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Mannopeptimycins, Novel Antibacterial Glycopeptides from *Streptomyces hygroscopicus*, LL-AC98

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Abstract: A series of novel antibiotics with activity against methicillin-resistant staphylococci and vancomycin-resistant enterococci has been purified, and their structures have been characterized using spectroscopic analyses and chemical conversions. These antibiotics, designated mannopeptimycins α−ϵ (**1**−**5**), are glycosylated cyclic hexapeptides containing two stereoisomers of an unprecedented amino acid, α-amino-β-[4′-(2′-iminoimidazolidinyl)]-β-hydroxypropionic acid (Aiha), as a distinguishing feature. The cyclic peptide core of these antibiotics is attached to a mannosyl monosaccharide moiety in **2** and to mannosyl monosaccharide and disaccharide moieties in **1**, **3**, **4**, and **5**. The presence and position of an isovaleryl group in the terminal mannose (Man-B) in **3**−**5** are critical for retaining antibacterial potency.

Introduction

Bacterial resistance to antibiotics is a serious public health problem. According to the World Health Organization,[1] more than 95% of the *Staphylococcus aureus* isolates worldwide are now resistant to penicillin and up to 60% are resistant to methicillin. Resistance is spreading from hospital-acquired infections to community-acquired pathogens, such as pneumococci and tuberculosis. The structurally related glycopeptides, vancomycin and teicoplanin, are considered the ultimate antibiotics of choice for treatment of methicillin-resistant *S. aureus*, but alarmingly, the rate of vancomycin-resistant enterococci has been increasing each year,[2] and there are cases of vancomycin-resistant *S. aureus* reported in industrial countries.[3]

As part of a program designed to discover new classes of agents to combat the rapid increase of bacterial resistance, a number of antibiotics discovered in the course of five decades of antibacterial research were reexamined.[4] One of the most promising of those was the AC98 complex,[5] which was an antibiotic mixture produced by a strain of *Streptomyces hygroscopicus*, LL-AC98. The complex was initially isolated in the late 1950s and shown to be effective against Gram-positive bacteria. In recent studies, the complex was demonstrated to have in vitro activity against methicillin-resistant staphylococci and vancomycin-resistant enterococci via inhibition of cell wall synthesis.[6a] On the basis of these findings a program was initiated to thoroughly investigate the antibacterial activity of the class and generate potent compounds for further development. In this paper, the structural characterization and antibacterial activity of the five glycopeptides, mannopeptimycins α−ϵ (**1**−**5**), purified from the complex are reported.

Results and Discussion

The AC98 complex, as previously described,[5] was separated by reverse phase HPLC using solvents containing trifluoroacetic acid (TFA) to afford TFA salts of mannopeptimycins α−ϵ (**1**−**5**). These compounds and other derivatives were also isolated from fermentation broths of *Streptomyces hygroscopicus* LL-AC98 and its mutant strains in various media.[6b] The majority of structural information for mannopeptimycins was derived from extensive nuclear magnetic resonance (NMR) analyses using D_2O, 1:1 mixture of D_2O/CD_3OD, or DMSO-d_6 as solvents.

The molecular formula of mannopeptimycin α (**1**) was determined by high-resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometry to be $C_{54}H_{78}N_{12}O_{25}$. The

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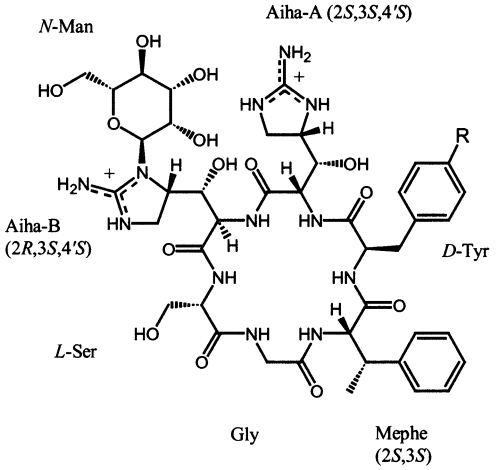

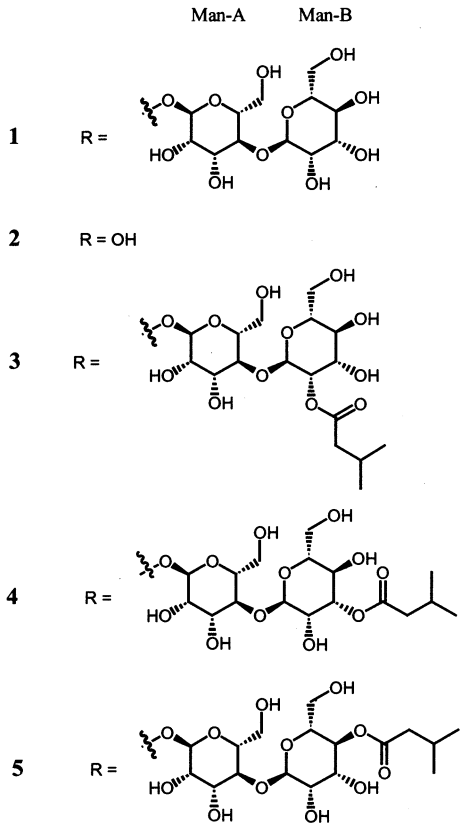

Figure 1. Structures of mannopeptimycins α−ϵ (**1**−**5**).

^{13}C NMR spectrum contained six carbonyl signals near δ 171, and seven methine signals between δ 55 and 63, typical for a peptide. The ^{13}C NMR spectrum also displayed signals for anomeric methine carbons at δ 104.2 and 100.5, together with 13 methine signals between 69 and 83, representing three possible sugar units in the molecule. The ^{1}H NMR spectrum contained signals for α-protons of the amino acid residues and for sugar moieties, all indicative of a glycopeptide structure.

Detailed analysis of two-dimensional (2-D) COSY, TOCSY, HMBC, and HSQC spectral data revealed the presence of several common amino acid residues, serine (Ser), glycine (Gly), β-methylphenylalanine (Mephe), and tyrosine (Tyr). In addition, 2-D NMR data identified two sugar moieties (Man-A and Man-B). For Man-A, the anomeric proton (H-1) at δ 5.47 was coupled to H-2 at 4.08, which was in turn coupled to H-3 at 4.14. H-3 was coupled to H-4 at δ 3.90 and H-4 was coupled to H-5 at 3.71, which was further coupled to H_2-6 at ~3.75. The small coupling constants (≤4.5 Hz) for $^{3}J_{H1-H2}$ and $^{3}J_{H2-H3}$ and large ones (9.5 Hz) for $^{3}J_{H3-H4}$ and $^{3}J_{H4-H5}$ defined Man-A as a mannose moiety. For Man-B, the seven-proton spin system from H-1 to H_2-6 was identified by analysis of COSY and TOCSY data; the coupling constants, however, were difficult to measure owing to the extensive signal overlap. Analyses of ^{1}H NMR spectra of the ester derivatives **3**, **4**, and **5**, which contained an isovaleryl group respectively at C-2, C-3, or C-4 of Man-B, enabled the determination of the ^{1}H−^{1}H coupling constants for this sugar. The coupling pattern, small $^{3}J_{H1-H2}$ and $^{3}J_{H2-H3}$ and large $^{3}J_{H3-H4}$ and $^{3}J_{H4-H5}$, defined the mannosyl stereochemistry for Man-B. The relatively large $^{1}J_{H1-C1}$'s (170−171 Hz) for both mannosyl moieties, as seen in the HMBC spectrum, indicated their α-anomeric configurations. A 4 → 1′ linkage between the two sugar moieties was required by ^{1}H−^{13}C correlations from H-4 (Man-A) at δ 3.90 to C-1 (Man-B) at 104.2 and from H-1 (Man-B) at 5.28 to C-4 (Man-A) at 76.7. The ^{13}C NMR data for Man-B were also consistent with those reported in the literature for α-mannose.[7]

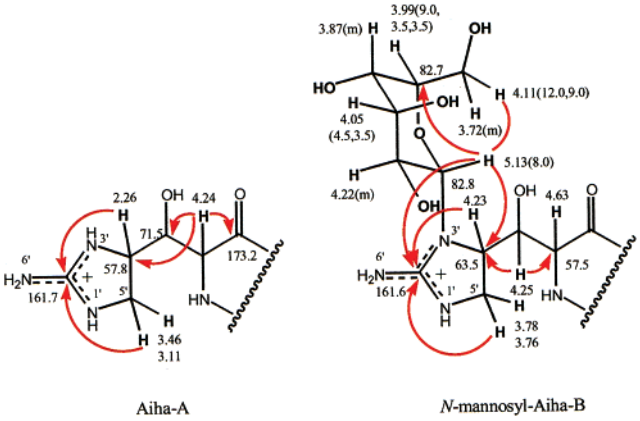

Figure 2. Aiha-A and *N*-mannosyl-Aiha-B moieties in **1**. Indicated are selected ^{1}H−^{13}C HMBC correlations (arrows) and ^{1}H−^{1}H ROESY correlation (arc).

The 2-D NMR data revealed the presence of two units of an unprecedented amino acid, the α-amino-β-[4′-(2′-iminoimidazolidinyl)]-β-hydroxypropionic acid (Aiha-A and -B), as illustrated in Figure 2. COSY and TOCSY data delineated the ^{1}H−^{1}H spin systems from H_α to H_2-5′, and the two- or three-bond ^{1}H−^{13}C correlations in the HMBC correlations provided evidence for the structures of these amino acid residues. For

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Aiha-A, the HMBC correlations from H_α at δ 4.24 to C═O at 173.2, to C_β at 71.5, and to C-4′ at 57.8, and from H-4′ at 2.26 and H_2-5′ at 3.46 and 3.11 to a guanidino carbon, C-2′, at 161.7, were observed. For Aiha-B, the HMBC correlations from H_β at δ 4.25 to C_α at 57.5 and to C-4′ at 63.5, and from H-4′ at 4.23 and H_2-5′ at 3.78 and 3.76 to C-2′ at 161.6, were observed.

Furthermore, a careful analysis of the TOCSY and COSY spectral data led to the identification for a third mannosyl moiety. The seven-proton spin system from H-1 to H_2-6 and the correlation from its anomeric proton signal (H-1) at δ 5.13 to C-5 at 82.7 in the HMBC spectrum were indicative of a pyranose moiety. The large $^3J_{H1-H2}$ (8.0 Hz), and small $^3J_{H2-H3}$ (3.0 Hz), $^3J_{H3-H4}$ (4.5 Hz), and $^3J_{H4-H5}$ (3.0 Hz), together with a ROESY cross-peak between H-1 at δ 5.13 and H-6 at 4.11, suggested a mannose moiety with an axial C-6 hydroxymethylene. A similar coupling pattern and corresponding ROESY cross-peak were observed in the spectra of compound **2**.

The attachment of C-1 (δ 82.8) of this sugar to N-3′ in Aiha-B was required by three-bond HMBC correlations from H-1 at δ 5.13 to C-4′ at 63.5 and to the guanidino carbon C-2′ at 161.6. In the electrospray ionization mass spectrum, the doubly charged molecular ion $(M + 2H)^{2+}$ was displayed as a prominent signal, supporting the presence of two basic groups in the molecule.

Evidence for the cyclic nature of the peptide core of **1** came from the molecular formula, which required an additional degree of unsaturation beyond those already accounted for in the substructures. The sequence of the peptide core was primarily determined by analysis of $^1H-^{13}C$ HMBC data. The respective three-bond correlations in an HMBC spectrum from α-protons of serine, glycine, β-methylphenylalanine, tyrosine, Aiha-A, and Aiha-B to carbonyls of Aiha-B, serine, glycine, β-methylphenylalanine, tyrosine, and Aiha-A established the amino acid sequence for the cyclic hexapeptide to be *c*-[Ser-Gly-Mephe-Tyr-Aiha-A-Aiha-B] (Table 1). In the HMBC spectrum, the $^1H-^{13}C$ correlation from H-1 in Man-A at δ 5.47 to C-4′ in Tyr at 157.5 required that the mannosyl disaccharide moiety be attached to the tyrosine. Thus, the elucidation of the planar structure for compound **1** was completed.

The molecular formula of mannopeptimycin β (**2**) was determined by high-resolution FTICR mass spectrometry to be $C_{42}H_{58}N_{12}O_{15}$, which was $C_{12}H_{20}O_{10}$ less than compound **1**. Compound **2** showed ^1H and ^{13}C NMR spectral data very similar to those of **1**, except that signals for the mannosyldisaccharide moiety on the tyrosine were lacking (for ^{13}C NMR data of **2**, see Table 3). In addition, compound **2** was obtained by hydrolysis of **1** with 5% aqueous HCl at 60 °C (Figure 3); therefore, the structure was confirmed.

Compound **2** was oxidized with potassium periodate to afford a dialdehyde **6**, which was reduced to the corresponding alcohol **7** by sodium borohydride. The hydrolysis of **7** with 5% aqueous hydrochloric acid at 80 °C afforded the aglycone (Figure 3). The TFA salt of the aglycone (**8**), obtained by HPLC with TFA-containing mobile phase, was subjected to NMR studies (spectral data acquired in DMSO-d_6). The respective two-bond $^1H-^{13}C$ correlations from NH proton signals of serine, glycine, β-methylphenylalanine, tyrosine, Ahia-A, and Ahia-B to carbonyls of Ahia-B, serine, glycine, β-methylphenylalanine, tyrosine, and Ahia-A in an HMBC spectrum confirmed the assigned sequence for the cyclic hexapeptide (Table 2). In addition, the respective strong ROESY cross-peaks between NH proton signals of serine, glycine, β-methylphenylalanine, tyrosine, Ahia-A, and Ahia-B, and the α-proton signals of Ahia-B, serine, glycine, β-methylphenylalanine, tyrosine, and Ahia-A in a ROESY spectrum also supported the sequence (Figure 5).

The molecular formula of mannopeptimycin γ (**3**) was determined by high-resolution FTICR mass spectrometry to be $C_{59}H_{86}N_{12}O_{26}$. Compared to **1**, the ^1H and ^{13}C NMR spectral data of **3** showed the presence of an isovaleryl group in addition to the cyclic peptide core and three mannosyl moieties. In a COSY spectrum, the signal of two methyl doublet signals at δ 0.96 (6H, d, 6.6 Hz) was coupled to a methine signal at 2.09 (m) which was in turn coupled to methylene protons at 2.40 (2H, m). The H-2 signal of Man-B at δ 5.18 (dd, 4.0, 1.8 Hz) was coupled to H-1 of the same sugar at 5.33 in the COSY spectrum and to C-1 of the isovaleryl group at 177.3 in an HMBC spectrum. The isovaleryl group was thus determined to be attached to C-2 of Man-B. Mannopeptimycin γ (**3**) was hydrolyzed with 5% sodium carbonate at ambient temperature to afford **1**.

Mannopeptimycins δ (**4**) and ϵ (**5**) were also assigned to be isovaleryl esters, differing from **3** only in the positions of the ester group, where the isovaleryl was connected to C-3 and C-4 of Man-B in **4** and **5**, respectively. The same hydrolysis condition was respectively applied to **4** and **5** to obtain **1**. For ^{13}C NMR data of **3**, **4**, and **5**, see Table 3.

Absolute Stereochemistry. Mannopeptimycin α (**1**) was subjected to standard peptide hydrolysis (6 N HCl, 100 °C) to yield the component amino acids. The absolute configurations of the tyrosine and serine were respectively established to be D and L by LC/MS analysis of the Marfey's derivatives.[8] The β-methylphenylalanine isolated from the hydrolysate was found to have a (2*S*, 3*S*) configuration by comparing its chemical shift data, $^1H-^1H$ coupling constants, and optical rotation value with literature data for the four isomers obtained by synthesis.[9] The configurations of the remaining chiral centers in Aiha-A and -B and the conformation of the cyclic hexapeptide core were determined by derivatization and further NMR experiments.

The mixture of the polar amino acids of the hydrolysate was reacted with benzyl chloroformate (CBZCl) to yield a benzyl carbamate mixture of diastereoisomeric Aiha-A and -B, not separable by HPLC. The mixture was treated with methyl iodide and the resulting methyl esters were carefully purified by reverse phase HPLC to afford **9** and **10**. The coupling constants, $^3J_{H\alpha-H\beta}$'s, were measured as 5.9 and 1.5 Hz respectively for **9** and **10** (Figure 4). This was indicative of an erythro configuration for the former and threo for the latter, compared with literature data for α-amide-β-hydroxycarboxylic acid derivatives.[10] It was interesting to note that the presence of a pair of L- and D-α-amino-β-[4′-(2′-iminoimidazolidinyl)]propionic acid residues, designated enduracididine and alloenduracididine, was reported in the cyclic peptide enduracidin.[11]

As indicated, the strong NOE cross-peaks between the α-H's of the amino acid residues and the NH's of their adjacent

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Table 1. ^{1}H and ^{13}C NMR Data of Mannopeptimycin α (**1**) (TFA Salt, D_2O, DSS as Reference)

amino acid residue	^{13}C (75 MHz)	^{1}H (500 MHz, mult, *J* in Hz)	2-D correln in HMBC (*J* = 8 Hz)
(2*S*,3*S*,4′*S*)-α-Amino-*β*-[4′-(2′-iminoimidazolidinyl)]-*β*-hydroxypropionic acid (Aiha-A)			
C═O (1)	173.2		$H_α$(Aiha-B), $H_α$
α (2)	55.7	4.24 (m)	$H_β$
β (3)	71.5	3.68 (m)	$H_α$, $H_α$, H_2-5′
2′	161.7		H-4′, H_2-5′
4′	57.8	2.26 (br dd, 9.5, 7)	$H_α$, $H_β$, H_2-5′
5′	44.6	3.46 (dd, 10, 7)	$H_β$
		3.11 (dd, 10, 10)	
(2*S*,3*S*,4′*R*)-α-Amino-*β*-[4′-(2′-iminoimidazolidinyl)]-*β*-hydroxypropionic acid (Aiha-B)			
C═O (1)	173.0		$H_α$(Ser)
α (2)	57.5	4.63 (d, 6)	$H_β$
β (3)	72.5	4.25 (m)	$H_α$
2′	161.6		H-4′, H_2-5′, H-1 (*N*-Man)
4′	63.5	4.23 (m)	$H_β$, H_2-5′, H-1 (*N*-Man)
5′	44.2	3.78 (m)	$H_β$
		3.76 (m)	
L-**Serine (Ser)**			
C═O (1)	174.1		$H_{α2}$(Gly)
α (2)	58.4	4.48 (dd, 5.5, 5.5)	$H_{β2}$
β (3)	63.6	3.88 (m)	$H_α$
		3.76 (m)	
Glycine (Gly)			
C═O (1)	173.54		$H_α$(MePhe), $H_{α2}$
α (2)	45.0	4.09 (d, 11)	
		3.82 (d, 11)	
(2*S*,3*S*)-*β*-Methylphenylalanine (Mephe)			
C═O (1)	174.8		$H_α$(Tyr), $H_α$
α (2)	63.1	4.33 (d, 10.5)	$H_β$, *β*-Me
β (3)	44.0	3.12 (m)	$H_α$, *β*-Me, H-2′, H-6′
1′	144.5		$H_β$, *β*-Me, H-3′, H-5′
2′, 6′	130.5	7.32 (2H, d, 7.5)	H-3′, H-5′
3′, 5′	131.5	7.41 (2H, dd, 7.5, 7.5)	H-2′, H-6′
4′	130.2	7.28 (t, 7.5)	H-2′, H-6′
β-Me	19.9	1.37 (3H, d, 7)	$H_β$
D-**Tyrosine (Tyr)**			
C═O (1)	173.4		$H_α$(Aiha-A), $H_α$, $H_{β2}$
α (2)	56.6	4.22 (d, 11)	$H_{β2}$
β (3)	38.4	2.42 (dd, 13.5, 11)	$H_α$, H-2′, H-6′
		1.98 (dd, 13.5, 5)	
1′	132.8		$H_{β2}$, H-2′, H-6′
2′, 6′	133.2	7.07 (2H, d, 8.5)	H-3′, H-5′
3′, 5′	119.7	7.05 (2H, d, 8.5)	H-2′, H-6′
4′	157.5		H-2′, H-6′, H-1 (Man-A)
***N*-Mannose (*N*-Man)**			
1	82.8	5.13 (d, 8.0)	H-2, H-3, H-5
2	67.8	4.22 (m)	H-1, H-3, H-4
3	73.3	4.05 (dd, 4.5, 3.5)	H-1, H-4, H-5
4	70.9	3.87 (m)	H-3, H-5, H-6 (3.72)
5	82.7	3.99 (ddd, 9, 3.5, 3.5)	H-1, H-3, H-6 (4.11)
6	61.7	4.11 (dd, 12, 9)	H-4, H-5
		3.72 (dd, 12, 3.5)	
α-Mannose-A (Man-A)			
1	100.5	5.47 (d, 1.5)	H-2
2	73.0[a]	4.08 (m)	
3	73.7	4.14 (dd, 9.5, 3.5)	
4	76.7	3.90 (dd, 9.5, 9.5)	H-1 (Man-B), H-2, H-3, H-5
5	74.5	3.71 (m)	H-1
6	63.6	3.75 (2H, m)	H-4, H-5
α-Mannose-B (Man-B)			
1	104.2	5.28 (d, 1.5)	H-4 (Man-A)
2	73.1[a]	4.09 (m)	
3	73.2	3.79 (m)	H-1
4	69.3	3.70 (m)	H-2, H-3, H-5
5	76.4	3.69 (m)	H-1
6	63.4	3.88 (m)	H-4, H-5
		3.78 (m)	

[a] Assignments may be reversed.

residues (toward the C-termini) were observed in the ROESY spectrum of the aglycone (**8**). The coupling constants between the α-H's and the NH's of the same residues ranged from 7.3 to 8.8 Hz (average ∼6.0 Hz for α-H's of glycine), indicative

Table 2. ^{1}H and ^{13}C NMR Data of Aglycone (**8**) (TFA salt, DMSO-d_6)

amino acid residue	^{13}C (75 MHz)	^{1}H (400 MHz, mult, *J* in Hz)	^{1}H–^{13}C correln in HMBC (400 MHz, *J* = 8 Hz)
(2*S*,3*S*,4′*S*)-α-Amino-*β*-[4′-(2′-iminoimidazolidinyl)]-*β*-hydroxypropionic acid (Aiha-A)			
C═O (1)	170.4		α-NH (Aiha-B), H_α
α (2)	53.6	4.39 (dd, 8.6, 8.6)	*β*-OH
α-NH		8.12 (d, 8.6)[a]	
β (3)	69.5	3.62 (m)	H_α, H_2-5′
β-OH		5.40 (d, 5.6)[a]	
1′		7.76 (m)a	
2′	159.2		H_2-5′
3′		8.04 (br s)[a]	
4′	55.8	3.42 (ddd, 9.7, 5.1, 2.2)	*β*-OH
5′	42.2	3.55 (m)	H_β
		3.22 (dd, 13, 5.1)	
6′		7.63 (2H, br s)[a]	
(2*S*,3*S*,4′*R*)-α-Amino-*β*-[4′-(2′-iminoimidazolidinyl)]-*β*-hydroxypropionic acid (Aiha-B)			
C═O (1)	169.1		α-NH (Ser), H_α
α (2)	55.7	4.22 (dd, 7.3, 2.5)	*β*-OH
α-NH		8.46 (7.3)[a]	
β (3)	70.5	3.95 (br dd, 7.1, 6.3)	H_2-5′, *β*-OH
β-OH		5.64 (6.3)[a]	
1′		8.00 (br s)[a]	
2′	159.2		H_2-5′
3′		7.79 (m)[a]	
4′	56.5	3.84 (ddd, 9, 7.1, 7.1)	H_2-5′, *β*-OH
5′	43.9	3.58 (2H, m)	
6′		7.77 (2H, m)[a]	
L-Serine (Ser)			
C═O (1)	169.8		α-NH (Gly), H_α, $H_{\beta 2}$
α (2)	54.0	4.31 (m)	$H_{\beta 2}$, *β*-OH
α-NH		7.93 (d, 7.5)[a]	
β (3)	61.0	3.62 (m)	H_α
		3.72 (m)	
β-OH		5.15 (t, 5.1)[a]	
Glycine (Gly)			
C═O (1)	168.4		α-NH (Mephe), H_α (Mephe), $H_{\alpha 2}$
α-NH		8.21 (t, 6.0)[a]	
α (2)	42.8	3.66 (2H, m)	
(2*S*,3*S*)-*β*-Methylphenylalanine (Mephe)			
C═O (1)	169.5		α-NH (Tyr), H_α
α (2)	57.4	4.49 (dd, 8.7, 6.5)	*β*-Me
α-NH		7.77 (m)[a]	
β (3)	40.0	3.19 (m)	H_α, *β*-Me, H-2′, H-6′
β-Me	16.3	1.06 (3H, d, 7.1)	H_α, H_β
1′	143.0		H_β, *β*-Me, H-3′, H-5′
2′, 6′	127.5	7.14 (2H, d, 7.1)	H_β, H-2′, H-6′
3′, 5′	127.9	7.21 (2H, dd, 7.5, 7.1)	H-3′, H-5′
4′	126.2	7.15 (t, 7.1)	H-2′, H-6′
D-Tyrosine (Tyr)			
C═O (1)	170.1		α-NH (Aiha-B), H_α, $H_{\beta 2}$
α (2)	54.3	4.30 (m)	$H_{\beta 2}$
α-NH		7.80 (8.8)[a]	
β (3)	36.1	2.65 (dd, 13.5, 7.6)	H_α, H-2′, H-6′
		2.41 (dd, 13.5, 6.3)	
1′	127.3		H_α, $H_{\beta 2}$, H-3′, H-5′
2′, 6′	129.9	6.83 (2H, d, 8.3)	H-2′, H-6′
3′, 5′	114.7	6.58 (2H, d, 8.3)	H-3′, H-5′, 4′-OH
4′	155.9		H-2′, H-6′, H-3′, H-5′, 4′-OH
4′-OH		9.20 (br s)[a]	

[a] D_2O exchangeable.

of trans conformations for all amide bonds and a zigzag orientation for the peptide backbone. Observation of several NOEs between the aromatic protons at *δ* 6.83 and 6.58 in the tyrosine and H-4′ and H-5′ at *δ* 3.42 and 3.22 in Aiha-A required an outward oriented side chain for Aiha-A. Likewise, the NOE between the signals of H-4′ of Aiha-B at *δ* 3.84 and *β*-OH of Aiha-A at 5.40 prohibited an inward oriented side chain for Aiha-B. These special relationships can only be established when Aiha-A and -B respectively have L (2*S*) and D (2*R*) configurations, resulting in a flat conformation for the peptide ring system with both side chains facing outward (Figure 5). It is known that a cyclic peptide containing an even number of alternating D- and L-α-amino acids adopts a flat confirmation that allows side chains to face outward.[12]

The relative stereochemical relationship of C_α, C_β, and C-4′ in the residues Aiha-A and -B was determined through an extension of the recently articulated *J*-configuration analysis

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Table 3. ^{13}C NMR Data of Mannopeptimycins $\beta-\epsilon$ (**2**−**5**) (TFA Salts, 75 MHz, CD_3OD/D_2O, 1:1)

	chemical shift (DSS as reference)			
	2	**3**	**4**	**5**
		(2*S*,3*S*,4′*S*)-Aiha-A		
CO (1)	173.2	173.2	173.2	173.3
α (2)	56.1	56.1	56.3	56.5
β (3)	71.9	72.0	72.1	72.1
2′	162.0	161.9	161.8	162.1
4′	58.3	58.3	58.4	58.5
5′	44.8	44.8	44.9	45.0
		(2*S*,3*S*,4′*R*)-Aiha-B		
CO (1)	173.0	173.0	173.0	173.1
α (2)	57.8	57.8	57.9	58.0
β (3)	72.2	72.2	72.1	72.3
2′	161.7	161.7	161.8	161.9
4′	63.8	63.8	63.8	63.8
5′	44.5	44.6	44.7	44.8
		L-**Ser**		
CO (1)	173.9	173.9	173.9	173.9
α (2)	58.3	58.4	58.4	58.6
β (3)	63.8	63.8	63.8	63.8
		Gly		
CO (1)	173.6	173.5	173.6	173.7
α (2)	45.1	45.0	45.1	45.1
		(2*S*,3*S*)-*β*-Mephe		
CO (1)	174.3	174.4	174.4	174.7
α (2)	62.8	62.7	62.7	62.6
β (3)	44.2	44.1	44.1	44.0
1′	144.7	144.8	144.9	144.9
2′, 6′	130.5	130.5	130.5	130.5
3′, 5′	131.3	131.2	131.2	131.2
4′	129.8	129.8	129.8	129.7
β-Me	19.7	19.7	19.6	19.5
		D-**Try**		
CO (1)	173.5	173.5	173.5	173.6
α (2)	56.8	56.8	56.9	57.0
β (3)	38.7	38.7	38.8	38.8
1′	130.0	133.1	133.1	133.1
2′, 6′	133.1	133.2	133.2	133.1
3′, 5′	117.8	119.4	119.5	119.4
4′	158.2	157.8	158.0	157.9
		***N*-Man**		
1	83.5	83.5	83.7	83.9
2	67.6	67.6	67.6	67.7
3	73.7	73.7	73.8	73.9
4	71.3	71.3	71.4	71.6
5	82.8	82.8	82.8	82.9
6	61.8	61.8	61.8	61.9
		α-Man-A		
1		100.8	101.1	101.0
2		73.6	73.6	73.6
3		73.9	74.2	74.2
4		76.7	76.6	76.7
5		74.8	75.0	75.0
6		63.9	63.9	63.9
		α-Man-B		
1		101.2	104.1	104.3
2		76.4	71.4	73.7
3		71.7	76.4	71.8
4		69.9	67.0	72.0
5		75.2	76.9	74.7
6		63.5	63.7	64.3
		Isovaleryl		
1		177.3	177.6	177.1
2		45.6	45.8	45.9
3		28.2	28.3	28.3
4, 5		24.27	24.3	24.36
		24.24	24.3	24.33

method.[13] The foundation of this method lies in the angular and substituent dependence of the three-bond homonuclear and two-

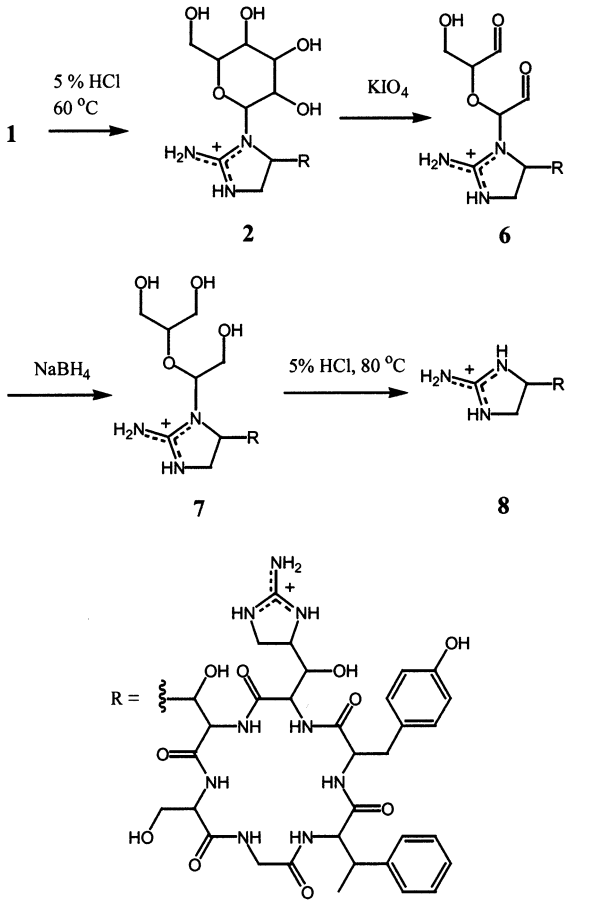

Figure 3. Conversion of mannopeptimycins α (**1**) and *β* (**2**) to the aglycone (**8**) by oxidation, reduction, and hydrolysis.

and three-bond heteronuclear coupling constants that are expressible by a Karplus-type equation to unambiguously determine the relationship of stereochemical centers in flexible systems.[14] Using this method, a series of staggered rotamers and their stereochemical association can be deduced through analysis of the magnitude of these coupling constants in conjunction with NOE data.[12] Generally, the most challenging aspect of this type of analysis is the measurement of the long-range ^{1}H−^{13}C heteronuclear coupling constants. In our case, this portion of the analysis was expedited by the use of the recently reported G-BIRD$_X$-HSQMBC NMR experiment that allows accurate measurement of the ^{1}H−^{13}C coupling constants of interest from antiphase slices taken through the particular carbon signal of interest.[15]All coupling constant values extracted through the use of this pulse sequence were subsequently checked for consistency by employing a gradient-selected decoupled HMBC experiment.[15b] All relevant homonuclear $^3J_{HH}$ scalar coupling constants were measured directly from the one-dimensional ^{1}H spectra or with a gradient selected e.COSY experiment. The selected $^3J_{HH}$, $^2J_{HC}$, $^3J_{HC}$, and NOE data regarding stereochemistry determination on Aiha-A and -B are listed in Table 4.

The Newman projections derived from the NMR data for the $C_\alpha-C_\beta$ bond (I) and the C_β−C-4′ bond (II) of the predominant

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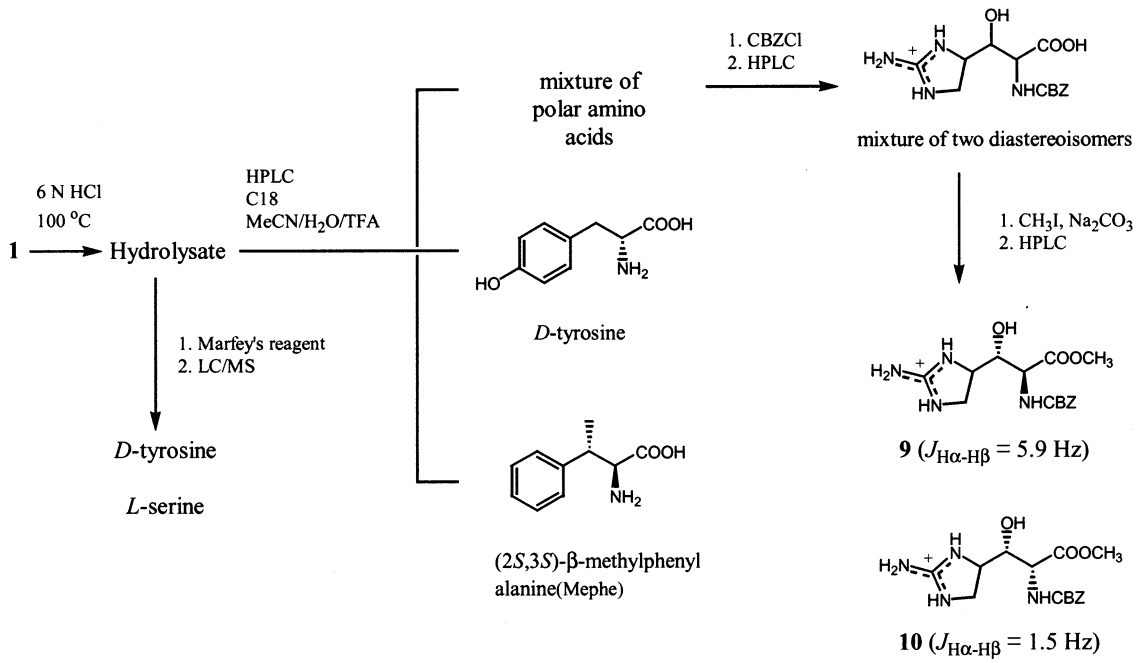

Figure 4. Derivatization and analysis of component amino acids of mannopeptimycin α (**1**).

Table 4. $^1H-^1H$ and $^1H-^{13}C$ Spin-Coupling Constants (J) and Nuclear Overhauser Effects (NOE) for Amino Acid Residues, Aiha-A and Aiha-B, in Aglycone (TFA Salt, **8**)

J (Hz)		NOE			
	Aiha-A	Aiha-B		Aiha-A	Aiha-B
$J_{H\alpha-H\beta}$	8.6	2.5	H_α and H_β	no	yes
$J_{H\alpha-C\beta}$	5.5	<2	H_α and H-4′	yes	yes
$J_{H\alpha-C4'}$	3.0	<1	H_α and H-5′	yes	NA[a]
$J_{H\beta-H4'}$	2.2	7.2	H_α and NH-3′		yes
$J_{H\beta-C\alpha}$	5.4	<1	H_β and H-4′	yes	
$J_{H\beta-C4'}$	<1	4.8	H_β and H-5′	no	yes
$J_{H\beta-C5'}$	3.8	2.8	β-OH and H-5′		yes
$J_{H4'-C\beta}$	1.8	NA[a]			
$J_{H4'-C\alpha}$	<1	2.4			

[a] Not measurable due to signal overlap.

rotamers for these two amino acid residues are depicted in Figure 6. For rotamer Aiha-A (I), the large coupling constant $^3J_{H\alpha-H\beta}$ (8.6 Hz) required an anti orientation between H_α and H_β. The large $^2J_{H\alpha-C\beta}$ and $^2J_{H\beta-C\alpha}$ (5.5 and 5.4 Hz, respectively) were indicative of gauche orientations between H_α and β-OH, and between H_β and the electronegative α-NH. The strong NOE correlations observed between α-NH and H-4′ required that these two protons be spatially close. For rotamer Aiha-A (II), the small $^3J_{H\beta-H4'}$ (2.2 Hz) required a gauche orientation between H_β and H-4′. The large $^2J_{H\beta-C5'}$ (3.8 Hz) and small $^2J_{H4-C\beta}$ (<1 Hz) were indicative of anti orientations between

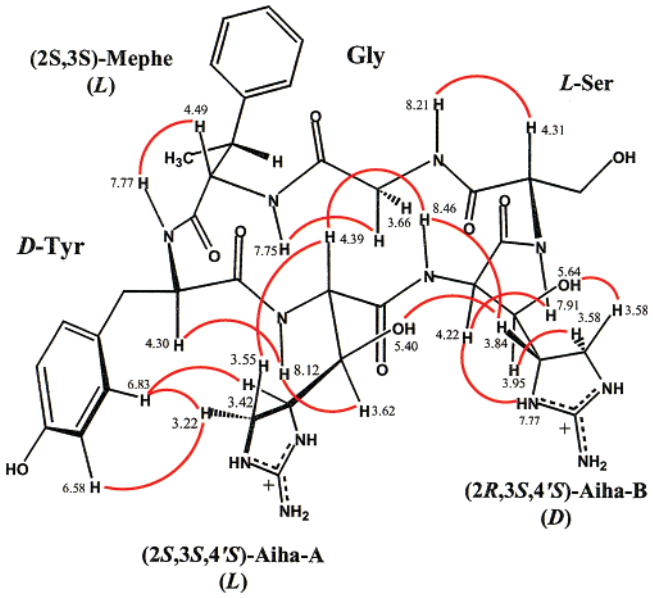

Figure 5. Stereo perspective of aglycone (**8**). Selected chemical shift data are labeled. Strong NOEs between α-protons and NH protons of the adjacent amino acid residues, and interresidue NOEs are indicated by arcs.

H_β and C-5′, and between the β-OH and H-4′. Finally, the small $^3J_{H4'-C\alpha}$ coupling (<1 Hz) suggested a gauche orientation between C_α and H-4′. These requirements defined Aiha-A to

Table 5. In vitro Antibacterial Activity for Mannopeptimycins α−ε (**1**−**5**)

organism	MIC[a] (μg/mL)				
	1	**2**	**3**	**4**	**5**
Staphylococcus aureus (7 strains, including methicillin-resistant strains)	128	64	8	48	4
Staphylococcus hemolyticus	>128	32	8	4	4
Enterococcus faecalis (5 strains)	>128	128	64−128	64	16−32
Enterococcus faecium (4 strains, including vancomycin-resistant strains)	>128	32−128	16−64	8−64	4−32
Escherichia coli (J2175)	>128	64	128	128	64
Bacillus subtilis (Bacto)	>128	>128	16	16	8
Micrococcus luteus (ID-3301)	64	16	2	2	1

[a] Broth dilution method in Mueller-Hinton II, incubated at 35 °C for 18 h.

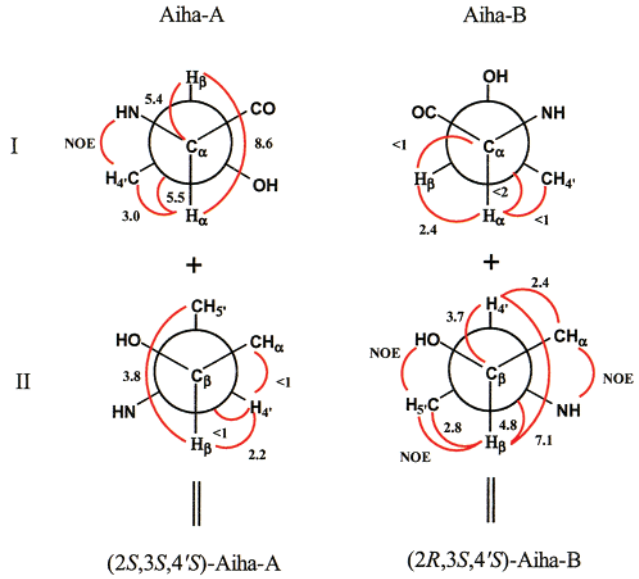

Figure 6. Newman projections of the $C_\alpha-C_\beta$ bond (I), and the C_β−C-4′ bond (II) for amino acid residues, Aiha-A and Aiha-B, in **8** determined by analyses of spin-coupling constants (numbers indicated; in Hz) and nuclear Overhauser effects (NOE).

be (2*S*,3*S*,4′*S*), considering its (2*S*) configuration determined by the previous assignment.

For rotamer Aiha-B (I), the analogous rotameric relationships could also be deduced from *J* values: gauche between H_α and H_β ($^3J_{H\alpha-H\beta} = 2.4$ Hz), gauche between H_α and C-4′ ($^3J_{H\alpha-C4'} < 1$ Hz), anti between H_α and β-OH ($^2J_{H\alpha-C\beta} < 2$ Hz), and anti between α-NH and H_β ($^2J_{H\beta-C\alpha} < 1$ Hz). For rotamer Aiha-B (II), *J* values required an anti relationship between H_β and H-4′ ($^3J_{H\beta-C4'} = 7.2$ Hz), a gauche orientation between H_β and NH-3′ ($^2J_{H\beta-C4'} = 4.8$ Hz, large), and also a gauche configuration between β-OH and H-4′ ($^2J_{H4'-C\beta} = 3.7$ Hz, large). Additionally, the strong NOEs observed between β-OH and H_2-5′ and between H_α and NH-3′ indicated the spatial proximities of these key protons. These relationships defined Aiha-B to be (2*R*,3*S*,4′*S*), considering its (2*R*) configuration from the previous discussion. In this fashion, the stereochemistry for Aiha-B was assigned. This assignment was additionally supported by observation of the interresidue NOE between β-OH (Ahia-A) and H-4′ (Aiha-B), illustrated in Figure 5.

Biological Activity. Mannopeptimycins α−ϵ (**1**−**5**) exhibited moderate to good antibiotic activity against Gram-positive bacteria, including methicillin-resistant streptococci and vancomycin-resistant enterococci, but showed poor activity against Gram-negative bacteria. MIC data in Table 5, obtained from the broth dilution method,[16] suggested that the presence and the position of an isovaleryl group in the terminal mannose (Man-B) in **3**−**5** are essential for retaining antibacterial potency, with compound **5** showing the best activity of the series. In an in vivo evaluation, compounds **3**−**5** exhibited good efficacy against *Staphylococcus aureus*.[6c]

In summary, mannopeptimycins α−ϵ (**1**−**5**), novel glycopeptides with activity against methicillin-resistant staphylococci and vancomycin-resistant enterococci, were purified and their structures characterized using spectroscopic analyses and chemical methods. The limited SAR data demonstrated the importance of an isovaleryl group in the terminal mannose. The enhancement of the antibacterial activity via introduction of lipophilicity at certain positions of the molecule was observed with teicoplanin derivatives, which could be attributed to the increase of membrane anchoring ability.[17] The results described herein for mannopeptimycins were helpful in directing a synthesis program that was aimed at improving antibacterial potency and maximizing therapeutic window by adding proper substituents at chosen positions of compound **1**.

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Supporting Information Available: Experimental details, including the purification and spectroscopic data of mannopeptimycins α−ϵ, the reaction of mannopeptimycin β with KIO_4 followed by acid hydrolysis to produce **8**, the LC/MS analysis of component amino acids, and the production of the new amino acid derivatives (**9** and **10**). (PDF) This material is available free of charge via the Internet at http://pubs.acs.org.

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