Antiretroviral Activity of Semisynthetic Derivatives of Glycopeptide Antibiotics

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A variety of semisynthetic derivatives of natural antibacterial glycopeptide antibiotics such as vancomycin, eremomycin, ristocetin A, teicoplanin A_2-2, DA-40926, their aglycons, and also the products of their partial degradation with a destroyed or modified peptide core show marked anti-retroviral activity in cell culture. In particular, aglycon antibiotic derivatives containing various substituents of a preferably hydrophobic nature displayed activity against human immunodeficiency virus type 1 (HIV-1), HIV-2, and Moloney murine sarcoma virus at a 50% inhibitory concentration in the lower micromolar (1−5 μM) concentration range while not being cytostatic against human lymphocytic cells at 250 μM or higher. The mode of anti-HIV action of the antibiotic aglycon derivatives could be ascribed to inhibition of the viral entry process.

Introduction

Glycopeptide antibiotics (i.e., vancomycin, teicoplanin) are vital therapeutic agents used worldwide for the treatment of serious life-threatening infections caused by Gram-positive bacteria. Other antibiotics of this type (eremomycin, chloroeremomycin, ristocetin, teicoplanin aglycon, and some others) are also highly active against Gram-positive microorganisms including methicillin-resistant staphylococci.[1] The antibacterial activity of natural glycopeptide antibiotics is based on their ability to inhibit bacterial cell wall biosynthesis by a reversible, noncovalent binding of the drugs to the -D-Ala-D-Ala fragments of the peptidoglycan precursor.[2] Emerging bacterial resistance to vancomycin, which has recently become a major public health threat, was a stimulus for the synthesis and investigation of various derivatives of glycopeptide antibiotics. This research has resulted in the discovery of the activity of hydrophobic derivatives of these antibiotics against glycopeptide-resistant enterococci (GRE).[3−5] Some hydrophobic derivatives of eremomycin or vancomycin also demonstrate antibacterial activity despite decreased binding to D-Ala-D-Ala and D-Ala-D-lactate.[6,8] This antibacterial activity appears to be due to inhibition of the transglycosylase reaction, which is of crucial importance in the bacterial peptidoglycan synthesis.[6−10] The nature of the hydrophobic substituent on these molecules plays a major role in the eventual antibacterial activity of the glycopeptide antibiotic derivatives against GRE.[8] In the present study, a wide variety of lipophilic glycopeptide antibiotic derivatives have been evaluated against the cytopathicity of HIV-1 and HIV-2 in human CEM cell cultures and against the transforming effect of Moloney murine sarcoma virus (MSV) on murine C3H/3T3 embryo fibroblast cell cultures. Several glycopeptide antibiotics proved to be highly efficacious in preventing HIV-induced cytopathicity in CEM cell cultures and MSV-induced transformation of murine fibroblast cells.

Results

The goal of our studies was the search for low toxicity anti-HIV compounds among various semisynthetic derivatives of natural vancomycin, eremomycin, teicoplanin, DA-40926 and ristocetin glycopeptide antibiotics, the aglycons thereof, and also glycopeptide antibiotics with the peptide core partially destroyed or modified. The methods for introducing chemical modifications in the sugar moieties, at the amide part, at the resorcinol fragment and at the N-end of the antibacterial glycopeptide antibiotics were elaborated earlier and used for the preparation of a variety of semisynthetic glycopeptides.[3,4] Here, we present for the first time the results of the investigations of the antiretroviral properties of these glycopeptide antibiotic derivatives. The structures of the compounds investigated are presented in Tables 1a−7a and 8. The cytotoxic and antiviral data are given in Tables 1b−7b and 8. They represent the inhibitory effects (IC_{50} in μM) of the compounds investigated on the proliferation of murine leukemia cells (L1210) and human T-lymphocytic cells (Molt4/C8, CEM), HIV-1 and HIV-2 infection (EC_{50} in μM) in human T-lymphocytic (CEM) cells, MSV-induced transformation of C3H/3T3 embryo murine fibroblasts (EC_{50} in μM), and cytotoxic activity against C3H/3T3 cell cultures (minimum inhibitory concentration (MIC) in μM).

Vancomycin and eremomycin were neither toxic to the human CEM and Molt4/C8 and murine embryo fibroblast cells nor inhibitory to HIV-1, HIV-2, and MSV. The introduction of a hydrophobic substituent on the vancomycin and eremomycin molecules resulted in the appearance of moderate anti-HIV-1 activity for some of the derivatives (e.g., **11**); however, their cytotoxicity was also increased in comparison with the unsubstituted antibiotics (Table 1). Compound **11** preferentially inhibited HIV-1 above HIV-2.

Interestingly, teicoplanin (**14**), but not ristocetin (**12**), was active against HIV-1 (EC_{50} = 17 μM). The deman-

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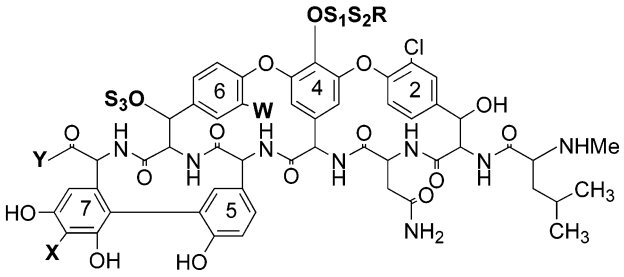
Table 1. Structures of Vancomycin, Eremomycin, and Their Derivatives and Inhibitory Data

(a) Vancomycin, Eremomycin, and Their Derivatives



compd	ref	X	Y	R	Brutto formula	MW
			Vancomycin (Van) and Its Derivatives W = CI, S_1 = Glc, S_2 = Vancosamine, S_3 = H			
1 (Van)		H	OH	H	$C_{66}H_{75}N_9O_{24}Cl_2$	1448
2	11	H	$NHC_{10}H_{21}$	H	$C_{76}H_{96}N_{10}O_{23}Cl_2$	1587
3	11	H	NHBn(PhCl-p)-p	H	$C_{79}H_{85}N_{10}O_{23}Cl_3$	1647
4	15, 16	H	OH	Bn(PhCl-p)-p	$C_{79}H_{84}N_9O_{24}Cl_3$	1648
			Eremomycin (Ere) and Its Derivatives W = H, S_1 = Glc, S_2 = S_3 = Eremosamine			
5 (Ere)		H	OH	H	$C_{73}H_{89}N_{10}O_{26}Cl$	1556
6	17	H	NHMe	H	$C_{74}H_{93}N_{11}O_{25}Cl$	1570
7	18	$CH_2NHC_{10}H_{21}$	OH	H	$C_{84}H_{112}N_{11}O_{26}Cl$	1725
8	19	H	$NHC_{10}H_{21}$	H	$C_{83}H_{110}N_{11}O_{25}Cl$	1693
9	8	H	NHBn(PhCl-p)-p	H	$C_{86}H_{100}N_{11}O_{25}Cl_2$	1756
10	19	H	NHBnPh-p	H	$C_{86}H_{100}N_{11}O_{25}Cl$	1721
11		$CH_2NHBn(PhCl-p)-p$	OH	H	$C_{87}H_{102}N_{11}O_{26}Cl_2$	1786

(b) Inhibitory Effects (IC_{50}, μM) of Vancomycin-Type Glycopeptides and Their Derivatives[a]

compd	IC_{50},[b] μM			EC_{50},[c] μM			MIC,[d] μM
	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
			Vancomycin Derivatives				
1 (vancomycin)	>500	>500	>500	>250	>250	>100	>100
2	**30 ± 22**	>**10**	**30 ± 2**	>10	>10	>4	**20**
3						>2	**20**
4						***7.7 ± 4.3***	≥25
			Eremomycin Derivatives				
5 (eremomycin)	>500	>500	>500	>250	>250	>100	>100
6	>500	>500	>500	>250	>250		
7	**19 ± 6**	**27 ± 8**	**24 ± 13**	>20	>20		
8	**29 ± 11**	**21 ± 1**	**9.4 ± 4.7**	>10	>10		
9	**15 ± 11**	**7.7 ± 0.5**	**8.6 ± 0.2**	>10	>10	>0.8	**4**
10	41 ± 5	34 ± 0.5	35 ± 1	>10	>10	>0.8	**4**
11	136 ± 12	72 ± 40	106 ± 65	22 ± 3	>50	>4	≥20

[a] Compounds with $IC_{50} \leq 30$ μM and MIC ≤ 30 μM are shown in bold. Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [b] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [c] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [d] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

nosyl ristocetin (**13**) and the methylamide of teicoplanin (**15**) were both active predominantly against HIV-1, and the last two compounds also proved to be inhibitory against MSV (Table 2).

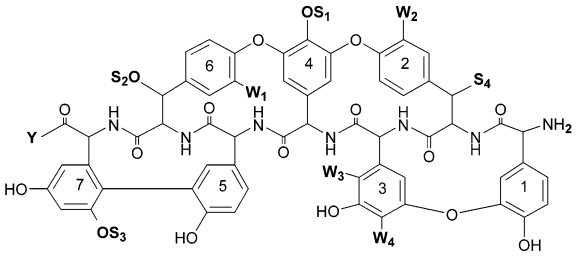
The antibiotic deacyl-A40926 (**16**) and its demannosylated derivative DMDA 40926 (**17**) were not cytotoxic and did not show marked antiviral properties, but the removal of the D-mannose residue and the introduction of hydrophobic substituents led to antibiotic derivatives with marked anti-HIV-1 activities (i.e., $EC_{50} = 3.5$ μM for **20** and **21**), though some of the compounds were toxic for the cells at rather low drug concentrations (Table 3). Compounds **20**, **21** also inhibited the replication of HIV-2, albeit at markedly higher (2.5- to 7-fold) concentrations than required for inhibition of HIV-1. Compounds **20** and **22** also proved to be inhibitory to MSV at subtoxic concentrations.

Removing all carbohydrates from vancomycin and eremomycin led to aglycon antibiotics (**23** and **24**), which were not cytotoxic (IC_{50} > 500 μM) but active against HIV-1 at rather high concentrations ($EC_{50} = 50-65$ μM) (Table 4). However, the hydrophobic derivatives of eremomycin aglycon **25**−**28** invariably inhibited both HIV-1 and HIV-2 to a comparable extent at EC_{50} values between 3.5 and 12 μM. These concentrations were at least 15- to 20-fold lower than required for the unsubstituted eremomycin aglycon to inhibit HIV replication. The aglycon antibiotic derivatives were invariably nontoxic for CEM cells (IC_{50} > 100−500 μM). Splitting off the first amino acid (*N*-methyl-D-leucine) from eremomycin aglycon led to the eremomycin aglycon hexapeptide (EAH) **29**, which had lost activity against HIV. However, its 2-aminoadamantane derivatives **30** and **31** were active against both HIV-1 and HIV-2 at EC_{50} values of 13−40 μM and were not cytotoxic at 250 μM (Table 4).

The aglycons of ristocetin, DA40926, and teicoplanin (Table 5, compounds **32**−**34**) were predominantly en-

Table 2. Structures of Teicoplanin-Type Glycopeptides and Their Derivatives and Inhibitory Data

(a) Teicoplanin-Type Glycopeptides and Their Derivatives



compd	ref	Y	Brutto formula	MW
		Ristocetin A		
		$W_1 = W_2 = W_3 = H$, $W_4 = Me$, S_1 = Tetrasaccharide, S_2 = Ristosamine, S_3 = Man, $S_4 = OH$		
12 (risto)	20	OMe	$C_{95}H_{110}N_8O_{44}$	2068
		Ristosaminylaglycon of Ristocetin		
		$W_1 = W_2 = W_3 = S_1 = S_3 = H$, $W_4 = Me$, S_2 = Ristosamine, $S_4 = OH$		
13	20	OMe	$C_{66}H_{64}N_8O_{21}$	1304
		Teicoplanin and Its Derivatives		
		$W_1 = W_2 = CI$, $W_3 = W_4 = H$, S_1 = GlcNAcyl, S_2 = GlcNAc, S_3 = Man, $S_4 = OH$		
14 (teico)		OH	$C_{88}H_{97}N_9O_{33}Cl_2$	2006
15	21	NHMe	$C_{89}H_{100}N_{10}O_{32}Cl_2$	1893

(b) Inhibitory Effects (IC_{50}, μM) of Teicoplanin-Type Glycopeptides and Their Derivatives[a]

	IC_{50},[b] μM			EC_{50},[c] μM			MIC,[d] μM
compd	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
12 (risto)	>500	>500	>500	>250	>250	>100	>100
13			>100	≥50	≥250	52 ± 30	≥100
14 (teico)	>500	>500	>500	***17*** ± ***3.5***	100 ± 0	>100	>100
15	>500	>500	>500	80 ± 28	190 ± 84	75 ± 3	>100

[a] Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [b] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [c] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [d] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

dowed with moderate anti-HIV-1 activity while not being toxic for mammalian cells. A large variety of teicoplanin aglycon derivatives with hydrophobic substituents were synthesized and explored for their anti-HIV and MSV activity (Table 5). Interestingly, all of them showed very pronounced anti-HIV-1 and anti-HIV-2 activity in cell culture, often with a tendency of being slightly more active against HIV-1 than HIV-2 (~2- to 5-fold). The methyl ester of the teicoplanin aglycon (**42**) was active against both HIV-1 and HIV-2 (EC_{50} = 9.5 and 15 μM, respectively) and MSV (EC_{50} = 12 μM). However, the most active congeners were inhibitory against HIV-1 in the range 0.75−2.5 μM (compounds **41**, **44**, **61**, and **62**). Compounds **41**, **61**, and **62** were not cytotoxic at 250 μM. This means that these compounds reached a selectivity index (ratio IC_{50}/EC_{50}) that was ≥100. The antiviral activity of the latter compounds (0.75−4.5 μM) was also at least 10- to 20-fold improved over that of the unsubstituted teicoplanin aglycon **34** (EC_{50} = 17−20 μM; IC_{50} > 500 μM). Many of the compounds active against HIV-1 were also potent inhibitors of MSV-induced transformation of murine fibroblast cell cultures at comparable drug concentrations (Table 5b).

The elimination of amino acids 1 and 3 from teicoplanin aglycon and the introduction of hydrophobic substituents was not accompanied by the disappearance of the antiviral properties, although the antiviral potency was ~5-fold reduced compared to that of the intact teicoplanin aglycon derivatives (Table 6, compounds **65**−**68**). Similarly, hydrophobic derivatives of teicoplanin aglycon with the first peptide bond disrupted (Table 7, compounds **69**, **70**) were still clearly active against HIV-1 and HIV-2, and also MSV. Even when the bond between amino acids 6 and 7 in the teicoplanin aglycon peptide core was disrupted (**71**, Table 8), this compound maintained measurable activity against both HIV strains and MSV (EC_{50} = 22−32 and 14 μM, respectively).

Discussion

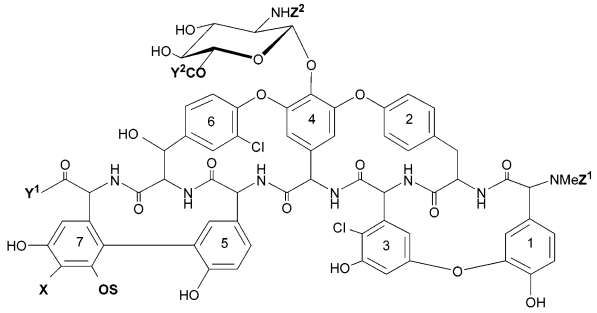
There are common structural features for glycopeptide antibiotics to be active against glycopeptide-resistant bacterial strains and retroviruses. The introduction of a hydrophobic substituent is beneficial for both types of activity. However, the presence of carbohydrate moieties in glycopeptides usually results in a dramatic decrease of anti-HIV activity, whereas for antibacterial activity the presence of sugars represents a critical determinant, although some hydrophobic derivatives of aglycons of eremomycin, teicoplanin, and des(*N*-methyl-D-leucyl)eremomycin also demonstrate rather good anti-GRE properties.[11]

It seems that the peptide framework formed by amino acids 2 and 4−7 containing a hydrophobic substituent is a prerequisite for the antiretroviral activity. Modifications of the aglycons resulting in the disruption of the macrocycles or deleting amino acid 1 leads to compounds with surprisingly high anti-HIV activity, though the antiviral efficacy is lower than for the derivatives with the intact peptide core.

A group of hexapeptide antibiotics with a peptide core similar to that of the antibacterial glycopeptides have

Table 3. Structures of *N*-Deacyl-A40926 (DA40), Demannosyl-*N*-deacyl-A40926 (DMDA40), and Their Derivatives and Inhibitory Data

(a) *N*-Deacyl-A40926 (DA40), Demannosyl-*N*-deacylA40926 (DMDA40), and Their Derivatives



compd	ref	X	$Y^1 = Y^2$	Z^1	Z^2	Brutto formula	MW
			DA40 and Its Derivatives S = Man				
16		H	OH	H	H	$C_{71}H_{66}N_8O_{28}Cl_2$	1551
			DMDA40 and Its Derivatives S = H				
17	22	H	OH	H	H	$C_{65}H_{56}N_8O_{23}Cl_2$	1389
18	23	H	$NH(CH_2)_3NMe_2$	p-BuOBn	p-BuOBn	$C_{97}H_{108}N_{12}O_{23}Cl_2$	1881
19	24	H	$NH(CH_2)_3NMe_2$	H	p-BuBn	$C_{86}H_{94}N_{12}O_{21}Cl_2$	1703
20	24	$CH_2N[CH_2CH_2]_2NBnPh$-p	OH	H	p-BuBn	$C_{94}H_{90}N_{10}O_{23}Cl_2$	1799
21	24	$CH_2N[CH_2CH_2]_2NBnPh$-p	$NH(CH_2)_3NMe_2$	H	p-BuBn	$C_{104}H_{114}N_{14}O_{21}Cl_2$	1967
22	23	$CH_2N[CH_2CH_2]_2NBnBu$-p	OH	H	H	$C_{81}H_{80}N_{10}O_{23}Cl_2$	1633

(b) Inhibitory Effects of DA40 and DMDA40 and Their Derivatives[a]

compd	IC_{50},[b] μM			EC_{50},[c] μM			MIC,[d] μM
	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
16 (DA40926)	>500	>500	>500	>250	>250	>100	>100
17 (DMDA40926)	>500	>500	>500	115 ± 21.2	≥250	>100	>100
18	**20 ± 7.5**	**18 ± 2.5**	80 ± 6	***5.0 ± 0.7***	>10	>4	**20**
19	36 ± 14	66 ± 20	>250	***12 ± 3.5***	>50	>20	100 to >20
20			81 ± 20	***3.5 ± 0.7***	22 ± 3.5	***6.9 ± 4.1***	≥**20**
21	**25 ± 0.7**	35 ± 6.1	212 ± 34	***3.5 ± 2.1***	***20*** ± 0.0	>***4***	20
22	**20 ± 7.1**	≥50	106 ± 2	***20 ± 7.1***	≥50	***6.9 ± 4.1***	100

[a] Compounds with $IC_{50} \leq 30$ μM and MIC ≤ 30 μM are shown in bold. Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [b] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [c] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [d] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

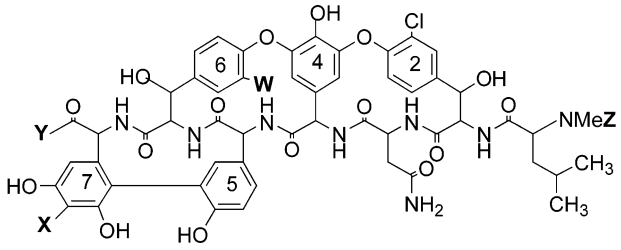
been reported to also exhibit antiviral properties. Chloropeptins I and II (complestatin) and kistamicins A and B (Figure 1) have been shown to inhibit in vitro binding of HIV-1 gp120 to the CD4 receptor and, consequently, HIV replication in human lymphocytes.[12−14] These antibiotics and the aglycons of antibacterial glycopeptides have a common structural motif, although the size of their macrocycles, i.e., the framework of these antibiotics, is different. Indeed, the structures of these hexa- or heptapeptide antibiotics and the structures of antibacterial glycopeptide antibiotics and their aglycons show profound differences in amino acid sequence, composition, and stereochemistry. The cycle formed with the participation of the C-terminal amino acid present in glycopeptide antibiotics is absent in chloropeptin and complestatin and is of a different type and size in kistamicin. Kistamicins, complestatin, and chloropeptin I contain a tryptophan moiety linked to the central amino acid 4 (F and D cycles), whereas a substituted phenylalanine moiety is present in the antibacterially active glycopeptides (Figure 1).

Preliminary mechanism of action studies on the anti-HIV activity of the modified antibiotic derivatives have been performed in "time-of-addition" experiments carried out with the glycopeptide antibiotic (**38**). In this study, the drug was added to the virus-infected cell cultures at different time points after infection. The results strongly suggest that inhibition of viral entry is the most likely molecular event for the anti-HIV activity of this type of compound. The compound (**38**) lost its antiviral activity when added at 1−2 h postinfection. The HIV adsorption inhibitor dextran sulfate (DS-5000) already lost its activity when added at 1 h postinfection, whereas addition of the reverse transcriptase inhibitor AZT and the HIV protease inhibitor ritonavir could be delayed for up to 3 and 18 h postinfection, respectively, before their antiviral efficacy was lost (data not shown). This is also in agreement with the report that related compounds interfere with gp120-CD4 binding.[12−14] Also, the inhibitory activity of **38** and **40** against fusion between uninfected Molt4/C8 and persistently HIV-1-infected HUT-78 cells (resulting in syncytium formation between both cell types) is in agreement with these findings (EC_{50} = 20 and 11 μM, respectively).

The viral entry process is the result of a specific interplay between viral glycoproteins (gp120, gp41) and cellular (co)receptors (CD4, CCR5, CXCR4). Given our preliminary findings that viral entry is a target for the anti-HIV activity of the glycopeptide antibiotics, it is not so surprising that we did not find a close correlation

Table 4. Structures of Vancomycin-Type Aglycons and Their Derivatives and Inhibitory Data

(a) Vancomycin-Type Aglycons and Their Derivatives



compd	ref	X	Y	Z	Brutto formula	MW
		Vancomycin Aglycon (VA) W = Cl				
23 (VA)	25	H	OH	H	$C_{53}H_{52}N_8O_{17}Cl_2$	1143
		Eremomycin Aglycon (EA) and Its Derivatives W = H				
24 (EA)	26	H	OH	H	$C_{53}H_{53}N_8O_{17}Cl$	1108
25		$CH_2N[CH_2CH_2]_2$ NBnPh-p	OH	H	$C_{71}H_{75}N_{10}O_{17}Cl$	1374
26		$CH_2N[CH_2CH_2]_2$NBnPh-p	OH	Boc	$C_{76}H_{83}N_{10}O_{19}Cl$	1474
27		$CH_2N[CH_2CH_2]_2$NBnPh-p	NHMe	Boc	$C_{77}H_{86}N_{11}O_{18}Cl$	1487
28		$CH_2N[CH_2CH_2]_2$NBnPh-p	NHMe	H	$C_{72}H_{78}N_{11}O_{16}Cl$	1387
		Eremomycin Aglycon Hexapeptide (EAH) and Its Derivatives W= H, First Amino Acid (*N*-Me-D-Leu) Replaced by H				
29 (EAH)	27	H	OH		$C_{46}H_{40}N_7O_{16}Cl$	981
30		CH_2NHAdam-2[a]	OH		$C_{57}H_{57}N_7O_{16}Cl$	1130
31		CH_2NHAdam-2[a]	NHMe		$C_{58}H_{60}N_8O_{15}Cl$	1143

(b) Inhibitory Effects of Vancomycin-Type Aglycons and Their Derivatives[b]

	IC_{50},[c] μM			EC_{50},[d] μM			MIC,[e] μM
compd	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
23 (aglycon Van)	>500	>500	>500	65 ± 7.1	≥250	>100	>100
24 (aglycon Ere)	>500	>500	>500	50 ± 28.3	≥250	>100	>100
			Aglycon Eremomycin Derivatives				
25	250 ± 39	>500	>500	***5.5 ± 0.7***	***12 ± 3.5***	***13 ± 2***	100 to >20
26			>100	***4.5 ± 0.7***	***4.5 ± 2.1***	***8.4 ± 3.0***	100 to >20
27	84 ± 22	>100	>100	***4.0 ± 0.0***	***3.5 ± 0.7***	>4	**20**
28	>100	>100	>100	***4.0 ± 1.7***	***5.5 ± 0.7***	***12 ± 1***	100 to >20
			EAH Derivatives				
29 (EAH)			>250	115 ± 21.2	>250	>100	≥100
30			>250	***22 ± 11***	40 ± 14.1	47 ± 5	≥100
31	>250	>250	>250	***13 ± 9.9***	***20 ± 7.1***	***14 ± 5***	≥100

[a] Adam-2 means admantyl-2. [b] Compounds with $IC_{50} \leq 30$ μM and MIC ≤ 30 μM are shown in bold. Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [c] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [d] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [e] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

between the anti-HIV activity and anti-MSV activity of the glycopeptide antibiotics in cell culture. In fact, the correlation coefficients (r) between the EC_{50} for MSV and the EC_{50} for HIV-1 and HIV-2 are 0.50 and 0.41, respectively, and the r value between the EC_{50} for HIV-1 and HIV-2 is 0.64 (figures not shown). These observations may point to a rather specific interaction of the compounds with a viral factor (i.e., gp120 or gp41 for HIV-1 and HIV-2 and an envelope protein for MSV) that has different structural requirements for interaction with the modified antibiotics. The elucidation of the antiretroviral target(s) of the glycopeptide aglycon antibiotics and of the molecular mode of interaction with their target is currently a subject of investigation in our laboratories.

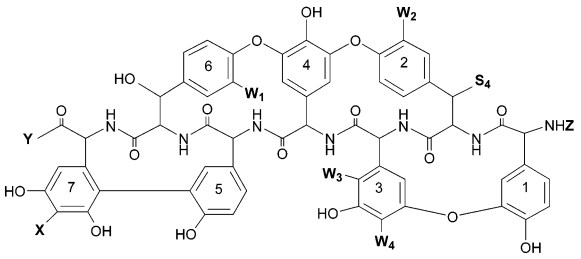
In conclusion, semisynthetic, lipophilic aglycon glycopeptide derivatives have been discovered to be selectively active against retroviruses including HIV-1, HIV-2, and MSV. In the case of HIV, the compounds seem to impede viral entry into the cells, and thus, these glycopeptide derivatives can be envisaged as potential lead compounds for application as microbicides against sexual HIV transmission.

Experimental Section

1. Chemistry. Eremomycin sulfate and ristocetin A were produced at the pilot plant of the Gause Institute of New Antibiotics, Moscow. Vancomycin hydrochloride was obtained from Sigma Corporation (St. Louis, MO). Teicoplanin, teicoplanin aglycon, and the antibiotic DA-40926 were kindly provided by Dr. R. Ciabatti and Dr. A. Malabarba (Biosearch S.p.A., Gerenzano (Varese), Italy). All reagents and solvents were purchased from Aldrich (Milwaukee), Fluka (Deisenhofen, Germany), and Merck (Darmstadt, Germany). The references for the methods of the preparation of the compounds previously described are presented in the Tables 1a−6a and 8. The novel compounds were obtained by the methods (e.g., amidation, Mannich reaction, N-acylation, alkylation) previously described for the synthesis of analogous glycopeptide derivatives.[4,5,8,17−19,30] The homogeneity and identity of the compounds obtained were assessed by HPLC and ESI mass spectrometry (see the Supporting Information).

Table 5. Structures of Teicoplanin-Type Aglycons and Their Derivatives and Inhibitory Data

(a) Teicoplanin-Type Aglycons and Their Derivatives



compd	ref	X	Y	Z	Brutto formula	MW
			Ristocetin Aglycon			
			W_1= W_2 = W_3 = H, W_4 = Me, S_4 = OH			
32	28	H	OMe	H	$C_{60}H_{52}N_7O_{19}$	1174
			Aglycon DA40			
			W_1 = W_3 = Cl, W_4 = S_4 = H,			
33	22	H	OH	Me	$C_{59}H_{47}N_7O_{18}Cl_2$	1212
			Teicoplanin Aglycon (TD) and Its Derivatives			
			W_1 = W_2 = Cl, W_3 = W_4 = S_4 = H			
34 (TD)	29	H	OH	H	$C_{58}H_{45}N_7O_{18}Cl_2$	1199
35	30	$CH_2NHC_{10}H_{21}$	$NH(CH_2)_3NMe_2$	H	$C_{74}H_{80}N_{10}O_{17}Cl_2$	1452
36	30	CH_2N(Me)BnCl-p	OH	H	$C_{67}H_{55}N_8O_{18}Cl_3$	1366
37	11	H	$NHC_{10}H_{21}$	H	$C_{68}H_{66}N_8O_{17}Cl_2$	1338
38	30	CH_2NHAdam-2[a]	$NH(CH_2)_3NMe_2$	H	$C_{74}H_{74}N_{10}O_{17}Cl_2$	1446
39	21	H	NHMe	H	$C_{59}H_{48}N_8O_{17}Cl_2$	1212
40		$CH_2N[CH_2CH_2]_2$NBnBu-p	$NH(CH_2)_3NMe_2$	H	$C_{79}H_{81}N_{11}O_{17}Cl_2$	1527
41		$CH_2N[CH_2CH_2]_2$NBnBu-p	NHMe	H	$C_{75}H_{72}N_{10}O_{17}Cl_2$	1456
42	31	H	OMe	H	$C_{59}H_{47}N_7O_{18}Cl_2$	1213
43		H	$NH(CH_2)_6NH_2$	H	$C_{64}H_{59}N_9O_{17}Cl_2$	1297
44		$CH_2NH(CH_2)_3N^+Me_2C_{10}H_{21}$	$NH(CH_2)_6NH_2$	H	$C_{80}H_{94}N_{11}O_{17}Cl_2$	1552
45		H	$NH(CH_2)_{10}NH_2$	H	$C_{68}H_{67}N_9O_{17}Cl_2$	1353
46		CH_2NHBnBu-p	OH	Boc	$C_{75}H_{70}N_8O_{20}Cl_2$	1474
47		CH_2NHBnBu-p	NHMe	Boc	$C_{76}H_{73}N_9O_{19}Cl_2$	1487
48		CH_2NHBnBu-p	NHMe	H	$C_{71}H_{65}N_9O_{17}Cl_2$	1387
49	32	H	OH	Boc	$C_{63}H_{53}N_7O_{20}Cl_2$	1299
50		H	OH	Fmoc	$C_{73}H_{55}N_7O_{20}Cl_2$	1421
51		H	OH	Adoc	$C_{69}H_{59}N_7O_{20}Cl_2$	1377
52	32	H	OH	Cbz	$C_{66}H_{51}N_7O_{20}Cl_2$	1333
53	21	H	NHMe	Boc	$C_{64}H_{56}N_8O_{19}Cl_2$	1312
54		H	NHMe	Adoc	$C_{70}H_{62}N_8O_{19}Cl_2$	1390
55	33	H	OH	C(S)NHPh	$C_{65}H_{50}N_8O_{18}Cl_2S$	1334
56		H	NHAdam-2[a]	H	$C_{68}H_{60}N_8O_{17}Cl_2$	1332
57		CH_2NHAdam-2[a]	OH	H	$C_{69}H_{62}N_8O_{18}Cl_2$	1362
58		CH_2NHAdam-2[a]	NHMe	H	$C_{70}H_{65}N_9O_{17}Cl_2$	1375
59		$CH_2NHC_{18}H_{37}$	OH	H	$C_{77}H_{84}N_8O_{18}Cl_2$	1480
60		$CH_2NHC_{18}H_{37}$	NHMe	H	$C_{78}H_{87}N_9O_{17}Cl_2$	1493
61		CH_2NHAdam-2[a]	NHAdam-2[a]	H	$C_{79}H_{77}N_9O_{17}Cl_2$	1495
62		H	perhydroisoquinolinyl-	H	$C_{67}H_{60}N_8O_{17}Cl_2$	1318
63		H	OH	(glyoxalyl-indol-3-yl)-	$C_{68}H_{50}N_8O_{20}Cl_2$	1368
64		H	OH	1-adamantoyl-	$C_{69}H_{66}N_7O_{19}Cl_2$	1366

(b) Inhibitory Effects of Teicoplanin-Type Aglycons and Their Derivatives[b]

	IC_{50},[c] μM			EC_{50},[d] μM			IC,[e] μM
compd	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
32 (aglycon risto)			>100	25 ± 7.1	≥50	***19* ± *1***	>100
33 (aglycon DA40)	>500	≥500	>500	40 ± 14.1	>50	82 ± 26	>100
34 (aglycon teico)	>500	>500	>500	***17* ± *3.5***	***20* ± *0.0***	25 ± 17	>100
				Aglycon Teicoplanin Derivatives			
35	48 ± 8	>100	>100	***1.4* ± *0.6***	***6.1* ± *3.9***		
36	>100	>100	>100	***17* ± *3.5***	***20* ± *7.1***	***11* ± *5***	100 to >20
37			21 ± 0.2	***2.6* ± *2.0***	***5.8* ± *0.4***		
38	>500	>500	>500/ >250	***2.5* ± *0.7/ 4.0* ± *0.0***	***8.0* ± *2.8/ 22* ± *3.5***	***12* ± *5***	**20**
39	>500	>500	>500	***15* ± *7.1***	***17* ± *3.5***	≥20	100 to >4
40	**29** ± **7**	108 ± 79	>500/ >250	***3* ± *0/ 4.0* ± *1.4***	***5* ± *1.4/ 13* ± *9.9***	***2.4/ 3.5* ± *0.7***	**20**
41	61 ± 10	>500	>500/ >250	***1.7* ± *0.42/ 3.5* ± *0.7***	***3.0* ± *1.4/ 6.3* ± *1.1***	***3.6/6.9* ± *0.4***	≥20
42	364 ± 192	>500	248 ± 1	***9.5* ± *7.8***	***15* ± *7.1***	***12* ± *2***	≥100
43	>500	>500	>500	***15* ± *0***	***17* ± *3.5***	>4	**20**
44	38 ± 1	72 ± 6.0	66 ± 2	***1.8* ± *0.49***	***7* ± *0***		
45	500	225 ± 8.0	402 ± 138	***6.5* ± *0.7***	***12* ± *3.5***	≥***4***	**20**
46			60 ± 11.9	***20* ± *0***	***8.3* ± *5.86***	***7.6* ± *6.1***	100 to >20
47	70 ± 23	>100	>100	***6* ± *1***	***12* ± *5.2***	>4	**20**
48	>100	>100	>100	***9.7* ± *9.0***	***12* ± *6.81***	>4	**20**
49			>250	***13* ± *9.9***	***20* ± *0.0***	***12* ± *0.0***	≥100
50			114 ± 1	***17* ± *3.5***	***15* ± *0.0***	***10* ± *0.0***	100
51	**22** ± **0.1**	**25** ± **0.99**	104 ± 3	***13* ± *9.9***	***6.0* ± *1.4***	***9.8* ± *1.8***	≥20
52			>250	***12* ± *3.5***	***8.0* ± *2.8***	***11* ± *2.0***	>100
53			229 ± 30	***13* ± *9.2***	***17* ± *3.5***	***11* ± *5.0***	>100
54			88 ± 16	***17* ± *17.7***	***7.8* ± *3.2***	***8.7* ± *4.6***	100
55			220 ± 43	***6.0* ± *1.4***	***8.0* ± *2.8***	***7.5* ± *5.0***	100

Table 5. (Continued)

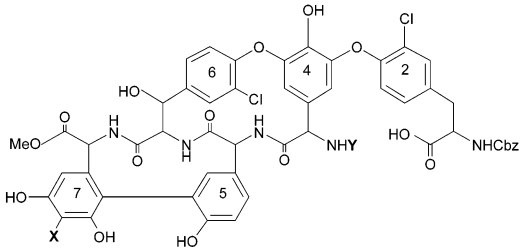
(b) Inhibitory Effects of Teicoplanin-Type Aglycons and Their Derivatives[b] (Continued)

compd	IC_{50},[c] μM			EC_{50},[d] μM			IC,[e] μM
	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
56	**30 ± 5.7**	**26 ± 6.0**	123 ± 6.0	***7.0 ± 4.2***	***6.0 ± 1.4***	***2.1 ± 0.1***	≥100
57			>250	25 ± 0.0	37 ± 17.7	***5.6 ± 3.9***	100
58	212 ± 54	>250	>250	***5.0 ± 1.4***	***17 ± 3.5***	***9.5 ± 0.8***	≥20
59			**22** ± 1.0	***4.5 ± 0.7***	***7.8 ± 3.2***	***1.7 ± 0.3***	100
60			60 ± 1.0	***4.5 ± 0.7***	***6.0 ± 1.4***	***4.7 ± 2.8***	100
61	202 ± 68	>250	>250	***2.5 ± 07***	***3.5 ± 2.1***	***2.0 ± 1.2***	≥20
62	>250	>250	>250	***0.75 ± 0.07***	***4.5 ± 0.7***	***>4***	**20**
63	192 ± 15	108 ± 5	108 ± 30	***10 ± 0.0***	***15 ± 0.0***	***4.4 ± 2.1***	≥100
64	33 ± 1.0	39 ± 13	83 ± 17	***10 ± 7.1***	***4.0 ± 1.4***	***11 ± 1.0***	100

[a] Adam-2 means admantyl-2. [b] Compounds with $IC_{50} \leq 30$ μM and MIC ≤ 30 μM are shown in bold. Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [c] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [d] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [e] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

Table 6. Structures of Teicoplanin Aglycon Derivatives with the Eliminated Amino Acids 1 and 3 and Inhibitory Data

(a) Teicoplanin Aglycon Derivatives with the Eliminated Amino Acids 1 and 3



compd	ref	X	Y	Brutto formula	MW
65	34	H	H	$C_{51}H_{43}N_5O_{16}Cl_2$	1053
66	34	H	Boc	$C_{56}H_{51}N_5O_{18}Cl_2$	1153
67		CH_2NHAdam-2[a]	Boc	$C_{67}H_{68}N_6O_{18}Cl_2$	1315
68		CH_2NHAdam-2[a]	H	$C_{62}H_{60}N_6O_{16}Cl_2$	1215

(b) Inhibitory Effects of Teicoplanin Aglycon Derivatives with the Eliminated Amino Acids 1 and 3[b]

compd	IC_{50},[c] μM			EC_{50},[d] μM			MIC,[e] μM
	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
65	>500	>500	>500	25 ± 7.1	85 ± 21.2		
66			>250	***17 ± 3.5***	***17 ± 3.5***	87 ± 19	>100
67	95 ± 10	122 ± 13	240 ± 13	***17 ± 3.5***	***11 ± 5.7***	26 ± 1	≥100
68	181 ± 4.0	>250	>250	***17 ± 3.5***	37 ± 17.7	39 ± 15	≥100

[a] Adam-2 means admantyl-2. [b] Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [c] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [d] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [e] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

1.1. Method A. Aminomethylated Derivatives 11, 25, 26, 28, 30, 36, 57, 59, 67, 68, 70. To a stirred solution of 0.5 mmol of an antibiotic or its degradation product and 4 mmol of an appropriate amine in 10 mL of an acetonitrile/water, 1:1, mixture was added 3 mmol of 37% aquous formaldehyde. If a salt of an amine was used, 1 N NaOH was added to attain pH 10. The reaction mixture was stirred at room temperature for 18 h, and then 100 mL of water was added. After adjustment of the reaction mixture at pH 3 with 1 N HCl, the resulting solution (or suspension) was extracted with *n*-BuOH (~25 mL × 2). The organic layer was washed with water (~15 mL × 2) and then concentrated at 45 °C in a vacuum to a small volume (~3 mL). On addition of ether (~100 mL), a solid that precipitated was collected and dried in a vacuum at room temperature for 4 h. Then it was dissolved in a minimal amount of MeOH and applied to a chromatographic column with Sephadex LH-20 (2 × 100 cm) preequilibrated with MeOH. The product was eluted with MeOH at a rate of 10 mL/h, while collecting 5 mL fractions. The suitable fractions were combined and concentrated to a small volume (~3 mL). After addition of ether (~100 mL), the formed precipitate was collected, rinsed with ether, and dried in a vacuum at room temperature.

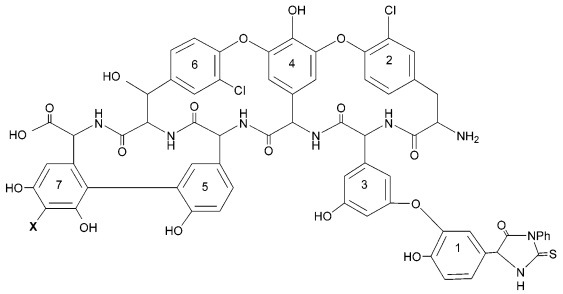
The starting compound for **67**, N^2-Cbz-N^4-Boc-TDTP-Me (**66**), was obtained as previously described.[34] Compound **68** was obtained from **67** by the removal of the Boc group in TFA as previously described for N^2-Cbz-N^4-Boc-TDTP-Me.[34]

The starting compound for **70**, the N-terminal phenylthiohydantoin derivative of teicoplanin aglycon, was obtained by Edman degradation of teicoplanin aglycon.

To a solution of teicoplanin aglycon (**34**) (100 mg, ~0.08 mmol) in a mixture of Py/H_2O (6:1, 4 mL), triethylamine (0.26 mL, 2 mmol) and PhNCS (0.02 mL, ~0.16 mmol) were added at room temperature under argon. The reaction mixture was stirred for 16 h, an amount of 8 mL of H_2O was added, and the reaction mixture was evaporated with *n*-BuOH to dryness. The precipitate was dissolved in a mixture of TFA/CH_2Cl_2, 1:1 (3 mL), at 0−5 °C and then was stirred at this temperature for 1 h. Water (3 mL) was then added, and the mixture was neutralized with 25% NH_4OH and washed with EtOAc (3 mL × 3), and the aqueous fraction was concentrated in a vacuum with the addition of *n*-BuOH and applied to a column of silanized silica gel (2 × 100 cm) previously equilibrated with

Table 7. Structures of Teicoplanin Aglycon with the Disrupted Bond between Amino Acids 1 and 2 and Inhibitory Data

(a) Teicoplanin Aglycon with the Disrupted Bond between Amino Acids 1 and 2



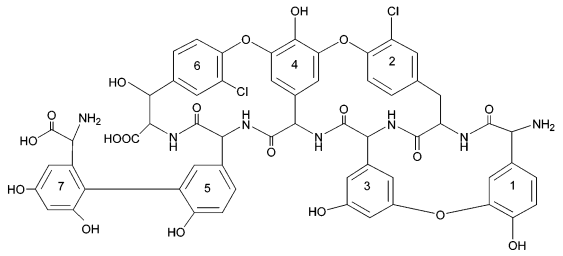
compd	X	Brutto formula	MW
69	H	$C_{65}H_{51}N_8O_{18}Cl_2S$	1335
70	$CH_2NHAdam\text{-}2^a$	$C_{76}H_{68}N_9O_{18}Cl_2S$	1498

(b) Inhibitory Effects of Teicoplanin aglycon with the Disrupted Bond between Amino Acids 6 and 7 and Their Derivatives[b]

	IC_{50},[c] μM			EC_{50},[d] μM			MIC,[e] μM
compd	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
69			>250	***15 ± 7.1***	37 ± 17.7	27 ± 0	>100
70	73 ± 24	≥250	242 ± 11	***13 ± 9.9***	***17 ± 3.5***	***2.9 ± 0.8***	≥100

[a] Adam-2 means admantyl-2. [b] Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [c] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [d] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [e] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

Table 8. Teicoplanin Aglycon with the Disrupted Bond between Amino Acids 6 and 7 and Its Inhibitory Effects[a]



		IC_{50},[b] μM			EC_{50},[c] μM			MIC,[d] μM
compd	ref	L1210	MOLT-4/C8	CEM	HIV-1	HIV-2	MSV	C3H/3T3
71	35			>250	***22 ± 3.5***	32 ± 3.5	***14 ± 2.0***	>100

[a] Compounds with $EC_{50} \leq 20$ μM are shown in bold and italic. [b] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%. [c] 50% effective concentration, or compound concentration required to protect cells against the cytopathogenicity of HIV or MSV by 50%. [d] Minimal inhibitory concentration, or compound concentration required to cause a microscopic detectable alteration of cell morphology.

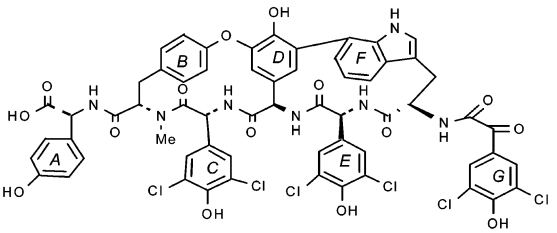
0.01 M acetic acid. Compound **70** was eluted with acetic acid (0.01M) at a flow rate of 30 mL/h. Fractions were pooled and concentrated with the addition of *n*-BuOH in a vacuum, and acetone (50 mL) was added to yield the precipitate, which was filtered off, washed with acetone, and dried to yield 68 mg (54%) of **70**.

1.2. Method B. Carboxamides 43, 45, 56, 62, 63, 64. To a mixture of an antibiotic or its degradation product (0.5 mmol) and 5 mmol of an amine hydrochloride dissolved in 5 mL of DMSO were added portionwise Et_3N to give pH 8.5−9 and afterward during 1 h 1 mmol of PyBOP reagent [(benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate] or HBPyU reagent [*O*-(benzotriazol-1-yloxy)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate]. The reaction mixture was stirred at room temperature for 3 h.

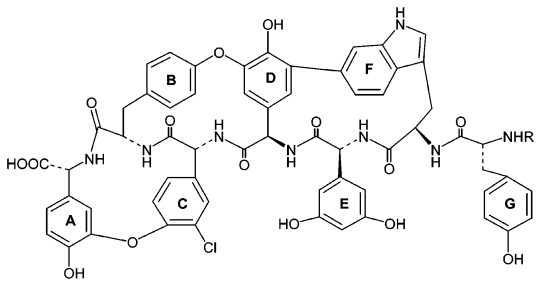
Addition of ether (∼100 mL) to the reaction mixture led to an oily residue, which was shaken successively with ether (15 mL × 2) and acetone (∼15 mL). After addition of 100 mL of acetone, a precipitate of crude amide was collected and dissolved in 50 mL of water and the pH was adjusted to 9 using a 1 N sodium hydroxide solution. The resulting solution (or suspension) was extracted with *n*-BuOH (∼25 mL × 3). The organic layer was washed with water (∼15 mL × 3) and then concentrated at 45 °C in a vacuum to a small volume (∼3 mL). On addition of ether (∼100 mL), a solid precipitate was collected and dried in a vacuum at room temperature for 4 h. Addition of 100 mL of acetone led to the formation of a precipitate, which was collected to give a pure carboxamide.

1.3. Method C. Carboxamides of Aminomethylated Derivatives 31, 40, 41, 44, 48, 58, 60, 61. The compounds were obtained by method B, starting from the aminomethylated derivatives obtained by the method A.

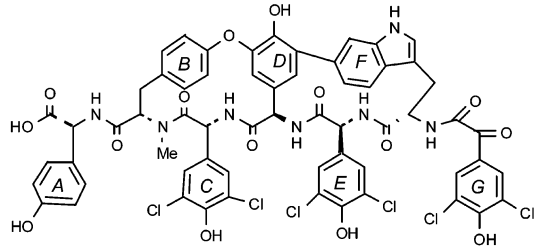
1.4. Method D. N-Carbamoylated Derivative 51. To a stirred solution of 0.5 mmol of teicoplanin aglycon (**34**) in 15 mL of THF/water, 1:1, mixture adjusted to pH 10 with 1 N NaOH 0.55 mmol of adamantyloxycarbonyl chloride was added. The reaction mixture was stirred at room temperature for 4 h, and then it was diluted with 100 mL of water. After adjustment of the reaction mixture at pH 3 with 1 N HCl, the resulting solution (or suspension) was extracted with *n*-BuOH (∼25 mL × 2). The organic layer was washed with water (∼15 mL × 2) and then concentrated at 45 °C in a vacuum to a small volume (∼3 mL). The precipitate that formed on addition of ether (∼100 mL) was collected and dried in a vacuum at room temperature for 4 h.



Chloropeptin I



R = H. Kistamicin A; R = $CONHCH_2CH_2Ph$ Kistamicin B



Complestatin

Figure 1. Structural formulas of chloropeptin I, kistamicins A and B, and complestatin.

1.5. Method E. N-Carbamoylated Derivatives of Carboxamides of Aminomethylated Derivatives 27, 47. The compounds were obtained by method D, using Boc_2O reagent starting from carboxamides of aminomethylated derivatives obtained by method C.

2. Antiviral and Cytostatic Assay Methods. 2.1. Viruses. The origins of MSV, HIV-1 (strain III_B) (kindly provided by Dr. R. Gallo and Dr. M. Popovic, at that time at the National Cancer Institute of the National Institutes of Health, Bethesda, MD), and HIV-2 (strain ROD) (kindly provided by Dr. L. Montagnier, at that time at the Pasteur Institute, Paris, France) have been described previously.[36−38] HIV-1(III_B) and HIV-2(ROD) stocks were obtained from supernatants of virus-infected MT-4 cell cultures. Moloney murine sarcoma virus (MSV) was obtained as described.[37]

2.2. Anti-HIV Activity Assays. Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing ~3 × 10^5 CEM cells/mL infected with 100 $CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4−5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

2.3. Cytostatic Activity Assays. All assays were performed in 96-well microtiter plates. To each well were added (5−7.5) × 10^4 cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and Molt4/clone 8 cells) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

2.4. Anti-Moloney Murine Sarcoma Virus (MSV) Assays. The inhibitory effect of the test compounds on MSV-induced transformation of murine embryo fibroblast C3H/3T3 cell cultures was examined microscopically at day 6 postinfection. MSV was added at 75 focus-forming units to monolayer cell cultures in 48-well microtiter plates. The detailed procedures for the antiretroviral evaluations have been previously described in detail.[36,37]

2.5. Time-of-Addition Assays. MT-4 cells were infected with HIV-1(III_B) at a multiplicity of infection (moi) of 0.5. The test compound **38** (final concentration of 100 μM) was added at different times after infection as described before.[39] Viral p24 Ag production was determined at 31 h postinfection by enzyme linked immunosorbent assay (NEN, Brussels, Belgium). The reference compounds, dextran sulfate (20 μM), AZT (1.87 μM), and ritonavir (2.84 μM), were added at 100 times their 50% inhibitory concentration (IC_{50}) as obtained in the MT-4/MTT assay.

2.6. Cocultivation Assays between Uninfected Molt4/C8 and Persistently HIV-1-Infected HUT-78/HIV-1 Cells. Persistently HIV-1-infected HUT-78 cells continuously releasing HIV-1 particles without dying (designated HUT-78/HIV-1) were washed to remove free virus from the culture medium, and 5 × 10^4 cells (50 μL) were transferred to 96-well microtiter plates (Sterilin). Then, 5 × 10^4 uninfected MOLT-4 (clone 8) cells (50 μL) and an appropriate concentration of test compound (100 μL) were added to each well. The mixed cell cultures were cultured at 37 °C in a CO_2-controlled atmosphere. The first syncytia (giant cell formation as a result of fusion between the HUT-78/HIV-1 and MOLT-4 cells) arose after about 6 h of cocultivation. After 16−20 h, marked syncytium formation was noted and the number of syncytia was examined and quantified under a microscope.

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Supporting Information Available: HPLC, molecular formulas, and mass spectral data (ESI MS) for compounds **11**, **25**−**28**, **30**, **31**, **40**, **41**, **43**−**48**, **50**, **51**, **54**, **56**−**64**, **67**−**70**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Nagarajan, R. *Glycopeptide Antibiotics*; Marcel Dekker: New York, 1994.
(2) Walsh, C. T. Vancomycin resistance: decoding the molecular logic. *Science* **1993**, *261*, 308−309.
(3) Malabarba, A.; Nicas, T. I.; Thompson, R. S. Structural Modifications of Glycopeptide Antibiotics. *Med. Res. Rev.* **1997**, *17*, 69−137.
(4) Pavlov, A. Y.; Preobrazhenskaya, M. N. Chemical Modification of Glycopeptide Antibiotics. *Russ. J. Bioorg. Chem.* **1998**, *24*, 570−587.
(5) 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.
(6) 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.

(7) 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.
(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) 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*, 361−364.
(11) 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.
(12) Matsuzaki, K.; Ikeda, H.; Ogino, T.; Matsumoto, A.; Woodruff, H. B.; Tanaka, H.; Omura, S. Chloropeptins I and II. Novel inhibitors against gp-120-CD4 binding from *Streptomyces* sp. *J. Antibiot.* **1994**, *47*, 1173−1174.
(13) Naruse, N.; Oka, M.; Konishi, M.; Oki, T. New antiviral antibiotics, kistamycins A and B. II. Structural determination. *J. Antibiot.* **1993**, *46*, 1812−1818.
(14) 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.
(15) Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L.; Felty-Duckworth, A. M. Syntesis and Antimicrobial Activity of N-alkyl Vancomycins. *J. Antibiot.* **1989**, *42*, 63−72.
(16) Nagarajan, R. Structure−activity relationships of vancomycin-type glycopeptides antibiotics. *J. Antibiot.* **1993**, *46*, 1181−1195.
(17) Pavlov, A. Y.; Berdnikova, T. F.; Olsufyeva, E. N.; Miroshnikova, O. V.; Filiposyanz, S. T.; Preobrazhenskaya, M. N.; Scottani, C.; Colombo, L.; Goldstein, B. P. Carboxamides and Hydrazide of Glycopeptide Antibiotic Eremomycin. Synthesis and Antibacterial Activity. *J. Antibiot.* **1996**, *49*, 194−198.
(18) Pavlov, A. Y.; Lazhko, E. I.; Preobrazhenskaya, M. N. A New Type of Chemical Modification of Glycopeptides Antibiotics; Aminomethylated Derivatives of Eremomycin and Their Antibacterial Activity. *J. Antibiot.* **1997**, *50*, 509−513.
(19) 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.
(20) Gerhard, U.; Mackay, J. P.; Maplestone, R. A.; Williams, D. H. The Role of the Sugar and Chlorine Substituents in the Dimerization of Vancomycin Antibiotics. *J. Am. Chem. Soc.* **1993**, *115*, 232−237.
(21) Malabarba, A.; Trani, A.; Strazzolini, P.; Cietto, G.; Ferrari, P.; Tarzia, G.; Pallanza, R.; Berti, M. Synthesis and Biological Properties of N^{63}-Carboxamides of Teicoplanin Antibiotics. Structure−Activity Relationships. *J. Med. Chem.* **1989**, *32*, 2450−2460.
(22) Hermann, R.; Ripamonti, F.; Romano, G.; Restelli, E.; Ferrari, P.; Goldstein, B. P.; Berti, M.; Ciabatti, R. Synthesis and Antibacterial Activity of Derivatives of the Glycopeptide Antibiotic A-40926 and Its Aglycone. *J. Antibiot.* **1996**, *49*, 1236−1247.
(23) Preobrazhenskaya, M. N.; Pavlov, A. Y.; Maffioli, S. I.; Romano, H.; Ciabatti, R. The Synthesis and Antibacterial Activity of Di-Alkylated Derivatives of the Glycopeptide Antibiotic A-40926 and Their Amides. *42nd ICAAC Abstracts*, San Diego, CA September 27−30, 2002; ICAAC: Washington, DC, 2002; p F-358.
(24) Maffiolli, S. I.; Romano, G.; Marzorati, E.; Pavlov, A. Y.; Preobrazhenskaya, M. N.; Ciabatti, R. The Synthesis and Antibacterial Activity of Monoalkylated Derivatives of the Glycopeptide Antibiotic A40926 and Their Amides. *42nd ICAAC Abstracts*, San Diego, CA, September 27−30, 2002; ICAAC: Washington, DC, 2002; p F-357.
(25) Kannan, R.; Harris, C. M.; Harris, T. M.; Waltho, J. P.; Skeiton, N. J.; Williams, D. H. Function of the amino sugar and N-terminal acid of the antibiotic vancomycin in its complexation with cell wall peptides. *J. Am. Chem. Soc.* **1988**, *110*, 2946−2953.
(26) 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. (Moscow)* **1991**, *36*, 28−31.
(27) 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.
(28) Bognar, R.; Sztaricskai, F.; Munk, M. E.; Tamas, J. Structure and Stereochemistry of Ristosamine. *J. Org. Chem.* **1974**, *39*, 2971−2974.
(29) Malabarba, A.; Ferrari, P.; Gallo, G. G.; Kettenring, J.; Cavalleri, B. Teicoplanin, Antibiotics from *Actinoplanes teichomyceticus* nov. sp. VII. Preparation and NMR Characteristics of the Aglycone of Teicoplanin. *J. Antibiot.* **1986**, *39*, 1430−1442.
(30) 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.
(31) Malabarba, A.; Trani, A.; Ferrari, P.; Pallanza, R.; Cavalleri, B. Synthesis and Biological Activity of Some Esters of the *N*-Acetylglucosaminyl Aglycone and of the Aglycone of Teicoplanin. *J. Antibiot.* **1987**, *40*, 1572−1587.
(32) Malabarba, A.; Ciabatti, R.; Kettenring, J.; Scotti, R.; Candiani, G.; Pallanza, R.; Berti, M.; Goldstein, B. P. Synthesis and Antibacterial Activity of a Series of Basic Amides of Teicoplanin and Deglucoteicoplanin with Polyamines. *J. Med. Chem.* **1992**, *35*, 4054−4060.
(33) Trani, A.; Ferrari, P.; Pallanza, R.; Ciabatti, R. Thiooureas and Isothiouronium Salts of the Aglycone of Teicoplanin. 1. Synthesis and Biological Activity. *J. Antibiot.* **1989**, *42*, 1268−1282.
(34) 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.
(35) Cavalleri, B.; Ferrari, P.; Malabarba, A.; Magni, A.; Pallanza, R.; Gallo, G. G. Teicoplanin, Antibiotics from *Actinoplanes teichomyceticus* nov. sp. VIII. Opening of the Polypeptide Chain of Teicoplanin Aglycone under Hydrolytic Conditions. *J. Antibiot.* **1987**, *40*, 49−59.
(36) 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.
(37) De Clercq, E.; Merigan, T. C. Moloney sarcoma virus-induced tumors in mice: inhibition of stimulation by (poly rI):(poly rC). *Proc. Soc. Exp. Biol. Med.* **1971**, *137*, 590−594.
(38) 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.
(39) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J. Potent and selective inhibition of HIV-1 replication by a novel series of TIBO derivatives. *Nature* **1990**, *343*, 470−474.

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