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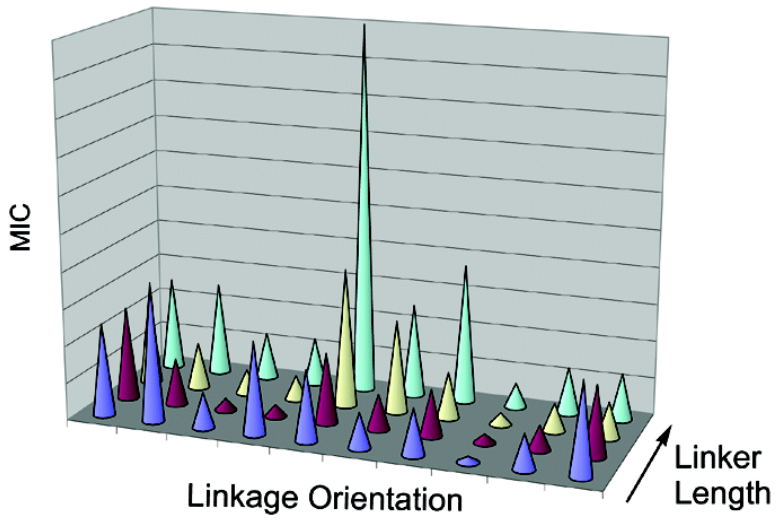

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Multivalent Drug Design. Synthesis and In Vitro Analysis of an Array of Vancomycin Dimers

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Abstract: The design, synthesis, and in vitro microbiological analysis of an array of forty covalently linked vancomycin dimers are reported. This work was undertaken to systematically probe the impact of linkage orientation and linker length on biological activity against susceptible and drug-resistant Gram-positive pathogens. To prepare the array, monomeric vancomycin synthons were linked through four distinct positions of the glycopeptide (C-terminus (C), N-terminus (N), vancosamine residue (V), and resorcinol ring (R)) in 10 unique pairwise combinations. Amphiphilic, peptide-based linkers of four different lengths (11, 19, 27, and 43 total atoms) were employed. Both linkage orientation and linker length were found to affect in vitro antibacterial potency. The V−V series displayed the greatest potency against vancomycin-susceptible organisms and vancomycin-resistant *Enterococcus faecalis* (VRE) of VanB phenotype, while the C−C, C−V, and V−R series displayed the most promising broad-spectrum activity that included VRE of VanA phenotype. Dimers bearing the shortest linkers were in all cases preferred for activity against VRE. The effects of linkage orientation and linker length on in vitro potency were not uniform; for example, (1) no single compound displayed activity that was superior against all test organisms to that of vancomycin or the other dimers, (2) linker length effects varied with test organism, and (3) whereas one-half of the dimers were more potent than vancomycin against methicillin-susceptible *Staphylococcus aureus* (MSSA), only one dimer was more potent against methicillin-resistant *S. aureus* (MRSA) and glycopeptide-intermediate susceptible *S. aureus* (GISA). In interpreting the results, we have considered the potential roles of multivalency and of other phenomena.

Introduction

The emergence and spread of antibiotic-resistant bacteria present an ongoing challenge to the healthcare community.[1] Of particular concern has been the emergence of high-level resistance to vancomycin and other glycopeptide antibiotics in virulent Gram-positive pathogens, first among enterococci (VRE)[2] and, very recently, in *Staphylococcus aureus* that are also resistant to methicillin (VRSA).[3] The basis for the biological effects exerted by vancomycin and other naturally occurring glycopeptides derives from their ability to bind to and prevent from use intermediates involved in the biosynthesis of bacterial cell wall peptidoglycan.[4] Specifically, glycopeptide antibiotics are receptors capable of forming complexes with terminal D-alanyl-D-alanine ligands that are displayed by lipid intermediate II (*N*-acetylglucosamine-β-1,4-(undecaprenyl diphospho-*N*-acetylmuramyl-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala)) and by immature peptidoglycan that has been polymerized through the action of transglycosylase enzymes but not yet cross-linked through the action of transpeptidases. Resistance to glycopeptides is most commonly associated with elaboration of altered peptidoglycan precursors. For example, VanA VRE employ an alternative biosynthetic pathway to generate peptidoglycan precursors terminating in D-alanyl-D-lactate rather than D-alanyl-D-alanine.[5] This simple replacement of an amide NH group for an ester oxygen results in the replacement of a stabilizing hydrogen bond with a destabilizing lone pair−lone pair repulsion within vancomycin/ligand complexes. This in turn leads to a decrease in relative stability by a factor of 1000 (Figure 1).[6]

Multiple approaches have been taken to address the challenges posed by vancomycin-resistant bacteria. First, novel antibiotics that act against targets other than cell wall biosynthesis have

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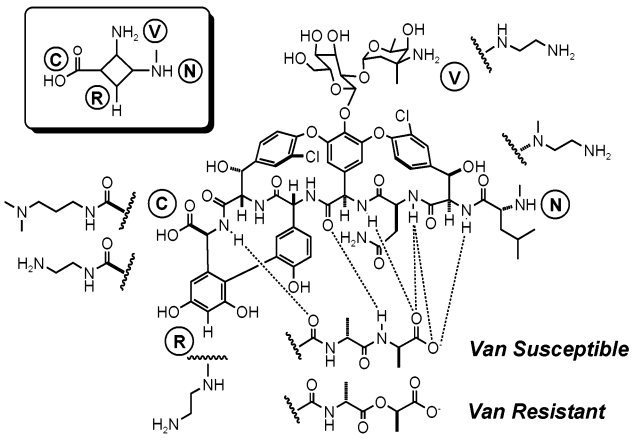

Figure 1. Structure of vancomycin (**1**) complexed to terminal D-Ala-D-Ala and D-Ala-D-Lac segments of ligands displayed by vancomycin-susceptible and -resistant bacteria, respectively. Hydrogen bonds are indicated as dotted lines. Also shown are various chemical modifications in synthons **2**−**5** (Scheme 1) used to prepare vancomycin dimers linked through the carboxyl terminus (C), the amino terminus (N), the vancosamine amino group (V), and the resorcinol side chain of amino acid residue 7 (R). The box presents a simplified depiction of vancomycin where the heptapeptide core is abbreviated as a diamond shape but with the functional groups at C-, N-, V- and R-positions explicated.

been developed. These include the ribosome-targeting protein synthesis inhibitors quinupristin/dalfopristin (Synercid) and linezolid (Zyvox), as well as the membrane-targeting antibiotic daptomycin.[7] A second approach has been to modify existing glycopeptides in order to regain activity against resistant bacterial strains.[8] Here, the most promising results have been obtained through addition of hydrophobic groups to the amino-disaccharide moieties of vancomycin and (chloro)eremomycin.[9]

A third approach has sought to improve the affinity of vancomycin for depsipeptide-containing glycopeptide precursors through multivalency. Multivalency is a phenomenon wherein multiple, simultaneous, energetically coupled receptor/ligand interactions enhance the overall affinity and selectivity of binding. Natural systems have evolved to make extensive use of multivalency, for example, in the functioning of immunoglobulins, in the adhesion of virus particles to target cells, and in controlling cell−cell interactions.[10] A diffuse yet growing literature suggests that multivalency may also find general applicability in the design of small molecule ligands,[11] including novel drugs for enzymes, receptors, transporters, and macromolecular structures representing the major classes of therapeutic targets.[12]

A series of studies in solution[13] and in the solid state[14] have shown that vancomycin and other glycopeptides self-associate via hydrogen bonding and hydrophobic interactions in an antiparallel, back-to-back fashion to form specific dimers. Noncovalent dimerization of glycopeptides can be both highly favorable and cooperative with the binding of peptide ligands and has been correlated with enhanced bacterial cell surface binding and in vitro antibacterial potency.[4f,15] Vancomycin self-associates only weakly in solution ($K_{dim} = 700\ M^{-1}$),[13b] which prompted the question of whether covalently linked dimers of this glycopeptide would display regained potency against VRE.[16] This hypothesis has been confirmed through the synthesis and study of vancomycin dimers linked through the C-terminal carboxyl group[16−18] as well as dimers of vancomycin and chloroeremomycin linked via their aminodisaccharide moieties.[19,20] A small number of heterodimers comprising two different glycopeptide subunits,[19,20] the synthesis of vancomycin dimers linked via the C-terminus of one subunit and the terminal *N*-methyl group of a second subunit,[21] and a single example of a covalently linked dimer of eremomycin[22] have also been reported.

In this paper, we report the design, synthesis, and in vitro biological evaluation of an array of forty covalently linked vancomycin dimers. This work was undertaken in order to systematically explore the structure−activity relationships among these compounds in the service of antibiotic lead discovery and as a platform on which to develop and illustrate the unique aspects of multivalent drug design.

Results

Multivalent Design. Our design process took into account the singular considerations of multivalent design, including the following:

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A. Which Glycopeptide(s) to Use as Monomeric Subunits. We chose to work with vancomycin, the best-studied of the 200+ known glycopeptides.[23] To simplify the synthesis of compounds and interpretation of data, we chose to study only dimers of vancomycin derivatives rather than to include heteromeric compounds comprising more than one distinct member of the glycopeptide family.

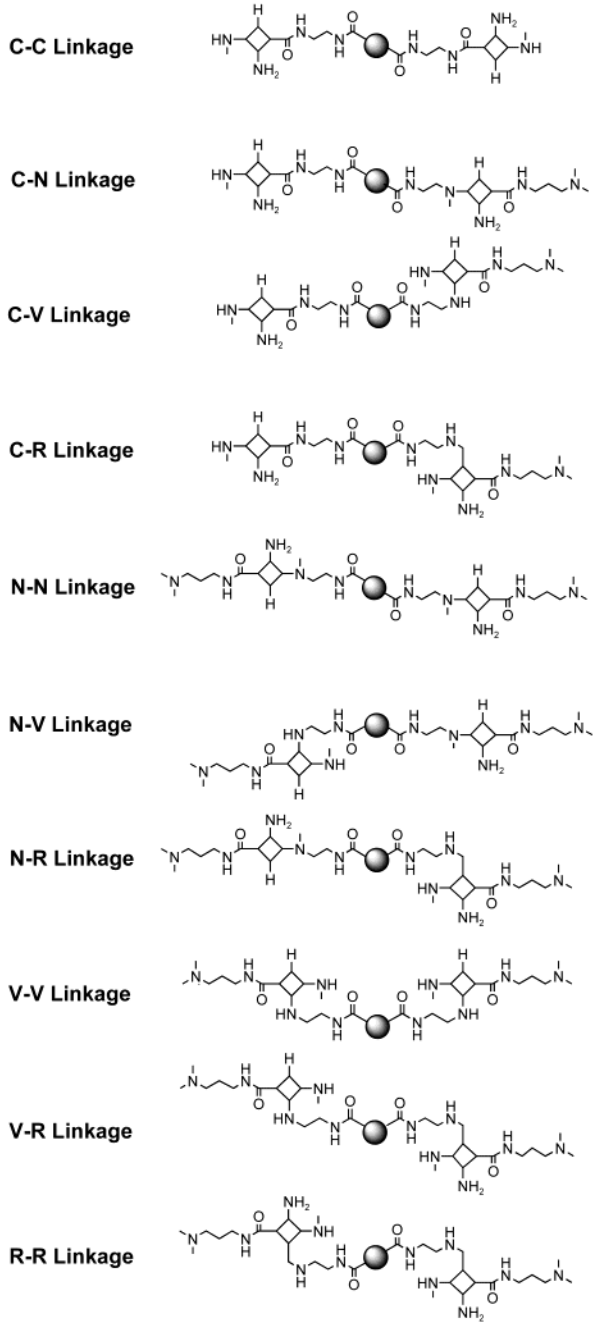
B. Valency of the Constructs. We chose to focus on dimeric constructs rather than higher-order structures in light of the prior art with dimers and in order to restrict molecular weights to below 5 kDa.

C. Linkage Orientation. The choice of linkage site dictates the relative orientations in which individual vancomycin subunits may be presented to their targets. We chose to link vancomycin subunits through four distinct sites: the carboxyl- (C) and amino- (N) termini of the dalbaheptide chain, along with the amino group of the vancosamine moiety (V) and an amino group that could be regioselectively installed at the 4′, resorcinol-like (R) position on the aromatic side chain of amino acid 7. These positions were chosen because they allow for linkage through sites around the perimeter of the vancomycin molecule: on the top (V), bottom (R), left (C), and right (N) of vancomycin when the glycopeptide is considered as oriented in Figure 1. Ten distinct linkage orientations are possible with these four distinct sites of attachment (Chart 1): C−C, C−N, C−V, C−R, N−N, N−V, N−R, V−V, V−R, R−R.

D. Linkage Chemistries. Reductive alkylation chemistry was chosen for attachment of the linkers at the N- and V-positions in order to preserve their basic character, which has been previously shown to be optimal for the antibacterial activity of vancomycin and chloroeremomycin.[8,9] Amide bond formation at the C-terminus of glycopeptides including vancomycin,[24] eremomycin,[25] and teicoplanin[26] provides derivatives that typically retain their antibacterial activity in full. It has also been shown that Mannich aminomethylation of vancomycin at the R-position provides compounds with in vitro activity similar to that of the parent compound.[8,27] Modified vancomycin synthons **2**−**5** which provide for these linkage chemistries are depicted in Scheme 1 along with methods by which they were synthesized.

E. Linker Characteristics (Length, Geometry, Composition, and Physical Properties). To assist in choosing linker lengths, we used two space-filling models of vancomycin arranged in the self-associated, back-to-back geometry observed for vancomycin and other glycopeptides in solution and in the solid state.[13,14] Linkers 11, 19, 27, and 43 atoms in length (Chart 2) were ultimately chosen to enforce, allow, or preclude this mode of self-association to the extent possible. For reference, a 19-atom linker in its fully extended conformation is approximately equal to the width and height dimensions of vancomycin when the glycopeptide is considered in the orientation presented in Figure 1. The following lists our estimates of the minimum linker lengths needed to span the two linkage sites in the 10 distinct linkage series without interfering with ligand binding. In the case of some V-linked series, two lengths are given because of the nonidentical conformations adopted by the disaccharide moieties of the two independent vancomycin subunits. Where informative, we also assign a preferred path for linkage, whether it be over the top or underneath the glycopeptide as oriented in Figure 1: for the C−C series, 27 atoms; C−N, 11 atoms; C−V, 11 or 27 atoms; C−R, 19 atoms (underneath); N−N, 27 atoms (underneath); N−V, 19 or 27

Chart 1. Depictions of the 10 Unique Vancomycin Dimer Linkage Orientations Produced by the Coupling of Vancomycin Synthons **2**−**5** (Scheme 1) through the C-, N-, V-, and R-Positions



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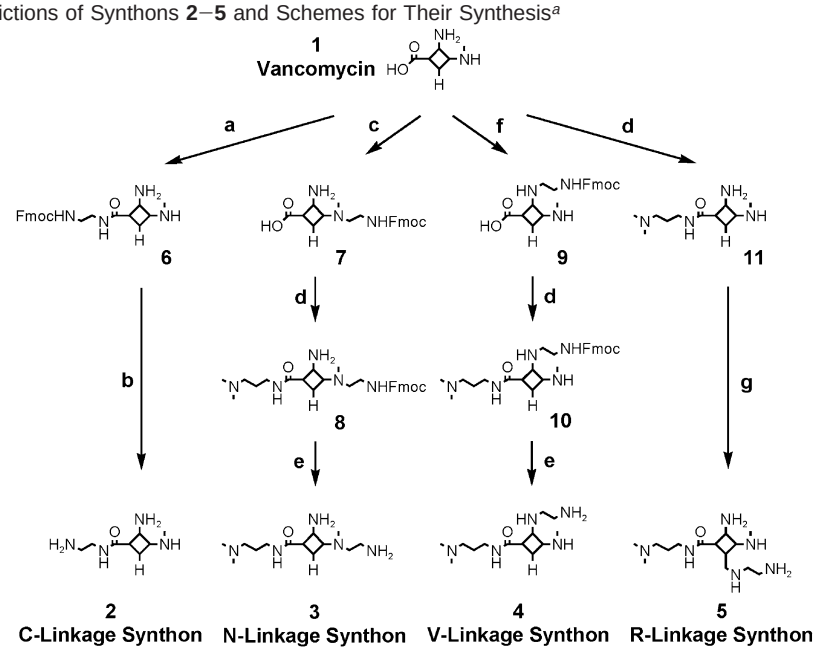
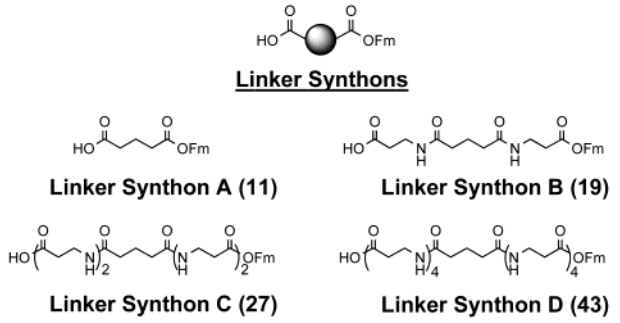
Scheme 1. Simplified Depictions of Synthons **2**−**5** and Schemes for Their Synthesis[a]



[a] Key to reagents: (a) 9-fluorenylmethyl *N*-(2-aminoethyl)carbamate hydrochloride/PyBOP/HOBT/ DIPEA/DMPU; (b) piperidine/DMF; (c) *N*-(9-fluorenylmethoxycarbonyl)glycinal/HOBT/ DIPEA/$NaCNBH_3$/MeOH/DMPU; (d) 3-(dimethylamino)propylamine/PyBOP/HOBT/DIPEA/ DMF; (e) quinuclidine/DMF; (f) *N*-(9-fluorenylmethoxycarbonyl)glycinal/DIPEA/TFA/BH_3-pyridine/DMF/MeOH; (g) ethylenediamine/formaldehyde/water/ACN.

Chart 2. Linker Synthons **A**−**D** Used to Couple Synthons **2**−**5** to Produce Dimers Linked by 11, 19, 27, or 43 Atoms



atoms; N−R, 11 atoms (underneath); V−V, 11 atoms; V−R, 19 or 27 atoms; R−R, 19 atoms (underneath).

We also estimated the minimum linker lengths needed to bridge attachment points without disrupting ligand binding when two vancomycin subunits were juxtaposed face-to-face as observed for vancomycin/ligand complexes in the solid state:[14,28] for the C−C series, 43 atoms (underneath); C−N, 11 atoms; C−V, 11 or 19 atoms; C−R, 27 atoms (underneath); N−N, 19 atoms (underneath); N−V, 27 atoms; N−R, 11 atoms; V−V, 19 atoms; V−R, 19 or 27 atoms; R−R, 11 atoms (underneath).

We chose neutral yet polar, peptide-based linkers in order to avoid substantial changes in the physical properties of the dimers with changes in linker length (Chart 2). These linkers comprise multiple rotatable bonds and, in conjunction with linker length, allow the dimeric constructs to adopt different conformations. We specifically avoided the use of hydrophobic linkers given that addition of hydrophobic appendages to the vancosamine residue or the C- and N-termini of the glycopeptide significantly impacts antibacterial potency, for example, imparting activity against VRE at low μg/mL levels.[8,9]

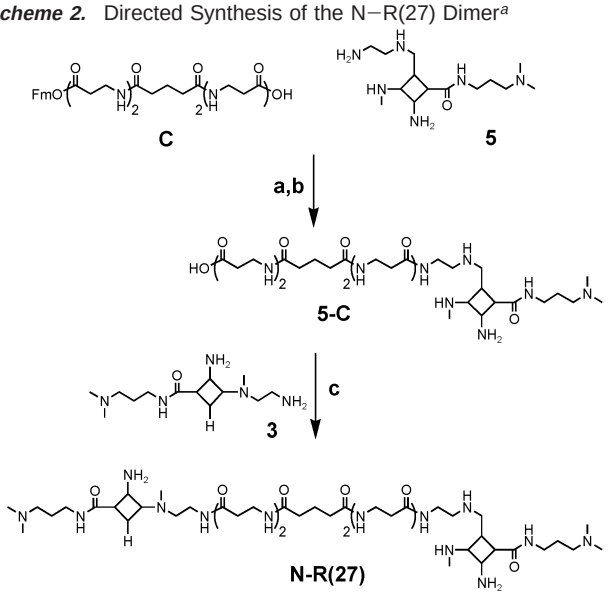
Synthesis. The covalently linked vancomycin dimers were synthesized through sequential, directed amide bond forming reactions. This was made possible by the use of the modified vancomycin synthons **2**−**5** bearing uniquely reactive, sterically unhindered primary amines in conjunction with the terminally differentiated dicarboxylic acid, *mono*-ester linker synthons **A**−**D** (Chart 2). In the case of compounds linked through the N-, V-, and/or R-positions (synthons **3**−**5**), the C-terminal carboxyl was capped as a 3-dimethylaminopropylamide in order to prevent its interference with subsequent amide bond coupling reactions. By way of example, the N−R linked dimer joined by a 27-atom linker (N−R(27)) was prepared as outlined in Scheme 2.

Coupling of R-linkage synthon **5** with linker synthon **C** followed by removal of the fluorenylmethyl ester afforded an intermediate **5-C** conjugate. Conjugate **5-C** was then coupled with N-linkage synthon **3** to afford the N−R(27) dimer. Details of the synthesis of **2**−**5**, the solution- and solid-phase synthesis of linker synthons **A**−**D**, and an example of the synthesis of one member of each of the 10 distinct dimer linkage series are described in the Experimental Section. The dimers are cationic in nature, bearing from four (C−C series) to eight basic centers (R−R series).

Bacterial Strains and In Vitro Susceptibility Studies. Bacterial strains were selected to represent the major Gram-positive human pathogens (streptococci, staphylococci, and enterococci) and various forms of drug resistance, including resistance to vancomycin: (1) a strain of penicillin-resistant *Streptococcus pneumoniae* (PRSP SU-10), (2) a strain of methicillin-susceptible *Staphylococcus aureus* (MSSA 13709), (3) a strain of methicillin-resistant *Staphylococcus aureus* (MRSA 33591), (4) a strain of methicillin-resistant *Staphylococcus aureus* that displays an intermediate level of resistance

(28) (a) Loll, P. J.; Bevivino, A. E.; Korty, B. D.; Axelsen, P. H. *J. Am. Chem. Soc.* **1997**, *119*, 1516−1522. (b) Loll, P. J.; Miller, R.; Weeks, C. M.; Axelsen, P. H. *Chem. Biol.* **1998**, *5*, 293−298.

Scheme 2. Directed Synthesis of the N−R(27) Dimer[a]



[a] Key to reagents: (a) R-linkage synthon **5**/linker synthon **C**/PyBOP/HOAT/DIPEA/DMF; (b) quinuclidine/DMF; (c) N-linkage synthon **3**/PyBOP/HOAT/DIPEA/DMF.

to glycopeptides (GISA CDC-5836), (5) a strain of vancomycin- and beta-lactam-susceptible *Enterococcus faecalis* (EFSVS 29212), (6) a strain of vancomycin-resistant *Enterococcus faecalis* of VanB phenotype (EFSVB 51575), (7) a strain of vancomycin-resistant *Enterococcus faecium* of VanA phenotype that is also highly resistant to beta-lactams (EFMVA KPB-01), and (8) a strain of vancomycin-resistant *Enterococcus faecalis* of VanA phenotype (EFSVA MGH-01).

The susceptibilities of these strains to vancomycin, the monomer synthons **2**−**5**, and the forty vancomycin dimers were determined by standard broth microdilution assays. The collected minimum inhibitory concentrations (MICs) are presented in Table 1. Here, it may be seen that against six of the eight test strains (MSSA, MRSA, GISA, EFSVS, EFMVA, EFSVA), no significant difference in potency is displayed by synthons **2**−**5** relative to vancomycin. Synthons **2**−**5** are 2.5- to 11-fold less potent than vancomycin against PRSP. In contrast, against the VanB strain of VRE tested, EFSVB, synthons **2** and **4** exceed the potency of vancomycin by factors in excess of 50 and 6, respectively.

The data for the covalently linked vancomycin dimers presented in Table 1 is coded such that MIC values for dimers that are at least 2-fold lower than those for vancomycin against the same organism are shown in bold type, while values that differ by less than a factor of 2 or are at least 2-fold higher than those for vancomycin are shown in normal or italic type, respectively. Of the 320 MIC values for dimers, 144 are at least 2-fold lower than the corresponding values for vancomycin. Visual inspection of the data coded in this way reveals that the effects of dimerization on in vitro potency are not uniform across all test organisms. For example, whereas many to most of the dimers are more potent than vancomycin against PRSP, MSSA, EFSVS, EFSVB, and EFMVA, only the V−V(11) dimer is even modestly more potent against MRSA and GISA.

The data for the covalently linked dimers are presented in graphical form as a function of linkage orientation in Figure 2 and as a function of test organism in Figure 3. These presentations allow for convenient visual analysis of the impact of variations in key structural parameters of vancomycin dimers on biological activity. In cases where only limiting experimental data were obtained (e.g., MIC < 0.1 μg/mL or > 200 μg/mL), the limiting values were used to make the graphs and in linear regression analyses. For reference, the data for vancomycin is also included in each figure panel.

Activity as a Function of Linkage Orientation and Linker Length. A. C−C Series. Relative to vancomycin, compounds in this series were as much as 30-fold more potent against PRSP, ESFVS, and the three VRE strains. C−C dimers were either slightly more or slightly less potent than vancomycin against MSSA. All compounds in this series were less potent than vancomycin against MRSA and GISA, by 3- to 8-fold. Greatest absolute potency was observed for all members of the series against PRSP. Lowest absolute potency was observed for C−C(27) and C−C(43) against VRE. Against the vancomycin-susceptible organisms, potency either was independent of linker length or, in the case of MSSA, was greatest at an intermediate linker length of 27 atoms. In contrast, potency against the three VRE strains decreased substantially with increasing linker length.

B. C−N Series. Relative to vancomycin, compounds in this series were as much as 20-fold more potent against PRSP and ESFVS, and some members of the series displayed measurable activity against EFSVB and EFMVA but not EFSVA. C−N dimers were also up to 4-fold more potent against MSSA. All compounds in this series were less potent than vancomycin against MRSA and GISA, by up to 12-fold. Greatest absolute potency was observed for C−N(11) and C−N(19) against PRSP. Lowest absolute potency was observed against EFSVA. Potency was greatest at shorter linker lengths for PRSP, EFSVS, and EFSVB and was either independent of linker length or maximal at intermediate length against the staphylococci.

C. C−V Series. Relative to vancomycin, compounds in this series were as much as 30-fold more potent against PRSP, MSSA, ESFVS, and the three VRE strains. The C−V(27) dimer was equipotent against MRSA, and the C−V(19) dimer was equipotent against GISA; otherwise, C−V dimers were less potent than vancomycin against these organisms. Greatest absolute potency was observed for C−V(11), C−V(19), and C−V(27) against PRSP and for C−V(11) against EFSVS. Lowest absolute potency was observed for C−V(19) and C−V(43) against VanA VRE. Potency was greatest at an intermediate linker length (19 atoms) against GISA but independent of linker length against all other vancomycin-susceptible organisms. Potency against the three VRE strains decreased substantially between the shortest and longest linker lengths.

D. C−R Series. Relative to vancomycin, compounds in this series were as much as 15-fold more potent against PRSP, MSSA, and ESFVS, and some members of the series were potent against EFSVB and EFMVA but not EFSVA. The C−R(27) dimer was equipotent against MRSA, and the C−R(19) dimer was equipotent against GISA; otherwise, C−R dimers were less potent than vancomycin against these organisms. Greatest absolute potency was observed against PRSP and EFSVS. Lowest absolute potency was observed against VanA VRE. Potency was greatest at intermediate linker lengths against MRSA and GISA, independent of linker length against PRSP,

Table 1. Analytical and In Vitro Susceptibility Data for Vancomycin (**1**), Monomer Synthons **2**−**5**, and Vancomycin Dimers

linkage orientation	linker length[a]	*m/z* (calcd)[b]	*m/z* (obsd)[c]	HPLC[d] rt	MIC[e] (μg/mL) PRSP	MSSA	MRSA	GISA	EFSVS	EFSVB	EFMVA	EFSVA
1	NA				0.58	0.78	1.6	6.3	3.1	>200	>200	>200
2	NA	1492.3	1492.2		1.6	0.78	1.6	2.3	3.1	4.7	>200	>200
3	NA	1577.5	1577.7		6.3	2.3	3.1	6.3	6.3	200	>200	>200
4	NA	1577.5	1577.7		2.3	0.78	1.6	3.1	3.1	38	>200	>200
5	NA	1606.5	1606.9		4.7	2.3	3.1	6.3	3.1	100	>200	>200
C−C	11	1539.0	1540.2	4.30	**<0.1**	1.2	*9.4*	*50*	**0.3**	**6.3**	**9.4**	**38**
C−C	19	1610.5	1611.3	4.27	**<0.1**	1.2	*9.4*	*50*	**0.3**	**6.3**	**15**	**50**
C−C	27	1681.5	1682.2	4.22	**0.1**	0.3	*7.8*	*50*	**0.2**	**13**	**75**	>200
C−C	43	1823.5	1824.5	4.02	**<0.15**	0.59	*4.7*	*50*	**0.4**	>150	>150	>200
C−N	11	1582.0	1582.8	3.90	**<0.1**	1.2	*4.7*	*75*	**0.4**	**19**	**120**	**200**
C−N	19	1653.0	1654.0	4.01	**<0.1**	0.2	*6.3*	*25*	**0.3**	**50**	**75**	>200
C−N	27	1724.1	1724.8	3.98	**0.2**	0.39	2.3	*25*	**0.4**	**200**	>200	>200
C−N	43	1866.3	1867.7	3.39	0.39	0.78	*4.7*	*50*	**1.2**	>200	>200	>200
C−V	11	1581.9	1582.5	3.99	**<0.15**	**0.39**	*3.1*	*19*	**0.2**	**4.7**	**19**	**38**
C−V	19	1653.0	1653.0	3.15	**<0.15**	**0.15**	*9.4*	6.3	**<0.1**	**75**	>150	>200
C−V	27	1724.1	1724.5	3.92	**<0.15**	**0.2**	1.2	*13*	**0.2**	**38**	**200**	**75**
C−V	43	1866.2	1867.4	3.92	**0.2**	**0.39**	2.3	*25*	**0.4**	**100**	>200	>200
C−R	11	1596.8	1597.2	3.95	0.39	0.59	*4.7*	*50*	**0.4**	**7.3**	**13**	>150
C−R	19	1667.6	1668.4	3.70	**0.2**	**0.2**	2.3	6.3	**0.2**	**150**	**150**	>150
C−R	27	1738.6	1739.5	3.70	**0.2**	**0.2**	1.2	*13*	**0.2**	>200	**200**	>200
C−R	43	1880.9	1881.8	3.83	0.39	**0.39**	*4.7*	*25*	**0.3**	**200**	>200	>200
N−N	11	1624.5	1625.4	4.15	**0.2**	0.59	*7.8*	*38*	**1.2**	**50**	**58**	>200
N−N	19	1695.0	1696.6	4.15	0.78	0.78	*4.7*	*38*	3.1	**100**	**150**	>200
N−N	27	1766.7	1767.7	4.04	0.78	1.2	*4.7*	*75*	3.1	**150**	>200	>200
N−N	43	1908.8	1909.7	4.03	*2.3*	*3.1*	*19*	*200*	6.3	>200	>200	>200
N−V	11	1624.5	1625.4	3.78	**0.2**	**0.3**	1.6	*19*	**0.3**	**6.3**	**19**	>200
N−V	19	1695.0	1696.3	3.68	**0.2**	**0.3**	1.6	*19*	**0.4**	**38**	**100**	>200
N−V	27	1766.7	1767.1	3.89	0.39	0.78	*3.6*	*50*	**0.8**	>150	> 150	>200
N−V	43	1908.8	1909.7	3.94	0.59	0.78	*4.7*	*50*	**0.8**	>200	>200	>200
N−R	11	1639.0	1639.8	3.95	0.59	*1.6*	1.6	*25*	**1.2**	**13**	**38**	**150**
N−R	19	1710.1	1711.3	3.65	0.78	**0.39**	*4.7*	*25*	**1.2**	**19**	**75**	>200
N−R	27	1781.2	1782.1	3.75	0.59	**0.39**	2.3	*25*	**0.8**	**75**	**150**	>150
N−R	43	1923.4	1923.5	3.84	*1.6*	1.2	*3.1*	*75*	**1.6**	**200**	>200	>200
V−V	11	1624.5	1625.1	3.37	**<0.1**	**0.15**	**0.4**	**3.1**	**0.2**	**<0.2**	**150**	>200
V−V	19	1695.0	1696.6	2.78	**<0.1**	0.59	0.9	4.7	**<0.1**	**100**	**170**	>200
V−V	27	1766.7	1767.7	3.63	**<0.15**	**0.2**	1.2	6.3	**0.2**	**150**	>200	>200
V−V	43	1908.8	1909.1	3.79	0.30	**0.2**	*3.1*	*13*	**0.3**	**150**	>200	>200
V−R	11	1639.0	1639.8	3.84	0.78	0.59	*3.1*	*19*	**0.8**	**4.7**	**9.4**	**75**
V−R	19	1710.1	1711.3	3.76	0.78	0.59	2.3	*13*	**0.8**	**6.3**	**38**	**200**
V−R	27	1781.2	1781.8	3.75	0.39	1.2	1.6	*15*	**0.8**	**25**	**150**	>200
V−R	43	1923.4	1924.7	3.78	0.59	**0.39**	*3.1*	*25*	**0.6**	**100**	>200	>200
R−R	11	1653.6	1654.9	3.63	*2.3*	*1.6*	*9.4*	*50*	**1.6**	**13**	**9.4**	**150**
R−R	19	1724.6	1725.7	3.49	*1.2*	**0.39**	*3.1*	*38*	**0.8**	**25**	**13**	>200
R−R	27	1795.7	1796.5	3.47	*1.2*	**0.39**	*3.1*	*19*	**1.2**	>200	**150**	>200
R−R	43	1937.9	1939.1	3.67	*1.2*	0.78	*9.4*	*25*	**0.6**	>150	>150	>150

[a] Total linker length in atoms. NA = Not applicable. [b] Calculated mass-to-charge ratios for singly charged parent ions for linkage synthons **2**−**5** ($[M + H^+]^+$) and for doubly charged parent ions for the dimers ($[M + 2H^+]^{2+}$). [c] Observed mass-to-charge ratios for singly charged parent ions for linkage synthons **2**−**5** ($[M + H^+]^+$) and for doubly charged parent ions for the dimers ($[M + 2H^+]^{2+}$). [d] Observed retention time in minutes in reversed-phase high-performance liquid chromatography carried out as described in the Experimental Section. [e] Minimum inhibitory concentration in micrograms per milliliter as determined by microdilution broth assays in vitro. Values for dimers indicated in bold type are lower than the corresponding values for vancomycin. Values for dimers in normal type are equal to the corresponding values for vancomycin. Values in italic are higher than the corresponding values for vancomycin. Strains are abbreviated as follows: PRSP, penicillin-resistant *Streptococcus pneumoniae* strain SU-10; MSSA, methicillin-susceptible *S. aureus* strain ATCC 13709; MRSA, methicillin-resistant *S. aureus* strain ATCC 33591; GISA, glycopeptide-intermediate resistant *S. aureus* strain CDC-5836; EFSVS, vancomycin-susceptible *E. faecalis* strain ATCC 29212; EFSVB, vancomycin-resistant *E. faecalis* strain ATCC 51575 (VanB phenotype); EFMVA, vancomycin-resistant *Enterococcus faecium* strain KPB-01 (VanA phenotype); EFSVA, vancomycin-resistant *Enterococcus faecalis* strain MGH-01 (VanA phenotype).

MSSA, and EFSVS, and greatest at the shortest linker length against EFSVB and EFMVA.

E. N−N Series. The N−N(11) dimer was the only member of this series that displayed even modestly enhanced potency against PRSP and EFSVS relative to vancomycin. Some compounds displayed measurable activity against EFSVB and EFMVA but not EFSVA. All compounds in this series were less potent than vancomycin against MRSA and GISA. Greatest absolute potency was observed for N−N(11) against PRSP. Lowest absolute potency was observed against EFSVA. Potency was greatest at shorter linker lengths against all organisms except MRSA, where potency appeared to be greatest at intermediate linker lengths.

F. N−V Series. Relative to vancomycin, compounds in this series were as much as 10-fold more potent against PRSP, MSSA, and ESFVS. N−V linked dimers were equipotent to as much as 8-fold less potent against MRSA and GISA. Some compounds displayed measurable activity against EFSVB and EFMVA but not EFSVA. Greatest absolute potency was observed for N−V(11) and N−V(19) against PRSP, MSSA, and EFSVS. Lowest absolute potency was observed against EFSVA. Potency was greatest at short linker lengths for PRSP,

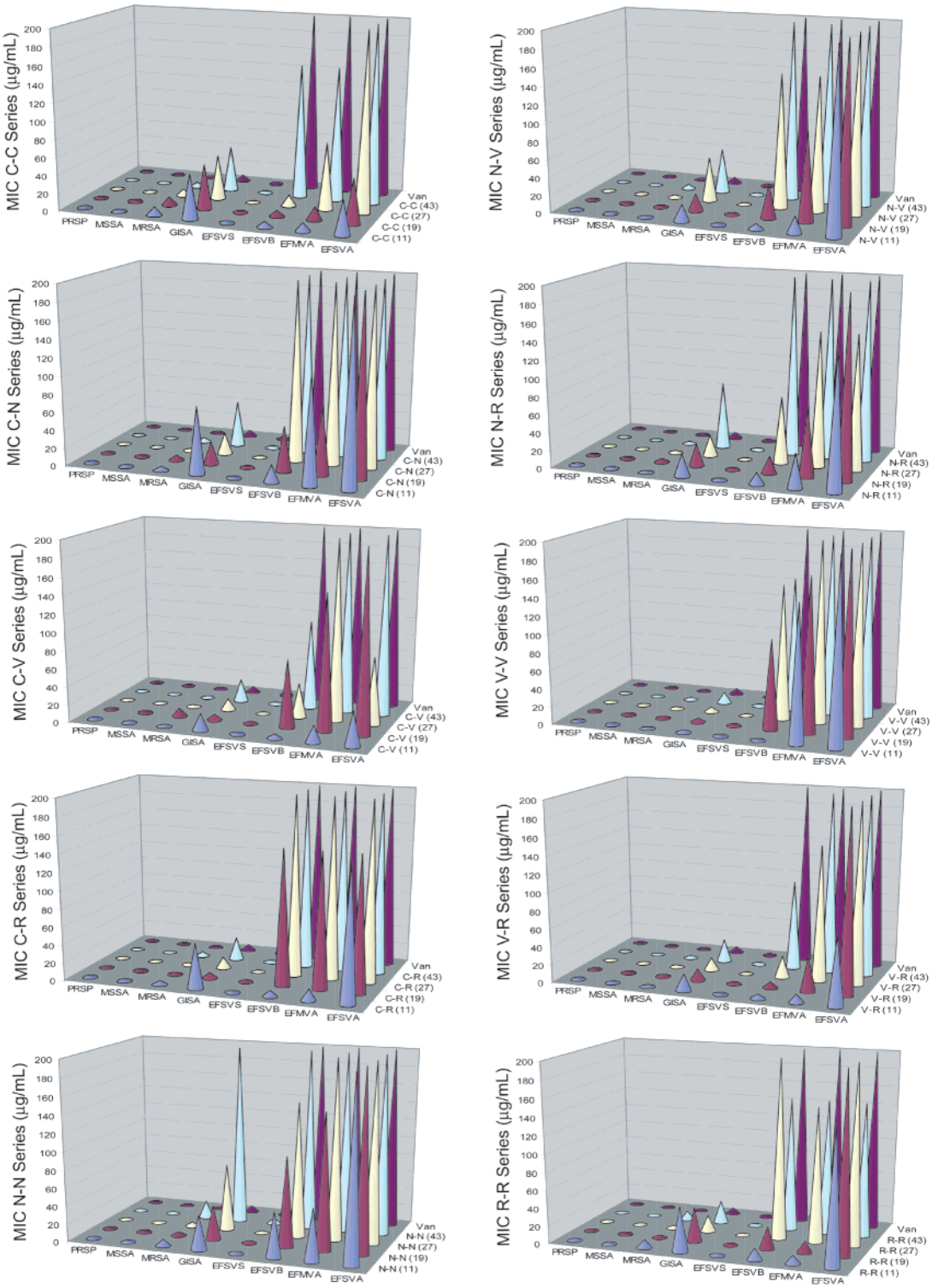


Figure 2. In vitro susceptibility data presented as a function of linkage orientation. In each plot, minimum inhibitory concentrations (MICs) are depicted as cones along the *y*-axis, the eight bacterial test strains are arranged along the *x*-axis, and linker length increases going back along the *z*-axis.

MRSA, EFSVB, and EFMVA but was independent of linker length for MSSA, GISA, and EFSVS.

G. N−R Series. Relative to vancomycin, compounds in this series were equipotent to slightly less potent against PRSP, slightly more to less potent against MSSA, and more potent against EFSVS. N−R linked dimers were equipotent to as much as 12-fold less potent against MRSA and GISA. Some compounds displayed potent activity against EFSVB, EFMVA, and, in one case, EFSVA. Greatest absolute potency was observed against PRSP, MSSA, and EFSVS. Lowest absolute potency was observed against EFSVA. Potency was greatest at short linker lengths for GISA and the three VRE strains, at intermediate linker lengths against MSSA, but was independent of linker length for PRSP, MRSA, and EFSVS.

H. V−V Series. V−V linked dimers were generally more potent than vancomycin against PRSP, MSSA, and EFSVS with

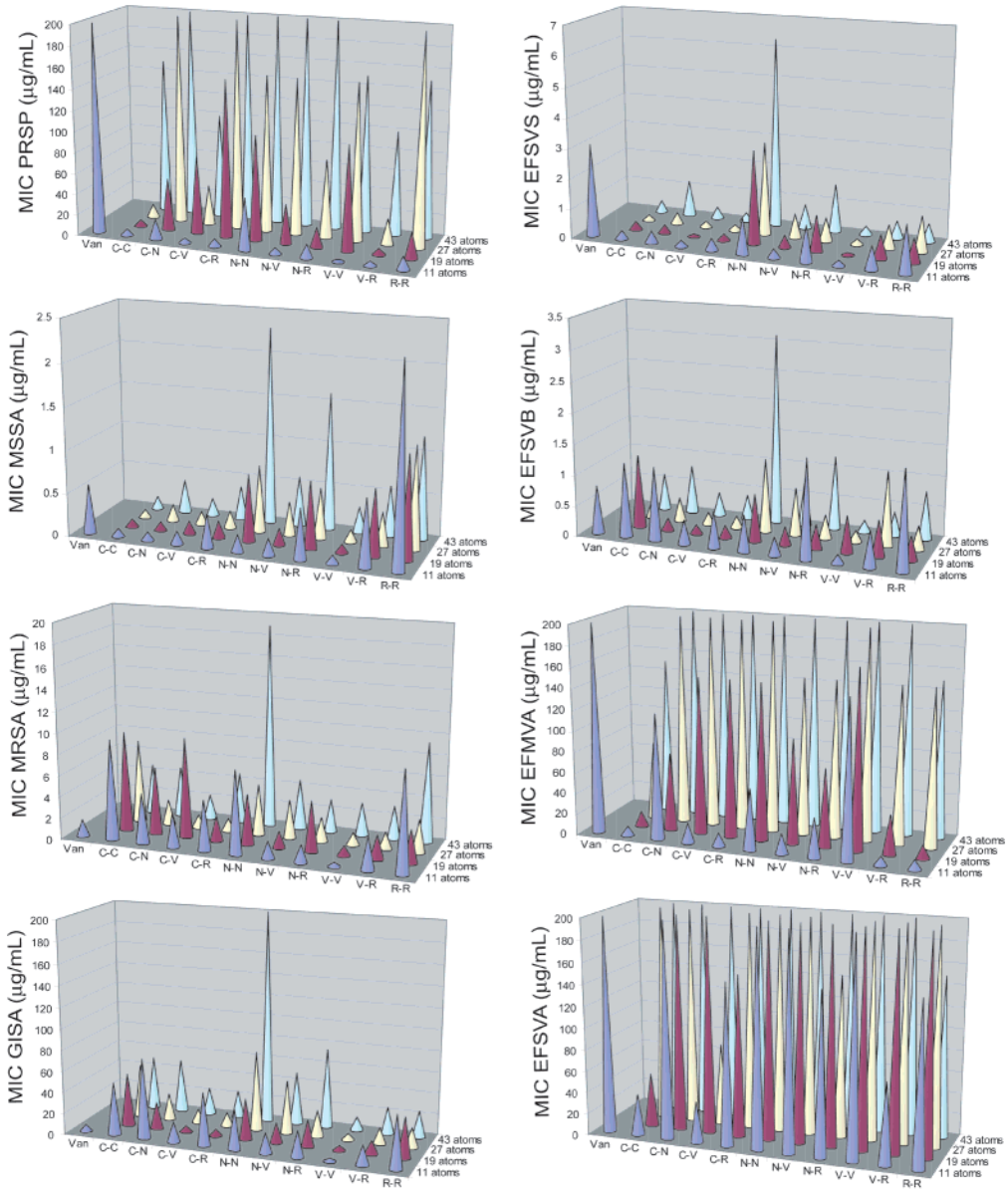


Figure 3. In vitro susceptibility data presented as a function of bacterial test strain. In each plot, minimum inhibitory concentrations (MICs) are depicted as cones along the *x*-axis, the 10 dimer linkage series are arranged along the *x*-axis, and linker length increases going back along the *z*-axis.

MIC values lower by up to a factor of more than 30. The V−V(11) compound was the only one of the forty dimers that displayed even modestly enhanced potency against MRSA and GISA. Other compounds in this series were equipotent to 2-fold less potent against these organisms. Against EFSVB, the compound bearing the shortest linker, V−V(11) was more than 400-fold more potent than vancomycin. In contrast, the V−V series was the least impressive overall against VanA VRE. Greatest absolute potency was observed with V−V(11) and V−V(19) against PRSP, EFSVS, and EFSVB. Lowest absolute potency was observed against EFSVA. Potency was greatest at short linker lengths against PRSP, MRSA, GISA, and EFSVB but was independent of linker length for MSSA and EFSVS. Activity against EFSVB decreased dramatically with increases in linker length.

I. V−R Series. Relative to vancomycin, compounds in this series were similarly potent against PRSP and MSSA and up to 5-fold more potent against EFSVS. V−R linked dimers ranged from equipotent to 4-fold less potent than vancomycin against MRSA and GISA. Some compounds displayed potent activity against EFSVB, EFMVA, and, in one case, EFSVA. Greatest absolute potency was observed against PRSP, MSSA, and EFSVS. Lowest absolute potency was observed for V−R(27) and V−R(43) against EFSVA. Potency was independent of linker length for all vancomycin-susceptible strains and greatest at the shortest linker length for the VRE.

J. R−R Series. Relative to vancomycin, compounds in this series were less potent against PRSP, slightly more to slightly less potent against MSSA, and as much as 5-fold more potent against EFSVS. R−R linked dimers were 2−8-fold less potent than vancomycin against MRSA and GISA. Some compounds displayed potent activity against EFSVB and EFMVA and, in one case, measurable activity against EFSVA. Greatest absolute potency was observed against MSSA and EFSVS. Lowest absolute potency was observed against EFSVA. Potency was greatest at the shortest linker length against VRE, at intermediate

Table 2. Linker Length Effects on the In Vitro Potency of Vancomycin Dimers[a]

	observed effect							
orientation	PRSP	MSSA	MRSA	GISA	EFSVS	EFSVB	EFMVA	EFSVA
C−C	U	I	U	U	U	S	S	S
C−N	S	I	U	U	S	S	U	X
C−V	U	U	U	I	U	S	S	S
C−R	U	U	I	I	U	S	S	X
N−N	S	S	I	S	S	S	S	X
N−V	S	U	S	U	U	S	S	X
N−R	U	I	U	S	U	S	S	X
V−V	S	U	S	S	U	S	X	X
V−R	U	U	U	U	U	S	S	S
R−R	U	I	I	U	U	S	S	X

[a] Legend: (U) in vitro antibacterial potency is relatively unaffected by changes in linker length in this linkage orientation series against this organism; (I) maximal potency is observed at an intermediate linker length in this linkage orientation series against this organism; (S) potency is maximal at short linker lengths and decreases with increasing linker length in this linkage orientation series against this organism; (X) data are insufficient to assign a linker length effect. Bacterial strains are abbreviated as described in the text and in the footnotes to Table 1. In making the assignments, we considered any difference in MICs of more than a factor of 3 to be outside the limits of experimental variation. In the series where the "I" linker effect was designated, the MIC value for one or both of the dimers joined by 19- or 27-atom linkers was at least 3 times lower than the average of the MICs for corresponding dimers linked with the shortest and longest linkers.

linker lengths against MSSA and MRSA, and was independent of linker length for PRSP, GISA, and EFSVS.

Effects of Linker Length. As described within each linkage orientation series, the effects of linker length on the in vitro antibacterial potency of dimers fell within one of three discernible categories as summarized in Table 2. Potency was relatively unaffected by changes in linker length within the range studied (U), was maximal at the shorter linker lengths and decreased with increasing linker length (S), or was greatest at an intermediate linker length (I). In the case of the V−V series against EFMVA and in seven of the 10 series against EFSVA, the data gathered for these essentially inactive compounds were insufficient to assign a linker length effect with confidence (X).

Linker effects varied with both linkage orientation and test organism. Maximum potency at intermediate lengths was only observed for staphylococci. Compounds bearing the shortest linkers were clearly superior against VRE. In no case did compounds joined by the longest linkers display the greatest potencies in their series. Each of the four C-linked series displayed maximum activity at an intermediate linker length against at least one strain. Three of the four R-linked series, except for V−R, also displayed this effect against at least one strain. In contrast, maximum activity at intermediate linker lengths was not preferred with V-linkage; only one V-linked series, C−V, displayed this behavior.

Activity as a Function of Test Strain. A. PRSP. Nearly one-half of the dimers were more potent than vancomycin against this strain: 19 dimers were more potent, 15 dimers were equipotent, and 6 dimers were less potent. The MIC values for 12 dimers were lower than that of vancomycin by a factor of more than 4. These results are especially impressive in light of the finding that all of the modified linkage synthons **2**−**5** were less potent than vancomycin against PRSP. The C−C, C−N, C−V, and V−V linkage series were the most potent overall, and the N−N, N−R, and R−R series were least potent overall against PRSP.

B. MSSA. One-half of the dimers were more potent than vancomycin against this strain: 20 dimers were more potent, 17 dimers were equipotent, and 3 dimers were less potent. The MIC values for 8 dimers were lower than that of vancomycin by a factor of at least 4. The N−N(43) dimer was 4-fold less potent. The C−V, C−R, and V−V linkage series were the most potent overall, and the N−N series was the least potent overall against MSSA. In 4 of the 10 series (C−C, C−N, N−R, R−R), dimers with intermediate-length linkers (19 or 27 atoms) displayed maximum relative potency.

C. MRSA. The majority of dimers were less potent than vancomycin against this strain: 1 dimer was more potent, 13 dimers were equipotent, and 26 dimers were less potent. The V−V(11) dimer was a factor of 4 more potent than vancomycin, and the N−N(43) dimer was 12-fold less potent than vancomycin against this strain. The V−V linkage series was the most potent overall, and the C−C, N−N, and R−R series were least potent overall against MRSA. In 3 of the 10 series (C−R, N−N, R−R), dimers with intermediate-length linkers displayed maximum relative potency.

D. GISA. Nearly all of the dimers were less potent than vancomycin against this strain: 1 dimer was more potent, 4 dimers were equipotent, and 35 dimers were less potent. The N−N(43) dimer was more than 30-fold less potent. In 2 of the 10 linkage series (C−V, C−R), dimers with intermediate-length linkers displayed maximum relative potency.

E. EFSVS. All but 3 dimers were more potent than vancomycin against this strain, and the MIC values for 15 compounds were lower by a factor of more than 10. Along with the V−V linkage series, each of the C-linked series (C−C, C−N, C−V, C−R) displayed impressive potency overall, while the N−N series was the least potent overall against EFSVS.

F. EFSVB. The majority of dimers were more potent than vancomycin against this strain: 32 dimers were more potent in that they displayed measurable activity in vitro, while 8 dimers, like vancomycin, did not display measurable activity at concentrations of 150 μg/mL or greater. Each dimer series has members that are at least a factor of 10 more potent than vancomycin against EFSVB. The V−V(11) dimer is remarkably potent, with an MIC value that is lower than those for other members of the array by at least 20-fold and lower than that for vancomycin by more than 400-fold. It must be considered that synthons **2** and **4** are significantly potent against EFSVB. In this respect, only the V−V(11) dimer is more potent than synthon **2**, by at least 24-fold. In each series, potency decreases substantially between members with the shortest linkers and those with the longest linkers, from 4-fold in the N−N series to 750-fold in the V−V series. The N−N series is least potent overall.

G. EFMVA. Out of 40 dimers, 25 displayed measurable activity (i.e., MIC less than or equal to 200 μg/mL) against this VanA strain, and 3 compounds (C−C(11), V−R(11), R−R(11)) displayed MIC values less than 10 μg/mL. In each series, potency decreases substantially as linker length increases. The least potent series overall was V−V.

H. EFSVA. This strain was the most resistant to the array; only 9 out of 40 dimers displayed measurable activity against this highly vancomycin-resistant organism. Compounds from the C−C and C−V series were the most potent, but no compound displayed an MIC value less than 38 μg/mL. Four

series (C−R, N−N, N−V, V−V) displayed no detectable activity at 200 μg/mL.

Statistical Correlations. Linear regression was used to make pairwise comparisons of the MIC data sets arranged by linkage series and by test organism (see Supporting Information). All 45 pairwise correlations between the 10 linkage series data sets were found to be highly statistically significant ($P < 0.0001$). The highest correlation coefficients were observed with the N−V and N−R linkage series, while the lowest correlation coefficients were observed with the C−C and C−V linkage series. With respect to comparisons between the test organism data sets, 9 out of 10 correlations involving the 5 vancomycin-susceptible organisms achieved statistical significance ($P < 0.05$), but none of the correlations between the data for these organisms and the data for vancomycin-resistant *E. faecalis* (VanB) reached significance.

Discussion

We have designed and synthesized an array of 40 covalently linked vancomycin dimers. In this array, the effects of varying the relative orientations of the individual glycopeptide subunits were systematically explored by varying two key parameters of multivalent design, the sites of linkage and the lengths of the linkers employed. We substantially fixed the parameters of linker chemical structure and physical properties by employing a nested set of amphiphilic, peptide-based linkers. The in vitro susceptibility data obtained with these compounds using a panel of representative Gram-positive bacterial strains are rich in content. Overall, the data indicate that dimerization with peptide-based linkers represents a useful but not fully general approach to enhancing the potency of vancomycin toward both susceptible and drug-resistant Gram-positive pathogens.

Both linkage orientation and linker length influence the in vitro activity of vancomycin dimers. With respect to linkage orientation, the V−V series displayed the greatest potency against vancomycin-susceptible organisms and VanB VRE, the C−C, C−V, and V−R series displayed the most promising broad spectrum of activity that included VanA VRE, while the N−N linked series was the least potent overall against the test organisms. Many to most of the dimers, including one or more compounds from each linkage series, were more potent than vancomycin against PRSP, MSSA, EFSVS, EFSVB, and EFMVA. At least 1 compound in 6 of the 10 linkage series also displayed measurable levels of activity against the highly resistant EFSVA strain. In addition, statistically significant correlations were observed for all pairwise comparisons of the data for each linkage series. It is possible to interpret this large subset of the results with simple models for the effects of dimerization. In one such model, many combinations of linkage orientation and linker length allow for multivalent interactions that improve affinity for intermediates involved in the biosynthesis of bacterial cell wall peptidoglycan, whether they terminate in either D-Ala-D-Ala or D-Ala-D-Lac, and this in turn provides for enhanced in vitro antibacterial potency. Alternatively, many combinations of linkage orientation and linker length may provide vancomycin dimers with the ability to exert a novel, potent mechanism of action against PRSP, MSSA, EFSVS, and VRE, such as direct inhibition of transglycosylase enzymes.[9e,29]

However, no single model in its simplest form appears to be sufficient to account for all of the results, including several nonuniformities in the observed effects. First, no one dimer is more potent than the other dimers or vancomycin against all target organisms. While the V−V(11) dimer is exceptionally potent against both EFSVS and EFSVB, this compound is among the least active against VanA VRE. Second, linker length effects for each linkage series vary depending upon test organism. Third, whereas the majority of dimers are more potent than vancomycin against MSSA, only the V−V(11) dimer is more potent than vancomycin against the other staphylococcal strains tested, MRSA and GISA.

That the V−V(11) dimer is exceptionally potent against both EFSVS and the VanB VRE strain but is at best only weakly active against VanA VRE suggests that this compound does not trigger the inducible resistance mechanism of VanB organisms which leads to the production of peptidoglycan precursors terminating in D-Ala-D-Lac rather than D-Ala-D-Ala.[30] This explanation may also account for the enhanced potency of synthons **2** and **4** toward EFSVB. More generally, the stark contrast in activity observed for V−V dimers joined by peptide linkers against vancomycin-susceptible organisms on one hand and against VanA VRE on the other may be considered in evolutionary terms.[31] The noncovalent self-association of glycopeptides into back-to-back dimers has been quantitatively correlated with in vitro antibacterial potency against vancomycin-susceptible bacteria that utilize peptidoglycan precursors terminating in D-Ala-D-Ala.[15] Accordingly, back-to-back self-association appears to be a naturally selected property of glycopeptides, and models indicate that back-to-back self-association is directly enforced by V−V linkage. It has also been established that glycopeptide-producing organisms, such as the vancomycin producer *Amycolatopsis orientalis*, possess homologues of the VanH, VanA, and VanX genes needed to elaborate peptidoglycan precursors terminating in D-Ala-D-Lac and to confer high-level resistance to glycopeptides upon enterococci and staphylococci.[32] If this mechanism is sufficient to confer upon producing organisms resistance to their own spontaneously self-associating secondary metabolites, it seems reasonable that such resistance would not be overcome by merely enforcing the self-associated state. Accordingly, in the absence of other effects, one would not expect significant activity for V−V linked dimers against VanA VRE. Consistent with this argument, against our VanA VRE test strains, we observed measurable activity with only one V−V dimer joined by an amphiphilic peptide linker. However, this result stands in contrast to the reports from the Lilly group and from Nicolaou

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et al. that V−V dimers joined by hydrophobic, benzyl-based linkers display potent activity against VanA VRE.[19,20] Linkers 12−34 atoms in length were employed in those studies, with maximum activity observed for compounds having linkers at least 18 atoms in length. Appending hydrophobic groups to the V-position of monomeric glycopeptides enhances their self-association, their association with bacterial cell surfaces and models thereof[4f,15] as well as their activity against VanA VRE.[9] In light of this and the findings with our V−V linkage series, we propose that the anti-VRE activity of V−V linked dimers joined by hydrophobic linkers is dominated not by effects that derive from enforced back-to-back self-association but rather by effects that derive from having added hydrophobic substituents to the amino disaccharide moiety.

Covalently linked glycopeptide dimers may self-assemble into extended, polyvalent networks of receptors for precursors and intermediates involved in peptidoglycan biosynthesis. This phenomenon, as envisioned by Staroske and Williams[21] and by Adamczyk et al.,[18] may be particularly critical in achieving activity against highly resistant organisms where individual vancomycin/D-Ala-D-Lac interactions are relatively weak ($K_a \approx 1000\ M^{-1}$).[6] Models indicate that, relative to dimers linked in other orientations, V−V linked dimers are hindered from engaging in the back-to-back interdimer interactions needed to form extended networks. Whitesides and co-workers observed enhanced binding of C−C linked dimers to surface displays of (N^{ϵ}-acetyl)-L-Lys-D-Ala-D-Ala, relative to vancomycin, as measured by surface plasmon resonance.[17b] A similar result was found by Adamczyk et al. who furthermore demonstrated that two V−V linked dimers did not display enhanced surface binding relative to vancomycin.[18]

The effects of linker length on in vitro potency vary with linkage series as well as with organism. We discern no correlation between the observed effects and the minimal linker lengths that models predicted would enforce the back-to-back or face-to-face associated geometries of vancomycin. That the effects of linker length would differ from one linkage series to another is not surprising. However, the finding that linker length effects for a given series vary from organism to organism suggests that the factors determining in vitro potency also vary among different organisms. Only one series (V−R) displays the same linker length effect across all of the non-VRE strains. Maximal potency at intermediate linker lengths is observed only with the three staphylococcal strains used (MSSA, MRSA, GISA), even though PRSP and EFSVS also utilize peptidoglycan precursors terminating in D-Ala-D-Ala. The linker length behavior observed with the staphylococci is independent of whether the compounds are generally more potent (MSSA) or less potent (MRSA, GISA) than vancomycin against the test organism.

Dimers bearing the shorter linkers are in all cases the most potent against the VRE strains (EFSVB, EFMVA, EFSVA), but dimers joined by short linkers are preferred in only 12 of the 50 cases involving EFSVS or nonenterococci. This could indicate that penetration of the cell wall to the sites at which glycopeptides exert antibacterial effects is hindered in VRE relative to vancomycin-susceptible organisms. However, significant changes in cell wall structure in vancomycin-resistant versus vancomycin-susceptible enterococci have not been reported, and in only 2 out of 10 cases (i.e., with the C−N and N−N linkage series) are dimers joined by the shortest linkers the most potent against EFSVS. We favor an alternative interpretation of the linker length effects observed with VRE, which involves the conformational entropy of the linker.[33] In the case of VRE, where individual vancomycin/D-Ala-D-Lac interactions are relatively weak, the absolute energetic benefits of multivalency can be offset by energy losses due to restricting the conformational entropy of longer linkers. Overall, our data suggest that one rational next step in seeking to optimize the activity of vancomycin dimers against VRE would be to develop focused structure−activity relationships around dimers joined with an array of shorter and more hydrophobic linkers. Whereas there is no evidence which indicates there is a difference in cell wall structure in vancomycin-susceptible enterococci versus vancomycin-resistant enterococci, it has been established that GISA strains elaborate an exceptionally thick, poorly cross-linked cell wall that presents many unprocessed D-Ala-D-Ala termini.[34] This structure presents a barrier to penetration by and a sequestering sink for vancomycin, and we expect that these effects would be exacerbated for the larger, multivalent vancomycin dimers.

Experimental Section

Chemistry. A. General. Reagents and solvents were used as received from commercial suppliers, and all reactions were carried out at room temperature and without rigorous exclusion of ambient atmosphere unless otherwise noted. Ion-spray mass spectra (IS-MS) were obtained on a PE Sciex API 150EX mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz. Chemical shifts (δ) are reported in parts per million downfield of tetramethylsilane. Analytical reversed-phase HPLC (RP-HPLC) was performed on an HP1100 instrument using a 2.1 mm × 50 mm, 3.5 μm C_{18} Zorbax Plus Bonus-RP column. For the analytical separations, a 0.5 min isocratic period was followed by a 4.5 min 2−30% gradient of 0.1% trifluoroacetic acid/acetonitrile (ACN) in 0.1% water at a flow rate of 0.5 mL/min. Preparative RP-HPLC was performed using trifluoroacetic acid (TFA) buffered ACN/water gradients on a Varian ProStar system using 2.5 or 10 cm × 25 cm, 8 μm C_{18} Rainin Dynamax columns and flow rates of 10 or 50 mL/min, respectively.

B. C-Linkage Synthon 2. This compound was prepared by the coupling of vancomycin with 9-fluorenylmethyl *N*-(2-aminoethyl)-carbamate, followed by deprotection.

Vancomycin hydrochloride (7.3 g, 4.7 mmol) was dissolved in 75 mL of dimethyl sulfoxide (DMSO). To this solution was added in sequence *N*,*N*-diisopropylethylamine (DIPEA, 4.1 mL, 24 mmol), 9-fluorenylmethyl *N*-(2-aminoethyl)carbamate hydrochloride (1.8 g, 5.6 mmol), and a solution of benzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP, 2.7 g, 5.2 mmol) and 1-hydroxybenzotriazole hydrate (HOBT, 0.63 g, 4.7 mmol) in 75 mL of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU). The resulting solution was stirred for 2 h at room temperature (rt) and then poured into 800 mL of diethyl ether (Et_2O), giving a gum. The liquid phase was decanted, and the gum containing crude vancomycin [9-fluorenylmethyl *N*-(2-amidoethyl)carbamate] (**6**) was washed with additional Et_2O. This compound was used without further purification.

Compound **6** was dissolved in 40 mL of *N*,*N*-dimethylformamide (DMF) and then treated with 10 mL of piperidine. The solution was left to stand at room temperature for 20 min and then added dropwise to 450 mL of ACN. The resulting precipitate was isolated by

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centrifugation/decantation, washed twice with 450 mL of ACN and once with 450 mL of Et_2O, and then air-dried. C-Linkage synthon **2** was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1492.3; obsd 1492.2.

C. N-Linkage Synthon 3. This compound was prepared by regioselective reductive alkylation at the amino terminus of vancomycin with *N*-(9-fluorenylmethoxycarbonyl)glycinal, followed by coupling with 3-(dimethylamino)propylamine and deprotection.

2-*N*-(9-Fluorenylmethoxycarbonyl)ethanol (20 g, 71 mmol) was suspended in 200 mL of dichloromethane (DCM), cooled under a nitrogen atmosphere to −40 °C (ACN/CO_2(s)), and treated with DIPEA (49.2 mL, 283 mmol). A freshly made solution of pyridine−SO_3 complex (45.0 g, 283 mmol) in 200 mL of DMSO was dropped into the reaction mixture over 10 min. The reaction was stirred for an additional 10 min and then poured into a mixture of 700 mL of saturated sodium chloride solution and 500 cm^3 of crushed ice. After the mixture was vigorously stirred, the DCM was removed under vacuum. The resulting white precipitate was isolated by filteration, washed with water, and dried under vacuum. The crude *N*-(9-fluorenylmethoxycarbonyl)-glycinal was used without further purification. ^{1}H NMR (DMSO-d_6) δ3.82 (d, 2H, J = 7 Hz), 4.21 (t, 2H, J = 7 Hz), 4.35 (d, 2H, J = 7 Hz), 7.31 (m, 4H), 7.70 (d, 2H, J = 7 Hz), 7.91 (d, 2H, J = 7 Hz), 9.21 (s, 1H).

Vancomycin hydrochloride (10 g, 6.8 mmol) was slurried in 100 mL of 1:1 methanol (MeOH)/DMPU. DIPEA (1.3 mL, 7.5 mmol) was added, and the mixture was stirred at rt until the solids dissolved. HOBT (2.1 g, 14 mmol) was added, followed by a solution of *N*-(9-fluorenylmethoxycarbonyl)glycinal in 20 mL of 1:1 MeOH/DMPU. The reaction was allowed to stir at room temperature for 4 h. Sodium cyanoborohydride ($NaCNBH_3$, 1.3 g, 21 mmol) was added as a solid. The reaction was stirred for another 2 h, at which point the MeOH was removed under vacuum, and the remaining solution was poured into 700 mL water. The resulting white precipitate was isolated by filtration, washed with water, and dried under vacuum to yield compound **7** as a white solid. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1715.6; obsd 1715.5. This compound was used without further purification.

Compound **7** (9.6 g, 5.6 mmol) was dissolved in 60 mL of DMF and treated sequentially with DIPEA (2.9 mL, 17 mmol) and 3-(dimethylamino)propylamine (1.1 mL, 8.34 mmol). A solution of PyBOP and HOBT (1 M in DMF, 6.1 mL, 6.14 mmol each) was added, and the reaction was stirred for 1 h at rt. The reaction mixture was poured into 300 mL Et_2O. The resulting white precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum to yield compound **8** as a white solid. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1798.7; obsd 1799.5. This compound was used without further purification.

Compound **8** (12 g, 5.6 mmol) was dissolved in 50 mL of DMF and treated with solid quinuclidine (3.0 g, 28 mmol). The reaction was stirred at rt for 1.5 h and then poured into 300 mL of Et_2O. The resulting white precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum. N-Linkage synthon **3** was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1577.5; obsd 1577.7.

D. V-Linkage Synthon 4. This compound was prepared by regioselective reductive alkylation at the vancosamine residue of vancomycin with *N*-(9-fluorenylmethoxycarbonyl)glycinal, followed by coupling with 3-(dimethylamino)propylamine and deprotection.

Vancomycin hydrochloride (82 g, 55 mmol) was slurried in 600 mL of anhydrous DMF and treated sequentially with DIPEA (29 mL, 170 mmol) and a solution of *N*-(9-fluorenylmethoxycarbonyl)glycinal in 100 mL of DMF. This solution was stirred at rt for 1.5 h, then treated with TFA (26 mL, 330 mmol) and 200 mL of MeOH, and stirred for another 40 min. A borane/pyridine complex (8 M, 7.6 mL, 61 mmol) was added, and the reaction was stirred at rt for 18 h. The mixture was concentrated under vacuum to a damp paste. Water (1.5 L) was added, and the pH was adjusted to 4 using 1 M sodium hydroxide. The resulting precipitate was filtered, washed extensively with water, and dried under vacuum. Compound **9** was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1715.6; obsd 1715.2.

Compound **9** (10 g, 5.1 mmol) was dissolved in 50 mL of DMF and treated sequentially with DIPEA (3.6 mL, 21 mmol) and 3-(dimethylamino)propylamine (0.97 mL, 7.7 mmol), followed by a solution of PyBOP (2.9 g, 5.6 mmol) and HOBT (0.87 g, 5.6 mmol) in 6 mL of DMF. The solution was stirred for 1 h at rt and then poured into 500 mL of Et_2O. The resulting precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum to yield crude compound **10**. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1798.7; obsd 1799.5. This compound was used without further purification

Compound **10** (5.14 mmol) was dissolved in 50 mL of DMF and treated with solid quinuclidine (2.9 g, 26 mmol). The solution was stirred at rt for 1.5 h and then added to 300 mL of Et_2O. The resulting precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum. V-Linkage synthon **4** was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1577.5; obsd 1577.7.

E. R-Linkage Synthon 5. This compound was prepared by Mannich reaction of vancomycin 3-*N*,*N*-dimethylaminopropanamide with ethylenediamine/formaldehyde.

Vancomycin hydrochloride (10 g, 6.7 mmol) was slurried in 75 mL of anhydrous DMF. DIPEA (4.7 mL, 30 mmol) was added, followed by 3-(dimethylamino)propylamine (1.3 mL, 10 mmol). A solution of PyBOP (3.9 g, 7.4 mmol) and HOBT (1.1 g, 7.4 mmol) in 7 mL of DMF was added, and the reaction was stirred at rt for 1 h. The reaction mixture was poured into 500 mL of Et_2O. The resulting precipitate was filtered, washed with Et_2O, and dried under vacuum. Vancomycin 3-*N*,*N*-dimethylaminopropanamide (**11**) was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1534.4; obsd 1534.5.

Compound **11** (12 g, 7.5 mmol) was dissolved in 200 mL of 1:1 water/ACN. Ethylenediamine (5.0 mL, 75 mmol) was added, followed by aqueous formaldehyde (37%, 0.53 mL, 7.1 mmol). The reaction was stirred at rt for 18 h. The ACN was removed under vacuum, and the solution was acidified with TFA and subjected to RP-HPLC. R-Linkage synthon **5** was isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1606.5; obsd 1606.9.

F. Linker Synthons A−D. The terminally differentiated linker synthons were prepared by a combination of solution- and solid-phase peptide synthesis approaches.

1. Linker Synthon A. Glutaric anhydride (10 g, 88 mmol) was dissolved in 100 mL of anhydrous DCM and treated with 9-fluorenylmethanol (19 g, 96 mmol), followed by DIPEA (17 mL, 96 mmol). The reaction was stirred at rt for 18 h, at which time thin-layer chromatography (TLC, 5% MeOH in DCM) indicated that the reaction was complete. The reaction mixture was washed twice with 100 mL of 1 M hydrochloric acid (HCl), dried over anhydrous magnesium sulfate ($MgSO_4$), and concentrated to a yellow oil. Linker synthon **A** was purified by chromatography on silica gel using 5% MeOH in DCM as eluent to afford a pale oil which crystallized upon standing. ^{1}H NMR ($CDCl_3$) δ 1.95 (qn, 2H, J = 7 Hz), 2.39 (t, 2H, J = 7 Hz), 2.49 (t, 2H, J = 7 Hz), 4.22 (t, 1H, J = 7 Hz), 4.44 (d, 2H, J = 7 Hz), 7.35 (m, 4H), 7.60 (d, 2H, J = 7 Hz), 7.78 (d, 2H, J = 7 Hz).

2. Linker Synthon B. This compound was prepared by condensation of glutaric acid with the fluorenylmethyl ester of β-alanine, followed by deprotection.

Boc β-Ala-OH (50 g, 0.26 mol) and 9-fluorenylmethanol (54.3 g, 0.28 mol) were dissolved in 800 mL of DCM and treated sequentially with 4-*N*,*N*-(dimethylamino)pyridine (DMAP, 1.6 g, 13.2 mmol) and a solution of 1,3-diisopropylcarbodiimide (DIPC, 43.4 mL, 0.28 mol) in 100 mL of DCM. The slightly exothermic reaction was stirred at rt for 18 h. The reaction volume was reduced to approximately 200 mL, and the white precipitate was filtered off, washed with 100 mL of DCM, and discarded. TFA (200 mL) was added to the filtrate, which was

stirred at rt for 1 h, concentrated under vacuum to a thick oil, and treated with 1 L of Et_2O. The resulting precipitate was filtered, washed with Et_2O, and dried under vacuum to yield the TFA salt of β-alanine, fluorenylmethyl ester (H-β-Ala-OFm TFA). IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 268.3; obsd 268.8.

Glutaric anhydride (5.0 g, 44 mmol) was dissolved in 70 mL of anhydrous DCM. H-β-Ala-OFm TFA (18 g, 48 mmol) was added, followed by DIPEA (8.4 mL, 48 mmol). The reaction mixture was stirred at rt for 24 h, diluted with 100 mL of DCM, and washed with 1 M HCl. The resulting precipitate was isolated by filtration, washed with a minimum of DCM, and dried to yield glutaryl-β-alanine, fluorenylmethyl ester (HO-Glut-β-Ala-OFm). IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 382.4; obsd 383.0. ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 2H, J = 7 Hz), 1.67 (qn, 2H, J = 7 Hz), 2.06 (t, 2H, J = 7 Hz), 2.16 (t, 2H, J = 7 Hz), 2.47 (t, 2H, J = 7 Hz), 3.21 (q, 1H, J = 6.7 Hz) 4.26 (t, 1H, J = 7 Hz), 4.37 (d, 2H, J = 7 Hz), 7.37 (m, 4H), 7.60 (d, 2H, J = 7 Hz), 7.85 (d, 2H, J = 7 Hz); ^{1}H NMR δ 2.64 (t, 2H, J = 7 Hz), 2.95 (t, 2H, J = 7 Hz), 4.21 (t, 1H, J = 7 Hz), 4.34 (d, 2H, J = 7 Hz), 7.30 (m, 4H), 7.58 (d, 2H, J = 7 Hz), 7.82 (d, 2H, J = 7 Hz), 7.89 (s, 3H).

HO-Glut-β-Ala-OFm (3.0 g, 7.9 mmol) and H-β-Ala-OtBu HCl (1.6 g, 8.6 mmol) were slurried together in 75 mL of DCM and treated sequentially with DIPEA (1.5 mL, 8.6 mmol), DMAP (~50 mg), and solid 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 1.7 g, 8.6 mmol). The reaction was stirred at rt for 45 min, treated with 10 mL of DMF, and then stirred for an additional 18 h, at which time TLC (10% MeOH in DCM) indicated that the reaction was complete. The reaction mixture was washed twice with 75 mL of 1 M HCl and twice with 75 mL of water. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The solid was purified by chromatography on silica gel using 10% MeOH in DCM eluent to afford the *mono*-*t*-butyl, *mono*-fluorenylmethyl ester of bis(β-alanyl)glutaramide (*t*BuO-β-Ala-Glut-β-Ala-OFm) as a pale yellow oil.

*t*BuO-β-Ala-Glut-β-Ala-OFm was dissolved in 100 mL of DCM and treated with 100 mL of TFA. The reaction was stirred for 5 h at rt and then concentrated. The residue was subjected to cycles of redissolution in DCM, concentration, and drying under vacuum until all TFA had been removed. Linker synthon **B** was purified by chromatography on silica gel using 10% MeOH in DCM as eluent to afford a colorless oil which crystallized upon standing. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 453.5; obsd 454.5.

3. Linker Synthons C and D. These compounds were prepared by application of solid-phase synthesis methodology using both C−N and N−C directional couplings.

Wang resin (6.1 g, 1.2 mequiv/g loading, 7.3 mmol) was swelled for 1 h in 150 mL of DCM. The solvent was drained, and 100 mL of fresh DCM was added. Fmoc-β-Ala-OH (11 g, 35 mmol) was added as a solid, followed by ~100 mg DMAP. Neat DIPC (5.5 mL, 35 mmol) was added, and the mixture was sparged with nitrogen for 5 h with occasional additions of DCM. The resin was then washed with 4 × 200 mL of DMF and 4 × 200 mL of DCM and then dried under vacuum to a constant weight which indicated 94% loading of the first β-Ala residue.

Linkers were initially elongated in the C−N direction using standard protocols for Fmoc-protected amino acids. Coupling and deprotection steps were carried out for 1 h, each followed by washing with 4 × 200 mL of DMF and 4 × 200 mL of DCM. Fmoc-β-Ala-OH/PyBOP/1-hydroxy-7-azabenzotriazole (HOAT)/DIPEA (3 equiv) was used for each coupling step, and deprotections were carried out with 20% piperidine in DMF. Couplings and deprotections were monitored on the resin by use of the qualitative Kaiser test.[35]

Once the addition of β-Ala residues in the C−N direction was complete (two residues for linker synthon **C** and four residues for linker synthon **D**), the direction (polarity) of chain elongation was reversed by the coupling of the N-terminus of the resin-bound chain with linker synthon **A** followed by deprotection.

The linkers were subsequently elongated in the N−C direction, again using standard Fmoc protocols. H-β-Ala-OFm TFA/PyBOP/HOAT/DIPEA (4 equiv) was used for each coupling step. At the end of each synthesis, the terminal fluorenylmethyl esters were not removed.

3.1. Linker Synthon C. Resin-bound linker synthon **C** was stirred in 100 mL of 1:1 TFA/DCM for 1 h at rt. The mixture was filtered, and the resin was washed with 50 mL of DCM. The filtrate was concentrated to a pale yellow oil which was treated with 100 mL of Et_2O. The resulting precipitate was recovered by filtration, washed with Et_2O, and dried under vacuum. Linker synthon **C** was purified by RP-HPLC and isolated by lyopholization. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 595.6; obsd 595.2.

3.2. Linker Synthon D. Resin-bound linker synthon **D** was stirred in 100 mL of 1:1 TFA/DCM for 1 h at rt. The mixture was filtered, and the resin was washed with 50 mL of DCM. The filtrate was concentrated to a pale yellow oil which was treated with 100 mL of Et_2O. The resulting precipitate was recovered by filtration, washed with Et_2O, and dried under vacuum. Linker synthon **D** was purified by RP-HPLC and isolated by lyopholization. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 879.9; obsd 879.5 $(M)^+$.

G. C−C Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by an 11-atom linker, C−C(11).

C-Linkage synthon **2** (2.0 g, 1.1 mmol) and glutaric acid (65 mg, 0.50 mmol) were dissolved in 10 mL of DMF and treated sequentially with DIPEA (0.86 mL, 5.0 mmol), and a solution of PyBOP (0.57 g, 1.1 mmol) and HOAT (150 mg, 1.1 mmol) in 1.5 mL of DMF. The reaction was stirred at rt for 2 h and then treated with 100 mL of Et_2O. The resulting precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum. The C−C(11) homodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1539.0; obsd 1540.2.

H. C−N Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 43-atom linker, C−N(43).

Linker synthon **D** (0.10 g, 0.11 mol) was dissolved in anhydrous DMSO with heating. The solution was cooled to rt and treated sequentially with N-linkage synthon **3** (0.30 g, 0.15 mmol), DIPEA (150 μL, 0.89 mmol), and a solution of PyBOP/HOAT (1 M each in DMF, 160 μL, 0.16 mmol). The solution was stirred at rt for 1 h and then treated with solid quinuclidine (0.1 g). The solution was stirred for an additional 1.5 h and then treated with 75 mL of Et_2O. The resulting precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum. The **3-D** conjugate was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1130.6; obsd 1130.4.

The **3-D** conjugate (45 mg, 0.017 mmol) and C-linkage synthon **2** (41 mg, 0.022 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (21 μL, 0.12 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 28 μL, 0.027 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The C−N(43**)** heterodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1866.3; obsd 1867.7.

I. C−V Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 19-atom linker, C−V(19).

V-Linkage synthon **4** (0.30 g, 0.19 mmol) and linker synthon **B** (50 mg, 0.15 mmol) were dissolved in 2 mL of DMF and treated sequentially with DIPEA (33 μL, 0.19 mmol) and a solution of PyBOP (90 mg, 0.17 mmol) and HOAT (30 mg, 0.17 mmol) in 0.5 mL of DMF. The solution was stirred at rt for 1 h and then treated with solid quinuclidine (0.11 g). The reaction was stirred for an additional 1.5 h

(35) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.

and then treated with 75 mL of Et_2O. The resulting precipitate was isolated by filtration, washed with Et_2O, and dried under vacuum. The **4-B** conjugate was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 1833.7; obsd 1833.8.

The **4-B** conjugate (16 mg, 0.0074 mmol) and C-linkage synthon **2** (18 mg, 0.0096 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (9 μL, 0.052 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 12 μL, 0.012 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The C−V(19) heterodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1653.0; obsd 1653.0.

J. C−R Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 27-atom linker, C−R(27).

The **5-C** conjugate was prepared as described in the following for the synthesis of the N−R(27) heterodimer.

The **5-C** conjugate (98 mg, 0.049 mmol) and C-linkage synthon **2** (110 mg, 0.059 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (16 μL, 0.10 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 79 μL, 0.079 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The C−R(27) heterodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1738.6; obsd 1739.5.

K. N−N Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 43-atom linker, N−N(43).

The **3-D** conjugate was prepared as described previously for the synthesis of the C−N(43) heterodimer.

The **3-D** conjugate (41 mg, 0.049 mmol) and N-linkage synthon **3** (42 mg, 0.021 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (22 μL, 0.10 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 25 μL, 0.025 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The N−N(43) homodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1908.8; obsd 1909.7.

L. N−V Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 43-atom linker, N−V(43).

The **3-D** conjugate was prepared as described previously for the synthesis of the C−N(43) heterodimer.

The **3-D** conjugate (34 mg, 0.049 mmol) and N-linkage synthon **3** (42 mg, 0.021 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (22 μL, 0.10 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 25 μL, 0.025 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The N−V(43) heterodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1908.8; obsd 1909.7.

M. N−R Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 27-atom linker, N−R(27).

R-Linkage synthon **5** (210 mg, 0.094 mmol) and linker synthon **C** (43 mg, 0.072 mmol) were dissolved in 1.5 mL of anhydrous DMF. DIPEA (110 μL, 0.66 mmol) was added, followed by a solution of PyBOP/HOAT (1 M each in DMF, 120 μL, 0.11 mmol). The solution was stirred at rt for 1 h and then treated with solid quinuclidine (40 mg, 0.50 mmol). The reaction was stirred for an additional 1.5 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The **5-C** conjugate was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + H^+]^+$: calcd 2004.9; obsd 2004.1.

The **5-C** conjugate (33 mg, 0.013 mmol) and N-linkage synthon **3** (34 mg, 0.017 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (20 μL, 0.12 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 21 μL, 0.021 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The N−R(27) heterodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1781.2; obsd 1782.1.

N. V−V Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 19-atom linker, V−V(19).

The **4-B** conjugate was prepared as described previously for the synthesis of the C−V(19) heterodimer.

The **4-B** conjugate (11 mg, 0.0049 mmol) and V-linkage synthon **4** (13 mg, 0.0064 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (6.9 μL, 0.039 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 7.9 μL, 0.0079 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The V−V(19) homodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1695.0; obsd 1696.6.

O. V−R Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 27-atom linker, V−R(27).

The **5-C** conjugate was prepared as described previously for the synthesis of the N−R(27) heterodimer.

The **5-C** conjugate (31 mg, 0.013 mmol) and V-linkage synthon **4** (34 mg, 0.017 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (20 μL, 0.12 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 21 μL, 0.021 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The V−R(27) heterodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1781.2; obsd 1781.8.

P. R−R Series. The synthesis of compounds in this series is exemplified by the synthesis of the dimer joined by a 27-atom linker, R−R(27).

The **5-C** conjugate was prepared as described previously for the synthesis of the N−R(27) heterodimer.

The **5-C** conjugate (98 mg, 0.049 mmol) and R-linkage synthon **5** (130 mg, 0.059 mmol) were dissolved in 1 mL of anhydrous DMF and treated sequentially with DIPEA (51 μL, 0.29 mmol) and a solution of PyBOP/HOAT (1 M each in DMF, 79 μL, 0.079 mmol). The reaction was stirred at room temperature for 1 h and then treated with 20 mL of Et_2O. The resulting precipitate was collected by centrifugation, washed with Et_2O, and dried under vacuum. The R−R(27) homodimer was purified by RP-HPLC and isolated by lyopholization as the TFA salt. IS-MS, *m*/*z* for $[M + 2H^+]^{2+}$: calcd 1795.7; obsd 1796.5.

Microbiology. Bacterial strains were obtained from the American Type Culture Collection (ATCC), Stanford University Hospital (SU), Kaiser Permanente Regional Laboratory in Berkeley (KPB), Massachusetts General Hospital (MGH), or the Centers for Disease Control and Prevention (CDC). Vancomycin-resistant enterococci were phenotyped as VanA or VanB based on their sensitivity to teicoplanin.

Minimum inhibitory concentrations (MICs) were measured in a microdilution broth procedure under NCCLS guidelines.[36] Compounds were 2-fold serially diluted into Mueller−Hinton broth in 96-well

(36) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard 5th Ed.* M7-A5. 20: 1−32. NCCLS.; **2000**.

microtiter plates. Overnight cultures of bacterial strains were diluted based on absorbance at 600 nm so that the final concentration in each well was 5×10^5 cfu/mL. Plates were returned to a 35 °C incubator. The following day (or 24 h in the case of enterococci), MICs were determined by visual inspection of the plates. Because of the inability of penicillin-resistant *Streptococcus pneumoniae* (PSRP) to grow well in Mueller−Hinton broth, MICs with this strain were determined using TSA broth supplemented with defibrinated blood.

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Supporting Information Available: Linear regression analysis comparison of MIC data sets. This material is available free of charge via the Internet at http://pubs.acs.org.

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