Multi-valent polymer of vancomycin: enhanced antibacterial activity against VRE

Hirokazu Arimoto,*[a] Kazuya Nishimura,[a] Tomoya Kinumi,[b] Ichiro Hayakawa[a] and Daisuke Uemura[c]

[a] *Department of Chemistry, Faculty of Science, Shizuoka University, Ohya, Shizuoka 422-8529, Japan. E-mail: scharim@ipc.shizuoka.ac.jp*
[b] *Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan*
[c] *Department of Chemistry, Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan*

Received (in Cambridge, UK) 4th May 1999, Accepted 14th June 1999

A multivalent polymer of vancomycin, synthesized *via* ring-opening metathesis polymerization (ROMP), exhibited significant enhancement of antibacterial activity against vancomycin-resistant enterococci (VRE).

The history of the fight against infectious bacteria centers around the recurring problem of drug resistance. For example, the evolution of MRSA (methicilin-resistant *Staphylococcus aureus*), which resists almost all established antibiotics, is now a serious worldwide problem. Currently the only viable treatment for MRSA is vancomycin. However, VRE (vancomycin resistant enterococci) has emerged, and the possibility of this resistance being transferred to *S. aureus* is a cause of major concern.[1] Although much effort has been put forth to locate new antibiotics, no alternatives to vancomycin-class glycopeptides have been found. Thus, novel strategies to modify the glycopeptides to enhance their potency against VRE are in great demand.

We have been interested in the rapidly growing molecular design principle of multivalent or cluster effects for the enhancement of weak non-bonding interactions.[2,3] Although successful applications of this concept with sugar ligands have been reported, it has not been further generalized to the recognition of complex natural products or peptides.[4] Vancomycin binds to the D-Ala-D-Ala residue of the pentapeptide terminal of a bacterial biosynthetic intermediate through five hydrogen bonds.[5] Since this binding interferes with bacterial peptidoglycan biosynthesis, it is widely believed that strengthening the association could enhance antibacterial activity. We describe herein our preliminary attempts to enhance the potency of vancomycin-class antibiotics against VRE by the formation of vancomycin-based polymers (Fig. 1).

Vancomycin contains a variety of functional groups sensitive to basic, acidic and oxidative conditions,[6,7] which necessitates careful consideration of strategies for both the modification and polymerizations steps. We chose to pursue a ring-opening metathesis polymerization (ROMP) approach. Among the known ROMP catalysts, Grubb's ruthenium catalyst[8] appeared to be well suited, as it is tolerant to polar functional groups, and applications to sugars and protected oligopeptides have appeared. However, deactivation of the catalyst by free amino groups has been demonstrated, and the functional group tolerance of the catalyst to primary amide, carboxylic acids, and phenols has not been fully established.[9]

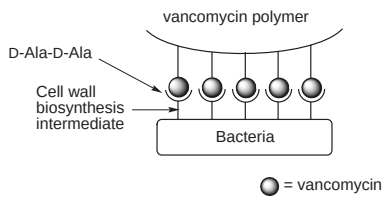
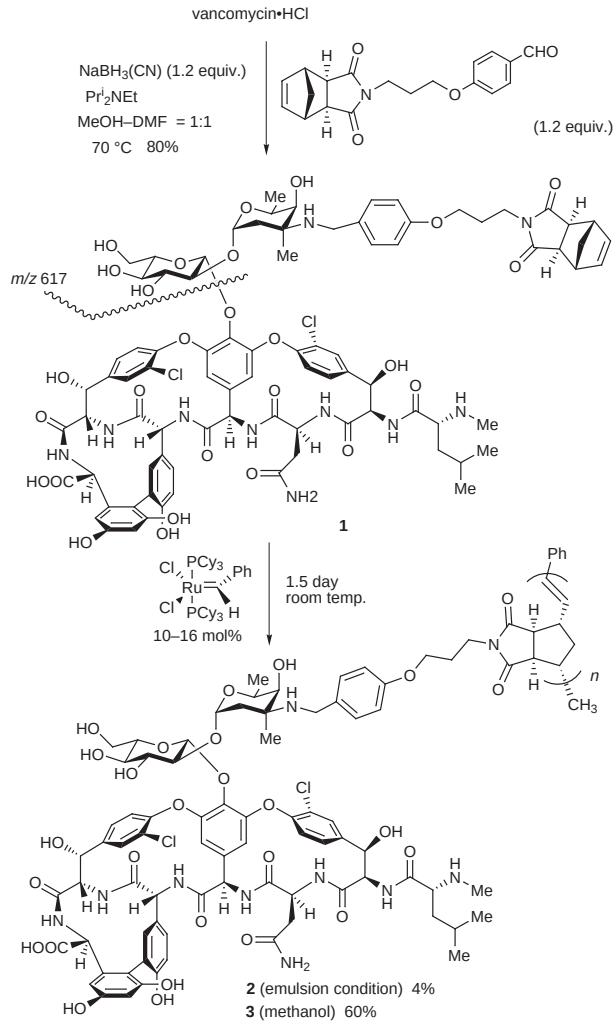


Fig. 1 Schematic presentation of the interaction of a vancomycin polymer with a bacterial cell wall biosynthesis intermediate.

Transformation of vancomycin to a ROMP monomer was accomplished as shown in Scheme 1. An aromatic aldehyde, which was linked to the metathesis-active norbornene unit, was appended onto vancomycin by regioselective reductive amination.[10] ^{1}H NMR and MALDI-TOF mass spectroscopy (*m/z* 1757, M + 1) data[11] were consistent with the structure of monomer unit **1**. The regioselectivity for the amino-sugar portion over the N-terminal secondary amine was further established through a fragmentation analysis of a PSD experiment on the MALDI-TOF mass spectra (*m/z* 617, average mass, see Scheme 1).

The ring-opening metathesis polymerization was investigated under two different sets of conditions. When the polymerization was conducted in aqueous emulsion condi-



Scheme 1

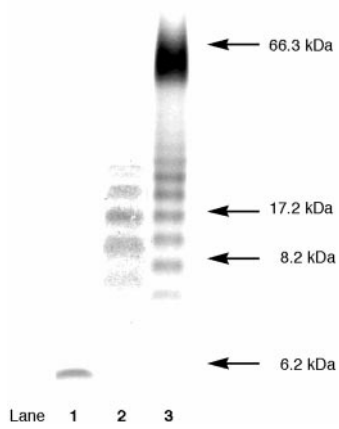


Fig. 2 Electrophoresis of compounds **1**–**3** [16% Tris–Tricine SDS–polyacrylamide gel (silver stained)]: lane 1 = monomer **1**; lane 2 = polymer **3**; lane 3 = polymer **2**.

tions,[12] the reaction was slow and the yield of polymer was only 4%. A significant improvement was observed with MeOH as the solvent in the standard homogeneous system. After 1.5 days reaction time, removal of unreacted monomer and catalyst by reversed phase column chromatography (Cosmosil 75C_{18}-OPN, MeCN–H_2O = 1:2, 1% TFA) afforded 60% yield of polymeric material **3**.[13] Polymerization was evidenced by broadened signals in the ^{1}H NMR spectra, as well as the disappearance of the norbornene olefinic proton signals. Polymer formation was also monitored by SDS-PAGE electrophoresis, which indicated the difference in molecular weight distribution of the two reactions (Fig. 2).

Antibacterial activities of monomer **1** and polymers **2** and **3** were thus evaluated (Table 1). The antibacterial properties of vancomycin were not affected by introduction of the norbornene unit present in **1**. In contrast, polymerization of **1** to **3** resulted in a significant (8 to 60 fold) enhancement of potency agaisnt VREs, with retention of practical MIC values against *S. aureus* and *Enterococci*. The reason for the marked differences in potency between **2** and **3** is not clear at this stage, but may be due either to the polymer weight distributions or to the substructural difference of each polymer. These results suggest that polyvalent polymers may be promising tools in the fight against multi-resistant bacteria.

Table 1 Antibacterial activities of compounds **1**–**3**

	MIC/μg ml^{-1}			
Compound	*S. aureus*[a]	*Entero-coccus*[b]	VRE (Van A)[c]	VRE (Van B)[d]
VCM	0.2	< 0.5	> 250	125
1	0.2	< 0.5	> 250	125
2	—	31	> 250	31
3	2.3	2	31	2

[a] Mean of six strains of *S. aureus*, which include clinically isolated MRSA. [b] *Enterococcus faecalis*. [c] NCB40. [d] RV1.

In conclusion, we have prepared multivalent polymers of vancomycin that displayed significant antibacterial activity enhancement against VRE. Further efforts towards the understanding of this enhancement will be reported in due course.

The authors thank Dr Yoshichika Arakawa, National Institute of Infectious Diseases, and Dr Kiyoshi Nakayama, Daiichi Pharmaceutical Co. Ltd., for antibacterial tests, Dr Masahiro Uritani, Shizuoka University, for SDS-PAGE analysis, and Professor William Moser of IUPUI for helpful discussions and for critiquing the manuscript. This research was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and a Sasakawa Scientific Research Grant from the Japan Science Society.

Notes and references

1 For recent reviews on this field, see D. Niccolai, L. Tarsi and R. J. Thomas, *Chem. Commun.*, 1997, 2333. See also D. H. Williams and B. Bardsley, *Angew. Chem., Int. Ed.*, 1999, **38**, 1173.
2 M. Mammen, S. K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754.
3 L. L. Kiessling and N. L. Pohl, *Chem. Biol.*, 1996, **3**, 71.
4 For some examples of multivalent polymers with non-sugar ligands: V. C. Gibson, E. L. Marshall, M. North, D. A. Robson and P. J. Williams, *Chem. Commun.*, 1997, 1095; S. C. G. Biagini, V. C. Gibson, M. R. Giles, E. L. Marshall and M. North, *Chem. Commun.*, 1997, 1097.
5 D. H. Williams, *Nat. Prod. Rep.*, 1996, **13**, 469. In VanA and VanB resistance, pentapeptide terminals are changed from -D-Ala-D-Ala to -D-Ala-D-Lac, which makes the binding of vancomycin considerably weaker.
6 C. M. Harris, H. Kopecka and T. M. Harris, *J. Am. Chem. Soc.*, 1983, **105**, 6915.
7 Vancomycin is also sensitive to acidic and oxidative conditions, see R. Nagarajan and A. A. Schabel, *Chem. Commun.*, 1988, 1306; M. Adamczyk, J. Grote and S. Rege, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 885 and references therein.
8 P. Schwab, M. B. France, J. W. Ziller and R. H. Grubbs, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 2039.
9 For compatibility of metathesis catalysts with various functional groups, see review: S. K. Armstrong, *J. Chem. Soc., Perkin Trans. 1*, 1998, 371. See also R. H. Grubbs and S. Chang, *Tetrahedron*, 1998, **54**, 4413; M. L. Randall and M. L. Snapper, *J. Mol. Catal. A: Chem.*, 1998, **133**, 29.
10 R. D. G. Cooper, N. J. Snyder, M. J. Zweifel, M. A. Staszak, S. C. Wilkie, T. I. Nicas, D. L. Mullen, T. F. Butler, M. J. Rodriguez, B. E. Huff and R. C. Thomspon, *J. Antibiot.*, 1996, **49**, 575; M. J. Rodriguez, N. J. Snyder, M. J. Zweifel, S. C. Wilkie, D. R. Starck, R. D. Cooper, T. I. Nicas, D. L. Mullen, T. F. Butler and R. C. Thompson, *J. Antibiot.*, 1998, **51**, 560.
11 Monoisotopic mass.
12 D. M. Lynn, S. Kanaoka and R. H. Grubbs, *J. Am. Chem. Soc.*, 1996, **118**, 784.
13 We believe that monomer **1** was used as a trifuoroacetic acid salt, since the chromatographic purification of **1** was done by a TFA-containing solvent system. Thus, the key to the success in the metathesis polymerization may be the ammonium salt formation of two *sec*-amines in **1**, which prevented the deactivation of catalyst.

Communication 9/03529J