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Structural determination of mannopeptimycin cyclic acetals

Haiyin He,[a,*] Ting-Zhong Wang,[b] Russell G. Dushin,[b] Xidong Feng,[a] Bo Shen,[a] Joseph S. Ashcroft,[a] Frank E. Koehn[a] and Guy T. Carter[a]

[a]*Department of Structural and Natural Products Chemistry, Chemical and Screening Sciences, Wyeth, Pearl River, NY 10965, USA*
[b]*Department of Medicinal Chemistry, Chemical and Screening Sciences, Wyeth, Pearl River, NY 10965, USA*

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Abstract—In structure–activity relationship (SAR) studies on mannopeptimycin antibiotics, mannopeptimycin α(**1**) was acetalized by reacting with certain dialkyl acetals under acidic conditions. The major products of these reactions were determined to be cyclic acetals at the 4,6-positions of the terminal mannose (Man-B), by exemplary spectroscopic analyses of two typical acetalization products **2** and **3**.

The burgeoning problem of bacterial resistance to antibiotics demands continuing efforts to discover novel types of antibacterial agents.[1,2] In a previous paper, we reported mannopeptimycins α–ε, a class of new antibiotics, produced by *Streptomyces hygroscopicus*, with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).[3] These compounds are glycosylated cyclic hexapeptides containing two stereoisomers of α-amino-β-[4′-(2′-iminoimidazolidinyl)]-β-hydroxypropionic acid[4] (Aiha-A and -B). Studies on the mode of action suggested that these antibiotics inhibited bacterial cell wall biosynthesis and the primary target appeared to be Lipid II.[5–7] SAR data for natural products,[3] and semi-synthetic esters and carbonates,[8] demonstrated that the attachment of a hydrophobic group on the terminal mannosyl moiety (Man-B) of mannopeptimycin α(**1**) significantly increased the potency against MRSA and VRE. In the process of achieving selective hydrophobic functionalization to enhance the antibacterial activity, a method for synthesizing cyclic acetal derivatives of **1** was developed.[9] It was found that the treatment of **1** with an appropriate dialkyl acetal under standard acid catalyzing conditions predominantly produced a cyclic acetal that exhibited potent antibacterial activity. Due to the complexity of these molecules and the presence of a number of 1,2- and 1,3-diol moieties, structural identification of the products was often difficult. Therefore, extensive spectroscopic analyses were employed to confirm the formation of the cyclic acetals and to define the regiochemistry of the acetal moieties. In this letter, the synthesis, characterization, and antibacterial activity of two cyclic acetal derivatives **2** and **3** are reported.

Cyclic acetal **2** was synthesized by treating **1** with cyclopentanecarboxaldehyde-diisopropylacetal in the presence of *p*-toluene sulfonic acid (TsOH, Fig. 1).[10] The newly formed cyclopentanecarboxacetal moiety (Cpca) was determined by analysis of 2-D NMR and ESI-MS data to locate at the 4,6-positions of the terminal mannose (Man-B).

The molecular formula of **2** was determined by high-resolution Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry to be $C_{60}H_{86}N_{12}O_{25}$. This was indicative of the addition of a cyclopentanecarboxacetal moiety (Cpca), by comparison with that of mannopeptimycin (**1**). The ^{1}H and ^{13}C NMR signals of **1** and **2**, assigned by analysis of 2-D NMR spectral data, including COSY, TOCSY, HSQC, and HMBC, were essentially identical for all the amino acid residues as well as the *N*-substituted mannose and mannose-A moieties (*N*-Man and Man-A). It was noticed, however, that the ^{1}H and ^{13}C signals for mannose-B moiety (Man-B) were substantially different between these two compounds. In particular, the C-4 and C-6 signals showed large downfield shifts while C-3 and C-5 signals exhibited upfield shifts upon acetalization. This implied that the cyclic acetal moiety was formed at the 4,6-positions on Man-B. The ^{13}C and relevant ^{1}H NMR data of **1** and

* Corresponding author. Tel.: +1-845-602-5687; fax: +1-845-602-3940; e-mail: heh@wyeth.com

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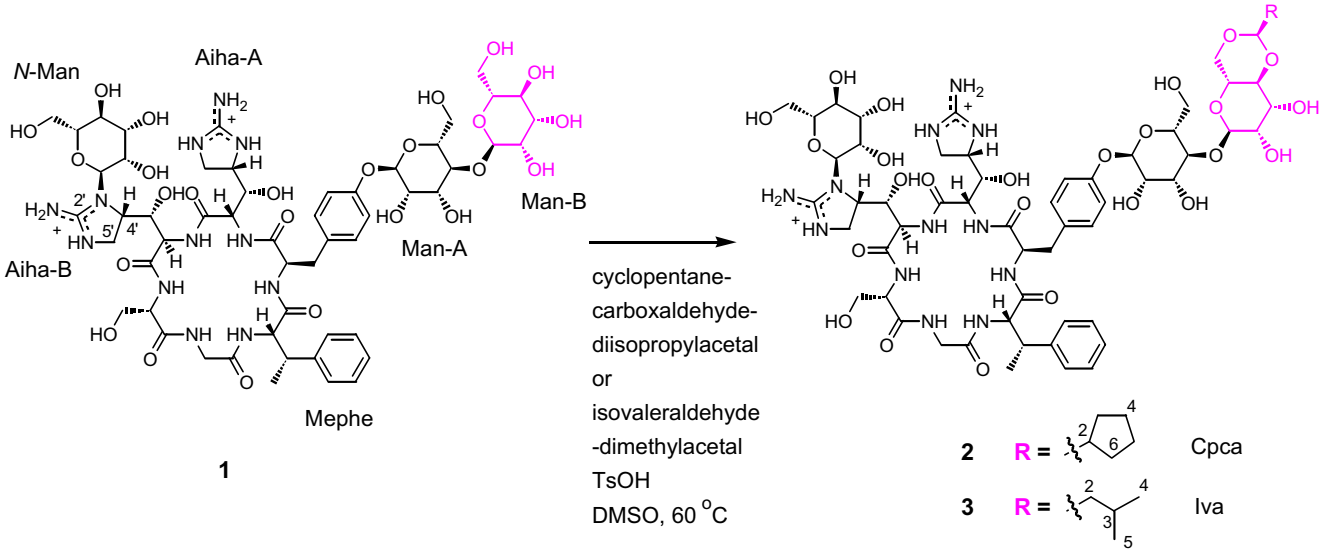

Figure 1. Acetalization of mannopeptimycin α(**1**).

Table 1. ^{13}C NMR spectral data of **1**, **2**, and **3** (TFA salts, 1:1 CD_3OD/D_2O, 400 MHz)

^{13}C	**1**	**2**	**3**
(2S,3S,4′S)-α-Amino-β-[4′-(2′-iminoimidazolidinyl)]-β-hydroxy-propionic acid A (Aiha-A)			
C=O (1)	173.2	173.2	173.2
α(**2**)	56.1	56.1	56.0
β(**3**)	72.0	71.9	71.9
2′	161.9	162.0	161.9
4′	58.3	58.3	58.2
5′	44.8	44.8	44.8
(2S,3S,4′R)-α-Amino-β-[4′-(2′-iminoimidazolidinyl)]-β-hydroxy-propionic acid B (Aiha-B)			
C=O (1)	173.0	173.0	173.0
α(**2**)	57.8	57.8	57.8
β(**3**)	72.2	72.2	72.3
2′	161.7	161.5	161.7
4′	63.8	63.8	63.8
5′	44.6	44.5	44.5
L-Serine (Ser)			
C=O (1)	173.9	173.9	173.9
α(**2**)	58.3	58.4	58.4
β(**3**)	63.8	64.0	64.0
Glycine (Gly)			
C=O (1)	173.5	173.5	173.5
α(**2**)	45.0	45.0	45.0
(2S,3S)-β-Methylphenylalanine (Mephe)			
C=O (1)	174.4	174.5	174.5
α(**2**)	62.7	62.8	62.8
β(**3**)	44.1	44.1	44.09
1′	144.8	144.7	144.6
2′,6′	130.5	130.5	130.5
3′,5′	131.3	131.3	131.3
4′	129.8	129.9	129.9
β-Me	19.9	19.7	19.7
D-Tyrosine (Tyr)			
C=O (1)	173.5	173.5	173.5
α(**2**)	56.8	56.7	56.7
β(**3**)	38.7	38.6	38.7
1′	133.0	133.0	132.9
2′,6′	133.1	133.2	133.2
3′,5′	119.4	119.3	119.3
4′	157.8	157.8	157.8
N-Mannose (N-Man)			
1	82.7	82.7	82.7
2	67.6	67.6	67.6
3	73.7	73.7	73.7
4	71.3	71.3	71.3
5	83.5	83.6	83.5
6	61.8	61.7	61.7
α-Mannose-A (Man-A)			
1	100.8	100.7	100.7
2	73.5	73.5	73.5
3	74.1	74.1	74.0
4	76.6	76.7	76.7
5	74.9	74.7	74.7
6	63.8	63.5	63.5
α-Mannose-B (Man-B)			
1	104.3	105.3	105.3
2	73.3	73.7	73.7
3	73.5	70.6	70.6
4	69.5	80.6	80.5
5	76.4	67.5	67.5
6	63.5	70.4	70.4
Acetal moiety		Cpca	Iva
1		109.3	105.0
2		45.7	45.2
3		30.2	26.5
4		28.1	24.8
5		28.1	24.5
6		30.3	

2 in 1:1 D_2O/Methanol-d_4 are listed Tables 1 and 2, and the HSQC spectra are illustrated in Figure 2. The ^1H and ^{13}C NMR resonances for Cpca were also observed for **2**.

Table 2. Selected ^{1}H NMR spectral data of **1**, **2**, and **3**[a]

^{1}H	Chemical shift (mult, J in Hz)[b]		
	1	**2**	**3**
α-Mannose-A (Man-A)			
1	5.44 (br s)	5.44 (br s)	5.44 (br s)
2	4.01 (m)	4.01 (m)	4.01 (m)
3	4.07 (m)	4.07 (br d, 10)	4.06 (br d, 10)
4	3.93 (dd, 9.5, 9.5)	3.90 (dd, 10, 10)	3.91 (dd, 10, 10)
5	3.63 (m)	3.62 (m)	3.62 (m)
6	3.74 (2H, m)	3.74 (2H, m)	3.75 (2H, m)
α-Mannose-B (Man-B)			
1	5.28 (br s)	5.26 (br s)	5.27 (br s)
2	4.02 (m)	4.08 (m)	4.08 (m)
3	3.74 (m)	3.87 (br d, 10)	3.88 (m)
4	3.69 (m)	3.75 (dd, 10, 10)	3.78 (dd, 10, 10)
5	3.62 (m)	3.65 (m)	3.67 (m)
6	3.79 (m)	4.06 (dd, 12, 2.5)	4.09 (m)
	3.72 (m)	3.64 (m)	3.65 (m)
		Cyclopentanecarb-oxacetal	*Isovaleracetal*
1		4.52 (d, 5.5)	4.79 (t, 5.8)
2		2.08 (m)	1.52 (2H, m)
3		1.71 (m)	1.77 (m)
		1.46 (m)	
4		1.58 (m)	0.92 (3H, d, 6.8)
		1.53 (m)	
5		1.58 (m)	0.92 (3H, d, 6.8)
		1.53 (m)	
6		1.71 (m)	
		1.46 (m)	

[a] TFA salts, 1:1 CD_3OD/D_2O, 400 MHz, DSS as reference.
[b] Due to extensive signal overlap, the multiplicities and coupling constants for many signals could not be measured directly from the 1-D ^{1}H NMR spectra.

In the HMBC spectrum of **2**, the 3-bond H-C correlations from the H-1 signal at δ 4.52 in Cpca to C-4 at δ 80.6 and C-6 at 70.4 in Man-B, indicated that the cyclic acetal moiety bridged the 4,6-positions of Man-B. The 3-bond H-C correlations from H-4 at δ 3.75 and H_2-6 at 4.06 and 3.64 in Man-B to C-1 at 109.3 in Cpca were also observed as supporting evidence.

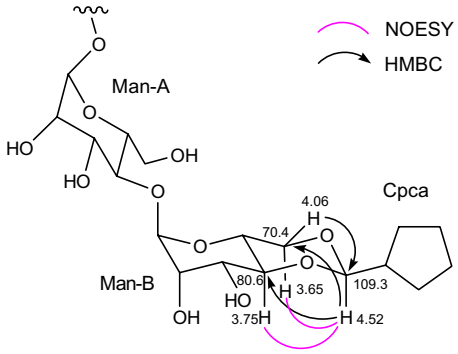

Figure 3. Selected HMBC and NOESY correlations that define the position and stereochemistry of Cpca moiety.

NOE correlations between H-1 in Cpca, and two axial protons H-4 (δ 3.65) and H-6 (δ 3.75) in Man-B were observed in NOESY spectrum, suggesting that these protons were proximal to each other. The 2-D NOE data not only confirmed the 4,6-cyclic acetal structure on Man-B but required the presence of an axial H-1 and thereby an equatorial cyclopentane group in Cpca (Fig. 3).

FT-ICR MS/MS data supported that the location of Cpca on the terminal mannose (Man-B) in **2**. The negative mode ESI-FT-ICR SORI-CID[11] mass spectrum of the singly charged parent ion showed three significant fragment ions at m/z 1260, 1098, and 985, due to the losses of the Aiha-A side chain, Aiha-B side chain (including *N*-Man), and both Aiha-A and Aiha-B side chains. The corresponding fragment ions for **1** were observed at m/z 1180, 1018, and 905. Positive mode ESI-FT-ICR SORI-CID mass spectrum of the doubly charged parent ion showed five significant fragment ions, three singly charged (m/z 1262, 1213, 1100), and two doubly charged (m/z 567, 486), respectively, corresponding to the losses of Aiha-A side chain, *N*-Man, Aiha-B side chain (including *N*-Man), Man-B with Cpca, and both Man-A and Man-B with Cpca. Detection of the fragment ion at m/z 985 in the negative ESI

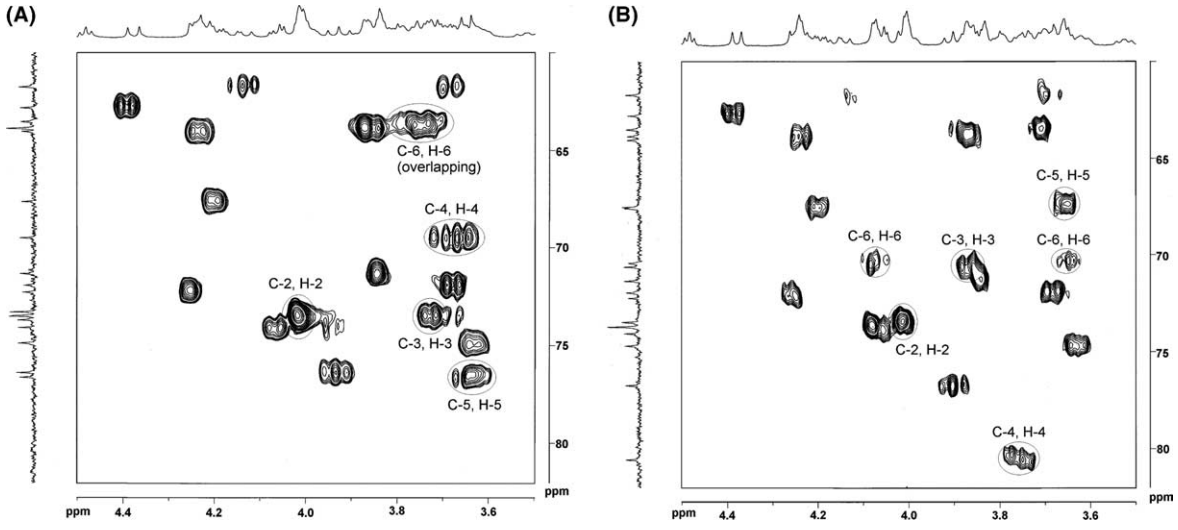

Figure 2. HSQC spectra of **1** (A) and **2** (B) in 1:1 D_2O/MeOH-d_4 (400 MHz). Assignments of ^{13}C and ^{1}H signals of Man-B are indicated.

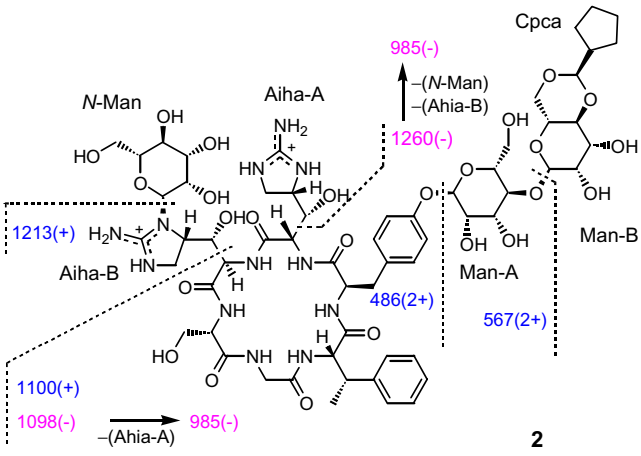

Figure 4. Significant fragment ions, observed in both positive and negative ESI-FT-ICR SORI-CID mass spectra.

mode eliminated the possibility of an *N*-mannose-attached Cpca, while detection of the doubly charged fragment ions at m/z 567, and 486 in the positive ESI mode unambiguously placed the Cpca moiety on Man-B. The origins of the significant ions observed in the FT-ICR-MS experiments are shown in Figure 4 and the accurate mass measurements for both the molecular ions and the significant fragment ions are listed in Table 3.

Cyclic acetal **3** was synthesized in a similar fashion as described for **2**, where the cyclopentanecarboxaldehyde-diisopropylacetal was replaced by isovaleraldehyde-dimethylacetal. The molecular formula of **3** was determined by FT-ICR-MS to be $C_{59}H_{86}N_{12}O_{25}$.[12] The ^{13}C and relevant ^{1}H NMR signals of **3** are listed in Tables 1 and 2. Apart from the isovaleracetal portion (Iva), it displayed nearly identical NMR data to those of compound **2**.

Based on the structural analyses of compounds **2** and **3**, we found that when **1** was treated with a dialkyl acetal under acidic conditions, the formation of six-membered cyclic acetals across the 4,6-positions of Man-B was favored to five-membered cyclic acetals bridging vicinal diols. Moreover, formation of the 4,6-cyclic acetals on *N*-Man was not observed, presumably because the C-4 hydroxyl and C-5 hydroxymethylene groups on *N*-Man adopted a diaxial orientation, allowing an equatorial conformation for the C-1 guanidinium group, as previously demonstrated by ROESY analysis.[3]

Table 4. MIC[a] and ED_{50} data for **1**, **2**, and **3**

	MIC (μg/mL)		ED_{50} (iv, mg/kg)[d]
	Staphylococcus aureus[b]	*Enterococcus faecalis*[c]	*Staphylococcus aureus* (Smith strain)
1	>64	>64	20
2	0.5–2	1–4	0.26
3	1–2	1–8	N/T[e]

[a] None of the tested compounds showed activity against Gram-negative bacteria, such as *Escherichia coli*, at concentrations $\leqslant 64$ μg/mL.
[b] 7 Strains, including MRSA strains.
[c] 5 strains, including VRE strains.
[d] Vancomycin as a control exhibited an ED_{50} of 1.0 mg/kg.
[e] Not tested.

Acetal derivatives **2** and **3** were tested against a panel of bacteria and their minimal inhibitory concentration (MIC) data, obtained by the broth dilution method,[13] are listed in Table 4. It is obvious that the addition of a hydrophobic acetal moiety to the terminal mannose (Man-B) significantly enhanced the activity against Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis*, including MRSA and VRE. Compound **2** also exhibited potent in vivo activity against *S. aureus* in a mouse model (Table 4). The enhancement of antibacterial activity by introducing hydrophobicity to Man-B was previously observed with natural and semisynthetic esters of this class,[3,7] which could be attributed to an increase of membrane anchoring ability.[14] Acetal derivatives **2** and **3** are potent antibiotics against Gram-positive bacteria, with MICs lower than not only mannopeptimycin α(**1**) but also its natural Man-B isovaleryl esters.[3]

In summary, potent antibacterial agents **2** and **3** were synthesized by acetalization of mannopeptimycin α(**1**) at the terminal mannose (Man-B). The structures of these products were identified by detailed spectroscopic analyses, using 2-D NMR and FT-ICR-MS methods. The syntheses and SAR studies of a series of cyclic acetal and ketal derivatives of mannopeptimycin are reported in a separate paper.[9] As has been demonstrated, the acetalization at mannose in a compound as

Table 3. Accurate mass measurement for **2** by FT-ICR-MS[a,b]

ESI mode	Experimental mass	Elemental formula	Predicted mass	Δ (mmu)	Ion assignment
–	1373.57571	$C_{60}H_{85}N_{12}O_{25}^{1-}$	1373.57543	−0.28	$[M-H]^{1-}$
–	1260.51551	$C_{56}H_{78}N_{9}O_{24}^{1-}$	1260.51652	−1.01	$[M-H-A]^{1-}$
–	1098.46413	$C_{50}H_{68}N_{9}O_{19}^{1-}$	1098.46369	0.44	$[M-H-A-NM]^{1-}$ and $[M-H-B]^{1-}$
–	985.40383	$C_{46}H_{61}N_{6}O_{18}^{1-}$	985.40478	−0.95	$[M-H-A-B]^{1-}$
+	688.29847	$C_{60}H_{88}N_{12}O_{25}^{2+}$	688.29863	−0.16	$[M+2H]^{2+}$
+	1262.53103	$C_{56}H_{80}N_{9}O_{24}^{1+}$	1262.53107	−0.04	$[M+H-A]^{1+}$
+	1213.53757	$C_{54}H_{77}N_{12}O_{20}^{1+}$	1213.53716	0.41	$[M+H-NM]^{1+}$
+	607.27221	$C_{54}H_{78}N_{12}O_{20}^{2+}$	607.27222	−0.01	$[M+2H-NM]^{2+}$
+	1100.47786	$C_{50}H_{70}N_{9}O_{19}^{1+}$	1100.47825	−0.39	$[M+H-A-NM]^{1+}$
+	567.24095	$C_{48}H_{70}N_{12}O_{20}^{2+}$	567.24092	0.03	$[M+2H-MBC]^{2+}$
+	486.21436	$C_{42}H_{60}N_{12}O_{15}^{2+}$	486.21451	−0.15	$[M+2H-MA-MBC]^{2+}$

[a] Abbreviations: A, Aiha-A side chain; B, Aiha-B side chain, including *N*-mannose; *N*M, *N*-mannose; MA, mannose-A; MBC, mannose-B with Cpca.
[b] Δ = experimental value − predicted mass.

complicated as **1** is relatively selective and changes in the NMR spectra are essentially localized to the reaction site. Therefore, this kind of reaction may be useful for SAR studies and structural analyses of other complicated natural products, particularly those with glycosyl moieties.

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10. Synthesis of **2**: A stirred solution of the bis-trifluoroacetate salt of **1** (0.5 g) in dimethylsulfoxide (35 mL) was treated with cyclopentanecarboxaldehyde-diisopropyl acetal (0.18 g) and *p*-toluene sulfonic acid mono-hydrate (0.15 g) and the mixture was stirred at 60 °C for 15 h. After it was cooled to rt, the reaction mixture was poured into 1:1 acetonitrile/ether (100 mL). The precipitate obtained by centrifugation was then purified by reverse phase HPLC using a gradient elution of acetonitrile in water containing 0.02% TFA. The major UV peak was collected and then lyophilized to provide the desired product, a white solid, as the bis-trifluoroacetate salt (21%). ^{1}H and ^{13}C NMR and HR-FT-ICR-MS data see Tables 1–3.
11. SORI-CID stands for sustained off-resonance irradiation with collision induced dissociation.
12. HR-FT-ICR-MS for **3** (calcd for $C_{59}H_{88}N_{12}O_{25}^{2+}$: 682.29863, found 682.29783, $[M+2H]^{2+}$, $\Delta = -0.80$ mmu).
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