# Hydrophobic Acetal and Ketal Derivatives of Mannopeptimycin-α and Desmethylhexahydromannopeptimycin-α: Semisynthetic Glycopeptides with Potent Activity Against Gram-Positive Bacteria

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**Abstract:** The effect of introducing hydrophobic groups onto the disaccharide portion of the mannopeptimycins has been examined. Under acid-catalyzed conditions dimethyl acetals and ketals react on the terminal mannose of the disaccharide moiety of mannopeptimycin-α and the cyclohexylalanyl analogue **2**. The preferentially formed monofunctionalized 4,6-acetals and -ketals display potent antibacterial activities against Gram-positive microorganisms, including MRSA, PRSP, and VRE pathogens.

The continued emergence of bacterial resistance to commonly used antibiotics is now recognized as a significant global health issue.[1−6] The growing resistance to glycopeptides of the vancomycin family exhibited by enterococci[7,8] and more recently to *Staphylococcus aureus*[9−11] is especially alarming given that these agents are frequently used as a last line of defense against serious infections. There remains an ever-increasing need for new antibiotics with activity against resistant and multiply resistant pathogens, particularly those that target essential pathogen-specific processes through novel mechanisms of action. Toward this end, second generation glycopeptides oritavancin and dalbavancin have emerged and are currently in advanced stages of development for the treatment of Gram-positive infections.[12,13] These and other glycopeptides of the vancomycin family, as well as the glycolipodepsipeptide ramoplanin, exert their antibacterial effects by binding to precursors of the bacterial cell wall and/or through inhibition of transglycosylation, ultimately disrupting cell wall biosynthesis. Hydrophobic moieties present in these agents appear to play a crucial role in every case, either by anchoring the agents in the bacterial cell membrane and thus bringing them in close proximity with their targets, or by promoting the formation of biologically relevant dimers.[14−19]

Recently, the isolation and identification of mannopeptimycins from a strain of *Streptomycetes hygroscopicus* (NRRL 3085) that is known to produce the AC98-complex[20] was described.[21] The naturally occurring mannopeptimycin isovalerate esters of the terminal mannose on the tyrosine-linked disaccharide were shown to possess a respectable degree of antibacterial activity against Gram-positive microorganisms, with the potency progressively increasing as the position of esterification moved from O-2 to O-3 to O-4. Additional acylated derivatives have been produced synthetically[22] or biosynthetically (through fatty-acid enrichment of the fermentation medium),[23] and these generally displayed a similar pattern of enhanced antibacterial activity. Notably, the naturally occurring isovalerate ester derivatives demonstrated little-to-no cross resistance with other known antibiotics,[24,25] were shown to have an apparently unique mechanism of action in targeting cell wall biosynthesis,[25,26] and were active in vivo in murine models of infection following intravenous administration.[25]

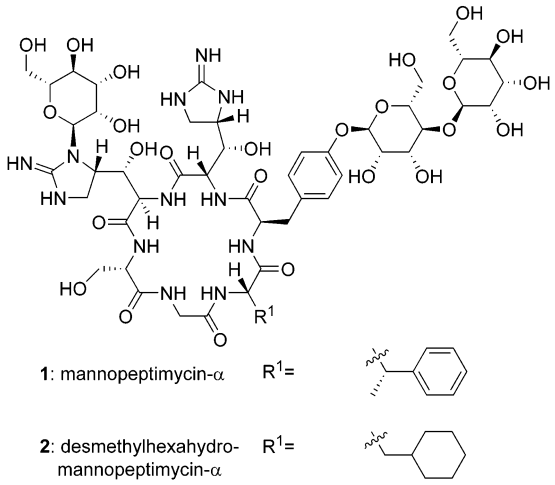


**Figure 1.** Natural and core-modified mannopeptimycins.

Herein we report that exposure of mannopeptimycin-α (**1**) and the cyclohexylalanyl analogue **2** (Figure 1) to typical acetal-forming reaction conditions results in the selective formation of biologically active acetal derivatives. Thus, **1** and **2** react with dimethyl acetals or ketals under the influence of acid catalysis to preferentially form cyclic acetals and ketals **3**−**13** derivatized on the 4,6-positions of the terminal mannose of the O-linked mannose disaccharide (Scheme 1). The acetal and ketal exchange reactions proceeded regioselectivity with minor formation of monoacetals and -ketals located on the 2,3-positions of the same mannose residue. The overall ratios of major:minor monoacetal products were on the order of ∼5:1 for unhindered aliphatic and aromatic aldehydes. In the case of ketals, the ease of isolation of the major product was generally improved using dimethyl ketals derived from symmetrical ketones, and the ratio of major:minor monoketal products were improved to ∼20:1 when using dialkyl ketals derived from hindered ketones such as adamantanone. Typically, the acetals and ketals were purified after precipitation using reverse phase HPLC techniques. In separate work, we have found that acetal mixtures of **1** and related substances can further be transformed by reductive ring opening into ether de-

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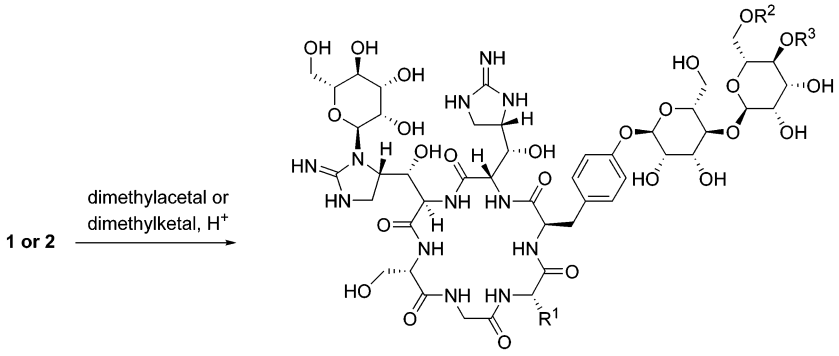
**Scheme 1.** Synthesis of Acetal and Ketal Derivatives of **1** and **2**




| Compound | $R^1$ | $R^2,R^3$ | Compound | $R^1$ | $R^2,R^3$ |
|---|---|---|---|---|---|
| **3** | | | **9** | | |
| **4** | | | **10** | | |
| **5** | | | **11** | | |
| **6** | | | **12** | | |
| **7** | | | **13** | | |
| **8** | | | | | |

rivatives which after purification also give analogues with potent antibacterial activities.[27,28]

The structures of the 4,6-acetal and -ketal derivatives of **1** and **2** were assigned by NMR and mass spectrometry. After determination of the molecular formula by exact mass FTICRMS, NMR methods were used to assign the specific $^1H$ and $^{13}C$ resonances of the peptide backbones and sugar moieties, followed by additional assignment of the acetal or ketal portion and determination of the point of linkage. The structure assignment of the 4,6-adamantyl ketal **10** is described as a typical example.

Electrospray FTICR mass spectrometry of **10** gave an $(M + 2H)^{2+}$ of 714.3143, corresponding to a molecular formula of $C_{64}H_{90}N_{12}O_{25}$ ($\delta$ 0.38 mmu), for a monoketalized product. Going through the 2D TOCSY data at short and long mixing times the spin systems of the backbone amino acids were assigned in a straightforward manner. These differ little from those values found for mannopeptimycin-α in DMSO.[21] A series of *z*-filtered 1D TOCSY experiments with mixing times of 17, 55, 80, 130, and 200 ms facilitated assignment of the $^1H$ spin systems of the mannose units, particularly the terminal mannose at: H-1 ($\delta$ 5.20), H-2 ($\delta$ 3.82), H-3 ($\delta$ 3.53), H-4 ($\delta$ 3.83), H-5($\delta$ 3.55), and H-6 ($\delta$ 3.67, 3.63).

The key NMR data showing the ketal linkage across the 4,6-positions on the terminal mannose of the O-linked disaccharide are (a) a three-bond gradient HMBC coupling from H-6 at $\delta$ 3.67 to the adamantyl ketal carbon at $\delta$ 100.6, and (b) strong ROESY cross-peaks from H-4 ($\delta$ 3.83), H-6 ($\delta$ 3.63), and H-3 ($\delta$ 3.53) to the adamantyl methine proton at $\delta$ 2.77, adjacent to the ketal carbon. The key NMR interactions are shown in Figure 2.

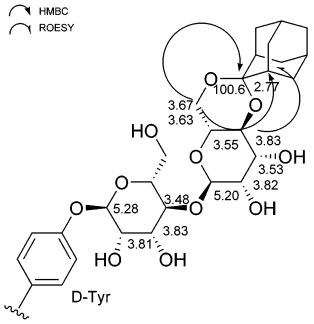



**Figure 2.** NMR interactions used for assignment of the site of ketal formation in compound **10**.

The in vitro activities of compounds **3**−**13** were determined by broth microdilution according to standard procedures.[29] The minimal inhibitory concentrations (MICs) against a spectrum of Gram-positive bacteria for the mannopeptimycin derivatives and vancomycin are shown in Table 1. In total, 30 sensitive and resistant strains were used to determine the inhibitory

**Table 1.** In Vitro Antibacterial Data for Compounds **1** and **3**−**14**

| compound | MIC[a] (μg/mL) | | |
|---|---|---|---|
| | *Staph.* spp.[b] | *Strep.* spp.[c] | *Ent.* spp.[d] |
| vancomycin | 0.5−2 | ≤0.12−0.5 | 0.5→128 |
| **1** | 128→128 | >32 | >128 |
| **3** | ≤0.5−1 | ≤0.5 | 1−2 |
| **4** | 0.5−2 | ≤0.12−0.25 | 2−4 |
| **5** | 0.5−1 | ≤0.12 | 0.5−2 |
| **6** | 0.5−2 | ≤0.06−0.25 | 1−2 |
| **7** | 1−2 | ≤0.06−0.12 | 2−4 |
| **8** | 1−4 | 0.12−1 | 4−8 |
| **9** | 1−4 | ≤0.06 | 4−8 |
| **10** | 0.25−1 | ≤0.06 | 0.5−1 |
| **11** | 0.5−2 | ≤0.12 | 2−4 |
| **12** | 1−2 | ≤0.06−0.12 | 0.5−4 |
| **13** | 0.25−1 | ≤0.06−0.12 | 0.5−2 |
| **13**[e,f] | ≤0.06−0.12 | ≤0.06 | 0.12−0.25 |

[a] Minimal inhibitory concentrations were determined by the broth microdilution method in Mueller-Hinton II broth (MHB)22. [b] Fourteen strains, including methicillin-sensitive and -resistant strains of *S. aureus* (nine strains) and coagulase-negative staphylococci (five strains). [c] Five strains, including penicillin-sensitive and -resistant strains of *S. pneumoniae* (three strains) and β-hemolytic streptococci (two strains). [d] Eleven strains, including vancomycin-sensitive and -resistant strains of *E. faecalis* (eight strains) and *E. faecium* (three strains). [e] Ten microliters of a 12.8 mg/mL DMSO stock solution of **13** was added to 1 mL of 30% BSA and adjusted to a volume of 8.00 mL by addition of 6.99 mL of MHB broth. The final concentration of drug and BSA after inoculation using an equal volume of inoculum was 8 μg/mL of compound **13** and 1.9% BSA in the first well with each subsequent well having half that concentration of the previous well. [f] Experiments conducted on an abbreviated panel of microorganisms that included six strains of *S. aureus*, including methicillin-sensitive and -resistant strains, three strains of streptococci, including penicillin-sensitive and -resistant strains, and five strains of enterococci, including vancomycin-sensitive and -resistant strains.

activities of the new semisynthetic acetal and ketal derivatives. Notably, the hydrophobic acetal and ketal derivatives presented herein invariably displayed potent antibacterial activities against both susceptible and resistant strains of Gram-positive microorganisms, including methicillin resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE). These activities compare favorably not only to vancomycin but also to the naturally occurring mannopeptimycin esters, for which activity against enterococci was substantially weaker than activities against staphyloccoci or streptococci.[21,24] Specifically, acetals **3**−**7** and ketals **8**−**10** of **1** exhibit potent activity against all species. Ketal **10** emerged with potent activity particularly against streptococci species. This trend was also noted with **13**, the corresponding ketal of **2.**

Compound **13**, designated as "AC98−6446," has been further evaluated against an expanded panel of recent clinical isolates alongside a number of comparative antibiotics.[30] In this study, we found that addition of blood products such as bovine serum albumin (BSA) to test preparations of compound **13** prior to dilution with broth improved the antibacterial activities by two or more dilutions in tests involving staphylococci and enterococci. This enhancement effect was not observed in tests with streptococci as these organisms are normally tested in the presence of 5% lysed horse blood.

Selected compounds were also evaluated in vivo in murine acute lethal infection models following IV

**Table 2.** In Vivo Efficacies of Selected Mannopeptimycin Derivatives

| compound | $ED_{50}$ (mg/kg)[a] | |
|---|---|---|
| | *S. aureus* Smith (GC 4543) | *E. faecalis* (GC 6189) |
| vancomycin[b] | 1 | >32 |
| **1** | 20 | n.t.[c] |
| **4** | 0.19 | 0.39 |
| **5** | 0.07 | 0.19 |
| **6** | 0.23 | 1.04 |
| **10** | 0.04 | 1.80 |
| **13** | 0.08 | 0.39 |

[a] Pooled mean from at least three separate tests using five dosages and five mice per dosage level. [b] Dosed in saline. [c] Not tested.

administration in D5W (5% dextrose in water) using previously published protocols.[25,31,32] As shown in Table 2, compounds **4**−**6**, **10**, and **13** showed potent protective effects against *S. aureus* and *E. faecalis* infections in murine models, again comparing favorably to vancomycin and the naturally occurring mannopeptimycins.[25] Additional studies that demonstrate the extraordinary in vivo activities and favorable pharmacokinetic properties of compound **13** will be the subject of a forthcoming publication.[33]

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**Supporting Information Available:** Details on the preparation, HRMS, HPLC, and NMR spectra for compounds **3**−**13** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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