Structure−Activity Relationship Studies of a Series of Antiviral and Antibacterial Aglycon Derivatives of the Glycopeptide Antibiotics Vancomycin, Eremomycin, and Dechloroeremomycin

Svetlana S. Printsevskaya,[#] Svetlana E. Solovieva,[#] Eugenia N. Olsufyeva,[#] Elena P. Mirchink,[#] Elena B. Isakova,[#] Erik De Clercq,[†] Jan Balzarini,[†] and Maria N. Preobrazhenskaya*[,#]

Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, B. Pirogovskaya 11, Moscow 119021, Russia, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

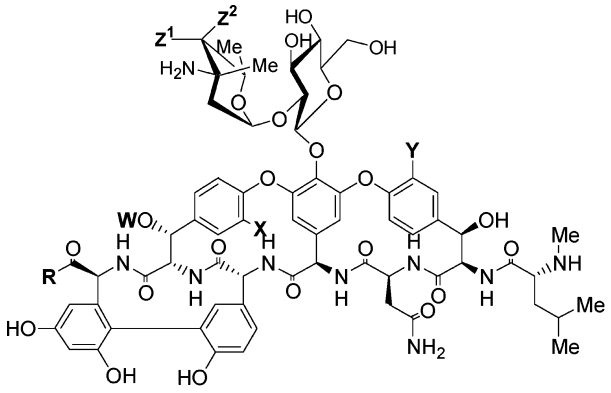
Received January 28, 2005

N-(Adamantyl-1)methyl, *N*-(adamantyl-2), and *N*-(ω-aminodecyl) amides of vancomycin, eremomycin, and dechloroeremomycin aglycons and their des-(*N*-Me-D-Leu) derivatives were synthesized and their antibacterial and anti-HIV activities were investigated. Carboxamides with an intact peptide core demonstrated activity against glycopeptide-susceptible and -resistant bacteria (1−32 μM). *N*-(Adamantyl-1)methylcarboxamide of eremomycin aglycons had good antiretroviral activity (1.6 μM against HIV-1). Compounds with destroyed peptide core [des-(*N*-Me-D-Leu)-aglycon amides] were inactive against both glycopeptide-sensitive and -resistant bacteria. (Adamantyl-1)methylamide of des-(*N*-Me-D-Leu)-eremomycin aglycon had good antiretroviral activity (EC_{50} of 5.5 μM for HIV-1 and 3.5 μM for HIV-2). (Adamantyl-1)-methylamides of eremomycin aglycon and its des-(*N*-Me-D-Leu)-derivative are the most promising and selective antiretroviral agents. Their ability to induce bacterial resistance to glycopeptide antibiotics during prolonged administration may be expected to be very low or absent. This might make the use of these derivatives feasible in the prolonged therapy or prophylaxis of HIV infections.

Introduction

Glycopeptide antibiotics (vancomycin, teicoplanin) are used worldwide for the treatment of infections caused by Gram-positive bacteria. There is an intensive search for novel antibacterial agents among semisynthetic derivatives of glycopeptides that exhibit enhanced antibacterial activity and improved pharmacological characteristics.[1] The antibacterial properties of these glycopeptide antibiotics (Figure 1) are based on the inhibition of bacterial peptidoglycan biosynthesis by the interaction with the D-Ala-D-Ala-containing pentapeptide precursors of nascent bacterial peptidoglycan.[2] It was shown earlier that some hydrophobic glycopeptide derivatives are active against glycopeptide-resistant enterococci and act in a manner different from that of parent antibiotics. Specific hydrophobic derivatives of eremomycin and vancomycin demonstrate antibacterial activity despite the absence of binding to D-Ala-D-Ala or D-Ala-D-lactate.[3−7] The latter depsipeptide fragment substitutes for the peptide fragment D-Ala-D-Ala in glycopeptide-resistant enterococci (GRE).

The antiretroviral activity of semisynthetic hydrophobic derivatives of antibacterial glycopeptides of the vancomycin and teicoplanin groups was recently discovered.[8] Natural antibacterial antibiotics are not active against HIV-1 and HIV-2, but their derivatives containing hydrophobic substituents of definite types demonstrate pronounced anti-HIV activity. Preliminary experiments strongly suggest that the inhibition of viral entry is the most likely molecular event responsible for the anti-HIV activity of this type of compound (J. Balzarini et al., unpublished data). The introduction of a hydrophobic substituent is beneficial for antibacterial and antiviral activity. However, the presence of carbohydrate moieties in these antibiotics usually results in a dramatic decrease of the anti-HIV activity,[8] whereas for antibacterial activity the presence of sugars in vancomycin or eremomycin represents a critical determinant.[9,10] The removal of the first amino acid in vancomycin or eremomycin (degradation of the binding



1. Vancomycin
R=OH; X=Y=Cl; Z^1=OH; Z^2=H; W = H
2. Eremomycin
R = OH; X = H; Y = Cl; Z^1= H; Z^2= OH; W = eremosaminyl-α
3. Dechloroeremomycin
R = OH; X = Y = H; Z^1 = H; Z^2 = OH; W = eremosaminyl-α

Figure 1. Glycopeptide antibiotics of the vancomycin group.

* To whom correspondence should be addressed. Phone: (7095) 245-37-53. Fax: (7095) 245-02-95. E-mail: mnp@space.ru.
[#] Gause Institute of New Antibiotics.
[†] Katholieke Universiteit Leuven.

10.1021/jm0500774 CCC: $30.25
Published on Web 05/05/2005

pocket) dramatically reduces antibacterial activity against sensitive Gram-positive bacteria.[6,7,9] The removal of chlorine in the aromatic ring of amino acid 2 in dechloroeremomycin diminishes the antibacterial activity by 2- to 10-fold.[9,10]

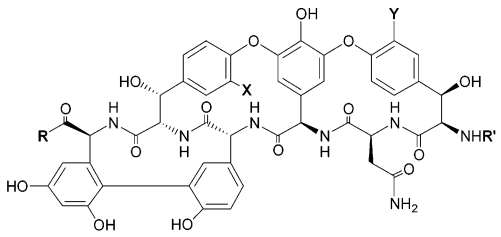
The use of antiretroviral agents possessing concomitant antibacterial activity might be dangerous because it can lead to shifts in bacterial populations and induction of bacterial resistance to glycopeptide antibiotics. It means that for this class of compounds to be used as antiretroviral agents the absence of antibacterial activity is preferable.

Earlier we have shown that *n*-decylamide and *p*-(chlorophenyl)benzylamide of eremomycin aglycon and similar amides of eremomycin aglycon with the destroyed peptide core still possess antibacterial activity (8−16 μg/mL) against sensitive Gram-positive bacteria and GRE.[7] They were also rather cytotoxic for eukaryotic cells. However, some glycopeptide derivatives with (adamantyl-2)amino and ω-aminodecylamino substituents possessed good anti-HIV-1 and HIV-2 activity without being cytotoxic.[8] Adamantane derivatives such as rimantadine are broadly employed as antiviral and neurological drugs, i.e., against Parkinson's disease. Recently it was shown that an adamantyl-containing derivative of globotriaosylceramide (ada-Gb_3), a semisynthetic analogue of Gb_3 in which the fatty acid chain is replaced with a rigid globular hydrocarbon frame (adamantane), has high affinity for the HIV-1 surface envelope glycoprotein gp 120.[11] The potential drawback of semisynthetic glycopeptide antibiotics containing a hydrophobic substituent is that they may bind to serum albumin, resulting in a serious decrease or even total loss of biological activity in the presence of serum. Interestingly it was demonstrated for ada-Gb_3 that serum albumin did not bind to this modified analogue of globotriaosylceramide and did not prevent the binding of ada-Gb_3 to its target.[11] These data made the modification of glycopeptide antibiotic derivatives by the introduction of adamantyl substituents worth pursuing.

The aim of this research was to study vancomycin-type aglycon derivatives and to select the compounds with the highest antiviral activity and no antibacterial properties. We synthesized the hydrophobic carboxamides of aglycons of the vancomycin group of antibiotics containing aminoadamantanes or ω-aminodecylamine substituents and compared their antibacterial and antiviral properties. We obtained *N*-(adamantyl-1)-methyl, *N*-(adamantyl-2), and *N*-(ω-aminodecyl) carboxamides of vancomycin, eremomycin, and dechloroeremomycin aglycons and investigated their antibacterial, anti-HIV-1, anti-HIV-2, and cytostatic activities in cell culture (Figure 2, Table 1 in Supporting Information, and Tables 2 and 3). To obtain novel derivatives that are devoid of antibacterial activity and that cannot induce resistance to vancomycin and related antibacterial antibiotics, we studied the amides of the antibiotics with a destroyed peptide core. To achieve this goal, we synthesized des-(*N*-Me-D-Leu)-aglycons of vancomycin, eremomycin, and dechloroeremomycin.

Chemistry

Vancomycin **1**, containing a disaccharide branch, and eremomycin **2**, containing both a disaccharide and a monosaccharide branch, differ also by the number of chlorine substituents (two chlorine atoms in the vancomycin molecule and a single chlorine atom in the eremomycin molecule (Figure 1)). Dechloroeremomycin **3** (orienticin C), which contains no chlorine substituents on the aromatic rings, was obtained by dechlorination of eremomycin over 5% Pd/C.[10] Antibiotic aglycons **4**−**6** were obtained by acidic hydrolysis of the corresponding antibiotics **1**−**3** using the methods described (1 N HCl, 70 °C, 5 min for **1** and concentrated HCl, 20 °C, 4 h for **2** or **3**)[10,12] and purified by ion-exchange column chromatography[8] in 45−50% yields. Eremomycin aglycon **5** was also obtained by deglycosylation of eremomycin in anhydrous HF in the presence of anisole in 95% yield. The des-(*N*-Me-D-Leu)-aglycons of vancomycin **7**, eremomycin **8**, and dechloroeremomycin **9** were prepared by cleaning the first amino acid from aglycons by Edman degradation.[13] Carboxamides were obtained by the condensation of antibiotic aglycons with hydrochlorides of (adamantyl-1)methyl, adamantyl-2, and ω-aminodecylamines, using PyBOP or HBPyU as condensing reagents, in yields of about 90%, as described earlier.[7,14] Alternative methods for the synthesis of carboxamides of eremomycin aglycon and des-(*N*-Me-D-Leu)-eremomycin aglycon (compounds **5a** and **8a**) were developed. The terminal amino group of eremomycin aglycon was blocked by a Boc group. The reaction of *N*-Boc-eremomycin aglycon with 1-methylaminoadamantane hydrochloride using DPPA in the presence of triethylamine followed by deblocking of the Boc group (TFA/CH_2Cl_2) led to amide **5a** in a 66% yield. A method of preparation of des-(*N*-Me-D-Leu)-eremomycin aglycon amide **8a** was developed starting from the eremomycin aglycon. Primarily *N*-(phenylaminothiocarbonyl)eremomycin aglycon **10** was synthesized by the reaction with phenyl isothiocyanate (the first step of Edman degradation). Then (adamantyl-1)methylamide of *N*-(phenylaminothiocarbonyl)eremomycin aglycon **11** was obtained using



4. R=OH; X=Y=Cl; R'=*N*-Me-*D*-Leu. **Vancomycin aglycon**
4a. R=(Adam-1)CH_2NH; **4b**. R=(Adam-2)NH; **4c**. R=$H_2N(CH_2)_{10}$NH
5. R=OH; X=H; Y=Cl; R'=*N*-Me-*D*-Leu. **Eremomycin aglycon**
5a. R=(Adam-1)CH_2NH; **5b**. R=(Adam-2)NH; **5c**. R=$H_2N(CH_2)_{10}$NH
6. R=OH; X=Y=H; R'=*N*-Me-*D*-Leu. **Dechloroeremomycin aglycon**
6a. R=(Adam-1)CH_2NH; **6b**. R=(Adam-2)NH ; **6c**. R=$H_2N(CH_2)_{10}$NH
7. R=OH; X=Y=Cl; R'=H. **Des-(*N*-methyl-*D*-leucyl)-vancomycin aglycon**
7a. R=(Adam-1)CH_2NH; **7b**. R=$H_2N(CH_2)_{10}$NH
8. R=OH; X=H; Y=Cl; R'=H. **Des-(*N*-methyl-*D*-Leu)-eremomycin aglycon**
8a. R=(Adam-1)CH_2NH ; **8b**. R=(Adam-2)NH ; **8c**. R=$H_2N(CH_2)_{10}$NH
9. R=OH; X=Y=H; R'=H. **Des-(*N*-methyl-*D*-Leu)-dechloroeremomycin aglycon**
9a. R=(Adam-1)CH_2NH ; **9b**. R=$H_2N(CH_2)_{10}$NH

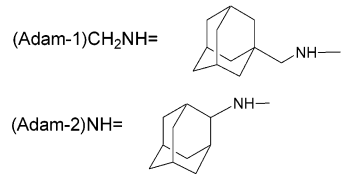
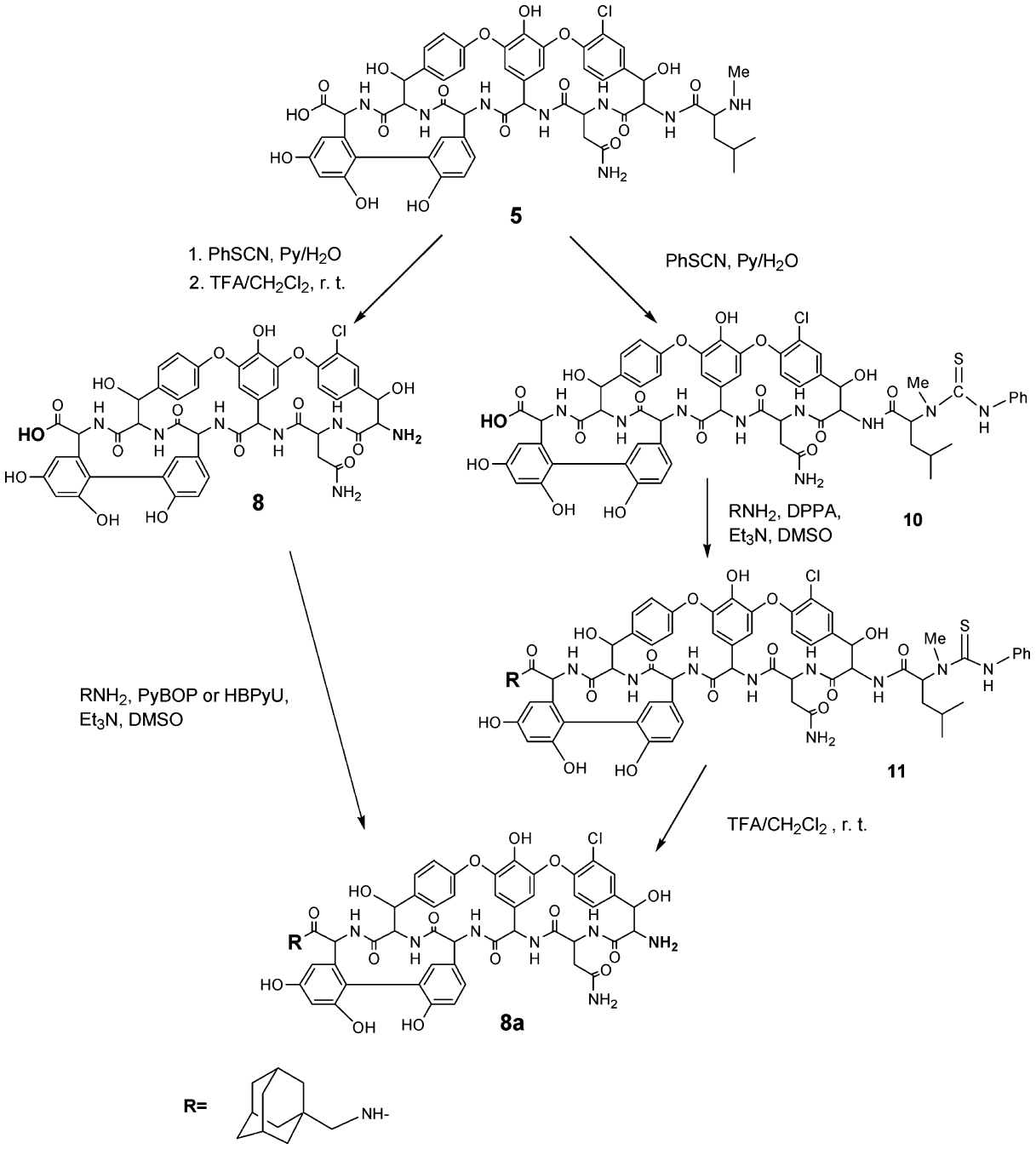


Figure 2. Amides of the glycopeptide antibiotic aglycons.

Scheme 1. Pathways of the Synthesis of **8a**



DPPA. Removal of the modified first amino acid of the peptide core (TFA/CH_2Cl_2) from this compound resulted in amide **8a** in a yield of 65% (Scheme 1). The purity and identity of the compounds obtained were assessed by HPLC and MALDI mass spectrometry (Table 1 in Supporting Information).

Results and Discussion

Removing carbohydrate moieties from glycopeptides of the vancomycin group led to a significant decrease or loss of antibacterial activity. Though the binding pocket of the aglycon was not altered, vancomycin aglycon **4** (MIC = 4−8 μg/mL) was less active than vancomycin (MIC = 1−2 μg/mL) against sensitive bacteria. Eremomycin aglycon **5**, which differs from **4** only by the absence of one chlorine substituent, was about 8-fold less antibacterially active than vancomycin aglycon (MIC of ~16 to >64 μg/mL). Dechloroeremomycin aglycon **6** had no antibacterial activity at concentrations below 32−64 μg/mL. Sugar substituents in the glycopeptide molecules stabilize the rigid conformation of the peptide core required for the interaction with the bacterial target D-Ala-D-Ala of peptidoglycan. Besides participating in antibiotic dimerization, the aminosugars are cooperative with binding D-Ala-D-Ala residues.[1] Removal of the sugars resulted therefore in a destabilization of the rigid heptapeptide conformation and a marked decrease of antibacterial activity. Another factor influencing the conformation of the antibiotic aglycons was the presence of the chlorine substituents in the nuclei of amino acids 2 and 6, which stabilized the conformation of the fragment consisting of three sub-

Table 2. Antibacterial and Antiviral Activities of Glycopeptide Aglycons and Their Derivatives[a]

	MIC (μg/mL)								EC_{50} (μM)		
compd	533 *S. epidermidis*	602 *S. haemolyticus*	3797 *S. aureus* (GISA)	3798 *S. aureus* (GISA)	568 *E. faecium* (GSE)	559 *E. faecalis* (GSE)	569 *E. faecium* (GRE)	560 *E. faecalis* (GRE)	HIV-1	HIV-2	MSV
					Vancomycin Aglycon						
4	4	8	4	4	4	2	>64	>64	65 ± 7.1	≥250	100
4a	2	2	2	2	2	1	16	16	3.0 ± 0	9.5 ± 3.5	10 ± 3
4b	4	4	4	4	2	2	32	32	3.0 ± 0	8.5 ± 2.1	13 ± 4
4c	2	1	2	2	2	0.5	16	16	2.5 ± 0.7	18.3 ± 5.8	5.0 ± 4.4
					Eremomycin Aglycon						
5	32	16	>64	>64	32	16	>128	>128	50 ± 28.5	≥250	>100
5a	8	8	8	8	8	4	8	8	1.6 ± 0.36	7 ± 0	12 ± 0.0
5b	16	16	16	16	16	8	32	32	8.5 ± 2.1	20 ± 7.1	>4
5c	8	8	8	8	8	8	32	32	8.5 ± 2.1	25 ± 0	>4
					Dechloroeremomycin Aglycon						
6	32	>32	>32	>32	32	32	>64	>64	>125	>125	≥100
6a	8	16	8	16	4	8	16	16	8.5 ± 2.1	12 ± 0	11 ± 2.0
6b	16	16	16	16	8	16	32	32	8.5 ± 2.1	21.7 ± 5.8	14 ± 3.0
6c	16	16	16	16	8	16	64	32	15 ± 0	>25	14 ± 5.0
					des-(*N*-Me-D-Leu)-vancomycin Aglycon						
7	>128	>128	>128	>128	>128	>128	>128	>128	≥125	≥125	88 ± 2.0
7a	>32	>32	>32	>32	>64	>64	>64	>64	20 ± 7.1	30 ± 7.1	27 ± 22
7b	>32	>32	>32	>32	>64	>64	>64	>64	≥25	≥25	9.3 ± 3.8
					des-(*N*-Me-D-Leu)-eremomycin Aglycon						
8	>128	>128	>128	>128	>128	>128	>128	>128	115 ± 21.2	>250	>100
8a	>32	>32	>32	>32	>64	>64	>64	>64	5.5 ± 0	3.5 ± 2.1	ND
8b	>32	>32	>32	>32	>64	>64	>64	>64	50 ± 0	50 ± 0	22 ± 7.0
8c	>32	>32	>32	>32	>64	>64	>64	>64	≥25	≥25	>4
					des-(*N*-Me-D-Leu)-dechloroeremomycin Aglycon						
9	>128	>128	>128	>128	>128	>128	>128	>128	ND	ND	ND
9a	>32	>32	>32	>32	>64	>64	>64	>64	30 ± 7.1	25.7 ± 7.5	2.6 ± 1.2
9b	>32	>32	>32	>32	>64	>64	>64	>64	>25	>25	7.8 ± 3.3

[a] GISA: glycopeptide intermediate-resistant *S. aureus*. GSE: glycopeptide susceptible enterococci. GRE: glycopeptide resistant enterococci. ND: no data.

stituted benzene rings in amino acids 2, 4, and 6. The absence of these chlorine atoms resulted in a disturbance of the binding pocket for the D-Ala-D-Ala moiety and subsequent decrease of antibacterial activity (Table 2). Introduction of an adamantyl or aminodecyl substituent into the aglycon antibiotic derivatives led to an increase of antibacterial activity. The derivatives of the vancomycin aglycon **4a**−**c** were active against vancomycin-sensitive and vancomycin-resistant bacteria with MIC values of 0.5−2 and 16−32 μg/mL, respectively. Amides of eremomycin aglycon **5a**−**c** and dechloroeremomycin aglycon **6a**−**c** are equally active against vancomycin-sensitive and vancomycin-resistant bacteria (MIC = 4−32 μg/mL), eremomycin aglycon amides **5a**−**c** being slightly more active than the dechloroeremomycin aglycon amides **6a**−**c** (Table 2). The difference between MIC values of vancomycin aglycon derivatives (0.5−4 μg/mL) and eremomycin and dechloroeremomycin aglycon derivatives (8−16 μg/mL) against vancomycin-sensitive bacteria may be explained by stronger binding of the glycopeptide molecule with D-Ala-D-Ala, contributing to the antibacterial activity.[7] The anti-GRE activities of the vancomycin-type aglycon derivatives are very similar. Anti-GRE activity of hydrophobic glycopeptide derivatives is not connected with the binding to D-Ala-D-Ala and-D-Ala-D-lactate of nascent bacterial peptidoglycan and strongly depends on the presence of a hydrophobic substituent of a definite size.[7] Aglycons with destroyed peptide cores **7**−**9** and their amides were inactive against both vancomycin-sensitive and -resistant bacteria at concentrations below 32 μg/mL.

The investigation of antiretroviral activity demonstrated that amides with an (adamantyl-1)methyl substituent **4a**, **5a**, and **6a** are, as a rule, equally if not more active than compounds with adamantyl-2 substituents (**4b**, **5b**, **6b**) and ω-aminodecyl substituents (**4c**, **5c**, **6c**) (Table 2). Vancomycin aglycon derivatives **4a**−**c** were generally more active than eremomycin aglycon **5b**,**c** and dechloroeremomycin aglycon **6a**−**c** derivatives except for the (adamantyl-1)methylamide of eremomycin aglycon **5a**, which was the most active compound within this series (EC_{50} of 1.6 μM against HIV-1 and of 7 μM against HIV-2). (Adamantyl-1)methylamides of des-(*N*-Me-D-Leu)-aglycons (**7a**, **8a**, **9a)** were less active against HIV-1 and HIV-2 than the corresponding derivatives **4a**, **5a**, **6a** with an intact peptide core. The most active was the (adamantyl-1)methylamide of des-(*N*-Me-D-Leu)-eremomycin aglycon **8a** (EC_{50} = 5.5 μM). This compound is of special interest because it is devoid of any antibacterial activity, keeping its pronounced anti-HIV activity. (Adamantyl-2)amides **7b**, **8b**, and **9b** and ω-aminodecylamides **7c**, **8c**, **9c** had rather moderate anti-HIV activity ($IC_{50} \geq 25$ μM).

There was in general a fairly good correlation between the anti-HIV activity of the test compounds and their inhibitory activity against Moloney sarcoma virus (MSV) induced transformation of murine C3H/3T3 embryo fibroblasts. As a rule, the most active anti-HIV compounds also showed the most pronounced anti-MSV activity in cell culture (i.e., compounds **4a**−**c**, **5a**, **6a**, **7a**, **9a**, **9b**) (Table 2).

Table 3. Cytostatic Activity of Glycopeptide Aglycons and Their Derivatives

compd	IC_{50} (μM)[a] L1210	Molt4/C8	CEM	MCC[b] (μg/mL) C3H/3T3
4	>500	>500	>500	>100
4a	≥250	72 ± 15	≥250	≥100
4b	≥250	≥250	>250	100
4c	>250	228 ± 32	≥250	≥20
5	>500	>500	>500	>100
5a	94 ± 15	126 ± 11	148 ± 3	100
5b	>250	>250	>250	20
5c	≥250	196 ± 76	202 ± 25	20
6	>250	>250	>250	≥100
6a	58 ± 18	44 ± 1	165 ± 60	100
6b	129 ± 11	188 ± 4	185 ± 92	100
6c	146 ± 2	106 ± 9	109 ± 21	100
7	>250	>250	≥250	>100
7a	≥250	178 ± 18	≥250	≥100
7b	>250	≥250	139 ± 10	100
8	> 250	>250	>250	≥ 100
8a	175 ± 37	>250	>250	ND
8b	>250	>250	>250	≥100
8c	≥250	≥250	124 ± 19	20
9	ND	ND	ND	ND
9a	>250	≥250	≥250	100
9b	≥250	144 ± 37	106 ± 14	≥20

[a] Concentration required to inhibit L1210, Molt4/C8, or CEM cell proliferation by 50%. [b] Minimal cytotoxic concentration to cause an alteration in C3H/3T3 cell morphology.

When evaluated for their inhibitory activity against murine (L1210) leukemia or human (Molt4/C8 and CEM) lymphocyte cell proliferation or cytotoxicity against murine C3H/3T3 embryo fibroblasts, none of the antibiotic derivatives demonstrated a marked cytostatic or cytotoxic activity (Table 3). In virtually all cases, cell proliferation was poorly or not at all inhibited at a compound concentration as high as 100−250 μM, that is, at a concentration that is markedly higher than that required to display antiviral activity. Therefore, the antibiotic aglycon derivatives shown in this study should be considered as selective anti-HIV agents.

Conclusion

Changes that do not disturb the binding pocket of the antibiotics of the vancomycin group (e.g., amidation of the carboxylic group) may lead to principal changes in the biological activity; introduction of a hydrophobic substituent makes them active against glycopeptide-resistant strains and deglycosylation, and introduction of a hydrophobic substituent renders them antivirally active. These observations suggest that it is possible to change the properties of a glycopeptide to interact with different receptors by chemical modification. Modification of the glycopeptide aglycon leads to decreased antibacterial activity; however, interestingly, it influences the antiretroviral properties to a much lesser extent. (Adamantyl-1)methylamides of eremomycin aglycon **5a** and des-(*N*-Me-D-Lys)-eremomycin aglycon **8a** demonstrate pronounced anti-HIV activity, while being poorly active (**5a**) or even completely inactive (**8a**) against Gram-positive bacteria. Because they cannot interact with the molecular target of the antibacterial glycopeptides (D-Ala-D-Ala of the bacterial peptidoglycan), their ability to induce resistance to glycopeptides during prolonged administration may be expected to be very low or even absent. Therefore, these novel glycopeptide aglycon derivatives should become promising candidate drugs to be explored further for the treatment and/or prophylaxis of HIV infections.

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 (St. Louis, MO). All reagents and solvents were purchased from Aldrich (Milwaukee), Fluka (Deisenhofen, Germany), and Merck (Darmstadt, Germany).

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, 1:1:1 (system B1) or 3:3:4 (system B2). Evaporations were performed in vacuo using a Büchi rotor evaporator. The precipitates thus obtained were dried in vacuo. For reverse-phase column chromatography, Merck silanized silica gel (0.063−0.2 mm) columns were used with a Uvicord 2138 detector supplied with a model 2065 (LKB, Sweden) recorder. For ion-exchange column chromatography, CM 32 carboxymethyl cellulose (Whatman, Great Britain) and Dowex 50 × 2 resin (H^+-form) were used. Analytical reversed-phase HPLC was carried out on a Shimadzu HPLC instrument on a LC-10 series (Japan) using a Diaspher C 18 column (4 mm × 250 mm, particle size 5 μm) at an injection volume of 10 μL and a wavelength of 280 nm. The sample concentration was 0.05−0.2 mg/mL. Three systems were used to control the identity of the final compounds. System A1 comprised 0.2% $HCOONH_4$ and acetonitrile, with a gradient of 10% → 40% for 30 min with a flow rate of 1.0 μL/min. System A2 comprised 0.2% $HCOONH_4$ and acetonitrile, with a gradient of 30% → 70% for 30 min with a flow rate of 1.0 μL/min. System A3 comprised 1% $H_2PO_4NH_4$ at pH 3.75 and acetonitrile, with a gradient of 5% → 40% for 25 min, where the ratio of acetonitrile was constant for 15 min with a flow rate of 1.0 mL/min. The retention times and other characteristics of the compounds obtained are presented in Table 1 in Supporting Inforamtion. MALDI mass spectra were recorded on a MALDI-MS Vision 2000 instrument (U.K.).

Chemistry. Eremomycin Aglycon 5. Anhydrous HF (10 mL) was condensed into the reaction vessel containing eremomycin sulfate (50 mg, 30 μmol) and anisole (0.5 mL) at −78 °C. The reaction mixture was warmed to −5 °C and stirred at this temperature for 1.2 h. Then HF and anisole were removed in a vacuum at 0 °C over 60 min. Addition of EtOAc−MeOH, 4:1 (10 mL) to the residue led to a light-pink precipitate, which was filtrated off and washed by EtOAc−MeOH, 4:1, three times. After the sample was dried, eremomycin aglycon **5** (32 mg, 95%) was obtained as a white solid with 87% purity (HPLC).

Synthesis of the Carboxamides 4a−c, 5a−c, 6a−c, 7a,b, 8a−c, 9a,b. Method A. General Procedure. To a stirred solution of 0.1 mmol of an antibiotic aglycon or its des-(*N*-Me-D-Leu) derivative in 4 mL of DMSO was added 0.5 mmol of a hydrochloride of the appropriate amine and Et_3N to give pH 8−8.5. Then the reaction mixture was cooled to 18 °C and a total of 0.15 mmol of HBPyU [*O*-benzotriazol-1-yl-*N,N,N′,N′*-bis(tetramethylene)uronium hexafluorophosphate] or PyBOP [benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate] was added in three portions with stirring over 45 min, while maintaining pH 8−8.5 with Et_3N. After 2 h of stirring at 18 °C, the reaction mixture was shaken with Et_2O to extract DMSO. The oily residue of corresponding amide was dissolved in a minimal volume of MeOH. Addition of Et_2O (∼20 mL) and acetone (∼10 mL) led to a precipitate that was collected, washed with acetone, and dried to give the corresponding amide in ∼90% yield.

Acknowledgment. We thank Marina I. Reznikova, Ph.D., and Natalia M. Maliutina (Gause Institutute of New Antibiotics, Moscow) for the HPLC studies and Ann Absillis and Lizette van Berckelaer for the antiviral and cytostatic activity evaluations. This research was supported by a grant from ANRS (to J.B.) and FWO (Grant G.0267.04) (to J.B. and E.D.C.).

Supporting Information Available: Details of the synthesis of carboxamides **5a** and **8a** and antiviral assay methods and Table 1 listing HPLC and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Preobrazhenskaya, M. N.; Olsufyeva, E. N. Patents on glycopepetides of the vancomycin family and their derivatives as antimicrobials. *Expert Opin. Ther. Pat.* **2004**, *14* (2), 141−173.

(2) Nicolau, K. C.; Boddy, C. N. C.; Brase, S.; Winssinger, N. Chemistry, biology, and medicine of the glycopeptide antibiotics. *Angew. Chem., Int. Ed*. **1999**, *38*, 2096−2152.

(3) Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, LL.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. *Science* **1999**, *284*, 507−511.

(4) 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 Microbiol. Lett.* **2000**, *183*, 209−214.

(5) 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.

(6) 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.

(7) Printsevskaya, S. S.; Pavlov, A. Y.; Olsufyeva, E. N.; Mirchink, E. P.; Preobrazhenskaya, M. N. The role of the glycopeptide framework in the antibacterial activity of hydrophobic derivatives of glycopeptide antibiotics. *J. Med. Chem*. **2003**, *46*, 1204−1209.

(8) Balzarini, J.; Pannecouque, C.; De Clercq, E.; Pavlov, A. Y.; Printsevskaya, S.; Miroshnikova, O. V.; Reznikova, M. I.; Preobrazhenskaya, M. N. Antiretroviral activity of semisynthetic derivatives of glycopeptide antibiotics. *J. Med. Chem*. **2003**, *46*, 2755−2764.

(9) Nagarajan, R. Antibacterial activity and mode of action of vancomycin and related glycopeptides. *Antimicrob. Agents Chemother.* **1991**, *35* (4), 605−609.

(10) 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. Khimiother. (Russia)* **1991**, *36*, 28−31.

(11) Mahfoud, R.; Mylvaganam, M.; Lingwood, C.; Fantini, J. A novel soluble analog of the HIV-1 fusion cofactor, globotriaosylceramide (Gb_3), eliminates the cholesterol requirement for high affinity gp120/Gb_3 interaction. *J. Lipid Res*. **2002**, *43*, 1670−1679.

(12) Tompson, C.; Ge, M.; Kahne, D. Synthesis of vancomycin from the aglycon. *J. Am. Chem. Soc*. **1999**, *121*, 1237−1244.

(13) 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.

(14) 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.

(15) 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 relationships. *J. Antibiot*. **1998**, *51*, 73−81.

(16) Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holý, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits retrovirus replication in vitro and simian immunodeficiency virus infection in rhesus monkeys. *AIDS* **1991**, *5*, 21−28.

(17) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **1984**, *224*, 497−500.

JM0500774