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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 2821–2824

Mannosidase chemistry of the mannopeptimycin complex

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Received 27 April 2004; accepted 13 July 2004
Available online 11 September 2004

Abstract—α-Mannosidase catalyzes the removal of the *O*-linked mannose units from mannopeptimycin-α. The reaction can be run on a preparative scale to allow isolation of mannopeptimycin-β or the mono-*O*-mannosyl intermediate according to the choice of catalyst.

1. Introduction

The mannopeptimycins (Fig. 1)[1] are a new class of glycopeptide antibiotics, which are active against a range of drug-resistant Gram-positive bacteria, including methicillin-resistant staphylococci and vancomycin-resistant enterococci, by a new mechanism of action.[2–5] The more active members of the naturally occurring series, and the semi-synthetic derivatives that have been prepared,[6–10] typically contain a tyrosine-*O*-linked α-mannosyl disaccharide functional group; we were interested in the manipulation of this moiety by mannosidase-catalyzed hydrolysis. Furthermore, the selective deglycosylation of other glycopeptide antibiotics has been reported to be a useful area of investigation.[11–13]

The main aims herein were to find a method for the conversion of mannopeptimycin-α to mannopeptimycin-β without the need for harsh conditions and to isolate the intermediate *O*-monosaccharide. We also sought to differentiate the *O*- and *N*-linked mannoses by this means, to provide a simple diagnostic probe to assist in the determination of the mannosylation pattern of new mannopeptimycin derivatives from mutant organisms and to indicate the general regiochemistry of synthetic modifications.

To our knowledge there are no examples in the literature of the α-mannosidase catalyzed hydrolysis of mannosyl or higher polymannosyl residues from a tyrosine or 4-hydroxyphenylglycine moiety, whether as an individual amino acid derivative or as part of a more complex peptide structure. However, there are many examples of α-mannosidase catalyzed hydrolyses of mannosyl residues from other phenol derivatives.[14–16] In fact, *p*-nitrophenyl α-mannopyranoside is the standard substrate for the enzyme.[14]

We therefore investigated the hydrolysis of mannopeptimycin-α catalyzed by the commercial α-mannosidases isolated from jack beans (*Canavalia ensiformis*)[15,17,18] and almonds (*Prunus amygdalus*).[19] On an analytical scale, both enzymes mediated a clean conversion to mannopeptimycin-β with the jack bean enzyme reaction progressing relatively quickly. No hydrolysis of the *N*-linked mannosyl residue was observed in either case.

These enzymes are known to be specific for terminal, unsubstituted α-mannopyranosides (and not the corresponding β-anomers). This result, together with the information that mannopeptimycin-α is not a substrate for the β-mannosidase from snails,[20,21] which has similar specificity for the β-anomer of mannopyranosides, corroborates the spectroscopic structural assignment (based upon ^{1}H–^{13}C coupling constants).[1] Similarly, the observation that mannopeptimycins-γ, δ- and -ε are not substrates for jack bean α-mannosidase is consistent with the sites of esterification being on the terminal ring.

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doi:10.1016/j.tetasy.2004.07.056

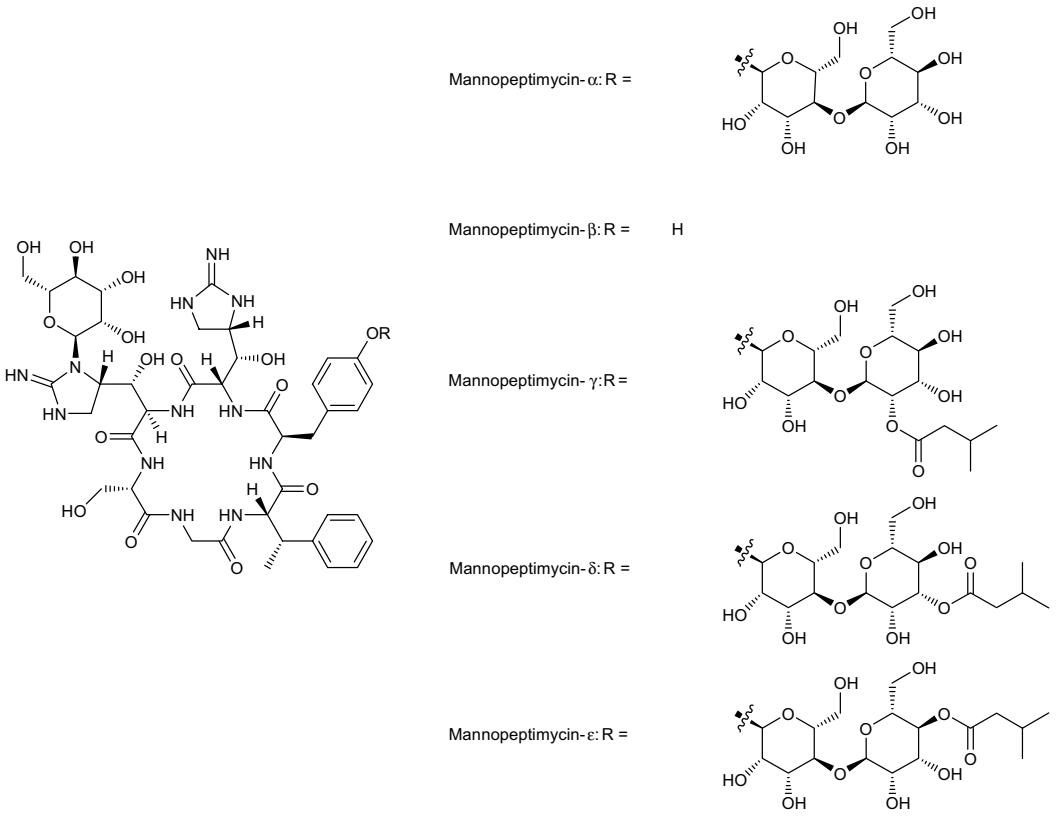

Figure 1. Structures of naturally occurring mannopeptimycins.

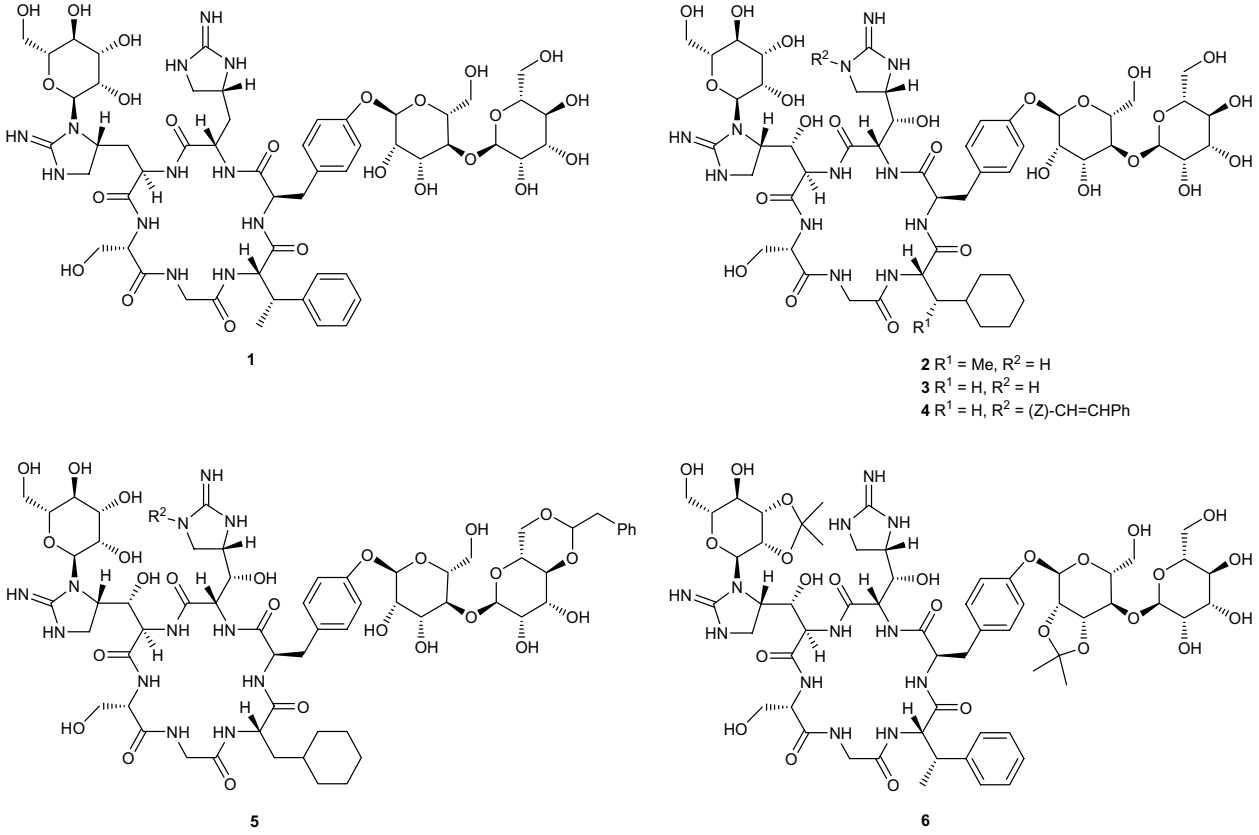

Figure 2. Modified mannopeptimycins investigated as potential substrates for jack bean α-mannosidase.

2. Results and discussion

As might be expected, derivatives of the mannopeptimycins, which retain an unfunctionalized *O*-mannosyldisaccharide were also found to be substrates for jack bean α-mannosidase (Fig. 2). Thus the dideoxy **1**,[22] cyclohexyl-substituted **2** and **3**[10,23,24] and *N*-alkylated **4** derivatives were all converted cleanly to the corresponding mannopeptimycin-β analogue. Compound **4** was a by-product in the synthesis of an acetal **5** at the 4,6-position[10] and this methodology allowed us to easily differentiate between the two. In fact none of the acetals formed at this terminal sugar were substrates for the enzyme—in common with the naturally occurring esters above. In contrast, the internal acetonide **6** (prepared by partial degradation of the pentaacetonide) was a substrate for the enzyme, with removal of only the terminal mannose unit.

The jack bean α-mannosidase mediated conversion of mannopeptimycin-α to -β was optimized for a preparative scale. The process was found to be most efficient at pH 5 while the addition of zinc chloride to a concentration of 0.02 M prolonged the lifetime of the enzyme. The addition of cosolvents, such as dimethyl formamide, acetonitrile and dioxane, had no beneficial effect, while the reaction ran optimally at a 0.002 M substrate concentration.[25] While this process compared favorably with the acid-catalyzed hydrolysis in terms of selectivity of reaction the catalyst cost was an issue. Fortunately, we found that the crude jack bean meal could be substituted for the isolated enzyme to give mannopeptimycin-β in an identical yield but with a greater than 1000-fold saving in catalyst cost (Fig. 3).[26]

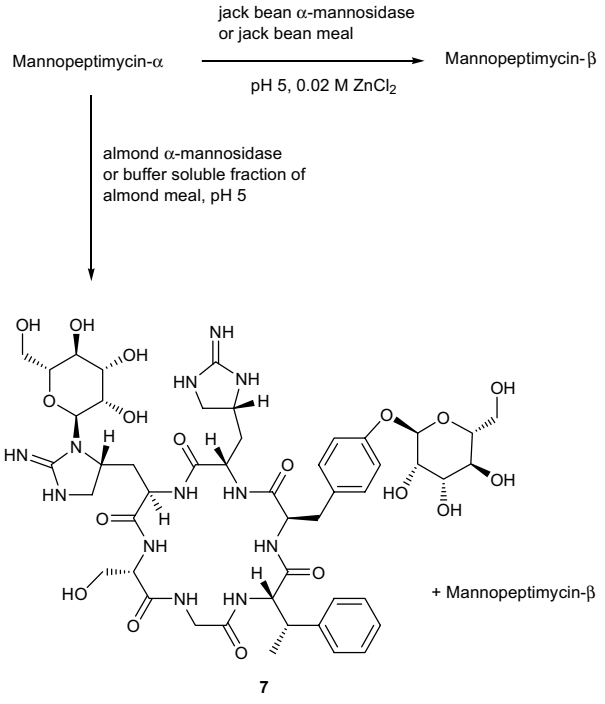

Figure 3. Preparative reactions of mannopeptimycin-α.

Our remaining goal was to prepare the intermediate *O*-monosaccharide **7**. Such 'interrupted' hydrolyses of poly-α-mannopyranosides have been reported with polymannosyl *N*-acetylglucosamines.[27] While little of **7** was accumulated during the acid or jack bean α-mannosidase catalyzed hydrolyses, significant amounts were observed in the almond α-mannosidase catalyzed process and could be isolated with reasonable efficiency if the reaction was terminated at ca. 20% conversion of the starting material. Again the isolated enzyme could be replaced with a relatively crude preparation as we found the buffer soluble fraction of the almond meal to be a satisfactory catalyst (in common with a recent report[28]) allowing us to isolate **7** in a scaleable fashion.[29]

3. Conclusion

In conclusion we have shown that both mannopeptimycin-β and the intermediate mono-*O*-mannosyl species **7** can be prepared by hydrolysis of mannopeptimycin-α under mild conditions using an appropriate source of α-mannosidase. The use of these products in the synthesis of novel mannopeptimycin derivatives will be reported elsewhere.

Acknowledgements

We would like to thank Guy T. Carter, Janis Upeslacis and Tarek S. Mansour for their support and the members of the Discovery Analytical Chemistry department, in particular Frank Koehn, Melissa Lin, Mark Tischler, Rachel Garlish and Murthy Damarla for their assistance in this work.

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25. Jack bean α-mannosidase (Sigma, 65u) was added to a solution of mannopeptimycin-α bis(trifluoroacetate) salt (0.60 g) in 0.1 M pH 5 sodium acetate buffer (200 mL, 0.02 M in $ZnCl_2$) and the solution stirred at room temperature for 18 h. The mixture was adjusted to pH 7 and then centrifuged. The supernatant was passed through a XAD-7 column (eluting with a solvent system of 1:1:0.001 water:acetonitrile:trifluoroacetic acid) and the fractions containing mannopeptimycin-β were collected. The solid was dissolved in 5% aq acetic acid solution and the pH adjusted to 7. This solution was filtered and the filtrate passed through a XAD-7 column (eluting with a solvent system of 1:1:0.001 water:acetonitrile:trifluoroacetic acid). The fractions containing mannopeptimycin-β were combined with those above, concentrated and further purified by elution through a C18 reverse phase preparative column (eluting with a solvent gradient of 6:1:0.0014 to 1:1:0.0014 water:acetonitrile:trifluoroacetic acid) to give mannopeptimycin-β bis(trifluoroacetate) salt (316 mg) as an off-white solid.
26. Jack bean meal (Sigma, 0.60 g) was substituted in an otherwise identical process to the above except that the reaction mixture was first filtered through diametaceous earth prior to application to the XAD-7 column; 312 mg of product was obtained.
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29. Almond meal (Sigma, 2.5 g) was suspended in 0.1 M pH 5 sodium acetate buffer (200 mL) and the suspension stirred for ca. 1 h and centrifuged. The supernatant was adjusted to pH 5 by the addition of acetic acid then recentrifuged. Mannopeptimycin-α bis(trifluoroacetate) salt (2 g) was added to 133 mL of the supernatant and the resultant solution stirred at room temperature for 18 h. The mixture was evaporated to dryness, resuspended in *N*,*N*-dimethylformamide, filtered and the filtrate was purified by chromatography on a C18 reverse phase preparative column (eluting with a solvent gradient of 6:1:0.0014 to 7:3:0.002 water:acetonitrile:trifluoroacetic acid) to give **7** bis(trifluoroacetate) salt (48 mg) as an off-white solid.