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Mannopeptimycin esters and carbonates, potent antibiotic agents against drug-resistant bacteria

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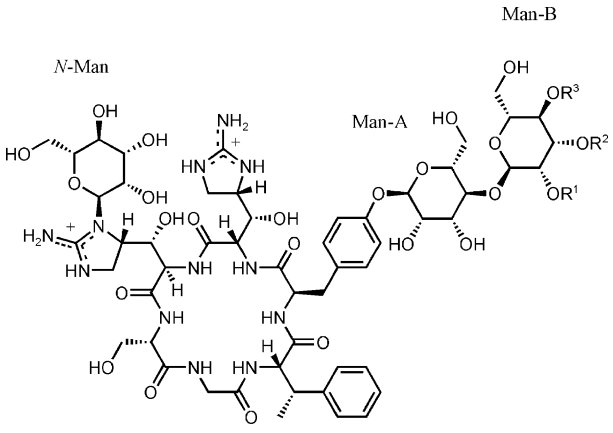
Abstract—A series of ester and carbonate derivatives of the glycopeptide mannopeptimycin α (**1**) with potent activity against G(+) bacteria, including the methicillin-resistant staphylococci and vancomycin-resistant enterococci, was synthesized. The SAR data obtained from natural and semisynthetic compounds demonstrated the importance of a hydrophobic group in the terminal mannosyl moiety for antibacterial activity.

The growing problem of bacterial resistance to antibiotics has spurred great efforts to discover novel types of antibacterial agents.[1,2] In a previous paper, mannopeptimycins α–ε (Fig. 1), a class of new antibiotics produced by *Streptomyces hygroscopicus* with activity against methicillin-resistant staphylococci (MRSA) and vancomycin-resistant enterococci (VRE) were repoted.[3] These compounds are glycosylated cyclic hexapeptides containing two stereoisomers of an unprecedented amino acid, α-amino-β-[4′-(2′-iminoimidazolidinyl)]-β-hydroxypropionic acid. The cyclic peptide core of these antibiotics is attached to mannosyl monosaccharide and disaccharide moieties in mannopeptimycins α (**1**), γ (**2**), δ (**3**), and ε (**4**). Mechanistic studies suggested that these antibiotics inhibited bacterial cell wall biosynthesis and the primary target appeared to be lipid II.[4–6]

It was discovered that the presence and position of an isovaleryl group on the terminal mannosyl moiety (Man-B) in **2**–**4** were critical for antibacterial potency. The minimal inhibitory concentrations (MICs) for compounds **1**, **2**, **3**, and **4** were measured as >64, 8, 4–8, and 4 μg/mL, respectively, against *S. aureus*. In an effort to obtain more potent antibacterial agents, an SAR study with synthetic esters was carried out. Random acylations were adopted as the initial approach, in which mannopeptimycin α (**1**) was reacted with limited amounts of acyl chlorides or anhydrides in dilute basic solution (Fig. 2). Owing to the multiple hydroxyl groups



1 $R^1 = R^2 = R^3 = H$

2 $R^2 = R^3 = H$, $R^1 =$

3 $R^1 = R^3 = H$, $R^2 =$

4 $R^1 = R^2 = H$, $R^3 =$

Figure 1. Structures of manopeptimycins α (**1**), γ (**2**), δ (**3**), and ε (**4**).

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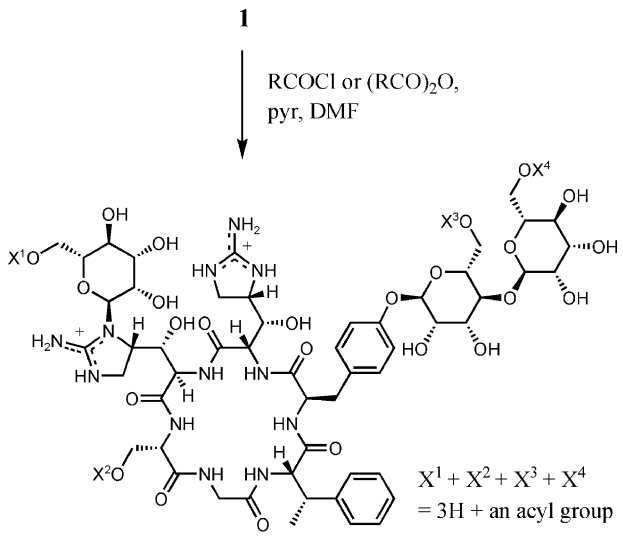


Figure 2. General acylation procedure.

in **1**, this procedure was expected to generate a mixture of esters, which could then be separated by HPLC to afford pure products.

The esterification mixture was generally worked up by precipitation with 1:1 acetone/ethyl ether. The precipitate was then separated by reversed-phase HPLC to afford pure esters (4–11% isolated yield). As an example, the hexanoate mixture, obtained by chromatography of the reaction mixture of **1** with hexanoic anhydride in pyridine,[7] contained four major products (**5**–**8**) as shown in Figure 3. These products, purified by further chromatography on a C-18 column, were identified by analysis of 2-D NMR spectral data. They were all monoester derivatives, with acylation at one of the four primary alcohol groups on *N*-Man, serine, Man-A, or Man-B of **1**.

High-resolution mass spectral data indicated that compounds **5**–**8** were all monohexanoyl products. For mannopeptimycin α (**1**), the ^{1}H chemical shift values of methylene signals were δ 4.10 and 3.70 for *N*-mannose (*N*-Man), between δ 3.88 and 3.70 for *O*-mannose moieties (Man-A and -B), and 3.90 and 3.78 for serine. One of the four pairs of these methylene signals was observed further downfield (0.5–0.8 ppm shift) in each of **5**–**8**, indicating that the primary alcohol groups had been acylated. The shifted methylene protons were identified by HSQCME,[8] a phase-sensitive $^1J_{CH}$ correlation experiment that distinguished CH_2's from CH's and CH_3's. These methylene protons were then correlated by TOCSY and COSY spectra to H-1 in *N*-Man at δ 5.07 in **5**, to H-2 in serine at 4.66 in **6**, to H-1 in Man-A at 5.47 in **7**, and to H-1 in Man-B at 5.15 in **8**. The chemical shift data for selected protons in compounds **5**–**8**, identified by 2-D NMR analyses to be in the same homonuclear spin systems, are summarized in Table 1.

An additional five esterification reactions each using a different acylating reagent to replace the hexanoic anhydride in preparing **5**–**8** were carried out.[9] Upon purification by reversed-phase HPLC, each of these

Table 1. The chemical shift data for selected protons of the same homonuclear spin systems for compounds **5**–**8**

Compound (selected moeity)	Position	^{1}H NMR (mult, *J* in Hz)[a]
5 (*N*-Man)	1	5.07 (d, 8.0)
	6	4.83 (2H, m)
6 (serine)	2	4.66 (t, 5.5)
	3	4.37 (2H, m)
7 (Man-A)	1	5.42 (br s)
	6	4.53 (br d, 11)
		4.56 (dd, 11, 5.2)
8 (Man-B)	1	5.15 (br s)
	6	4.45 (br d, 11.5)
		4.46 (dd, 11.5, 5.5)

[a] 400 MHz, 1:1 CD_3OD/D_2O, DSS as internal ref.

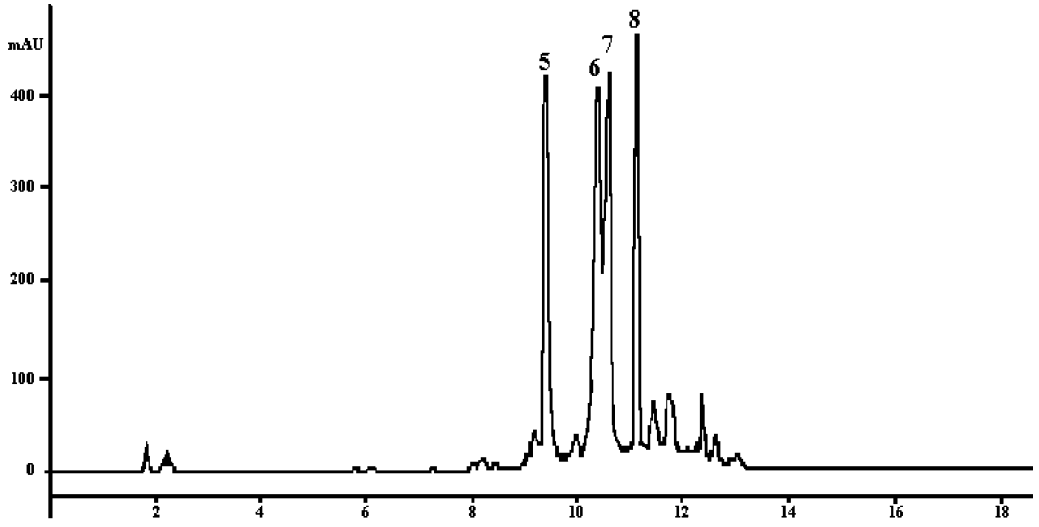


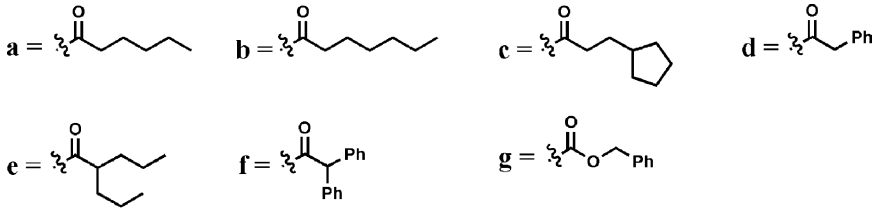
Figure 3. HPLC chromatogram of monohexanoate ester mixture.

Table 2. Monoesters derived from mannopeptimycin α (**1**)

Entry	Formula I (Fig. 2)				Molecular formula	High-resolution FTICRMS[a] $[M+2H]^{2+}$		Reagent
	X^1	X^2	X^3	X^4		Measured	Calculated	
5	a	H	H	H	$C_{60}H_{88}N_{12}O_{26}$	697.30315	697.30391	Hexanoic anhydride
6	H	a	H	H	$C_{60}H_{88}N_{12}O_{26}$	697.30333	697.30391	Same as above
7	H	H	a	H	$C_{60}H_{88}N_{12}O_{26}$	697.30320	697.30391	Same as above
8	H	H	H	a	$C_{60}H_{88}N_{12}O_{26}$	697.30349	697.30391	Same as above
9	b	H	H	H	$C_{61}H_{90}N_{12}O_{26}$	704.31086	704.31174	Heptanoic anhydride
10	H	b	H	H	$C_{61}H_{90}N_{12}O_{26}$	704.31205	704.31174	Same as above
11	H	H	b	H	$C_{61}H_{90}N_{12}O_{26}$	704.31054	704.31174	Same as above
12	H	H	H	b	$C_{61}H_{90}N_{12}O_{26}$	704.31094	704.31174	Same as above
13	c	H	H	H	$C_{62}H_{90}N_{12}O_{26}$	710.31165	710.31174	3-Cyclopentylpropanoic chloride
14	H	c	H	H	$C_{62}H_{90}N_{12}O_{26}$	710.31242	710.31174	Same as above
15	H	H	c	H	$C_{62}H_{90}N_{12}O_{26}$	710.31228	710.31174	Same as above
16	H	H	H	c	$C_{62}H_{90}N_{12}O_{26}$	710.31186	710.31174	Same as above
17	d	H	H	H	$C_{62}H_{84}N_{12}O_{26}$	707.28849	707.28826	Phenylacetyl chloride
18	H	d	H	H	$C_{62}H_{84}N_{12}O_{26}$	707.28876	707.28826	Same as above
19	H	H	d	H	$C_{62}H_{84}N_{12}O_{26}$	707.28827	707.28826	Same as above
20	H	H	H	d	$C_{62}H_{84}N_{12}O_{26}$	707.28876	707.28826	Same as above
21	e	H	H	H	$C_{62}H_{92}N_{12}O_{26}$	711.31890	711.31956	2-Propyl-pentanoyl chloride
22	H	e	H	H	$C_{62}H_{92}N_{12}O_{26}$	711.31861	711.31956	Same as above
23	H	H	e	H	$C_{62}H_{92}N_{12}O_{26}$	711.31916	711.31956	Same as above
24	H	H	H	e	$C_{62}H_{92}N_{12}O_{26}$	711.31858	711.31956	Same as above
25	f	H	H	H	$C_{68}H_{88}N_{12}O_{26}$	745.30339	745.30391	Diphenylacetyl chloride
26	H	f	H	H	$C_{68}H_{88}N_{12}O_{26}$	745.30358	745.30391	Same as above
27	H	H	f	H	$C_{68}H_{88}N_{12}O_{26}$	745.30349	745.30391	Same as above
28	H	H	H	f	$C_{68}H_{88}N_{12}O_{26}$	745.30337	745.30391	Same as above
29	H	H	g	H	$C_{62}H_{84}N_{12}O_{27}$	715.28570	715.28572	Benzyl chloroformate
30	H	H	H	g	$C_{62}H_{84}N_{12}O_{27}$	715.28560	715.28572	Same as above

[a] Fourier transform ion cyclotron resonance mass spectrum.

Wherein



reactions generated four primary ester derivatives. Structures of the purified compounds, identified by analogous 2-D NMR methods and high-resolution mass spectral data are summarized in Table 2.

The esters thus prepared were tested against a panel of bacteria and their MIC data, obtained by the broth dilution method,[10] are listed in Table 3. By examining these data, it was obvious that the substitution of a hydrophobic acyl group on *N*-Man or serine moieties suppressed antibacterial activity, whereas the hydrophobic acylations on the two *O*-mannoses, especially the terminal Man-B, significantly enhanced the activity. Several derivatives with ester chains on position-6 of the terminal sugar, such as compounds **8**, **12**, and **16**, were potent antibacterial agents for MRSA and VRE, superior to the most potent natural product **4**. However, the two linear acyl derivatives, **8** and **12**, showed only moderate activity against *S. aureus* infection in mice (Table 3).

To test the hypothesis that weaker than expected in vivo activity of **8** and **12** might be owing to the hydrolysis by mouse serum esterases,[11] several ester derivatives were incubated in serum obtained from CD-1 mice (0.1 mg/mL) at 37 °C for 1 h. Upon analysis of the resulting samples by reversed-phase HPLC, it was found that compounds **8** and **12** with unbranched acyl chains were readily hydrolyzed to **1** (>60%), whereas those with α- or β-branched acyl groups showed no apparent hydrolysis, as in the cases of **3**, **24**, and **28**. The stable esters **24** and **28** were therefore evaluated in the mouse model against *S. aureus* infection and exhibited excellent antibacterial activity, with respective ED_{50} values of 0.52 and 0.29 mg/kg. The in vivo potencies of these compounds exceeded that of vancomycin (1.0 mg/kg, Table 3). The serum incubation experiment also illustrated that the mouse serum esterases were not effective in hydrolyzing α- and β-branched esters.

Two carbonate derivatives, **29** and **30**, were synthesized by treating **1** with benzyl chloroformate in pyridine and purified by HPLC. These compounds showed comparable antimicrobial potencies to their ester counterparts **19** and **20**.

In summary, a series of ester and carbonate derivatives of mannopeptimycin α (**1**) were synthesized by hydrophobic

Table 3. MIC[a] and ED_{50} data for mannopeptimycin esters derivatives

Entry	MIC (μg/mL)		ED_{50} (iv, mg/kg)[d]
	Staphylococcus aureus[b]	*Enterococcus faecalis*[c]	*Staphylococcus aureus*
1	>64	>64	20
2	8	64–>64	3.5
3	4–8	64	2.6
4	4	16–32	0.6
5	>64	>64	
6	NT[e]	NT[e]	
7	4–8	16–32	
8	1–2	4–8	4.0
9	>64	>64	
10	NT[e]	NT[e]	
11	0.5–2	4–16	
12	0.5–1	1–4	4.0
13	>64	>64	
14	>64	>64	
15	2	8	
16	1	2–4	
17	NT[e]	NT[e]	
18	NT[e]	NT[e]	
19	8–16	64–>64	
20	2–4	8–16	
21	NT[e]	NT[e]	
22	NT[e]	NT[e]	
23	4	16–32	
24	1–2	4	0.52
25	>64	>64	
26	>64	>64	
27	1–2	4–8	
28	0.5–1	1–2	0.29
29	4–8	16–64	
30	2–8	8–16	

[a] None of the tested compounds showed activity against Gram negative bacteria, such as *Escherichia coli,* at concentrations ≤64 μg/mL.
[b] 5–7 strains, including MRSA strain(s).
[c] 3–5 strains, including VRE strain(s).
[d] Vancomycin as a control exhibited an ED_{50} of 1.0 mg/kg.
[e] These compounds showed no antibacterial activity in a plate assay (25 μg/spot). No attempt was made to obtain their MICs.

functionalizations of the primary alcohols and purified by HPLC. The SAR data obtained from natural and semisynthetic compounds demonstrated the importance of a hydrophobic group in the terminal mannosyl moiety (Man-B) for activity against MRSA and VRE. The enhancement of antibacterial activity via introduction of lipophilicity at certain positions was observed with teicoplanin derivatives and was attributed to an increase of membrane anchoring ability.[12] Although the synthesis of mannopeptimycin derivatives reported in this paper was not selective and the purification of the products was generally difficult, our data revealed the potential of this antibiotic class. Furthermore, the SAR data were helpful in directing a semisynthetic program, which resulted in the efficient introduction of hydrophobic functionalities to **1** at the terminal mannosyl moiety and dramatically improved antibacterial activity.[13]

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7. **Esterification with hexanoic anhydride**. To a solution of bis-TFA (trifluoroacetic acid) salt of 1 (348 mg) and pyridine (400 mg) in dry DMF (48 mL) was slowly added hexanoic anhydride (600 mg) at room temperature with stirring. The reaction mixture was stirred for an additional 16 h before 1:1 acetone/ethyl ether (120 mL) was added. The grayish precipitate, obtained by centrifugation, was then separated by reversed-phase HPLC using a large C18 column (YMC ODS-A, 70×500 mm in size, 10 μm particle size) and a gradient solvent system (20–70% MeCN/H_2O, containing 0.01% TFA) to obtain an ester mixture as a broad peak after the elution of the unreacted 1. This mixture was further purified by a smaller C18 column (YMC ODS-A, 20–30×250 mm in size, 5 μm particle size) and a shallow gradient system (15–35% MeCN/H_2O, containing 0.01% TFA) to afford pure esters **5** (7% isolated yield), **6** (6%), **7** (6%), and **8** (8%), all as white amorphous powders.
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