SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF *N*-ACYL VANCOMYCINS†

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(Received for publication April 25, 1988)

Several glycopeptides containing *N*-acyl groups have been isolated recently. We undertook the synthesis of *N*-acyl vancomycins, using the active ester method. The *in vitro* and *in vivo* antibacterial activity were evaluated, and structure-activity relationship of this series of semisynthetic vancomycins is discussed.

Vancomycin is produced by *Amycolatopsis orientalis*[1] (previously designated *Nocardia orientalis* and *Streptomyces orientalis*). The antibiotic has been marketed for the past 30 years and continues to be marketed as the hydrochloride salt to treat deep-seated Gram-positive infections. It is the drug of choice for *Staphylococcus aureus* strains especially those resistant to methicillin (MRSA). Vancomycin is bactericidal to most Gram-positive organisms, but Gram-negative organisms are resistant. Cross resistance with other antibiotics is unknown and there have been few, if any, reports of the emergence of resistant organisms[2] during therapy, in spite of its long usage. Vancomycin is not absorbed from the gastrointestinal tract, and the antibiotic is used to treat enterocolitis caused especially by *Clostridium difficile* in the gut.

Several glycopeptides containing a long chain aliphatic acyl residue on one of the amino sugars have been reported[3~7]. Some of these compounds have been claimed to be superior in antibacterial activity and pharmacokinetics to vancomycin. These glycopeptide antibiotics contain seven aromatic rings and belong to the ristocetin class of glycopeptide antibiotics. Vancomycin contains five aromatic rings and is structurally unique in having *N*-methyl leucine as the *N*-terminal residue. However, the recently discovered orienticins also contain a *N*-methyl leucine as the *N*-terminal amino acid[8]. Until now no *N*-acyl vancomycins have been reported. In this paper we report the synthesis and antibacterial activity of this new class of semisynthetic *N*-acyl vancomycins.

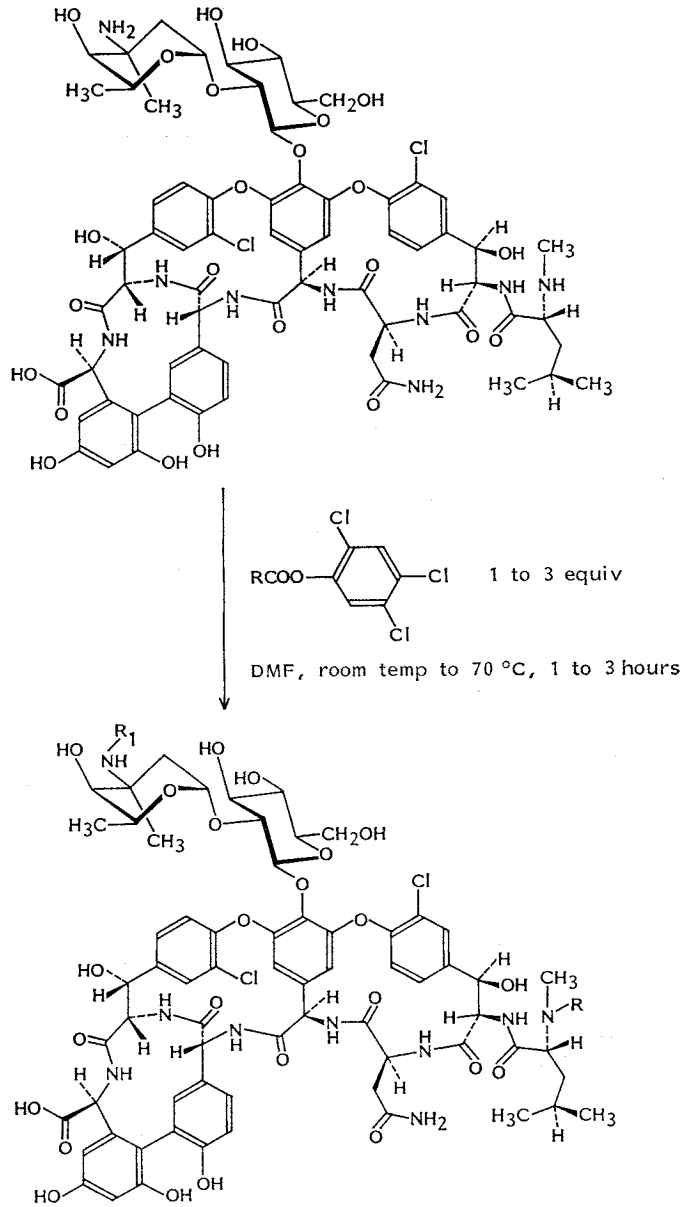
Chemistry

Several methods are available for the synthesis of *N*-acyl vancomycins. We chose the reaction of vancomycin base with the 2,4,5-trichlorophenyl active ester due to the versatility of the reaction and its mild reaction conditions. Two mono-*N*-acyl derivatives substituted at the amino groups of the vancosamine and *N*-methyl leucine moieties, respectively, and one di-*N*-acyl derivative were obtained. The ratio of these three *N*-acyl vancomycins obtained depended on the reaction conditions. These semi-synthetic vancomycins were purified by preparative HPLC.

The HPLC retention time of these *N*-acyl vancomycins were diagnostic of the site of substitution. Accordingly, the mono-*N*-substituted vancomycin on the sugar eluted first, followed by the mono-*N*-

† The SAR of *N*-acyl vancomycins were presented at the 26th Intersci. Conf. on Antimicrob. Agents Chemother., Sept. 26~Oct. 1, 1986, New Orleans, LA., U.S.A., abstract No. 224.

Fig. 1. Synthesis of *N*-acyl vancomycins.

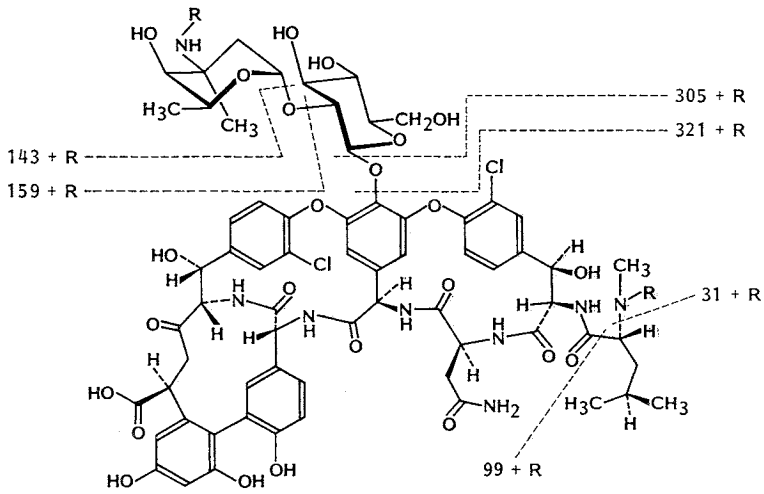


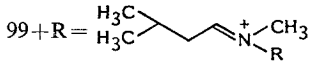
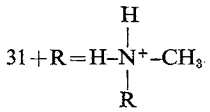
Two mono-*N*-acyl and one di-*N*-acyl vancomycins

acyl derivative on *N*-methyl leucine, and lastly the di-*N*-substituted vancomycin was obtained.

The fast atom bombardment mass spectral (FAB-MS) analysis of the *N*-substituted vancomycins was the single most useful physical-chemical method in the structural assignment of the three *N*-acyl vancomycins. The molecular ion clearly distinguishes the mono-*N*-acyl vancomycin from the di-*N*-acyl vancomycin. Analysis of the fragmentation patterns of the two mono-*N*-acyl vancomycins helps to establish the identity between these two alternative structures. Accordingly, the mono-*N*-acyl vancomycin substituted on the vancosamine affords devancosamine vancomycin and aglucovanco-

Fig. 2. Structure assignment of *N*-acyl vancomycins FAB-MS fragmentation pattern.



R on vancosamine	R on leucine
M+H=Vancomycin+R+H=1,447+R+H	M+H=Vancomycin+R+H=1,447+R+H
1,305=Devancosamine vancomycin+H	1,305+R=Devancosamine vancomycin+H+R
1,143=Aglucovancomycin+H	1,143+R=Aglucovancomycin+H+R
143+R=Vancosamine+R	
159+R=Vancosamine+R+oxygen	99+R= 
305+R=Vancosamine+glucose+R	
	31+R= 

mycin fragments, but the vancosaminyl-*O*-glucose and vancosamine fragments contain the additional mass representing the incorporation of an acyl residue in these two fragments. Similarly, mono-*N*-acyl vancomycin substituted on the amino acid yields the vancosaminyl-*O*-glucose and vancosamine fragments, but the aglucovancomycin and *N*-methyl leucine fragments showed increased mass corresponding to the acyl residue, thereby establishing that it is acylated at the *N*-methyl leucine moiety.

Scope and Structure-activity Relationship (SAR) of *N*-Acyl Vancomycins

All of the naturally occurring *N*-acyl glycopeptides contain the long chain fatty acid side chains. Consequently, we first undertook the preparation of a series of aliphatic *N*-acyl vancomycins. We subsequently included in the SAR several aryl and heterocyclic acids to give the corresponding *N*-aracyl derivatives of vancomycin.

In the aliphatic *N*-acyl vancomycins, the mono-*N*-acyl derivative functionalized on the amino group of the vancosamine sugar is more active than the mono-*N*-acyl vancomycin substituted on the amino acid, *N*-methyl leucine. Both the above mono-*N*-acyl vancomycins are more active than the di-*N*-acyl vancomycin (Table 1).

A comparison of the aliphatic mono-*N*-acyl vancomycins substituted on the amino group of vancosamine reveals that increasing the length of the side chain increases activity. The optimum activity is found when the side chain is between C_9 and C_{11} straight chain fatty acid residue. When

Table 1. *N*-Alkanoyl vancomycins.

Compound	FAB-MS[a]	HPLC[b]		MIC (μg/ml)[c]									
		Gr	RT	S.A. 1	S.A. 2	S.A. 3	S.A. 4	S.E. 1	S.E. 2	S. Py	S. Pn	S.D. 1	S.D. 2
Vancomycin (**1**)	1,448	A	6.66	0.5	0.5	1	1	2	1	0.5	0.5	1	2
2 R=H, R_1=*n*-$C_7H_{15}CO$	1,574	A	13.9	1	2	2	2	8	2	0.5	0.5	2	4
3 R=*n*-$C_7H_{15}CO$, R_1=H	1,574	A	15.72	8	8	8	8	32	16	4	2	8	16
4 R=R_1=*n*-$C_7H_{15}CO$	1,699	A	19.32	32	32	32	32	32	64	32	32	32	32
5 R=H, R_1=*n*-$C_9H_{19}CO$	1,602	A	11.62	0.5	0.5	0.5	0.5	2	1	0.5	1	0.5	1
6 R=*n*-$C_9H_{19}CO$, R_1=H	1,602	A	12.64	0.5	0.5	0.5	1	4	2	4	4	2	4
7 R=R_1=*n*-$C_9H_{19}CO$	1,755	A	16.55	2	4	4	8	32	8	4	16	8	8
8 R=H, R_1=CH_2=$CHC_7H_{15}CO$	1,614	A	12.46	0.5	0.5	0.5	0.5	2	0.5	0.25	0.25	0.25	1
9 R=CH_2=$CHC_7H_{15}CO$, R_1=H	1,614	A	14.15	4	4	4	4	32	8	1	2	2	4
10 R=R_1=CH_2=$CHC_7H_{15}CO$	—	A	18.22	8	8	16	8	64	16	2	4	8	16

[a] Column show molecular ion as M^+ or M^++1.

[b] HPLC: Waters micro Bondapak C_{18} column, UV detection at 254 nm, gradient (Gr); acetonitrile - water, 0.2% triethylamine buffer solvent systems in the following gradients

System	Gradient
A	5% CH_3CN→80% CH_3CN
B	10% CH_3CN→60% CH_3CN

retention time (RT) in minutes.

[c] MICs determined by standard agar dilution method.

S.A. 1: Benzylpenicillin-sensitive *Staphylococcus aureus* X 1.1, S.A. 2: benzylpenicillin-resistant *Staphylococcus aureus* V41, S.A. 3: methicillin-resistant *Staphylococcus aureus* X400, S.A. 4: methicillin-resistant *Staphylococcus aureus* S13E, S.E. 1: macrolide-resistant *Staphylococcus epidermidis* 270, S.E. 2: sensitive *Staphylococcus epidermidis* 222, S. Py: *Streptococcus pyogenes* C203, S. Pn: *Streptococcus pneumoniae* Park, S.D. 1: *Streptococcus faecium* X66, S.D. 2: *Streptococcus faecalis* 2041.

Table 2. Mono-*N*-alkanoyl vancomycins.

Compound (R=H)	MIC (μg/ml)										ED_{50} (mg/kg×2, sc)		
	S.A. 1	S.A. 2	S.A. 3	S.A. 4	S.E. 1	S.E. 2	S. Py	S. Pn	S.D. 1	S.D. 2	S.A. 1	S. Py	S. Pn
Vancomycin (**1**)	0.5	0.5	1	1	2	1	0.5	0.5	1	2	1.8	0.8	0.9
11 *n*-C_3H_7CO	2	2	2	2	8	4	1	1	2	8	6.3	5.5	3.8
12 *n*-$C_5H_{11}CO$	2	4	4	4	16	8	2	2	4	16	11.2	12.9	3.5
2 *n*-$C_7H_{15}CO$	1	2	2	2	8	2	0.5	0.5	2	4	6.9	4.4	4.8
5 *n*-$C_9H_{19}CO$	0.5	0.5	0.5	0.5	2	1	0.5	1	0.5	1	5	2.9	1.4
13 *n*-$C_{11}H_{23}CO$	0.25	0.25	0.5	0.5	2	0.5	0.5	0.5	0.5	0.5	6.8	1.6	0.9
14 *n*-$C_{13}H_{27}CO$	2	2	2	2	8	2	2	2	2	2	6.6	1.4	1.3
15 $(CH_3)_2CHCH_2CO$	2	2	2	2	8	4	1	1	2	8	3.5	3.7	3.9
16 *N*-Valinyl	8	8	32	8	32	32	8	8	8	32			
17 $Br(CH_2)_5CO$	2	2	4	2	8	4	2	1	2	8			
18 $CH_3CO(CH_2)_2CO$	2	2	2	4	16	4	1	1	4	8	3.7	4.0	3.8

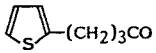
Abbreviations: See footnote in Table 1.

Table 3. *N*-Aracyl vancomycins.

Compound	FAB-MS[a]	HPLC[b]		MIC (μg/ml)[c]									
		Gr	RT	S.A. 1	S.A. 2	S.A. 3	S.A. 4	S.E. 1	S.E. 2	S. Py	S. Pn	S.D. 1	S.D. 2
19 R=H, R_1=$C_6H_5SCH_2CO$	1,598	B	17.1	1	1	1	1	4	2	0.5	0.5	1	4
20 R=$C_6H_5SCH_2CO$, R_1=H	1,598	B	20.6	4	4	8	4	16	8	4	2	8	16
21 R=R_1=$C_6H_5SCH_2CO$	1,748	B	24.7	16	16	32	32	128	64	8	8	32	64
22 R=H, R_1=*p*-$C_4H_9C_6H_4CO$	1,664	A	20.5	0.5	0.5	0.5	0.5	8	1	0.25	0.5	0.125	0.125
23 R=*p*-$C_4H_9C_6H_4CO$, R_1=H	1,664	A	25.0	2	2	2	2	16	2	1	1	1	1
24 R=H, R_1=*p*-$CH_3OC_6H_4CO$	1,582	A	12.8	2	2	2	2	8	2	1	0.5	2	8
25 R=*p*-$CH_3OC_6H_4CO$, R_1=H	1,582	A	15.0	2	2	4	4	16	4	2	2	4	8

[a~c] and abbreviations: See footnote in Table 1.

Table 4. Mono-*N*-aracyl vancomycins.

Compound (R=H)	MIC (μg/ml)										ED_{50} (mg/kg×2, sc)		
	S.A. 1	S.A. 2	S.A. 3	S.A. 4	S.E. 1	S.E. 2	S. Py	S. Pn	S.D. 1	S.D. 2	S.A. 1	S. Py	S. Pn
Vancomycin (**1**)	0.5	0.5	1	1	2	1	0.5	0.5	1	2	1.8	0.8	0.9
26 C_6H_5CO	1	1	2	1	4	2	1	0.125	2	4	5.3	4.2	1.7
27 $(C_6H_5)_2CHCO$	1	1	1	1	4	2	0.5	0.25	1	4	3.9	7.1	2.0
28 $C_6H_5(CH_2)CO$	2	2	2	4	8	2	1	1	2	4	3.7	4.4	3.7
29 $C_6H_5OCH_2CO$	1	1	2	1	4	2	0.5	0.125	1	4	3.3	4.0	3.1
19 $C_6H_5SCH_2CO$	1	1	1	1	4	2	0.5	0.5	1	4	2.3	4.5	1.9
22 p-$C_4H_9C_6H_4CO$	0.5	0.5	0.5	0.5	2	1	0.25	0.125	0.5	1	2.5	5.8	1.2
30 p-$C_8H_{17}C_6H_4CO$	1	1	2	1	16	4	2	2	0.5	1	3.9	1.0	0.9
24 p-$CH_3OC_6H_4CO$	2	2	2	2	8	2	1	0.5	2	8	5.4	6.3	3.7
31 p-$C_4H_9OC_6H_4CO$	0.5	0.5	0.5	0.5	2	1	0.25	0.125	0.5	1	3.1	6.6	2.7
32 p-$C_8H_{17}OC_6H_4CO$	0.5	0.5	0.5	0.5	8	1	0.25	0.5	0.125	0.125	1.4	1.3	1.2
33 p-$CH_3OC_6H_4OCH_2CO$	1	2	2	1	4	2	0.5	0.25	2	4	3.5	5.0	2.3
34 	1	1	1	2	8	2	1	0.5	2	4	3.1	3.4	2.3

Abbreviations: See footnote in Table 1.

the chain length is above C_{11}, the activity drops off. Modification of the side chain with branched chain, or introduction of amino, bromo or carbonyl group does not alter the activity (Table 2).

The SAR of the *N*-aracyl vancomycins follow a pattern similar to the aliphatic *N*-acyl vancomycins. Accordingly, the mono-*N*-acyl vancomycins substituted on vancosamine are more active than the mono-*N*-acyl derivatives functionalized on *N*-methyl leucine; and both the above mono-*N*-acyl vancomycins are more active than the corresponding di-*N*-acyl vancomycins. Having established firmly that the di-*N*-acyl derivatives are the least active, the reaction conditions were adjusted to yield mainly the two mono-*N*-acyl vancomycins. As can be seen in the case of *p*-butylbenzoyl (compounds **22** and **23**) and *p*-methoxybenzoylvancomycins (compounds **24** and **25**), no di-*N*-acyl derivatives were isolated (Table 3).

A comparison of the aliphatic mono-*N*-acyl and the aromatic mono-*N*-aracyl vancomycins substituted on the vancosamine sugar (Tables 2 and 4), show that the mono-*N*-aracyl vancomycins are in general more active than the aliphatic *N*-acyl derivatives. The most active compounds in the mono-*N*-aracyl series are the *p*-octylbenzoyl (compound **30**) and *p*-octyloxybenzoyl (compound **32**) derivatives, with a hydrocarbon attached to the aromatic ring. (Table 4).

Finally, a comparison of the antibacterial activity of the parent antibiotic with its *N*-acyl derivatives shows, that even though in some cases there is a slight increase in the *in vitro* spectrum of the mono-*N*-acyl derivatives, the *in vivo* activities do not exhibit any increase over vancomycin.

Experimental

Chemistry

General procedure for the preparation of *N*-acyl vancomycins: Vancomycin base was reacted with varying amounts of the appropriate active ester of the acid in DMF solution. Reaction times varied from 1 hour to 2 days, and the temperature from ambient to 65°C. A shorter reaction time and slight excess of the active ester favored the formation of the mono-*N*-acyl vancomycin. A longer reaction time and a large excess of the active ester gave mainly the di-*N*-acyl vancomycins. Thus, except in a few cases, a single reaction did not yield all the three *N*-acyl vancomycins. Two typical examples are given below.

Example 1

Vancomycin base (4 g, 2.76 mmol) was dissolved in 150 ml of DMF. To this solution, *n*-decanoyl-2,4,5-trichlorophenyl active ester (4 g, 11.39 mmol) was added and the reaction stirred at ambient temperature for 2 days. The reaction mixture was transferred to a Virtis jar and mixed with Celite to form a very thick paste, and dried under vacuum overnight.

The residue was stirred in methanol and filtered. The insoluble residue was again stirred in methanol and filtered. The methanol filtrates were pooled and evaporated under vacuum. The residue was triturated with CH_2Cl_2 and filtered to remove unreacted active ester. The insoluble residue was purified by preparative HPLC using a Waters C_{18} Prep Pak column. The column was eluted with an acetonitrile - water gradient system containing 1% pyridinium acetate and was monitored using a UV detector at 280 nm. There was obtained 709 mg (16% yield) of mono-*N*-acyl **5** and 498 mg (10% yield) of diacylated **7** decanoyl derivatives of vancomycin.

Example 2

To a solution of vancomycin base (5.14 g, 3.55 mmol) in 50 ml of DMF was added 2,4,5-trichlorophenylthiophenoxyacetyl ester (1.63 g, 4.71 mmol). The reaction mixture was stirred at 70°C for 2 hours. The reaction mixture was evaporated to about 10 ml, then 10 ml of water was added and the resulting solution freeze-dried. The lyophilized residue was triturated with CH_2Cl_2, filtered, and yielded 6.5 g of insoluble residue. Two g of this insoluble residue was purified by preparative

HPLC on a Waters C_{18} Prep Pak column using an acetonitrile - water gradient system containing 1% pyridinium acetate. This separation afforded 570 mg (33% yield) of **19**, 178 mg (10% yield) of **20**, and 396 mg (23% yield) of **21**.

Chromatography

The reaction was monitored by analytical HPLC using a Waters micro Bondapak C_{18} column with acetonitrile - water, 0.2% triethylamine buffer solvent system. The gradients were: System A, gradient 5% $CH_3CN \rightarrow 80\%$ CH_3CN; system B, gradient 10% $CH_3CN \rightarrow 60\%$ CH_3CN. The UV was monitored at 254 nm.

The reaction mixture was purified by HPLC using a Waters C_{18} Prep Pak. The column was eluted with an acetonitrile - water system containing 1% pyridinium acetate and was monitored using UV detection at 280 nm. The purity of the separated products was established by analytical HPLC, and their structures confirmed by FAB-MS.

FAB-MS

FAB-MS spectra were determined using a VGZ AB-3F mass spectrometer. Samples were dispersed in thioglycerol and introduced into the mass spectrometer on a cooled FAB target.

Antibacterial Activity *In Vitro*

The MICs for the aerobic bacteria strains were determined in an agar dilution assay. Mueller-Hinton agar containing 1% supplement C (Difco Laboratories, Detroit, Michigan) was used. The dilutions of the antibiotics were made in water and mixed with the melted agar prior to pouring the plates. The various bacteria were inoculated onto the medicated plates using a Cotlara replicator at an inoculum of 10^4 cfu/spot. The plates were then incubated for 20~24 hours at 35°C. End points were read to discrete colonies.

Antibacterial Activity *In Vivo*

The therapeutic efficacy of the vancomycin derivatives were determined in standard mouse protection tests. An experimental systemic infection was produced using ICR random sex mice (Harland Laboratories, Cumberland, Indiana), by intraperitoneal inoculation of a suitable diluted broth culture of the infecting organism. The test compounds (*N*-acyl vancomycins) were administered subcutaneously at one and 5 hours post-infection. Five 2-fold dilutions of each antibacterial agent were tested and there were 8 mice for each dose level. All the mice were observed for a period of 7 days, after which the effective dose (ED_{50}) was calculated by the method of REED and MUENCH[9]. Under the conditions of the test, all infected and untreated mice died within 48 hours.

Acknowledgments

We thank DONNIS BERRY, DALE DUCKWORTH, LYELL HUCKSTEP, STEVEN LAWRENCE, LARRY SACHS and REGINALD THOMAS for analytical and preparative HPLC separations. We also thank MRS. VIRGINIA SANTERRE for typing the manuscript.

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