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Novel Ether Derivatives of Mannopeptimycin Glycopeptide Antibiotic

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Abstract—Novel ether derivatives of mannopeptimycin glycopeptide were synthesized to probe their SAR. Many of these derivatives exhibited potent antibacterial activity against methicillin resistant and vancomycin resistant strains. These ether derivatives were prepared via reductive ring cleavage of acetals to give a mixture of 6-*O*, 4-*O*, 3-*O*, and 2-*O*-ether isomers. Both 6-*O*-ether and 4-*O*-ether showed significantly enhanced antibacterial activity over the parent and the isovalerate esters.

The emergence of bacterial resistance to commonly used antibiotics, and more recently to the glycopeptides vancomycin and teicoplanin, has become a great concern to the medical professionals. There is an urgent need for new antibiotics to battle these unmet problems.[1] In an effort to search for compounds with improved activity against resistant bacteria, we investigated a class of novel mannopeptimycin glycopeptides. These compounds were first isolated from a strain of *Streptomyces hygroscopicus* over 30 years ago and have been referred to as the 'AC98 complex'.[2] The chemical structures of these compounds were recently elucidated through extensive NMR spectroscopic and chemical degradations studies.[3]

Structurally, the mannopeptimycins contain a cyclic hexapeptide core comprised of alternating L- and D-amino acids that include a β-methylphenylalanine and two epimeric residues of a previously unknown amino acid containing a cyclic guanidino moiety. One of the residues is *N*-glycosylated with mannopyranose while the D-tyrosine residue is *O*-glycosylated with a 1-4-α-linked mannose disaccharide. In mannopeptimycin-α (**1**), a mannose disaccharide unit is attached to the tyrosine phenolic group and in mannopeptimycin-γ (**2**), mannopeptimycin-δ (**3**) and mannopeptimycin-ε (**4**), an isovaleryl group is attached to the terminal mannose at various positions as shown in Figure 1. Mannopeptimycin-α (**1**) is bactericidal and the mechanism of action appears to be inhibition of cell wall synthesis, probably via the inhibition of lipid II and peptidoglycan production.[4]

Mannopeptimycins **2**, **3** and **4** showed better antibacterial activity than the parent compound **1**, indicating that substitution at the terminal mannose could enhance the antibacterial activity. The in vitro antibacterial activities of mannopeptimycins are shown in Table 1.[5]

Since the overall activity of **1**–**4** is only moderate, the goal of our research efforts was to improve the biological activity of these compounds by investigating new semi-synthetic derivatives. We envisioned that the introduction of chemically stable functional groups at the terminal mannose positions 2-,3-,4-, and 6- might help clarify the structure–activity relationships of these regioisomers. One of our initial semi-synthetic strategies was to focus on identifying the pharmacophore, followed by functional group modifications and the synthesis of compounds with improved stability.

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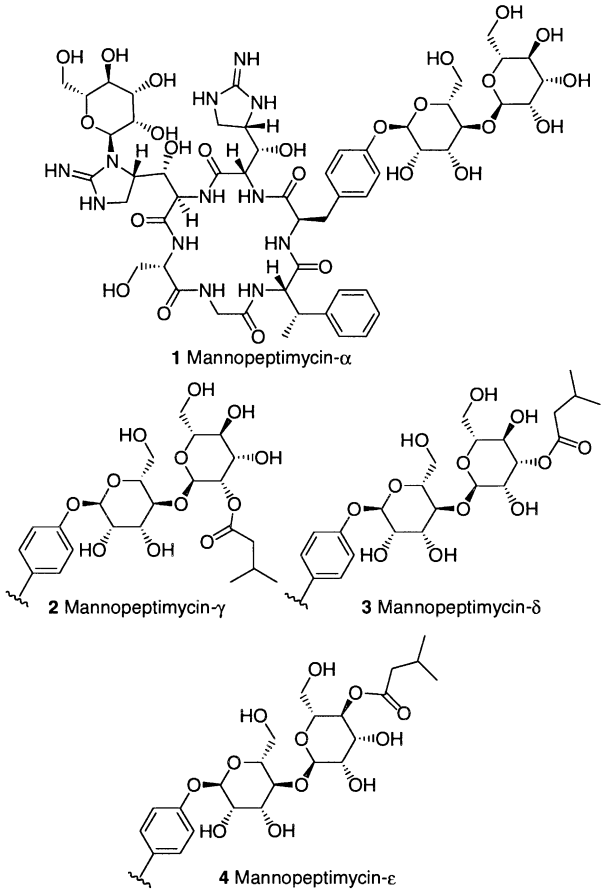


Figure 1. Principle components of the mannopeptimycin.

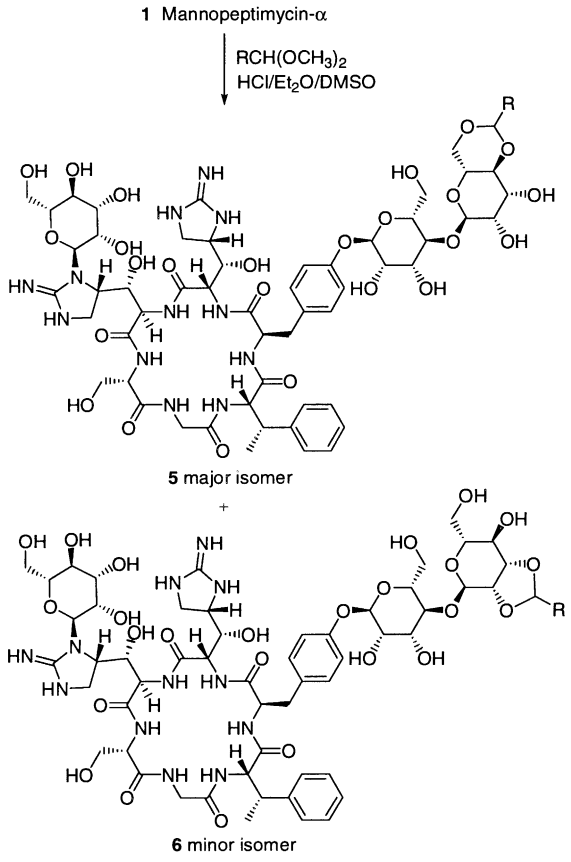
Table 1. In vitro antibacterial activity of the mannopeptimycins

Organism; minimum inhibitory concentration (MIC)[a] μg/mL			
Compd	*S. aureus*	*Streptococcus* spp.	*Enterococcus* spp.
1	>128	>32	128
2	8	8	16–>128
3	4–8	4–8	8–64
4	4	2	4–32

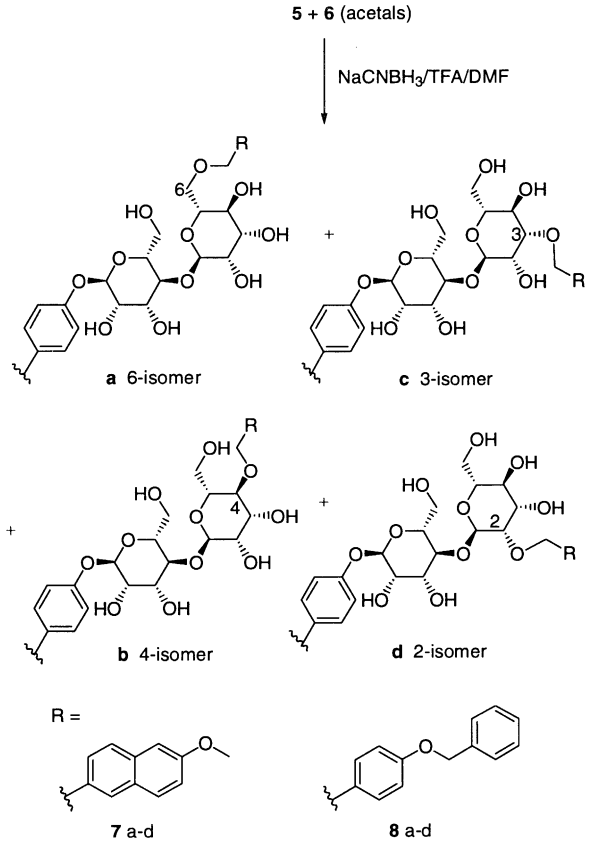
[a]Range of MICs for *Staph.* (7 strains, including MRSA); *Strep.* Species (8 strains, including PRSP); *Enterococcus* species (8 strains, including VRE).

Introduction of functional groups into the molecule presented a synthetic challenge. Numerous reactions have been investigated. Most were not selective, generating a number of regioisomers in various ratios. While formation of benzylidene acetals in simple carbohydrates and their ring opening reactions to *O*-benzyl ethers is reported,[6,7] the unusual glycopeptide nature of our mannopeptimycin posed some potential complications. Based on this precedent, we decided to investigate the synthesis of ether derivatives of the mannose function via reductive acetal cleavage.

The arylmethylidene acetals were prepared using DMSO as solvent and a catalytic amount of anhydrous HCl/Et_2O or *p*-toluenesulfonic acid at 50 °C for a few hours. A mixture of regioisomers was obtained under these conditions, with the 4,6-*O*-arylmethylidene on the



Scheme 1. Synthesis of mannopeptimycin acetals.



Scheme 2. Reductive ring cleavage of acetals.

Table 2. In vitro antibacterial activity of selected ether derivatives

Organism; (MIC) μg/mL		Compd				
		Van	**7a**	**7b**	**7c**	**7d**
S. aureus (GC 1131)	MRSA	1	2	0.5	4	128
S. aureus (GC 4541	MRSA	0.5	1	0.5	2	64
S. aureus (GC 4542)	MRSA	1	2	0.25	1	>128
S. aureus (GC 4543)	MSSA	1	1	0.5	2	64
S. aureus (GC 4544)	MSSA	1	2	1	4	128
S. aureus (GC 4545)	MSSA	0.5	1	0.5	2	128
S. aureus (GC 2216)	MSSA	1	2	1	2	128
S. hemolyticus (GC 4546)	MRCNS	1	1	0.25	1	64
SCN (GC 4547)	MRCNS	2	1	0.5	2	64
SCN (GC 4548)	MRCNS	2	0.5	0.12	1	64
SCN (GC 4549)	MSCNS	0.5	1	0.12	1	64
SCN (GC 6257)	MSCNS	0.5	0.5	0.25	1	32
SCN (GC 4551)	MSCNS	2	2	0.25	2	64
E. faecalis (GC 4552)	VSE	1	8	2	8	>128
E. faecalis (GC 4553)	VSE	0.5	8	2	8	128
E. faecalis (GC 4554)	VSE	0.5	8	2	8	>128
E. faecalis (GC 2242)	VRE	>64	8	2	8	>128
E. faecalis (GC 4555)	VSE	2	8	2	8	>128
E. faecium (GC 2243)	VRE	>64	8	2	8	128
E. faecium (GC 4556)	VSE	1	8	2	8	>128
E. faecium (GC 4557)	VSE	0.5	2	1	0.25	32
E. avium (GC 4558)	VRE	>64	8	2	2	128
S. pyogenes (GC 4563)		0.25	0.25	≤0.06	0.5	64
S. agalactiae (GC4564)		0.5	0.5	≤0.06	0.5	32
S. pneumoniae (GC 4565)	PSSP	0.25	0.12	≤0.06	0.25	32
P. aeruginosa (GC 2214) control		>64	>64	>64	>128	>128
M. morganii (GC 4381)		>64	>64	64	>128	>128
E. coli (GC 4564)		>64	>64	32	128	>128
E. coli (GC 4560)		0.5	1	0.25	1	32
E. coli (GC 2203)		>64	64	32	128	>128
B. cereus (GC 4561) assay organism		1	2	1	2	128
S. lutea (GC 4562)		1	0.25	≤0.06	0.25	16
E. faecalis (GC 2691)	VSE	1	4	2	8	>128
E. faecalis (GC 6189)	VRE	>64	4	1	4	>128
E. faecalis (GC 3059)	VRE	>64	8	2	8	>128
S. pneumoniae (GC 1894)	PRSP	0.12	≤0.06	≤0.06	0.12	16
S. pneumoniae (GC 6242)	PSSP	0.5	0.12	≤0.06	0.25	32
S. aureus (GC 3051)	GISA	1	2	1	2	128
S. aureus (GC 3066)	GISA	4	2	1	2	128
C. albicans GC 3066	Yeast	>64	>64	>64	>64	>64

MIC (minimal inhibitory concentration); MRSA (methicillin resistant *Staph. aureus*); MSSA (methicillin susceptible *Staph. aureus*); MRCNS (methicillin resistant Coagulase-negative *Staphylococci*); MSCNS (methicillin susceptible Coagulase-negative *Staphylococci*); VRE (vancomycin resistant Enterococci); PRSP (penicillin resistant *Strept. pneumoniae*); GISA (glycopeptide intermediate *Staph. aureus*); Van (vancomycin).

terminal mannose as major product, and the 2,3-*O*-arylmethylidene, also on the terminal mannose as minor product (Scheme 1). The two acetals were first separated by reverse phase HPLC (acetonitrile-water-0.02% trifluoroacetic acid). Subsequent reductive ring cleavage was carried out to obtain the corresponding ether isomer pairs.

The acetal derivatives of mannopeptimycin-α (**1**) synthesized for the reductive ring opening included 6-methoxy-2-naphthaldehyde acetal and *p*-benzyloxybenzaldehyde acetal (Scheme 2). Both were selected based on the fact that an electron donating group at the para position could lead to cleaner reductive cleavage products.[6] Reductive ring opening of the acetals was found to be somewhat regioselective using $NaCNBH_3$/TFA in DMF. The 6-*O*-ether derivative was the major component. Reduction of the 6-methoxy-2-naphthaldehyde acetals (4,6-acetal and 2,3-acetal) gave a mixture of 6-, 4-, 3-, and 2-*O*-ether isomers. The ratio of 6-*O*-ether to 4-*O*-ether was about 1.5:1, and the ratio of 3-*O*-ether to 2-*O*-ether was about 1:1 (by HPLC) respectively. The regioselectivity of the reaction was not crucial at this stage since our intent was to make all four possible isomers for structure–activity relationship study.

To isolate all four regioisomes, the crude acetal mixture was subjected to the reductive ring opening reaction without purification. The resulting ether derivatives were separated by HPLC (10–60% acetonitrile in water containing 0.02% trifluoroacetic acid) and regioisomers were identified by extensive ^{1}H and ^{13}C NMR experiments (DEPT, DQFCOSY, HMQC, HMBC, and ROESY) using DRX-500 and DRX-400 spectrometers.[8]

The chemical and metabolic stability of the isolated mannopeptimycin ether derivatives was determined. No decomposition or conversion to the parent compound was observed after incubation in the mouse and rat serum. These ethers were tested in vitro to determine their MIC (minimum inhibitory concentration) against a spectrum of Gram-positive and Gram-negative

pathogens. Compounds (**7a**, **7b**, **8a**, and **8b**) exhibited potent in vitro activities were also tested in vivo (single intravenous dose) in mice. As the natural ester isomers may undergo 1,2-acyl migration in the testing media, the data from the more stable ethers were very useful in deciding the effect of modification at these positions. In vitro antibacterial activities of the acetals were also tested and extensive structure–activity relationships were determined. These results will be disclosed in a separate communication. The in vitro data of compounds **7a–d** is shown in Table 2, MIC data of vancomycin is included for comparison.[9]

The regioisomers of methoxy-naphthyl ethers **7a–d** shared a distinct SAR. The activity order of the positions of ether attachment is as follows: **7b** > **7a** > **7c**, **7d** (4-*O*-ether > 6-*O*-ether > 3-*O*-ether > 2-*O*-ether). The most active compound, **7b**, showed good gram-positive activity against the staphylococcal and streptococcal isolates, and moderate activity against enterococcal isolates including vancomycin resistant strains.

Compounds **8a–d** were tested in vitro with the same spectrum of organisms. The structure–activity relationship for **8a–d** was similar to that observed for **7a–d**. These results are summarized in Table 3. All ether derivatives showed good to moderate activity against a number of Gram-positive bacteria. Both 6-*O*-ether (**8a**) and 4-*O*-ether (**8b**) showed activity better than **8c** and **8d**, with excellent activity shown against the staphylococcal isolates, including methicillin resistant strains. Similar good activity was shown against enterococcal isolates, including vancomycin resistant strains and streptococcal strains.

Compounds **7a**,**b** and **8a**,**b** were tested in vivo and results are shown in Table 4. All of these compounds demonstrated excellent efficacy when tested intravenously (iv) in an acute lethal infection model in mice with *S. aureus* Smith.[9] It is evident that these ether derivatives show promise and warrant further studies.

In summary, substitution at the 6- and 4-position (**7a**, **7b**, **8a**, **8b**) of the terminal mannose generally produced compounds with better in vitro antibacterial activity than the corresponding 3-, or 2-positional isomers among the ether derivatives. These ethers showed significantly enhanced antibacterial activities against a variety of Gram-positive pathogens when compared to the esterified compounds **2**, **3** and **4** and the parent compound **1**. The potent in vitro and in vivo activity of these compounds against resistant Gram-positive bacteria make them potential candidates for the development of new antibiotics.

Table 3. In vitro antibacterial activity of selected ether derivatives[a]

Organism; (MIC) μg/mL	Compd				
	Vancomycin	**8a**	**8b**	**8c**	**8d**
MRSA	0.5–1	4	1–2	4–8	32–64
MSSA	0.5–1	2–4	1–4	4–8	32–64
MRCNS	1–4	1–4	0.25–1	4	32
MSCNS	0.5–2	0.5–1	≤0.12–0.5	1–8	16–32
E. faecallis	0.5–>128	4	2–4	8–16	64–128
E. faecium	1–>128	2–4	2–4	8–16	16–128
Streptococcus	≤0.12–0.5	≤0.12	≤0.12	≤0.12–2	2–8
Gram (−)	>128	>128	32–>128	>128	>128
C. albicans	>128	>128	>128	>128	128

[a]Range of MICs for *S. aureus* (10 strains, including MRSA); *Strep.* Species (8 strains, including PRSP); *Enterococcus* species (8 strains, including VRE).

Table 4. In vivo activity of **7a**, **7b**, **8a** and **8b** in mice (iv)

Compd	Organism: *S. aureus* Smith		
	Route	ED_{50}, mg/kg	MIC (μg/mL)
7a	SIV	0.12–0.5	1
7b	SIV	0.06–0.12	0.5
8a	SIV	0.25–0.5	2
8b	SIV	0.06–0.12	1
Vancomycin	SIV	0.5–1.0	1

SIV (single intravenous dose).

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8. The results from the NMR experiments will be communicated in a separate paper. A typical experimental procedure for the preparation of acetals and ethers is as follows: The dihydrochloride salt of mannopeptimycin-α (**1**) (4 mmol) was dissolved in 25 mL of DMSO, 6-methoxy-2-naphthaldehyde dimethyl acetal (8 mmol) was added, followed by 1 mL of 1.0 M HCl/Et_2O in DMSO. The mixture was heated at 50 °C for 1 h completion of reaction was followed by MS. After work-up, the acetals were used in the next step without further purification. A typical reduction was as follows: Trifluoroacetic acid (18 mmol) in DMF (5 mL) was added at 0 °C dropwise to a mixture containing the acetal (1.4 mmol), and

sodium cyanoborohydride (14 mmol) in DMF. The reaction was stirred for 24 h and the mixture poured into water, and the pH was adjusted to ca. 10 by adding aqueous NaOH. The resultant solid was filtered, washed with water, then with acetonitrile to give a crude mixture of **7a–d**. Purification was done by HPLC (acetonitrile/water/0.02% TFA). MS (ES) m/z: 733.2 $(M+2H)^{2+}$; HPLC retention time (min.): 6-*O*-ether **7a**, 9.394; 4-*O*-ether **7b**, 9.747; 3-*O*-ether **7c**, 9.198; and 2-*O*-ether **7d**, 8.813. Analytical HPLC conditions: 4.6×150 mm YMC ODS-A, 120A, 5 micron column, eluted with a linear gradient of 10–60% acetonitrile in water with 0.02% TFA over 15 min. For **8a–d**, MS ES) m/z: 746.3 $(M+2H)^{2+}$. HPLC retention time (min.): **8a**, 10.22; **8b**, 10.64; **8c**, 10.07; **8d**, 9.72.

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