

Fig. 5. The effect of 15-min exposure to monochromatic light (509 nm) on pineal melatonin content in *rd/rd cl* and wild-type mice. Compared with unpulsed animals (A), *rd/rd cl* mice showed irradiance-dependent suppression of pineal melatonin content after exposure for 15 min to 509-nm light (B). Data represent mean \pm SEM for six to eight animals per genotype at each irradiance; $^{***}P < 0.01$ compared with unpulsed controls; post hoc Bonferroni's test after one-way ANOVA. Although the data suggest that the production of melatonin in *rd/rd cl* mice may be less sensitive to inhibition by light, this is not supported by statistical analysis (two-way ANOVA, $P > 0.05$).

acterized ocular photoreceptors might form the basis of a general non-image forming photoreceptive pathway mediating many, if not all, nonvisual responses to light.

References and Notes

- R. G. Foster, *Neuron* **20**, 829 (1998).
- B. G. Soni, A. Philp, B. E. Knox, R. G. Foster, *Nature* **394**, 27 (1998).
- R. J. Thresher *et al.*, *Science* **282**, 1490 (1998).
- R. W. Rodieck, *The First Steps in Seeing* (Sinauer, Sunderland, MA, 1998).
- D. C. Klein and J. L. Weller, *Science* **177**, 532 (1972).
- T. Deguchi and J. Axelrod, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2547 (1972).
- _____, *ibid.*, p. 2208.
- J. Arendt, *Melatonin and the Mammalian Pineal Gland* (Chapman & Hall, Cambridge, 1995).
- R. J. Lucas and R. G. Foster, *Endocrinology*, in press.
- H. Sun, J. P. Macke, J. Nathans, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8860 (1997).
- G. H. Jacobs, J. Neitz, J. F. Deegan, *Nature* **353**, 655 (1991).
- Y. Wang *et al.*, *Neuron* **9**, 429 (1992).
- The *cl* transgene was previously characterized in C57BL/6 mice (12). However, because this strain of mice does not produce pineal melatonin [S. Ebihara, T. Marks, D. J. Hudson, M. Menaker, *Science* **231**, 491 (1986)], we introduced this transgene into the C3H/He strain by at least nine generations of backcrossing.
- E. Soucy, Y. Wang, S. Nirenberg, J. Nathans, M. Meister, *Neuron* **21**, 481 (1998). Our reference to the *cl* transgene corresponds to the h.red DT-A construct in this paper.
- Total RNA was extracted from ($n = 4$ to 6) eyes collected from 80-day-old wild-type, *cl*, and *rd/rd cl* mice with the Promega (Southampton, UK) SV RNA isolation kit. For Northern (RNA) blot analysis, 5 μ g of total RNA from each genotype was size-separated on a 1.2% agarose-formaldehyde electrophoresis gel and blotted overnight onto Hybond N+ membrane (Amersham). Three blots were hybridized with ³²P-labeled probes specific for mouse rod opsin [cDNA probe after W. Baehr *et al.*, *FEBS Lett.* **238**, 253 (1988)] and green and UV cone opsins [cRNA probes after M. von Schantz *et al.*, *Mol. Brain Res.* **770**, 131 (1997)], and subsequently with a ³²P-labeled cDNA probe for glyceraldehyde phosphate dehydrogenase

(GAPDH) (positive control). For reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, cDNA was synthesized from 2 μ g of total RNA from each genotype. PCR amplification was then undertaken over 35 cycles under optimized conditions with primers designed against the published sequences for mouse rod opsin, green cone opsin, UV cone opsin, cone arrestin, and tubulin (positive control). Appropriate negative controls (total RNA without reverse transcriptase step) were included for all genotypes. PCR reactions were separated on a 1.5% agarose gel and blotted overnight onto Hybond N+ membrane. Blots were hybridized with appropriate ³²P-labeled cDNA probes.

- Mice stably entrained to a 12 hour light:12 hour dark cycle were individually either sham-pulsed (no light exposure) or exposed to 15 min of defined irradiance (1.2×10^{-4} , 1.2×10^{-3} , or 2.6×10^{-2} μ W/cm²) with monochromatic light ($\lambda_{max} = 509$ nm, half band width 10 nm) at zeitgeber time 20-21. The melatonin content of pineal homogenates was assessed by radioimmunoassay as described (9).
- C. Bowes *et al.*, *Nature* **347**, 677 (1990).
- S. J. Pittler and W. Baehr, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8322 (1991).
- L. D. Carter-Dawson, M. M. LaVail, R. L. Sidman, *Investig. Ophthalmol. Vis. Sci.* **17**, 489 (1978).
- We used polyclonal antisera (12) to the human blue

cone pigment (used at 1:8000 dilution) and human red-green pigments (1:8000 dilution) and a monoclonal antibody to rat rod opsin [D. Hicks and R. S. Molday, *Exp. Eye Res.* **42**, 55 (1986); 1:20,000 dilution].

- R. J. Lucas, M. S. Freedman, M. Muñoz, J.-M. Garcia-Fernández, R. G. Foster, data not shown.
- D. C. Klein and R. Y. Moore, *Brain Res.* **174**, 245 (1979).
- I. Provencio, H. M. Cooper, R. G. Foster, *J. Comp. Neurol.* **395**, 417 (1998).
- D. S. Hsu *et al.*, *Biochemistry* **35**, 13871 (1996).
- Y. Miyamoto and A. Sancar, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6097 (1998).
- I. Provencio, G. Jiang, W. J. DeGrip, W. P. Hayes, M. D. Rollag, *ibid.*, p. 340.
- L. Wetterberg, Ed., *Light and Biological Rhythms in Man* (Pergamon, Oxford, 1993), vol. 62.
- M. Freedman *et al.*, *Science* **284**, 502 (1999).
- We thank J. Nathans and Y. Wang (Johns Hopkins University) for donation of the original C57BL/6 *cl* mouse colony and the polyclonal antisera to cones and D. Hicks for the monoclonal antisera to rods. This work was supported by research grants from UK Biotechnology and Biological Sciences Research Council and European Union BioMed 2 program.

21 December 1998; accepted 15 March 1999

Vancomycin Derivatives That Inhibit Peptidoglycan Biosynthesis Without Binding D-Ala-D-Ala

Min Ge,¹ Zhong Chen,¹ H. Russell Onishi,² Joyce Kohler,² Lynn L. Silver,² Robert Kerns,¹ Seketsu Fukuzawa,¹ Christopher Thompson,¹ Daniel Kahne^{1*}

Vancomycin is an important drug for the treatment of Gram-positive bacterial infections. Resistance to vancomycin has begun to appear, posing a serious public health threat. Vancomycin analogs containing modified carbohydrates are very active against resistant microorganisms. Results presented here show that these carbohydrate derivatives operate by a different mechanism than vancomycin; moreover, peptide binding is not required for activity. It is proposed that carbohydrate-modified vancomycin compounds are effective against resistant bacteria because they interact directly with bacterial proteins involved in the transglycosylation step of cell wall biosynthesis. These results suggest new strategies for designing glycopeptide antibiotics that overcome bacterial resistance.

Vancomycin is a glycopeptide antibiotic that kills bacterial cells by inhibiting peptidoglycan biosynthesis (1). It is the most important drug in current use for the treatment of Gram-positive bacterial infections, representing the final option for curing infections that are resistant to other antibiotics. The emergence of vancomycin-resistant bacterial strains is a very serious public health problem. Recently, a set of carbo-

hydrate derivatives of vancomycin that are active against resistant bacterial strains was discovered (2). We now show that the modified carbohydrates alone are specific inhibitors of the transglycosylation step of peptidoglycan biosynthesis. This finding changes the picture for how modified glycopeptides kill resistant bacteria.

Vancomycin functions by binding to the terminal D-Ala-D-Ala dipeptide of bacterial cell wall precursors (Fig. 1), thereby impeding further processing of these intermediates into peptidoglycan (3, 4). The vancomycin complex involves a set of complementary hydrogen bonds between the peptide portion

¹Department of Chemistry, Princeton University Princeton, NJ 08544, USA. ²Infectious Diseases, Merck Research Laboratories, Rahway, NJ 07065, USA.

*To whom correspondence should be addressed. E-mail: dkahne@princeton.edu

REPORTS

of vancomycin and the D-Ala-D-Ala dipeptide (4). Walsh and co-workers have shown that vancomycin resistance arises when bacteria acquire the ability to substitute D-Ala-D-Ala with D-Ala-D-Lac (5). This structural change results in the loss of a critical hydrogen bond between the binding pocket of vancomycin and the peptide substrate. The binding affinity of vancomycin for the D-Ala-D-Lac substrate decreases by

three orders of magnitude, with a concomitant loss of biological activity.

The complexity of the peptide portion of vancomycin makes it virtually impossible to reengineer the peptide backbone to include new contacts to the modified substrate (6). However, glycopeptides containing hydrophobic substituents on the vancosamine nitrogen (such as compounds **1** and **2**, Fig. 2 and Table 1) are

very active against vancomycin-resistant strains (2, 7). Although it is believed that these carbohydrate derivatives must bind the dipeptide termini of cell wall precursors to function (8–10), they do not show increased affinity for D-Ala-D-Lac (9); therefore, it is not obvious how they overcome resistance. It has been proposed that the hydrophobic substituents compensate for the decreased affinity of D-Ala-D-Lac binding by facilitating dimerization (9, 11) and by anchoring the glycopeptides to the bacterial surface in close proximity to the cell wall precursors (9, 10). Although some elegant experiments support the hypothesis that membrane-anchoring and dimerization can significantly increase D-Ala-D-Ala binding avidity (10, 12), there is little evidence that these phenomena enhance binding to D-Ala-D-Lac sufficiently to explain the biological activity of the substituted glycopeptides against resistant bacteria.

We compared vancomycin and carbohydrate-modified glycopeptides **1** and **2** in an assay designed to establish how different compounds inhibit peptidoglycan biosynthesis. Peptidoglycan biosynthesis takes place in three stages (Fig. 1) (1). The first two stages occur inside the bacterial cell and involve the assembly of a lipid-linked GlcNAc-MurNAc-pentapeptide (Lipid II). The third stage, which takes place on the exterior surface of the bacterial membrane, involves the polymerization of the GlcNAc-MurNAc disaccharide by transglycosylases and the cross-linking of the peptide side chains by transpeptidases. In the Gram-negative bacterium *Escherichia coli*, polymerization and cross-linking take place sequentially, producing immature (uncross-linked) peptidoglycan first and then mature (cross-linked) peptidoglycan (13). Because of the sequential nature of the polymerization and cross-linking reactions, it is possible to use permeabilized *E. coli* strains to determine at which step peptidoglycan biosynthesis is blocked (14) simply by monitoring how much radioactivity is incorporated into Lipid II, immature, and mature peptidoglycan after treatment of the cells with [¹⁴C]UDP-GlcNAc and [¹⁴C]UDP-MurNAc-pentapeptide in the pres-

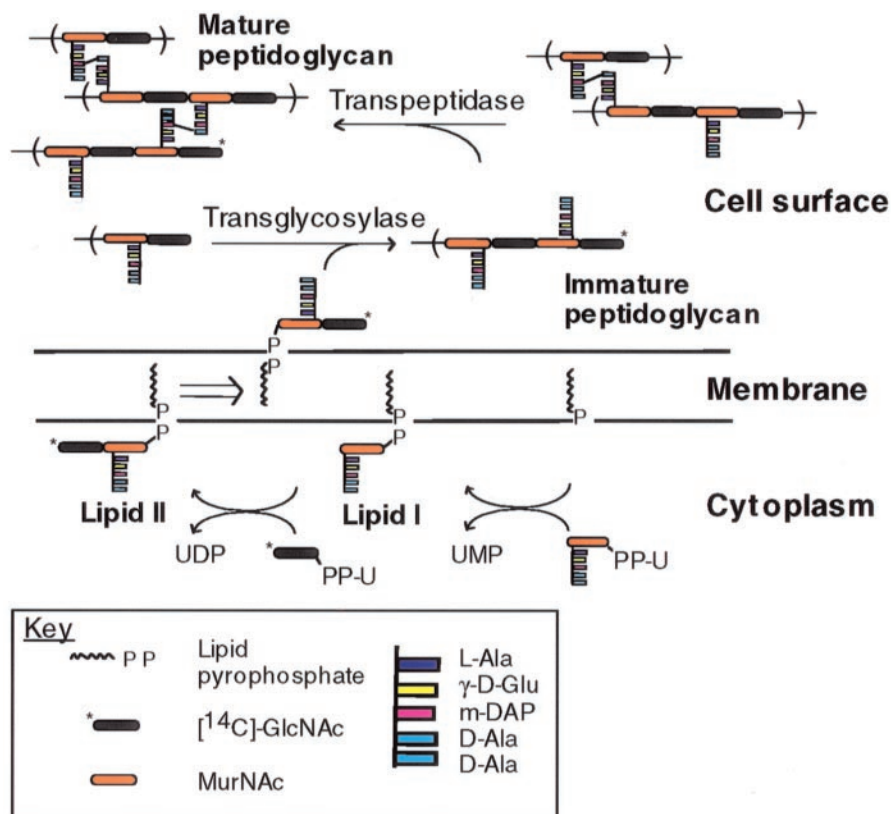


Fig. 1. Proposed pathway for peptidoglycan synthesis in Gram-negative bacteria. Lipid II is assembled in the cytoplasm and then transported through the membrane where it is polymerized by transglycosylases to form immature peptidoglycan and then cross-linked by transpeptidases.

Table 1. Twenty-two-hour minimum inhibitory concentrations (MICs) ($\mu\text{g/ml}$) for selected bacterial strains (*Enterococcus faecium* and *Enterococcus faecalis*). MICs were determined against strains grown in brain-heart infusion broth in a microdilution format according to NCCLS guidelines (23). The inoculum was 5 to 10 times higher than the recommended 3×10^5 to 7×10^5 colony-forming units per milliliter. The MIC is defined as the lowest antibiotic concentration that resulted in no visible growth after incubation at 35°C for 22 hours.

	<i>E. faecium</i>		<i>E. faecalis</i>	
	Sensitive	Resistant (VanA)	Sensitive	Resistant (VanB)
Vancomycin	1	2048	4	2048
Chlorobiphenyl	0.03	16	0.25	16
Vancomycin (1)				
<i>n</i> -decyl	0.03	32	0.12	32
Vancomycin (2)				
Des-leucyl chlorobiphenyl	10	40	20	80
Vancomycin (4)				
Chlorobiphenyl	128	128	128	128
Disaccharide (6)				
Chlorobiphenyl	0.03	32	0.06	64
Des-methyl vancomycin (8)				

Table 2. Twenty-two-hour MICs ($\mu\text{g/ml}$) of vancomycin and compounds **1** and **8** for selected staphylococcal strains. MSSA: methicillin-sensitive *Staphylococcus*; MRSA: methicillin-resistant *Staphylococcus*.

	MSSA		MRSA	
	MB2985	CL3033	COL	
Vancomycin	0.5	1	1	
Chlorobiphenyl	0.03	0.12	0.25	
Vancomycin (1)				
Chlorobiphenyl				
Des-methyl				
Vancomycin (8)	0.016	0.016	0.03	

REPORTS

ence of increasing concentrations of an inhibitor. As shown in Fig. 3A, compounds such as ramoplanin that inhibit the formation of Lipid II cause a decrease in all the products (15); compounds such as bambamycin that inhibit transglycosylase activity cause a decrease in both immature and mature peptidoglycan (13); and compounds such as cefoxitin that inhibit transpeptidase activity cause a decrease in the formation of mature peptidoglycan (16).

Marked differences were observed in the inhibition patterns obtained for vancomycin and carbohydrate derivatives **1** and **2** (Fig. 3B). Vancomycin causes a decrease in mature peptidoglycan and an increase in immature peptidoglycan, consistent with a mechanism of action in which the primary site of inhibition is the transpeptidation step. The carbohydrate derivatives, in contrast, cause a decrease in both immature and mature peptidoglycan, and an increase in the lipid intermediates, consistent with transglycosylase inhibition. Hence, the carbohydrate derivatives **1** and **2** block a different step in peptidoglycan biosynthesis than does vancomycin.

We have considered two possible explanations for the above result. One explanation is that the hydrophobic substituent on the vancosamine sugar helps anchor the glycopeptide to the cell membrane, as proposed previously (8, 10). In vitro assays have shown that vancomycin itself can block either transglycosylation or transpeptidation depending on whether Lipid II or immature peptidoglycan is bound (3, 17). If **1** and **2** are anchored to the cell membrane, they might preferentially bind membrane-bound Lipid II over immature peptidoglycan (Fig. 1), thus blocking transglycosylation more effectively than

vancomycin itself, which binds to all cell wall precursors containing exposed D-Ala-D-Ala dipeptides. An alternative explanation for the change in the inhibition pattern is that the carbohydrate-modified glycopeptides interact with a target other than D-Ala-D-Ala, such as the enzymes involved in transglycosylation. If so, dipeptide binding might not be required for activity.

To evaluate the role of peptide binding in activity, we prepared des-leucyl vancomycin **3** (18) and its chlorobiphenyl derivative **4**. Des-leucyl vancomycin **3** does not bind D-Ala-D-Ala (19) and has no activity against either vancomycin-sensitive or -resistant strains (2); in contrast, chlorobiphenyl derivative **4** has good activity against both sensitive and resistant bacterial strains (Table 1). Derivative **4** is almost as active as **1** and **2** against resistant bacterial strains even though it is missing an important portion of the dipeptide-binding pocket. Although the lower minimum inhibitory concentrations (MICs) of **1** and **2** compared with **4** in D-Ala-D-Ala-producing microorganisms indicate that dipeptide binding enhances antibiotic activity, it is not essential.

The preceding results imply that the modified disaccharide is a key determinant of biological activity, with a mechanism of action independent of peptide binding. To evaluate the activity of the carbohydrate portion alone, we synthesized disaccharide **5** and the substituted analog **6** (20). Disaccharide **5** was completely inactive, but **6** had antibiotic activity (Table 1). Indeed, derivative **6** was more than 10 times as effective as vancomycin itself against vancomycin-resistant strains. Moreover, **6** specifically inhibited the incorporation of radiolabeled precursors into pep-

tidoglycan but not into DNA or protein, consistent with a mechanism of action involving inhibition of peptidoglycan biosynthesis (21). In addition, in the site of inhibition assay **6** was found to inhibit the transglycosylation step (Fig. 3B). These results suggest a direct interaction between the substituted carbohydrates and proteins involved in transglycosylation.

On the basis of the results presented above, we propose that the modified vancomycin analogs **1** and **2** have a complex mechanism of action involving specific interactions with at least three targets: immature peptidoglycan, Lipid II, and proteins involved in transglycosylation. In vancomycin-sensitive strains, the aglycon binds the D-Ala-D-Ala dipeptides of Lipid II and immature peptidoglycan; the modified carbohydrates can also interact with one or more proteins involved in transglycosylation. In resistant strains where the aglycon does not effectively bind D-Ala-D-Lac, the modified carbohydrates can still inhibit bacterial transglycosylases.

The hypothesis that peptide binding is not required for activity against resistant bacterial strains suggests new strategies for designing better glycopeptide antibiotics. For example, instead of trying to increase binding to D-Ala-D-Lac, it might be simpler to optimize the carbohydrate portion of vancomycin for transglycosylase inhibition. We have recently developed chemistry for glycosylating the pseudoaglycon of vancomycin (20). This chemistry allows us to replace the vancosamine sugar of vancomycin with other sugars. Because we have no information about how specific the requirements are for carbohydrate recognition, we decided to first re-

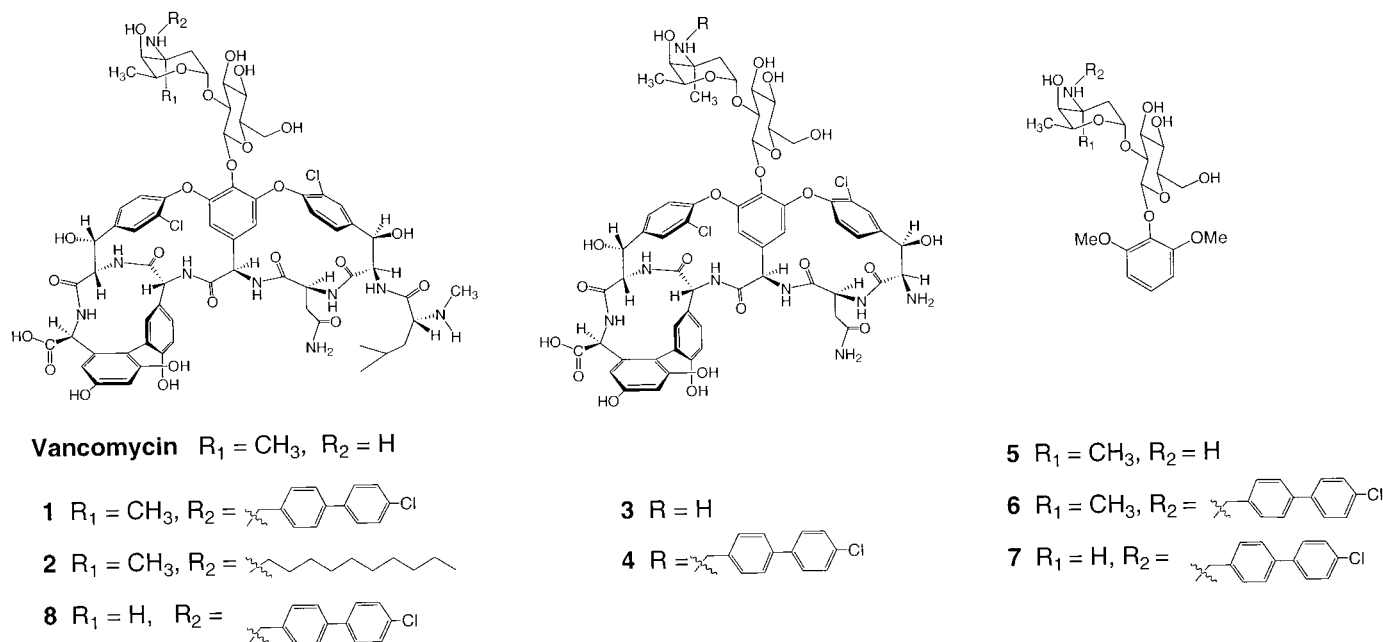


Fig. 2. Structures of vancomycin and compounds **1** through **8**.

REPORTS

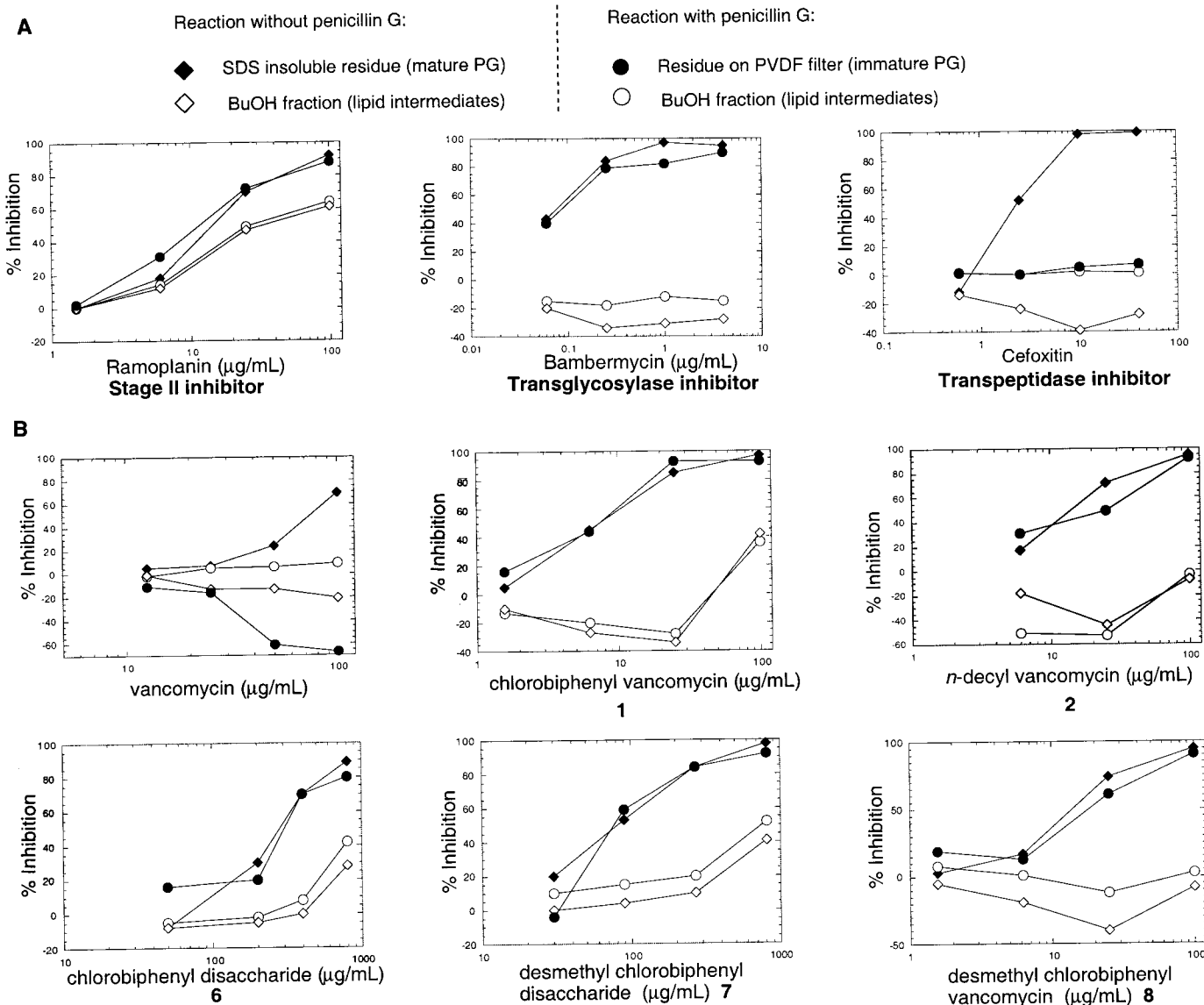


Fig. 3. (A) Inhibition patterns of three reference compounds with known mechanisms of action in the site of inhibition assay (13, 24, 25). (B) Inhibition patterns of vancomycin and compounds 1, 2, 6, 7, and 8 in the site of inhibition assay. PG, peptidoglycan.

place vancosamine with its des-methyl analog, daunosamine. After verifying that the des-methyl disaccharide 7 inhibits transglycosylase activity (Fig. 3B), which shows that the methyl group does not play an essential role in the recognition process, we attached daunosamine to the vancomycin pseudoaglycon to make 8. Although 8 differs from 1 only in a single methyl group on the terminal sugar, it shows some notable differences in activity. The activity against methicillin-resistant staphylococcal strains is particularly good (Table 2). This is only the first attempt to explore unnatural sugars by chemical glycosylation, and it is quite likely that a wide-ranging investigation of different sugars will lead to more significant improvements across a range of bacterial strains. Because we have also developed chemistry to attach both sug-

ars sequentially to the vancomycin aglycon (22), it is now possible to explore the effects of replacing either or both sugars.

The finding that peptide binding is not required for the biological activity of carbohydrate-modified glycopeptides should change the way scientists think about how to overcome vancomycin resistance. The challenge now is to determine the specific molecular targets of the substituted carbohydrates.

References and Notes

1. E. F. Gale, E. Cundliffe, P. Reynolds, M. H. Richmond, M. J. Waring, *The Molecular Basis of Antibiotic Action* (Wiley-Interscience, New York, ed. 2, 1981).
2. R. Nagarajan, *J. Antibiot.* **46**, 1181 (1993).
3. H. R. Perkins, *Biochem. J.* **111**, 195 (1969).
4. J. C. J. Barna and D. H. Williams, *Annu. Rev. Microbiol.* **38**, 339 (1984).
5. T. D. H. Bugg, S. Dutka-Malen, M. Arthur, P. Courvalin, C. T. Walsh, *Biochemistry* **30**, 2017 (1991); M. Arthur

and P. Courvalin, *Antimicrob. Agents Chemother.* **37**, 1563 (1993); C. T. Walsh, *Science* **261**, 308 (1993).

6. For recent total syntheses of the vancomycin aglycon, see D. A. Evans *et al.*, *Angew. Chem. Int. Ed.* **37**, 2700 (1998); K. C. Nicolaou *et al.*, *ibid.*, p. 2708. For a recent review of modifications to the aglycon, see A. Malabarba, T. I. Nicas, R. C. Thompson, *Med. Res. Rev.* **17**, 69 (1997).
7. R. Nagarajan *et al.*, *J. Antibiot.* **42**, 63 (1989).
8. N. E. Allen, D. L. LeTourneau, J. N. Hobbs Jr., *Antimicrob. Agents Chemother.* **41**, 66 (1997).
9. N. E. Allen, D. L. LeTourneau, J. N. Hobbs Jr., *J. Antibiot.* **50**, 677 (1997).
10. G. J. Sharman *et al.*, *J. Am. Chem. Soc.* **119**, 12041 (1997).
11. D. H. Williams, A. J. Maguire, W. Tsuzuki, M. S. Westwell, *Science* **280**, 711 (1998).
12. Covalently linked dimers and trimers of vancomycin show cooperative enhancements in binding to dimeric and trimeric D-Ala-D-Ala peptides. See U. N. Sundram, J. H. Griffin, T. I. Nicas, *J. Am. Chem. Soc.* **118**, 13107 (1996); J. Rao and G. M. Whitesides, *ibid.* **119**, 10286 (1997); J. Rao, J. Lahiri, L. Isaacs, R. M. Weis, G. M. Whitesides, *Science* **280**, 708 (1998).

REPORTS

13. Y. Van Heijenoort, M. Derrien, J. Van Heijenoort, *FEBS Lett.* **89**, 141 (1978).
14. Many studies have used particulate enzyme preparations to study the site of inhibition. However, the inhibitory activity of vancomycin in cells depends on the availability and distribution of various D-Ala-D-Ala binding sites on the cell surface. We use a permeabilized cell assay rather than a particulate enzyme assay so that both the enzymes and the cell wall precursors included in peptidoglycan synthesis are as close to the native context as possible. For studies in which particulate enzyme preparations are used to evaluate the mechanism of action of vancomycin, see (3, 8, 17).
15. E. A. Somner and P. E. Reynolds, *Antimicrob. Agents Chemother.* **34**, 413 (1990).
16. H. R. Onishi, S. B. Zimmerman, E. O. Stapley, *Ann. N.Y. Acad. Sci.* **235**, 406 (1974).
17. J. S. Anderson, M. Matsuhashi, M. A. Haskin, J. L. Strominger, *Biochemistry* **53**, 881 (1965); J. S. Anderson, P. M. Meadow, M. A. Haskin, J. L. Strominger, *Arch. Biochem. Biophys.* **116**, 487 (1966); W. P. Hammes and F. C. Neuhaus, *Antimicrob. Agents Chemother.* **6**, 722 (1974); for a recent review see P. E. Reynolds, *Eur. J. Clin. Microbiol. Infect. Dis* **8**, 943 (1989).
18. Des-leucyl vancomycin was prepared as described [P. M. Booth, D. J. M. Stone, D. H. Williams, *J. Chem. Soc. Chem. Commun.* **1987**, 1694 (1987); R. Nagarajan and A. A. Schabel, *ibid.* **1988**, 1306 (1988)].
19. M. Cristofaro, D. A. Beauregard, H. Yan, N. J. Osborn, D. H. Williams, *J. Antibiot.* **48**, 805 (1995).
20. M. Ge, C. Thompson, D. Kahne, *J. Am. Chem. Soc.* **120**, 11014 (1998).
21. M. Ge *et al.*, unpublished results.
22. C. Thompson, M. Ge, D. Kahne, *J. Am. Chem. Soc.* **121**, 1237 (1999); Nicolaou's group has also reported chemical glycosylation of the vancomycin aglycon [K. C. Nicolaou *et al.*, *Angew. Chem.* **111**, 253 (1999)]. The vancomycin aglycon has been enzymatically glycosylated [P. J. Solenberg *et al.*, *Chem. Biol.* **4**, 195 (1997)].
23. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically* (approved standard, NCCLS Document M7-A4, National Committee for Clinical Laboratory Standards, Wayne, PA, ed. 4, 1997).
24. The assay was performed as follows: Parallel reactions were run with ether-treated *E. coli* W7 (ETB). Both reactions contained the test compound, 5% dimethylsulfoxide (DMSO), 50 mM tris-HCl (pH 8.3), 50 mM NH₄Cl, 25 mM MgCl₂, 0.5 mM 2-mercaptoethanol, 0.05 mM UDP-MurNAc-pentapeptide, and 0.005 mM UDP-[¹⁴C]GlcNAc (2 μCi/μmol) and ETB in a final volume of 0.1 ml. The reaction for the formation of uncross-linked peptidoglycan contained penicillin G (1 mg/ml). The reaction for the formation of cross-linked peptidoglycan lacked the penicillin. After incubating at 30°C for 30 min, 0.05 ml of 6 M pyridinium acetate (pH 4.2) and 0.2 ml of *n*-BuOH were added to terminate the reactions and to extract the bactoprenyl-linked intermediates. Phases were separated by centrifugation. The upper layer was isolated and the lower layer was reextracted with *n*-BuOH. The extracts were combined and back-extracted with 0.2 ml of distilled water. The radioactivity in a sample of the *n*-BuOH phase was determined. The residue from the reaction run in the presence of penicillin G was resuspended in DMSO by sonication.
25. The site of inhibition assay was developed by combining methods for analyzing individual steps and products involved in peptidoglycan biosynthesis [D. Mirelman, Y. Yashouv-Gan, U. Schwartz, *Biochemistry* **15**, 1781 (1976); J. N. Umbreit and J. L. Strominger, *J. Bacteriol.* **112**, 1306 (1972); Y. Van Heijenoort and J. Van Heijenoort, *FEBS Lett.* **110**, 241 (1979); M. Di Berardino, A. Dijkstra, D. Stuber, W. Keck, M. Gubler, *ibid.* **392**, 184 (1996)].
26. Supported by Intercardia Research Laboratories, Merck Research Laboratories, The National Institutes of Health (National Research Service Award to R.K.), and the Japan Society for the Promotion of Science Postdoctoral Fellowship for Research Abroad (to S.F.).

22 February 1999; accepted 23 March 1999

Science ~~ONLINE~~

Take a hike!

In our Enhanced Perspectives, we navigate the virtual forest for you. Each week, one Perspective from *Science's Compass* links readers to the best related Web-based content:

- research databases
- tutorials
- glossaries
- abstracts
- other online material

Take your virtual hike at www.sciencemag.org/misc/e-perspectives.shtml