Role of the Glycopeptide Framework in the Antibacterial Activity of Hydrophobic Derivatives of Glycopeptide Antibiotics

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The antibacterial properties of glycopeptide antibiotics are based on their interaction with the D-Ala-D-Ala containing pentapeptide of bacterial peptidoglycan. The hydrophobic amides of vancomycin (**1**), teicoplanin (**2**), teicoplanin aglycon (**3**), and eremomycin (**4**) were compared with similar amides of minimally or low active des-(*N*-methyl-D-leucyl)eremomycin (**5**), eremomycin aglycon (**6**), des-(*N*-methyl-D-leucyl)eremomycin aglycon (**7**), and a teicoplanin degradation product TB-TPA (**8**). All hydrophobic amides of **1**, **3**, **4**, and **6** were almost equally active against glycopeptide-resistant enterococci (GRE) [minimum inhibitory concentrations (MIC) ≤ 4 μg/mL] and had better activity against Gram-positive strains sensitive to glycopeptides than against GRE. Extensive degradation of the glycopeptide framework in amides of **7** and **8** led to a decrease of anti-GRE activity (MIC = 16−64 μg/mL), and for these derivatives MIC values for bacterial strains sensitive and resistant to glycopeptides were very close. These results suggest that in sensitive bacteria two mechanisms of action are operating for the hydrophobic derivatives of glycopeptide antibiotics with the nondamaged peptide core—interaction with the D-Ala-D-Ala moiety and the inhibition of bacterial membrane bound enzymatic reactions, whereas for GRE lacking the D-Ala-D-Ala fragment, only the second mechanism is operating. It appears that a minimal glycopeptide core is required for activity, and that more extensive degradation results in a serious decrease of antibacterial activity.

Introduction

The search for derivatives of glycopeptide antibiotics active against glycopeptide-resistant enterococci (GRE) resulted in the discovery of the anti-GRE activity of hydrophobic derivatives of vancomycin,[1,2] eremomycin,[3−5] and chloreremomycin[6] among which the most active is *N′-p-*(*p*-chlorophenyl)benzylchloreremomycin (LY 333328).[7] Specific hydrophobic derivatives of eremomycin or vancomycin demonstrate antibacterial activity despite the absence of binding to -D-Ala-D-Ala and -D-Ala-D-lactate,[2,8] and this activity appears due to inhibition of transglycosylation process.[2,9−12]

Previous investigations showed that the hydrophobic substituents play a major role in the antibacterial activity against GRE.[8,9,12] The structural demands for the size, type, and position of a hydrophobic substituent are relatively clear: it must be an C_{10-12} alkyl or diaryl substituent,[13] while the position of a hydrophobic substituent on the periphery of the glycopeptide does not seriously influence its antibacterial properties.[8] It was also shown that a vancosaminylglucose derivative (disaccharide component of vancomycin) containing a *p*-(*p*-chlorophenyl)benzyl substituent blocks the transglycosylation step of cell wall synthesis, although its antibacterial activity was low [minimum inhibitory concentrations (MIC) = 128 μg/mL];[2] and it was concluded that peptide binding is not required for the biological activity of carbohydrate-modified glycopeptides. The 3,4-dichlorobenzyloxybenzyl putrescine is also antibacterial (MIC ≤ 64 μg/mL) and was able to interact directly with bacterial membrane proteins.[9] However, the role and structural demands for the peptide core remain unclear. Earlier it was shown that splitting off the first amino acid of a hydrophobic vancomycin or eremomycin derivative does not result in a serious decrease of antibacterial properties.[2,8] The role of the peptide framework of hydrophobic glycopeptide derivatives in antibacterial activity can be clarified by comparison of the antibacterial activities of hydrophobic derivatives with more extensively degraded peptide cores.

Results

The goal of our research was synthesis and comparison of the antibacterial properties of hydrophobic derivatives of various active antibiotics and those of poorly active glycopeptide degradation products. We compared antibacterial activities of *N*-*n*-decyl- or *N*-[*p*-(*p*-chlorophenyl)benzyl] carboxamides of natural antibiotics (vancomycin **1**, teicoplanin **2**, its aglycon **3**, and eremomycin **4**) with the corresponding carboxamides of poorly active or inactive eremomycin degradation products [des-(*N*-methyl-D-leucyl)eremomycin **5**, eremomycin aglycon **6**, des-(*N*-methyl-D-leucyl)eremomycin aglycon **7**, and the teicoplanine degradation product TB-TPA (Figure 1)].

Des-(*N*-methyl-D-leucyl)eremomycin (**5**),[8] aglycon of eremomycin (**6**),[14] and des-(*N*-methyl-D-leucyl)eremomycin aglycon (**7**)[15] were obtained by the methods previously described. TB-TPA (**8**) was obtained by double Edman degradation of the product of teicoplanin

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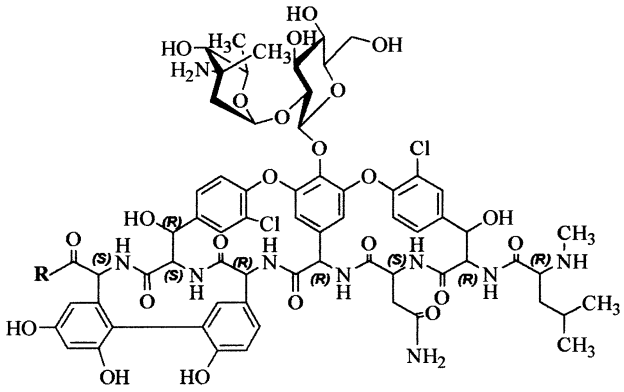
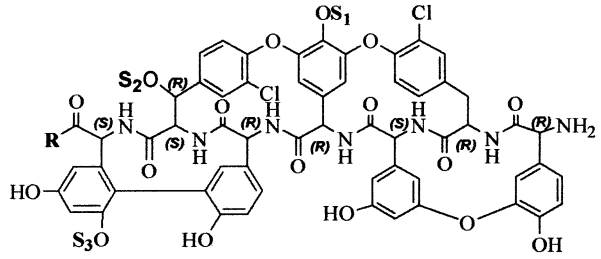
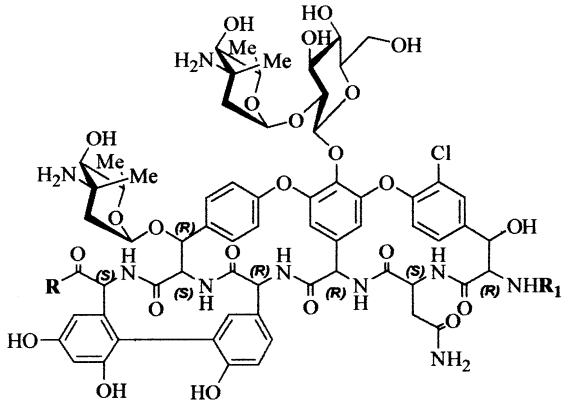


1. R=OH Vancomycin
1a. R=NH-*n*$C_{10}H_{21}$
1b. R=NH-[*p*-(*p*-ClPh)Bn]



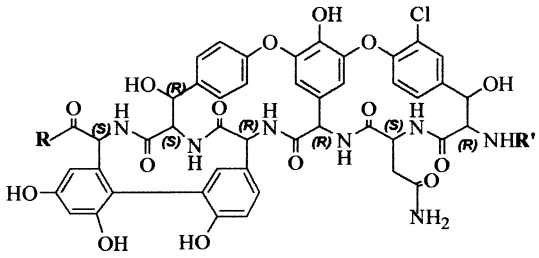
2. R=OH Teicoplanin
S_1=NAcyl-β-D-Gln; S_2=NAc-β-D-Gln; S_3=α-D-Man
2a. R=NH-*n*$C_{10}H_{21}$
2b. R=NH-[*p*-(*p*-ClPh)Bn]

3. R=OH Teicoplanin aglycon
S_1=S_2=S_3=H
3a. R=NH-*n*$C_{10}H_{21}$
3b. R=NH-[*p*-(*p*-ClPh)Bn]



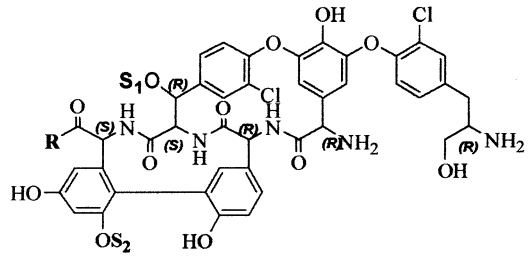
4. R=OH; R'=N-Me-D-Leu Eremomycin
4a. R'=N-Me-D-Leu; R=NH-*n*$C_{10}H_{21}$
4b. R'=N-Me-D-Leu; R=NH-[*p*-(*p*-ClPh)Bn]

5. R=OH; R'= H Des-(N-methyl-D-leucyl) eremomycin
5a. R'=H; R=NH-*n*$C_{10}H_{21}$
5b. R'=H; R=NH-[*p*-(*p*-ClPh)Bn]



6. R=OH; R'=N-Me-D-Leu Eremomycin aglycon
6a. R'=N-Me-D-Leu; R=NH-*n*$C_{10}H_{21}$
6b. R'=N-Me-D-Leu; R=NH-[*p*-(*p*-ClPh)Bn]

7. R=OH; R'=H Des-(N-methyl-D-leucyl) eremomycin aglycon
7a. R'=H; R=NH-*n*$C_{10}H_{21}$
7b. R'=H; R=NH-[*p*-(*p*-ClPh)Bn]



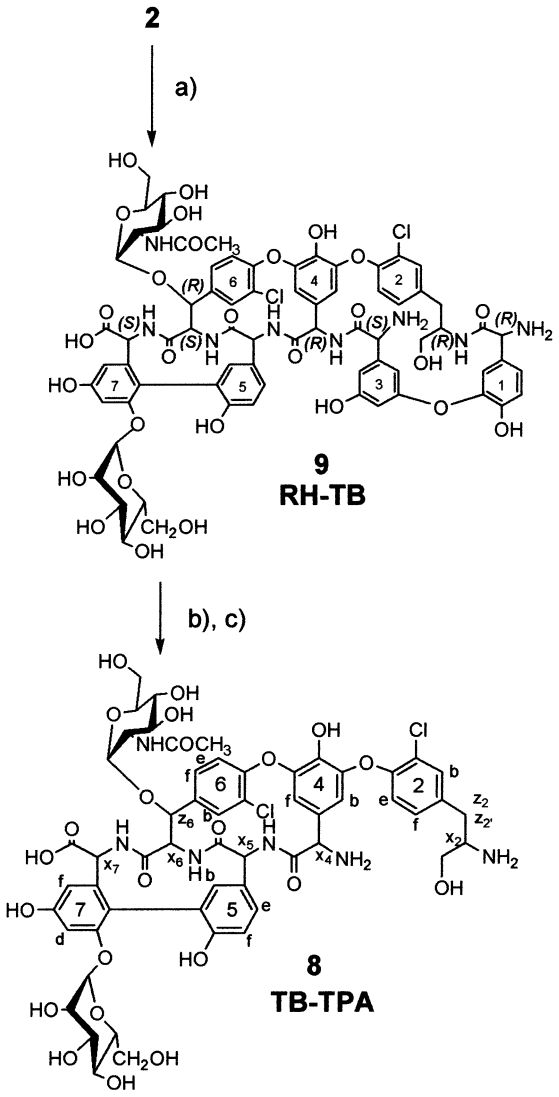
8. R=OH TB-TPA
S_1=NAc-β-D-Gln; S_2= α-D-Man
8a. R=NH-*n*$C_{10}H_{21}$

Figure 1. Glycopeptide antibiotics and their derivatives.

reductive hydrolysis RH-TB [des-(*N*-acylglucosamine)-teicoplanin with the reduced 2,3-peptide bond][16] (Scheme 1). Edman degradation of RH-TB was performed by the method previously described for RH-TD (deglycosylated RH-TB).[17] The structure of TB-TPA was confirmed by ESI-MS and ^{1}H NMR.

Decyl- and *p*-(*p*-chlorophenyl)benzyl amides of eremomycin (**4a** and **4b**)[8] and des-(*N*-methyl-D-leucyl)eremo-

Scheme 1. Degradation of Teicoplanin (**2**) to TB-TPA (**8**)[a]



[a] (a) $NaBH_4$, H_2O/EtOH (65/35), r.t.;[16] (b) PhNCS, Py/H_2O (6/1), Et_3N;[17] (c) TFA/CH_2Cl_2 (1/1).

mycin (**5a** and **5b**)[8] were obtained as previously described. Syntheses of decyl and *p*-(*p*-chlorophenyl)-benzyl amides of vancomycin, teicoplanin, teicoplanin aglycon, eremomycin aglycon, des-(*N*-methyl-D-leucyl)-eremomycin aglycon, and TB-TPA were performed using standard methods.[5] The purity and identity of the compounds obtained were assessed by HPLC and ESI mass spectrometry (Table 1).

Natural glycopeptides are active against glycopeptide sensitive 533 *Staphylococcus epidermidis* and 602 *S. haemolyticus*, with MIC values in the range 0.25−2 μg/mL for vancomycin (**1**), teicoplanin aglycon (**3**), eremomycin (**4**), and 8−16 μg/mL for teicoplanin (**2**). The MIC values against sensitive 568 *Enterococcus faecium* and 559 *E. faecalis* are similar in range (Table 2). Glycopeptide antibiotics are inactive against GRE strains 569 *E. faecium* and 560 *E. faecalis* (MIC > 128 μg/mL). Their activities against glycopeptide intermediate-resistant *S. aureus* (GISA) strains are in the range of 8−16 μg/mL. Decyl- or *p*-[*p*-(chlorophenyl)benzyl] amides of these antibiotics maintain good activity against sensitive bacteria (MIC = 0.13−2 μg/mL) and are also active against GRE (MIC ≤4 μg/mL). Their activity against GISA strains is also improved (MIC ≤4 μg/mL).

Table 1. Properties of Carboxamides of Glycopeptide Antibiotics and Their degradation products

compd	TLC, R_f	HPLC (system) A[a]	HPLC (system) B[b]	molecular formula	molecular weight M calcd	molecular weight $[M+H]^+$ found
1a	0.24	17.55	8.8	$C_{76}H_{97}Cl_2N_{10}O_{23}$	1587.6	1588.3
1b	0.22	16.0	6.9	$C_{79}H_{86}Cl_3N_{10}O_{23}$	1647.5	1648.2
2a	0.25	19.79	5.53	$C_{98}H_{119}Cl_2N_{10}O_{31}$	2000.7	2002.0
2b	0.24	17.12	5.82	$C_{101}H_{107}Cl_3N_{10}O_{31}$	2060.6	1062.3
3a	0.25	20.02	23.28	$C_{68}H_{66}Cl_2N_8O_{17}$	1336.4	1338.0
3b	0.24	18.18	12.8	$C_{71}H_{55}Cl_3N_8O_{17}$	1396.3	1396.7
6a	0.30	19.49	21.44	$C_{63}H_{75}ClN_9O_{16}$	1247.5	1249.2
6b	0.29	20.06	12.22	$C_{66}H_{63}Cl_2N_9O_{16}$	1307.4	1309.0
7a	0.28	19.19	12.13	$C_{56}H_{62}ClN_8O_{15}$	1120.4	1120.8
7b	0.28	19.33	16.49	$C_{59}H_{50}Cl_2N_8O_{15}$	1180.3	1182.5
8	0.03	2.72	2.42	$C_{56}H_{60}Cl_2N_6O_{23}$	1254.3	1255.3
8a	0.15	13.02	4.87	$C_{66}H_{81}Cl_2N_7O_{22}$	1393.5	1394.5

[a] Analyses were performed on a Shimadzu HPLC instrument of the LC 10 series. [b] Analyses were performed on a Milichrom-5 HPLC instrument (Orel, Russia).

Eremomycin with the degraded dipeptide binding pocket (**5**) and eremomycin aglycon (**6**) have poor activity against sensitive strains (MIC = 16−32 μg/mL), but are inactive against GRE and GISA bacteria. However, their hydrophobic amides (**5a**, **5b**, **6a**, **6b**) are almost equally active against both sensitive and resistant microorganisms (MIC ≤ 8 μg/mL). Similar antibacterial activities against both sensitive and resistant strains are demonstrated for hydrophobic carboxamides of inactive des-(*N*-methyl-D-leucyl)eremomycin aglycon (**7a**, **7b**) and *n*-decylamide of TB-TPA (**8a**), but their MIC values are in the range 16−64 μg/mL.

Discussion

In general MIC values of hydrophobic derivatives of poorly active glycopeptide degradation products (**5**−**8**) for sensitive bacteria are close to MIC values for GRE strains in which the -D-Ala-D-Ala moiety in nascent peptidoglycan is substituted by -D-Ala-D-lactate, and GISA strains that overproduce -D-Ala-D-Ala. This demonstrates that the antibacterial activity of these compounds is not based on dipeptide or depsipeptide binding. As the MIC values against resistant and sensitive strains are close, it follows that the mechanism of action may be similar for the resistant and sensitive strains. Earlier it was shown that hydrophobic derivatives of vancomycin[2,9−11] and eremomycin[8] inhibit the peptidoglycan biosynthesis at the transglycosylation stage, most likely due to direct binding to membrane proteins involved in peptidoglycan polymerization.[9]

Since the MIC values for amides of active antibiotics (**1**, **2**, **4**) against sensitive strains are lower than MIC values against resistant strains, it appears that the functional binding pocket is responsible for the strong activity against sensitive strains due to dipeptide binding. We concluded that the activity of these compounds against sensitive strains is based both on the inhibition of membrane proteins involved in peptidoglycan polymerization and the interaction with the -D-Ala-D-Ala peptidoglycan moiety. In GRE strains, in which this moiety is substituted by -D-Ala-D-lactate only one mechanism (transglycosylase and other bacterial membrane protein inhibition) is working.

Table 2. Antibacterial Activity of Glycopeptide Antibiotics and Their Derivatives (MIC, μg/mL)

strain/compound	533 *S. epidermidis*	602 *S. haemolycus*	3797 *S. aureus* (GISA)[a]	3798 *S. aureus* (GISA)[a]	568 *E. faecium* (GSE)[b]	559 *E. faecalis* (GSE)[b]	569 *E. faecium* (GRE)[c]	560 *E. faecalis* (GRE)[c]
vancomycin 1	2	2	16	8	2	1	>128	>128
vancomycin decylamide **1a**	0.5	1	1	1	0.5	0.5	8	8
vancomycin [*p*-(*p*-ClPh)Bn]amide **1b**	1	1	2	2	0.5	0.5	8	8
teicoplanin 2	8	16	16	8	0.25	0.5	>128	>128
teicoplanin decylamide **2a**	0.25	2	1	1	1	1	8	8
teicoplanin [*p*-(*p*-ClPh)Bn]amide **2b**	0.5	4	1	1	1	2	8	16
teicoplanin aglycon 3	0.25	0.25	1	1	0.13	0.13	>128	>128
teicoplanin aglycon decylamide **3a**	0.13	0.5	1	1	0.25	0.5	4	4
teicoplanin aglycon [*p*-(*p*-ClPh)Bn]amide **3b**	0.13	0.13	1	1	0.25	0.25	4	8
eremomycin 4	0.25	0.25	8	8	0.25	0.25	>128	>128
eremomycin decylamide **4a**	0.13	0.13	4	4	0.5	1	2	4
eremomycin [*p*-(*p*-ClPh)Bn]amide **4b**	2	1	4	4	1	2	4	4
des-(*N*-Me-D-Leu)-eremomycin 5	16	16	64	64	16	16	>128	>128
des-(*N*-Me-D-Leu)-eremomycin decylamide **5a**	0.5	1	4	8	2	2	2	4
de-(*N*-Me-D-Leu)-eremomycin [*p*-(*p*-ClPh)Bn]amide **5b**	4	4	8	8	4	4	2	4
eremomycin aglycon 6	32	16	>64	>64	32	16	>128	>128
eremomycin aglycon decylamide **6a**	4	4	4	4	4	4	4	4
eremomycin aglycon [*p*-(*p*-ClPh)Bn]amide **6b**	4	8	8	8	8	8	8	8
de-(*N*-methyl-D-leucyl)-eremomycin aglycon 7	>128	>128	>128	>128	>128	>128	>128	>128
des-(*N*-methyl-D-leucyl)-eremomycin aglycon decylamide **7a**	16	64	32	32	64	64	64	>128
des-(*N*-methyl-D-leucyl)-eremomycin aglycon [*p*-(*p*-ClPh)Bn]amide **7b**	32	64	64	64	32	64	64	64
TB-TPA 8	64	64	>64	>64	32	64	>128	>128
decylamide of TB-TPA **8a**	16	32	32	16	16	16	16	64

[a] GISA = glycopeptide intermediate-resistant *S. aureus*. [b] GSE = glycopeptide susceptible enterococci. [c] GRE = glycopeptide resistant enterococci.

Eremomycin aglycon **6**, as opposed to teicoplanin aglycon **3**, has poor antibacterial activity (MIC ∼ 32−64 μg/mL) although the binding pocket in this compound is not damaged. This may be due to the change of heptapeptide conformation in deglycosylated compound **6** in comparison with the parent antibiotic.[18] Eremomycin aglycon **6** is also less active then vancomycin aglycon (which MIC values against sensitive staphylococci and enterococci are in the range of 4−8 μg/mL). The single difference between eremomycin aglycon and vancomycin aglycon is the absence of chlorine atom in the nucleus of the sixth amino acid. The introduction of hydrophobic substituents into eremomycin aglycon (**6a,b**) and the eremomycin derivative with the damaged binding pocket (**5a,b**) leads to a dramatic increase in activity against both sensitive and resistant bacteria. As these compounds cannot act in the manner of natural glycopeptide by binding -D-Ala-D-Ala, we suppose that they act by inhibiting transglycosylation process, like the other hydrophobic glycopetide derivatives investigated.[2,8−10] The derivatives of inactive glycopeptides derivatives **7** and **8** represent the most interesting examples. A hydrophobic substituent introduced into the damaged glycopeptide molecule (**7a,b** and **8a**) is still able to increase the antibacterial activity, although not to the same extent as in the case of **5a,b**.

Conclusion

As shown earlier, the interaction of a glycopeptide antibiotic with the -D-Ala-D-Ala- pentapeptide target is very strictly controlled by the three-dimensional structure of the binding pocket. Any change in the proximity of the binding pocket leads to a decrease of antibacterial activity. For example, splitting off the *N*-methyl-D-leucine in eremomycin or vancomycin, or the substitution of this amino acid by the 2-amino-4-methylpentyl group in eremomycin, yield inactive compounds.[19]

The structural demand is much less stringent for the specific hydrophobic derivatives of glycopeptide antibiotics. Even compounds with major degradative changes in the peptide backbone (**7a,b** and **8a**) maintain antibacterial activity; however, the more the heptapeptide is destroyed the poorer is the antibacterial activity. Further degradation leads to further decrease of activity. Earlier it was shown that the *p*-(*p*-chlorophenyl)-benzyl derivative of a vancosaminyl-containing disaccharide derivative has weak activity against sensitive and vancomycin resistant enterococci (MIC = 128 μg/mL), and retained the ability to inhibit transglycosylation step of peptidoglycan biosynthesis.[2] 1-[4-(3,4-Dichlorobenzyl)oxybenzyl]-4-acetylputrescin had poor activity against sensitive and resistant strains (MIC = 32−64 μg/mL).[9] It appears that antibacterial activity is influenced by not only the structure of a hydrophobic substituent that interacts with membrane proteins, but also the structure of a glycopeptide carrier or its surrogate. Recently, it was hypothesized that the disaccharide moiety carrying a hydrophobic substituent is a determinant of anti-VanA activity of vancomycin derivatives.[20] In our study, we demonstrate that the hydrophobic derivatives of various glycopeptide aglycones have antibacterial properties. It shows that a

particular carbohydrate structure is not necessary for biological activity against resistant bacterial strains Further research directed to determining the role and structural demands for the framework upon which hydrophobic groups are attached may lead to derivatives possessing the appropriate antibacterial and phamacokinetic properties for clinical use.

Experimental Section

Eremomycin sulfate was produced at the pilot plant of the Gause Institute of New Antibiotics, Moscow. Vancomycin hydrochloride was obtained from Sigma Corporation. Teicoplanin, teicoplanin aglycon, and RH-TB were kindly supplied by Dr. R. Ciabatti and Dr. A. Malabarba (Biosearch S.p.A., Italy). All reagents and solvents were purchased from Aldrich, Fluka, and Merck. *p*-(*p*-Chlorophenyl)benzaldehyde was kindly provided by Advanced Medicine East, Inc (N. J. USA). *p*-(*p*-Chlorophenyl)-benzylamine was obtained by the reduction of *p*-(*p*-chlorophenyl)benzaldehyde oxime with the use of $LiAlH_4$. The progress of the reactions, column eluates, and all final samples were analyzed by TLC using Merck Silica Gel $60F_{254}$ plates in EtOAc/*n*-PrOH/25% NH_4OH (2:1:1) with UV control. Reaction products were purified by reverse-phase chromatography on Merck Silanized Silica Gel (0.063 ~ 0.2 mm).

Analytical reverse phase HPLC was carried out on a Shimadzu HPLC instrument of the LC 10 series on a Diasorb C16 column (4 × 250 mm, particle size 7 μm) at an injection volume of 10 μL and a wavelength 280 nm. The sample concentration was 0.05−0.2 mg/mL. System A comprised 0.1 M $NH_4H_2PO_4$ at pH 3.75 and acetonitrile, the proportion of acetonitrile varied from 15 to 40% for 15 min and then the ratio of acetonitrile was constant during 25 min with flow rate 1.0 mL/min. HPLC was also carried out on a Milichrom-5 HPLC instrument (Orel, Russia) on a Diasorb C16 column (2 × 120 mm, particle size 7 μm). System B comprised 0.2% $HCOONH_4$ and 45% acetonitrile, with flow rate 70 μL/min. The retention times and other characteristics of the compounds obtained are presented in Table 1. ^{1}H NMR spectra were recordered on a Varian VXR-400 spectrometer (USA). Mass spectra were determined by Electrospray ionization (ESI) on a Finnigan SSQ7000 single quadrupole mass spectrometer.

Chemistry

TB-TPA (8). To a solution of RH-TB (**9**) (200 mg, 0.13 mmol) in a mixture of Py/H_2O (6:1, 7 mL), triethylamine (0.94 mL, 13 mmol), and PhNCS (0.08 mL, 0.65 mmol) were added at room temperature under argon. The reaction mixture was stirred for 16 h, then 8 mL of H_2O were added and the reaction evaporated with *n*-BuOH to dryness. The precipitate was dissolved in the mixture of TFA/CH_2Cl_2 (1:1) (3 mL) at 0−5 °C and the mixture was stirred at this temperature for 1 h. Water (3 mL) was then added and the mixture was neutralized with 25% NH_4OH, washed with EtOAc (3 mL × 3), and the aqueous fraction was concentrated in vacuo with the addition of *n*-BuOH and applied to a column of silanized silica gel (2 × 100 mL), previously equilibrated with 0.01 M acetic acid. The column was eluted with acetic acid (0.01 M) at a flow rate of 30 mL/h for elution of compound **8**. Fractions were pooled, concentrated with the addition of *n*-BuOH in vacuo, and acetone (50 mL) was added to yield the precipitate, which was filtered off, washed with acetone, and dried to yield 49 mg (30%) of **8**.

^{1}H NMR (400 MHz, D_2O): δ **2b**, 7.44 (d, J = 2 Hz, 1H); **2e**, 6.94 (d, J = 8 Hz, 1H); **2f**, 7.16 (dd, J = 8 Hz, J = 2 Hz, 1H); **4b**, 6.71 (d, J = 2 Hz, 1H); **4f**, 5.65 (d, J = 2 Hz, 1H); **x4**, 5.08 (s, 1H); **5b**, 7.03 (s, 1H); **5e**, 6.85 (d, J = 5, 1H); **5f**, 6.96 (m, 1H); **x5**, 4.47 (s, 1H); **6b**, 7.67 (s, 1H); **6e**, 7.11 (m, 1H); **6f**, 7.19 (m, 1H); **z6**, 5.49 (s, 1H); **x6**, 4.17 (s, 1H); **7d**, 6.75 (d, J = 2 Hz, 1H); **7f**, 6.55 (d, J = 2 Hz, 1H); **x7**, 4.72 (s, 1H); carbohydrate hydrogen atoms: 2.8−3.8; 4.5; 5.3 (m, 14H); −$NCOCH_3$:1.74 (s, 3H).

***n*-Decylamides of eremomycin aglycon (6a), des-(*N*-methyl-D-leucyl)eremomycin aglycone(7a), TB-TPA (8a) and *p*-(*p*-chlorophenyl)benzylamides of eremomycin aglycon (6b) and of des-(*N*-methyl-D-leucyl)eremomycin aglycon (7b). General Procedure.**[5] To a solution of eremomycin aglycon (**6**) or des-(*N*-methyl-D-leucyl)eremomycin aglycon (**7**) or TB-TPA (**8**) (0.03 mmol) in DMSO (2 mL) hydrochlorides of *n*-decylamine or *p*-(*p*-chlorophenyl)benzylamine (0.3 mmol), Et_3N (0.3 mmol), and HBPyU [*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate] (0.06 mmol) were added at room temperature in three portions with stirring over 1 h. After 4 h acetone (100 mL) was added to give a white solid, the sample was washed with acetone and dried in vacuo to give the corresponding amide in ca. 90% yield.

Determination of Antibacterial Activity. Minimum inhibitory concentrations (MIC) were determined by broth microdilution method using Mueller Hinton broth as recommended by NCCLS procedures. Results were usually identical, and always within 2-fold. The strains tested were kindly provided by Dr. R. Ciabatti and G. Romano from Biosearch Italia S.p.A (Gerenzano, Italy). Resistant strains with the confirmed genotype for vancomycin-resistant enterococci are the same as used in the previously published paper.[21] 533 *S. epidermidis* and 602 *S. haemoliticus* are clinical isolates. GISA strains are 3797 *S. aureus* (GISA HIP-5836 New Jersey) and 3798 *S. aureus* (GISA HIP-5827 Michigan). The samples of the compounds were dissolved in 10% DMSO in water and then diluted by broth. The final concentration of DMSO in the media was from 1.28% (corresponding to antibiotic concentration 128 μg/mL) to 0.0013% (corresponding to antibiotic concentration 0.13 μg/mL).

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References

(1) Malabarba, A.; Nicas, T. I.; Thompson, R. S. Structural Modifications of Glycopeptide Antibiotics. *Med. Res. Rev.* **1997**, *17*, 69−137.
(2) Ge, M.; Chen, Zh.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. Vancomycin Derivatives That Inhibit Peptidoglycan Synthesis Without Binding D-Ala-D-Ala. *Science* **1999**, *284*, 507−511.
(3) Pavlov, A. Y.; Preobrazhenskaya, M. N. Chemical Modification of Glycopeptide Antibiotics. *Russ. J. Bioorg. Chem.* **1998**, *24*, 570−587.
(4) Olsufyeva, E. N.; Berdnikova, T. F.; Miroshnikova, O. V.; Reznikova, M. I.; Preobrazhenskaya, M. N. Chemical Modification of Antibiotic Eremomycin at the Asparagin Side Chain. *J. Antibiot.* **1999**, *52*, 319−324.
(5) Miroshnikova, O. V.; Printsevskaya, S. S.; Olsufyeva, E. N.; Pavlov, A. Y.; Nilius, A.; Hensey-Rudloff, D.; Preobrazhenskaya, M. N. Structure−Activity Relationships in the Series of Eremomycin Carboxamides. *J. Antibiot.* **2000**, *53*, 286−293.
(6) Cooper, R. D. G.; Snyder, N. J.; Zweifel, M. J.; Staszak, M. A.; Rodrigues, M. J.; Huff, B. E.; Thompson, R. C. Reductive Alkylation of Glycopeptide Antibiotics: Synthesis and Antibacterial Activity. *J. Antibiot.* **1996**, *49*, 575−581.
(7) Nicas, T. I.; Mullen, D. L.; Flokowitsch, J. E.; Preston, D. A.; Snyder, N. J.; Zweifel, M. J.; Wilkie, S. C.; Rodriguez, M. J.; Thompson, R. C.; Cooper R. D. G. Semisynthetic glycopeptide antibiotics derived LY264826 active against vancomycin resistant enterococci. *Antimicrob. Agents Chemother.* **1996**, *40*, 2194−2199.
(8) Printsevskaya, S. S.; Pavlov, A. Y.; Olsufyeva, E. N.; Mirchink, E. P.; Isakova, E. B.; Reznikova, M. I.; Goldman, R. C.; Branstrom, A. A.; Baizman, E. R.; Longley, C. B.; Sztaricskai, F.; Batta, G.; Preobrazhenskaya, M. N. Synthesis and Mode of Action of Hydrophobic Derivatives of the Glycopeptide Antibiotic Eremomycin and des-(*N*-methyl-D-leucyl)eremomycin Against Glycopeptide-Sensitive and Resistant Bacteria. *J. Med. Chem.* **2002**, *45*, 1340−1347.
(9) Roy, R. S.; Yang, P.; Kodali, S.; Xiong, Y.; Kim, R. M.; Griffin, P. R.; Onishi, R,; Kohler, J.; Silver, L. L.; Chapman, K. Direct Interaction of a Vancomycin Derivative with Bacterial Enzymes Involved in Cell Wall Biosynthesis. *Chem. Biol.* **2001**, *8*, 1095−1106.

(10) Goldman, R. C.; Baizman, E. R.; Longley, C. B.; Branstrom, A. A. Chlorobiphenyl-Desleucyl-Vancomycin Inhibits the Transglycosylase Required for Peptidoglycan Synthesis in Bacteria. *FEMS Microb. Lett.* **2000**, *183*, 209−214.
(11) Eggert, U. S.; Ruiz, N.; Falcone, B. V.; Branstrom, A. A.; Goldman, R. C.; Silhavy, T. J.; Kahne, D. Genetic Basis for Activity Differences Between Vancomycin and Glycolipid Derivatives of Vancomycin. *Science* **2001**, *294*, 361−366.
(12) Sun, B.; Chen, Z.; Eggert, U. S.; Shaw, S. J.; LaTour J. V.; Kahne, D. Hybrid Glycopeptide Antibiotics. *J. Am. Chem. Soc.* **2001**, *123*, 12722−12723.
(13) Allen, N. E.; LeTourneau, D. L.; Hobbs, J. N. The Role of Hydrophobic Side Chains as Determinants of Antibacterial Activity of Semisynthetic Glycopeptide Antibiotics. *J. Antibiot.* **1997**, *50*, 677−684.
(14) Berdnikova, T. F.; Lomakina, N. N.; Olsufyeva, E. N.; Alexandrova, L. G.; Potapova, N. P.; Rozinov, B. V.; Malkova, I. V.; Orlova, G. I. Structure and Antimicrobial Activity of Products of Partial Degradation of Antibiotic Eremomycin. *Antibiot. Chemother. (Russ.)* **1991**, *36*, 28−31.
(15) Miroshnikova, O. V.; Berdnikova, T. F.; Olsufyeva, E. N.; Pavlov, A. Y.; Reznikova, M. I.; Preobrazhenskaya, M. N.; Ciabatti, R.; Malabarba, A.; Colombo, L. A Modification of the *N*-Terminal Amino Acid in the Eremomycin Aglycone. *J. Antibiot.* **1996**, *49*, 1157−1161.
(16) Malabarba, A.; Ciabatti, R.; Kettenring, J.; Ferrari, P.; Vekey, K.; Bellasio, E.; Denaro, M. Structural Modifications of the Active Site in Teicoplanin and Related Glycopeptides. 1. Reductive Hydrolysis of the 1,2- and 2,3-Peptide Bonds. *J. Org. Chem.* **1996**, *61*, 2137−2150.
(17) Malabarba, A.; Ciabatti, R.; Maggini, M.; Ferrari, P.; Vekey, K.; Colombo, L.; Denaro, M. Structural Modifications of the Active Site in Teicoplanin and Related Glycopeptides. 2. Deglucoteicoplanin-Derived Tetrapeptide. *J. Org. Chem.* **1996**, *61*, 2151−2157.
(18) Kaplan, J.; Korty B. D.; Axelsen P. H.; Loll, P. J. The Role of Sugar Residues in Molecular Recognition by Vancomycin. *J. Med. Chem.* **2001**, *44*, 1837−1840.
(19) Printsevskaya, S. S.; Olsuf'eva, E. N.; Lazhko, E. I.; Preobrazhenskaya, M. N. Antibiotic *N′N″*-Dibenzyleremomycin with Reduced 1,2-Peptide Bond. *Russ. J. Bioorg. Chem.* **2002**, *28*, 65−73.
(20) Chen, Z.; Eggert, U. S.; Shaw, S. S.; Sun, B.; LaTour, J. V.; Kahne, D. *Tetrahedron* **2002**, *58*, 6585−6594.
(21) Pavlov, A. Y.; Preobrazhenskaya, M. N.; Malabarba, A.; Ciabatti, R.; Colombo, L. Mono and Double Modified Teicoplanin Aglycon Derivatives on the Amino Acid No. 7; Structure−Activity Relationship. *J. Antibiot.* **1998**, *51*, 73−81.

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