2** to complex with the antibiotic's target peptide D-Ala-D-Ala, was investigated by measuring their binding to the synthetic analog Ac_2-L-Lys-D-Ala-D-Ala by both UV and NMR methods. Using the diffential UV assay,[40] no detectable variation in absorbance was observed on adding the test peptide. Nevertheless, with the only exception of *epi*-**TDHPA**, the compounds were retained by a resin of D-Ala-D-Ala covalently linked with an inert matrix,[41] thus showing affinity for the dipeptide. This apparent discrepancy could be interpreted as being due to a different complexation which does not cause any detectable difference in the UV absorbance of the open molecule. Limited to **2,3-RH-derivatives**, preliminary investigation using the 2D NMR spectroscopy seems to indicate that no binding exists between the two amide-NH groups of residues 2 and 4, and the free NH_2 of residue 3 and the carboxylate anion of target peptide model, but that a weak binding still exists which involves the amide-NH proton of residue 7 and the lysyl-CO of the synthetic tripeptide. Further NMR studies are in progress to assess this unusual mechanism. In fact, for

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Table 8. *In Vitro* Activity of RH-Glycopeptides in Comparison with Parent Unmodified Glycopeptides

	MIC (μg/mL)				
compd	*Staphylococcus aureus* Tour	*Staphylococcus epidermidis* ATCC 12228	*Streptococcus pyogenes* C 203	*Streptococcus pneumoniae* UC 41	*Enterococcus faecalis* ATCC 7080
RH-CTA	8	2	1	4	4
RH-TD	4	2	8	16	16
RH-A-40	16	64	2	4	16
RH-V	16	32	8	8	16
RH-HR			>128		
CTA	0.125	0.125	0.063	0.063	0.125
TD	0.063	0.016	0.125	0.125	0.125
A-40	0.063	0.063	0.063	0.063	0.125
V	0.25	0.5	0.125	0.125	0.5
R	4	4	0.5	1	1
HR	4	8	1	1	2

Table 9. Antibacterial Activity of TDHPA, RC-1, and RC-2 in Comparison with TD and RH-TD

	MIC[a] (μg/mL)				
test organisms	**TDHPA**	**RC-1**	**RC-2**	**RH-TD**[b]	**TD**[b]
S. aureus Tour L165	2	1	16	32 (4)	0.063
S. epidermidis L147	0.5	1	32	8 (2)	0.063 (0.016)
S. epidermidis L533	1	0.5	8	16 (8)	0.063
S. haemolyticus L602	16	8	>128	16	0.25
S. pyogenes L49	8	4	32	32 (8)	0.25 (0.13)
S. pneumoniae L44	4	4	16	16	0.13
E. faecalis L149	8	4	32	16	0.13
Escherichia coli L47	>128	>128	>128	>128	64

[a] Values given were obtained with final inoculum of 5×10^5 cfu/mL. [b] Values in parentheses refer to inocula of 10^4 cfu/mL.

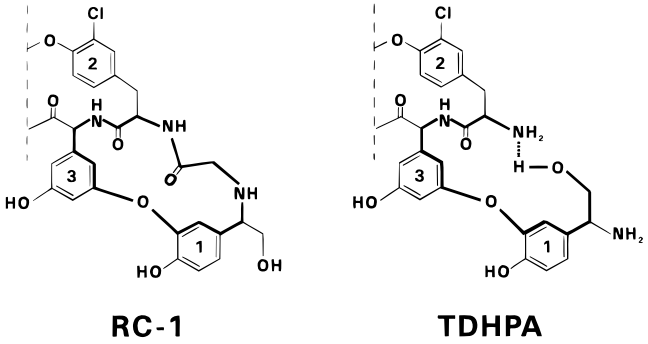

Figure 7. Structure of the 1,2,3-macrocyclic ring in **RC-1** compared with that generated by a hydrogen bonding system in **TDHPA**.

the antibiotics of the vancomycin family it has been reported[42] that this secondary binding only exists as a consequence of the primary interaction. However, a mechanism of action different from that of "natural" glycopeptides cannot be excluded in principle.

The drastic structural modification of the binding pocket accounts for the decreased, though interesting,[43] *in vitro* activity of **2,3-RH-dalbaheptides** against a panel of selected Gram-positive bacteria (Table 8). The lack of activity of **RH-HR** (MIC >128 μg/mL) is likely related to the poor activity of **R**. Although inactive against Gram-negative bacteria and less active than **TD** against Gram-positive organisms, **TDHPA** was significantly more active than **RH-TD** against the majority of tested strains (Table 9). In contrast, *epi*-**TDHPA** was inactive at a concentration of up to 128 μg/mL.[44] The activity of ring-closed compound **RC-1** was comparable to that of **TDHPA**, while homologous cyclic derivative **RC-2** was markedly less active. The activity of **TDHPA** was unexpected considering that the vancomycin-derived hexapeptide aglycon **VAHP** was devoid of antibacterial activity.[6] This is very likely due to the different conformation of their hexapeptide backbone in the active site region. While **TDHPA** almost maintains the original conformation of the peptide chain and in particular the orientation of the 2,3-amide as **TD**, in **VAHP** the 2,3-*trans* peptide unit exhibits a 180° flipping motion, from having the NH-proton at the front of the molecule, as in **VA**, **TD**, and the other active glycopeptides, to having it at the rear. This results in the loss of the ability by **VAHP** to bind to dalbaheptide's target peptide D-Ala-D-Ala[6] and hence in the loss of the antibiotic activity. In *epi*-**TDHPA**, the change in the configuration of the 1,3-diphenyl ether amino acid is the same as that occurring at the chiral center of amino acid 3 of **TD** in the epimerization to *epi*-**TD**. Like *epi*-**TD**, the molecule of *epi*-**TDHPA**, instead of having the typical pocket-like conformation in the binding region, has two half-pockets pointing in opposite directions. It follows a perturbation in the active site which results in the loss of antibacterial activity. Also in **RH-TD**, due to the opening of the 2,3-amide bond, the right-hand part of the molecule is folded backward, but in this case the "natural" *S* configuration of amino acid 3 is in part maintained.[45] This could explain the residual antibacterial activity of **RH-TD**. The comparable activity between **TDHPA** and its cyclic derivative **RC-1** was expected considering that the overall conformation of the two compounds is very similar and not very different from that of **TD**, as drawn from NMR conformational studies. One possibility is the presence in **TDHPA** of a hydrogen bonding, favored at the neutral/physiological pH of the *in vitro* tests, between the peptide N-terminal amino group of residue 2 and the newly formed hydroxyl group of residue 1 (Figure 7). The resulting 16-membered hydrogen bonding system should have similar conformation and geometry as that of the corresponding 16-membered 1,2,3-macrocyclic ring present in the structure of **RC-1**. However, the lower activity of these two compounds compared with that of **TD** indicates that the enlargement of the 1,2,3-macrocyclic ring has a negative effect on their binding properties to the target

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(43) The unexpected activity of RH-glycopeptides is of particular interest considering that their mode of action is very likely based on the interaction (Chart 1) with a target moiety, the lysyl-CO which is present in the peptidoglycan portion of the bacterial cell wall of both glycopeptide-susceptible (L-Lys-D-Ala-D-Ala) and resistant (L-Lys-D-Ala-D-hydroxy acid) organisms.

(44) Data not reported.

(45) **RH-TD**, as obtained by reductive hydrolysis of **TD**, is an unresolved mixture of two diasteroisomers *S* (**RH-TD**, as the main product, ~60%) and *R* (*epi*-**RH-TD**) at the C^3-chiral center.

dipeptide. This is even more evident with a further ring enlargement by one additional methylene group, as shown by the poor activity of **RC-2**.

Experimental Section

The ^1H NMR experiments were recorded at 500 MHz in DMSO-d_6 solution, added or not with CF_3COOH. The phase-sensitive double quantum filter (PHDQ) $^1H-^1H$ COSY spectra were run using time-proportional-phase-incrementation in f_1. In the relayed-coherence-transfer (RCT) $^1H-^1H$ COSY, the fixed delay was 25 ms. The phase-sensitive NOE spectra were obtained with a mixing time of 200 ms. In the HMQC experiments for $^1H-^{13}C$ correlations, the sweep width in t_2 was 5500 Hz and t_1 11300 Hz; 512 increments with 64 transitions each were collected. For the HMBC experiments with *epi*-**TDHPA**, 196 transitions were collected. For the $^1H-^{15}N$ correlations, the sweep width in t_1 was 2000 Hz; 128 increments with 200 experiments each were collected.

FAB MS positive ion spectra were obtained using 8 kV accelerating voltage. The samples were dissolved in DMF or H_2O, and then 1 μL of the solution was mixed with 1 μL of thioglycerol matrix containing 0.1 N AcOH on the target.

Reaction products were purified by reversed-phase column chromatography on silanized silica gel (0.063−0.2 mm), according to the following procedure: 1 g of crude compound was dissolved in 20−30 mL of a MeCN/H_2O (1/1) mixture, the solution was adjusted at pH 5.5 with solid HCO_2NH_4, and then H_2O was added dropwise under stirring until precipitation started; after a few drops of MeCN were added, the resulting cloudy solution was loaded on a column of 50 g of silanized silica gel in a same solvent mixture; elution was carried out according to a linear step-gradient from 5−10% to 40−70% of MeCN in H_2O, in 10−15 h, at a flow rate of 200−300 mL/h, while 20 mL fractions were collected; those containing pure compound were pooled, and enough 1-BuOH was added to obtain, after evaporation of most solvent at 40 °C under reduced pressure, a concentrated dry butanolic solution (or suspension); on adding Et_2O the precipitated solid was collected, washed with Et_2O, and dried *in vacuo* at room temperature overnight.

Reactions, column eluates, and final products were checked by HPLC performed on a column (125 × 4 mm) prepacked with LiChrospher RP-8 (5 μm). Chromatograms were recorded at 254 nm. Elutions were carried out by mixing eluent a, MeCN, with eluent b, 0.2% aqueous HCO_2NH_4, according to linear step gradients programmed as follows:

time (min)	0	10	20	30	35	40
Method A: % **b** in **a**	5	23	26	35	75	5
Method B: % **b** in **a**	20	33	46	60	75	20

All RH-derivatives were analyzed for C, H, N, and Cl on samples previously dried at 140 °C under N_2 atmosphere. The analytical results obtained for the above elements were within ±0.4% of the theoretical values. The solvent content (<12%, mainly water) and inorganic residue (<0.2%) were determined by thermogravimetry (TG), at 140 °C, after the samples were heated at 900 °C in O_2 atmosphere, respectively.

The **V**-aglycon (**VA**) was prepared by reaction of **V** with TFA, according to the procedure described by Nagarajan and Schabel.[6] **BOC** and **CBZ-TD** were prepared as previously described.[36]

Reductive Hydrolysis of Glycopeptides Teicoplanin (CTA and Its Single Factor A2-2), Its Pseudoaglycons (TB and TC) and Its Aglycon (TD), Vancomycin (V), Its Aglycon (VA), Ristocetin (R), and A-40,926 (A-40) (General Procedure, Method a). A suspension of 10 mmol of a dalbaheptide in 600 mL of a H_2O/EtOH 65/35 mixture was stirred at 10−15 °C for 90 min, while a suitable amount (Tables 1 and 2) of $NaBH_4$ pellets was added portionwise. A clear solution formed which was stirred at room temperature over a period of time depending on the starting compound. After 1 L of MeOHand 0.5 L of EtOH were added, the resulting solution was slowly poured into a solution of an excess (10%) of AcOH in 0.5 L of MeOH, and then the solvents were evaporated at 35 °C under reduced pressure. The jelly residue, dissolved in 1 L of H_2O, was purified by reversed-phase column chromatography as described above. Fractions containing pure (HPLC) RH-derivatives were pooled and solvents were evaporated, at 40 °C under reduced pressure, in the presence of 1-BuOH to avoid foaming. The solid residue was collected, washed with 200 mL of Et_2O, and dried at room temperature *in vacuo* (over KOH) for 3 days, yielding the final product.

Preparation of RH-TB from RH-CTA (Method b). A solution of 2 g (∼1 mmol) of **RH-CTA** in 20 mL of dry trifluoroacetic acid (TFA) was stirred at room temperature for 30 min, and then 130 mL of Et_2O was added. The precipitated solid was collected and redissolved in 100 mL of H_2O. The resulting solution was adjusted at pH 7.5 with 1 N NaOH. Purification by reversed-phase column chromatography under usual conditions yielded 1.2 g of the title compound.

Preparation of RH-TC from RH-CTA or RH-TB (Method c). Dry HCl was bubbled at room temperature into a stirred suspension of 1 mmol of **RH-CTA**, or **RH-TB**, in 100 mL of dimethoxyethane (DME) for 6 h. Then, the solvent was evaporated at 40 °C under reduced pressure, and the solid residue, dissolved at pH 7.5 in 100 mL of H_2O, was chromatographed to give (55−60%) the title compound.

Preparation of RH-TD from RH-CTA, RH-TB, or RH-TC (Method d). Dry HCl was bubbled at 70 °C into a stirred suspension of 1 mmol of one of the above **RH-teicoplanins** in 100 mL of trifluoroethanol (TFE) for 16 h. The insoluble matter was collected and dissolved in 200 mL of H_2O at pH 7.0. After purification by column chromatography, the title compound was obtained (20−25%).

Reductive Hydrolysis of TD-Carbamates. The comparative experiments (Table 3) were carried out as follows. To a stirred solution of 4 mmol of **BOC** or **CBZ-TD** in 150 mL of a proper aqueous alcohol mixture, was added 30 g of $NaBH_4$ (pellets) portionwise in 90 min, while the temperature was maintained at 20−25 °C. Stirring was continued at room temperature until the starting compound was completely transformed (the course of the reaction was monitored by HPLC every 2 h), and then the reaction mixture was slowly poured into 300 mL of a MeOH/EtOH/AcOH 3/2/1 solution. After 1-BuOH was added to prevent foaming, the solvents were evaporated at 45 °C under reduced pressure. The solid residue was collected and chromatographed to yield pure final products.

Synthesis of TDHPA (and *epi*-TDHPA). (a) From TD. To a stirred solution of 100 g (∼83 mmol) of **TD** in 3.5 L of a H_2O/EtOH 6/4 mixture was added 600 g (∼16 mol) of $NaBH_4$ (pellets) portionwise in 2 h, with cooling to 15−25 °C. The reaction mixture was stirred at room temperature for 22 h, and then it was poured into a solution of 960 mL of glacial AcOH in 5.5 L of a MeOH/EtOH 65/35 mixture, with cooling to 10 °C. The resulting suspension was centrifuged, and the insoluble matter (5 g, *epi*-**TD**)[46] was separated. The clear solution was concentrated at 45 °C under reduced pressure to a final volume of ∼500 mL in the presence of 1-BuOH to prevent foaming. The precipitated solid (mainly boron salts) was filtered off, and the filtrated solution was loaded on a column of 2.5 kg of silanized silica gel in H_2O. The column was eluted with 10 L of H_2O and then with 10 L of a MeCN/H_2O 1/1 mixture while 500 mL fractions were collected. Fractions 9−16 contained crude **RH-TD** (∼55 g, HPLC titer 63%). Fractions 21−30, containing a mixture of the title compound and its C^3-epimer, were pooled, and 9 L of 1-BuOH was added. The resulting mixture was concentrated at 40 °C under reduced pressure to a small (∼200 mL) volume. Upon addition of Et_2O (500 mL), the precipitated solid was collected and washed with 100 mL of Et_2O, yielding 30 g of a powder containing **TDHPA** (83%) and *epi*-**TDHPA** (17%). Resolution of the above mixture by reversed-phase chromatography yielded 18 g of pure **TDHPA** and 0.7 g of pure *epi*-**TDHPA**.

(b) From BOC-TD. To a stirred solution of 10.5 g (∼8 mmol) of **BOC-TD** in 300 mL of a EtOH/H_2O 9/1 mixture was added 60 g of $NaBH_4$ (powder) portionwise at 20 °C in 2 h.

(46) Precipitated *epi*-**TD** (20% over the total amount formed) was enough pure for analysis.

After being stirred at room temperature for 72 h, the reaction mixture was worked up as described above, yielding 5.6 g of **BOC-TDHPA**. This compound was dissolved at room temperature in 50 mL of dry TFA. After 5 min of stirring, the solvent was evaporated and the oily residue was slurried with 50 mL of EtOAc and then with 150 mL of Et_2O. The solid which separated was chromatographed under the usual conditions, yielding 4.9 g of pure **TDHPA**.

Preparation of TDHPO *via* Intermediate CBZ-TDHPA. A stirred solution of 10.7 g (~8 mmol) of **CBZ-TD** in 300 mL of a EtOH/H_2O 9/1 mixture was treated with 60 g of $NaBH_4$ as described above. After 48 h, the reaction mixture was divided into two portions of 150 mL each. One portion was worked up as usual to yield 1.7 g of pure **CBZ-TDHPA**. The other aliquot was allowed to react for additional 72 h until **CBZ-TDHPA** completely transformed into **TDHPO**, which was recovered and chromatographed,[47] obtaining 1.4 g of pure title compound.

Synthesis of VAHP from BOC-VA. **(a) Preparation of BOC-VA.** A stirred solution of 1.15 g (~1 mmol) of **VA** in 25 mL of a dioxane/water 1/1 mixture was adjusted at pH 6.5 with solid $NaHCO_3$, and then a solution of di-*tert*-butyl dicarbonate in 3 mL of dioxane was added dropwise in 15 min. After being stirred at room temperature for 24 h, the reaction mixture was adjusted at pH 3 with 1 N HCl and 50 mL of H_2O was added. Extraction with 50 mL of a 1-BuOH/EtOH 1/1 mixture and evaporation of the organic solvents yielded 1.2 g of **BOC-VA** pure enough for the next step.

(b) Reductive Hydrolysis of BOC-VA. The compound (**BOC-VA**, 1.2 g) was dissolved in 50 mL of a EtOH/H_2O 8/2 (or 2/8) mixture and then treated with 6 g of $NaBH_4$ as described above for the reductive hydrolysis of **BOC-TD**, yielding 0.85 g of pure title compound.

Synthesis and Reductive Hydrolysis of Ac-VAHP. The compound was prepared (98%) by reaction of 1 mmol of **VAHP** with 1.1 mmol of MeCOCl in 20 mL of DMF at room temperature (4 h), in the presence of 1 mmol of TEA. Then it was submitted to reductive hydrolysis (reaction time: 35 h) under the same conditions described above for BOC-VA, yielding[48] **VAHP** (36%) and **Ac-RH-VAHP** (24%).

Synthesis of RC-1 and RC-2. Step a: BOC-Ac-1 and BOC-Ac-2. To a stirred solution of 1.3 g (~1 mmol) of **BOC-TDHPA** and of 84 mg of $NaHCO_3$ in 30 mL of a Me_2CO/H_2O 1/1 mixture was added dropwise a solution of 1 mmol of bromoacetyl (for **BOC-Ac-1**) or 3-bromopropionyl (for **BOC-Ac-2**) chloride in 2 mL of Me_2CO with cooling to 0−3 °C. After 15 min, the reaction mixture was poured into 40 mL of H_2O, and after the pH was adjusted to 4.5 with glacial AcOH, the resulting mixture was extracted with 100 mL (2 × 50 mL) of EtOAc (or 1-BuOH). The organic layer was washed with 25 mL of H_2O, and then it was concentrated at 35 °C (or 45 °C) under reduced pressure to a small volume (~5 mL). On addition of Et_2O (~50 mL), the precipitated solid was collected and dried *in vacuo* at room temperature overnight, yielding **BOC-Ac-1** (0.79 g) or **BOC-Ac-2** (1.3 g).

Step b: Ac-1 and Ac-2. A solution of 0.7 mmol of one of the above BOC-acyl derivatives in 10 mL of dry TFA was stirred at room temperature for 10 min, and then the solvent was evaporated (30 °C, reduced pressure). The oily residue was redissolved in 50 mL of H_2O, and the resulting solution was adjusted at pH 7 with 1 N NaOH then extracted with 50 mL of 1-BuOH. The organic layer was washed with 15 mL of H_2O and concentrated at 40 °C under reduced pressure to a small (~5 mL) volume. Upon addition of 50 mL of Et_2O, the precipitated solid was collected, washed with 20 mL of Et_2O, and then dried *in vacuo* at room temperature overnight, yielding (100%) **Ac-1** or **Ac-2**.

Step c: RC-1 and RC-2. To a stirred solution of 0.5 mmol of one of the above compounds in 40 mL of DMF was added 100 mg (~0.8 mmol) of K_2CO_3, and the resulting suspension was stirred at room temperature for 2 h. Then the reaction mixture was poured into 100 mL of H_2O. The resulting solution was adjusted at pH 3 with 1 N HCl, and then it was extracted with 70 mL of 1-BuOH. The organic layer was washed with 50 mL of H_2O, and then it was concentrated at 35 °C under reduced pressure to a small (~10 mL) volume. On addition of 50 mL of Et_2O, the precipitated solid was collected and purified by reversed-phase chromatography under the usual conditions, yielding pure **RC-1** (85%) or **RC-2** (90%).

NMR Data (DMSO-d_6, δ in ppm) for Some Representative Compounds. RH-TD: ^{1}H NMR δ 2.67 (m, 1H), 2.87 (m, 1H), 3.51 (br s, 2H), 3.96 (m, 1H), 4.17 (br s, 1H), 4.20 (dd, 1H), 4.33 (br s, 1H), 4.38 (d, 1H), 4.59 (br s, 1H), 5.11 (s, 1H), 5.49 (s, 1H), 5.65 (d, 1H), 5.83 (s, 1H), 6.36 (s, 1H), 6.39 (s, 1H), 6.45 (s, 3H), 6.58 (d, 1H), 6.62 (s, 1H), 6.70 (d, 2H), 6.77 (d, 1H), 6.83 (d, 1H), 6.93 (d, 1H), 6.96 (d, 1H), 7.16 (d, 2H), 7.23 (s, 1H), 7.42 (d, 1H), 7.74 (d, 1H), 7.89 (s, 1H), 8.39 (d, 1H), 8.62 (br s, 1H), 8.64 (d, 1H).

BOC-RH-TD (·TFA): ^{1}H NMR δ 1.38 (s, 9H), 2.70 (m, 1H), 2.90 (m, 1H), 3.40−3.50 (m, 2H), 4.00 (m, 1H), 4.23 (dd, 1H), 4.42 (d, 1H), 4.62 (br s, 1H), 4.85 (br s, 1H), 4.92 (br s, 1H), 5.11 (s, 1H), 5.50 (s, 1H), 6.32 (s, 1H), 6.75 (br s, 1H), 7.18 (s, 1H), 7.31 (br s, 1H), 7.42 (d, 1H), 7.89 (s, 1H), 8.60 (d, 1H, and NH_3^+), 8.90 (br s, 1H), 9.20 (d, 1H).

CBZ-RH-TD (·TFA): ^{1}H NMR δ 2.75 (m, 1H), 2.90 (m, 1H), 3.38−3.48 (dd, 2H), 4.00 (m, 1H), 4.28 (dd, 1H), 4.46 (d, 1H), 4.65 (br s, 1H), 4.90 (br s, 1H), 5.00 (br s, 1H), 5.05 (br s, 2H), 5.14 (s, 1H), 5.50 (s, 1H), 5.64 (d, 1H), 6.00 (s, 1H), 6.32 (s, 1H), 6.48 (s, 1H), 6.70 (s, 1H), 6.80 (br s, 1H), 7.35 (m, 5H), 7.45 (d, 1H), 7.85 (br s, 1H), 7.90 (s, 1H), 8.60 (d, 1H, and NH_3^+), 8.88 (br s, 1H), 9.22 (d, 1H).

TDHPA: ^{1}H NMR δ 2.74 (dd, 1H), 3.21 (dd, 1H), 3.58 (d, 1H), 3.62 (d, 1H), 3.71 (m, 1H), 4.10 (m, 1H), 4.12 (dd, 1H), 4.37 (d, 1H), 4.42 (d, 1H), 5.08 (d, 1H), 5.25 (s, 1H), 5.48 (d, 2H), 5.96 (d, 2H), 6.26 (s, 2H), 6.35 (s, 1H), 6.40 (s, 1H), 6.63 (d, 1H), 6.68 (d, 1H), 6.72 (d, 1H), 6.94 (d, 1H), 7.07 (s, 1H), 7.10 (d, 1H), 7.12 (d, 2H), 7.16 (s, 1H), 7.28 (d, 1H), 7.43 (d, 1H), 8.42 (d, 1H), 8.42 (br s, 1H), 8.52 (br s, 1H); ^{13}C NMR δ 39.72 (t), 54.14 (d), 54.70 (d), 55.99 (d), 56.36 (d), 57.29 (d), 58.03 (d), 62.09 (d), 63.76 (t), 71.89 (d), 102.40 (d), 102.95 (d), 104.80 (d), 106.47 (d), 107.36 (d), 107.69 (d), 116.64 (d), 117.56 (d), 118.49 (s), 121.63 (d), 123.36 (d), 124.96 (d), 125.69 (s), 125.88 (d), 126.25 (s), 127.36 (d), 127.54 (s), 127.91 (d), 128.64 (s), 131.24 (d), 134.39 (s), 135.28 (s), 136.45 (s), 136.73 (d), 137.90 (s), 142.15 (s), 148.31 (s), 149.73 (s), 150.47 (s), 155.46 (s), 156.76 (s), 157.68 (s), 158.56 (s), 159.16 (s), 161.56 (s), 167.85 (s), 168.96 (s), 169.33 (s), 169.63 (s), 172.84 (s), 173.03 (s); ^{15}N NMR δ 87.7 (d), 95.8 (d), 105.2 (d), 107.5 (d).

***epi*-TDHPA**: ^{1}H NMR δ 2.69 (dd, 1H), 3.42 (dd, 1H), 3.60 (m, 1H), 3.67 (dd, 2H), 4.11 (dd, 1H), 4.14 (br s, 1H), 4.31 (d, 1H), 4.42 (d, 1H), 4.85 (s, 1H), 5.12 (br s, 2H), 5.51 (s, 1H), 5.87 (d, 1H), 5.92 (s, 1H), 6.00 (d, 1H), 6.19 (s, 2H), 6.25 (s, 1H), 6.47 (s, 1H), 6.65 (d, 1H), 6.71 (d, 2H), 6.80 (br s, 1H), 6.94 (d, 1H), 6.97 (d, 1H), 7.06 (d, 1H), 7.12 (d, 3H), 7.24 (d, 1H), 7.43 (d, 1H), 7.82 (s, 1H), 7.84 (s, 1H), 8.31 (br s, 1H), 8.48 (br s, 1H), 8.59 (d, 1H), 8.95 (d, 1H); ^{13}C NMR δ 36.58 (t), 53.22 (d), 54.11 (d), 54.33 (d), 56.92 (d), 60.25 (d), 62.09 (d), 63.02 (t), 71.71 (d), 102.58 (d), 103.69 (d), 104.25 (d), 105.91 (d), 106.10 (d), 107.58 (d), 109.24 (d), 116.45 (d), 117.38 (d), 118.12 (s), 121.44 (d), 123.48 (d), 124.96 (d), 125.14 (s), 125.70 (s), 125.76 (d), 126.06 (s), 126.44 (s), 127.54 (d), 127.91 (d), 128.47 (s), 131.43 (d), 131.98 (d), 134.20 (s), 134.94 (s), 136.05 (d), 136.23 (s), 137.90 (s), 142.52 (s), 147.51 (s), 148.25 (s), 149.18 (s), 149.55 (s), 150.10 (s), 155.28 (s), 156.76 (s), 157.50 (s), 158.97 (s), 158.98 (s), 165.03 (s), 167.85 (s), 168.40 (s), 169.22 (s), 172.43 (s); ^{15}N NMR δ 21.0 (t), 22.5 (t), 87.0 (d), 100.2 (d), 103.0 (d), 106.5 (d), 107.3 (d).

BOC-TDHPA: ^{1}H NMR δ 1.34 (s, 9H), 2.70 (dd, 1H), 3.22 (dd, 1H), 3.42 (m, 2H), 3.69 (m, 1H), 4.12 (dd, 1H), 4.38 (d, 1H), 4.40 (m, 2H), 5.10 (d, 1H), 5.26 (s, 1H), 5.47 (s, 1H), 5.51 (d, 1H), 5.90 (s, 1H), 5.96 (d, 1H), 6.22 (s, 1H), 6.30 (s, 1H), 6.55 (d, 1H), 7.81 (s, 1H), 8.35 (br s, 1H), 8.43 (d, 1H), 8.50 (br s, 1H).

TDHPO: ^{1}H NMR δ 2.76 (dd, 1H), 3.21 (dd, 1H), 3.69 (m, 1H), 3.94 (m, 1H), 4.12 (dd, 1H), 4.39 (d, 2H), 4.58−4.80 (dd, 2H), 5.09 (s, 1H), 5.26 (s, 1H), 5.47 (s, 1H), 5.50 (d, 1H), 5.70 (d, 1H), 5.92 (s, 1H), 6.24 (s, 1H), 6.33 (s, 1H), 6.63 (d, 1H),

(47) Purification yield, ~60%.
(48) Overall yields from **VAHP**.

7.29 (d, 1H), 7.43 (d, 1H), 7.81 (s, 1H), 8.05 (s, 1H), 8.37 (d, 1H), 8.43 (d, 1H), 8.52 (br s, 1H).

BOC-TDTPDA (·TFA): ^{1}H NMR δ 2.78 (dd, 1H), 3.18 (dd, 1H), 3.42−3.60 (dd, 4H), 4.13 8m, 1H), 4.23 (dd, 1H), 4.41 (m, 1H), 4.46 (d, 1H), 4.63 (d, 1H), 4.78 (m, 1H), 5.14 (br s, 1H), 5.57 (s, 1H), 6.04 (s, 1H), 6.28 (s, 1H), 6.39 (s, 1H), 6.42 (s, 1H), 6.49 (s, 1H), 6.85 (d, 1H), 6.91 (s, 1H), 7.12 (br s, 1H), 7.27 (s, 1H), 7.56 (s, 1H), 7.89 (s, 1H), 8.10 (br s, NH_3^+), 8.50 (br s, NH_3+), 8.60 (d, 1H), 9.01 (d, 1H), 9.18 (d, 1H).

Determination of Antibacterial Activity. Antibacterial activity expressed as MIC (minimal inhibitory concentration in μg/mL) was determined by the microdilution method in Difco Todd-Hewitt broth (streptococci) or Oxoid Iso-Sensitest broth (other organisms). The final inoculum was about 5×10^5 cfu[49] /mL. MIC was read as the lowest concentration which showed no visible growth after 18−24 h incubation at 37 °C.

Acknowledgment. We are indebted to G. Tamborini for acid−base titrations and IR spectra and to R. Scotti for the determination of antimicrobial activity. Dr. L. Colombo is acknowledged for his contribution (FAB MS spectra) in the structure elucidation of some RH-products.

(49) Colony forming unit.

Supporting Information Available: Combustion analysis data; abundance of fragment ions formed by sugar losses, as observed in FAB MS of **RH-CTA**, **RH-TB**, and **RH-TC**; assignments of the ^{1}H NMR spectra of **RH-TD**, **BOC** and **CBZ-RH-TD**, **BOC-TDHPA**, **TDHPO**, and **BOC-TDHPA** in comparison with **TD**; NOE's observed in **RH-TD**; assignments of the ^{1}H, ^{13}C, and ^{15}N NMR spectra of **TDHPA** and *epi*-**TDHPA**; long-range couplings observed by HMBC, and NOE's for **TDHPA** and *epi*-**TDHPA** (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO941809V