Synthesis and Biological Evaluation of Vancomycin Dimers with Potent Activity against Vancomycin-Resistant Bacteria: Target-Accelerated Combinatorial Synthesis

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Abstract: Based on the notion that dimerization and/or variation of amino acid 1 of vancomycin could potentially enhance biological activity, a series of synthetic and chemical biology studies were undertaken in order to discover potent antibacterial agents. Herein we describe two ligation methods (disulfide formation and olefin metathesis) for dimerizing vancomycin derivatives and applications of target-accelerated combinatorial synthesis (e.g. combinatorial synthesis in the presence of vancomycin's target Ac_2-L-Lys-D-Ala-D-Ala) to generate libraries of vancomycin dimers. Screening of these compound libraries led to the identification of a number of highly potent antibiotics effective against vancomycin-suspectible, vancomycin-intermediate resistant and, most significantly, vancomycin-resistant bacteria.

Keywords: antibiotics · biological evaluation · combinatorial synthesis · synthesis design · vancomycin

Introduction

The emergence of microorganisms resistant to antibiotics poses a serious threat to public health.[1] In particular, the rise of bacterial resistance to vancomycin (**1**, Figure 1), the drug of last resort for the treatment of many Gram-positive infections, has spurred vigorous research activities into discovering new classes of antibacterial agents, as well as toward modifying existing types of antibiotics[2] in order to check the latest bacterial moves. In the preceding paper[3] we described the synthesis and biological evaluation of a series of monomeric vancomycin derivatives, some of which exhibited good to excellent activity against vancomycin-intermediate susceptible *Staphylococcus aureus* (VISA) and vancomycin-resistant *Enterococci* (VRE). In this article we describe the synthesis and biological evaluation of dimeric derivatives of vancomycin (**1**) by parallel and target-accelerated combinatorial synthesis in a study that led to the identification of a number of highly potent antibiotics effective against vancomycin-resistant bacteria.

Figure 1. Chemical structure of vancomycin (**1**).

A number of glycopeptide antibiotics form non-covalent dimers[4] and the tendency of these compounds to dimerize has been correlated with their ability to eradicate bacteria.[5] The reasons for this phenomenon are manifold. First, there is a benefit from the multivalency.[6] Second, in the case of head-to-tail, back-to-back dimer formation, the hydrogen bonds that form the dimer interface are mediated by the same amide units which are responsible for binding to the terminal D-Ala-

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D-Ala, vancomycin's binding site, through a different network of hydrogen bonds[7] (see Figure 2). The net result of this cooperative interaction is that the dimer has a higher affinity for the D-Ala-D-Ala ligand than the monomer, and ligand-bound monomer has a higher propensity to dimerize than the free monomer. In the case of vancomycin, the dimerization constant[8] for free vancomycin (**1**) is about 700 M^{-1}, while the dimerization constant for the vancomycin/ligand complex[7] is about $10^4 M^{-1}$. Given these considerations, we designed a strategy for the generation and identification of highly potent vancomycin dimers employing target-accelerated combinatorial synthesis (TACS). Similar dynamic combinatorial strategies have been proposed and advanced in different contexts by Lehn,[9] Sanders,[10] Benner,[11] and others.[12]

Attempting to harness the benefits of multivalency and cooperativity for the generation of potent vancomycin-derived antibiotics with activity against vancomycin-resistant strains, several groups have synthesized dimeric derivatives of vancomycin. Griffin and co-workers[13] synthesized covalent dimers linked through the C-terminus of vancomycin (Figure 3). Significantly, in this first example of covalent dimerization of this antibiotic, compounds were produced which displayed respectable antibacterial activity against vancomycin-resistant strains. Additionally, the Whitesides group constructed head-to-head dimers.[14] Perhaps, however, of more biological relevance are the head-to-tail dimers. Constructs of this type were synthesized by the Williams group,[15] linking from the C-terminus to the N-terminus, and by groups at Abbott[16] and Eli Lilly,[17] both bridging their monomeric units through the vancosamine nitrogen. Finally, a vancomycin-derived trimer[18] has been reported as well as an oligomeric system,[19] the latter exhibiting improved bacteriacidal activity against vancomycin-resistant strains.

Results and Discussion

Having decided to explore the compelling dimer approach to improving vancomycin's biological activity, we faced the following issues: a) choice of the site of dimerization; b) choice of the appropriate bridge to join the two monomeric units; and c) the type of reaction to be used for the ligation. On the basis of the expected ligand binding enhancement, due to cooperative effects of the head-to-tail, back-to-back dimer, we reasoned that the saccharide domain, specifically the vancosamine nitrogen, would be the most appropriate site at which to construct the bridge between the two vancomycin units. It should be noted that dimerization from the C-terminus to the N-terminus, as has been accomplished by Williams,[15] could also generate a back-to-back dimer capable of similar cooperative effects. However, as we envisioned a one-step dimerization process, this arrangement seemed overly complicated. With the location of the tether decided upon, the more challenging problem of selecting the tether itself that would maximize the cooperative binding to the ligand needed to be addressed. To this end, we envisioned a target-accelerated combinatorial synthesis (TACS) strategy for the construction of vancomycin dimers to simultaneously evaluate the effect of tether length and binding pocket modifications on the overall activity of vancomycin dimers (see below). This approach required a ligation method that was compatible with vancomycin's polyfunctional structure, able to operate at ambient temperature and in aqueous solution, and reversible, at least as long as the TACS reaction was in progress, so as to allow for dynamic equilibration toward the thermodynamically most stable assemblies. It appeared that both the classical formation of a disulfide bond and the more contemporary olefin metathesis reaction could meet these stringent requirements.

Disulfide dimers: During our initial investigation of the two chosen dimerization processes (disulfide formation and olefin metathesis) several dimeric compounds were synthesized and biologically evaluated. As depicted in Scheme 1, saponification of the vancomycin-derived thioacetates (**2**, prepared as described in the preceding article)[3] with NaOH (10.0 equiv) in H_2O at 23 °C proceeded smoothly to give, after spontaneous aerial oxidation, disulfides **3**. Special care was necessary in this procedure in order to balance the increased rate of reaction under basic conditions with the decomposition which appeared to take place at high pH. The antibacterial activities against a variety of vancomycin-susceptible, vancomycin-

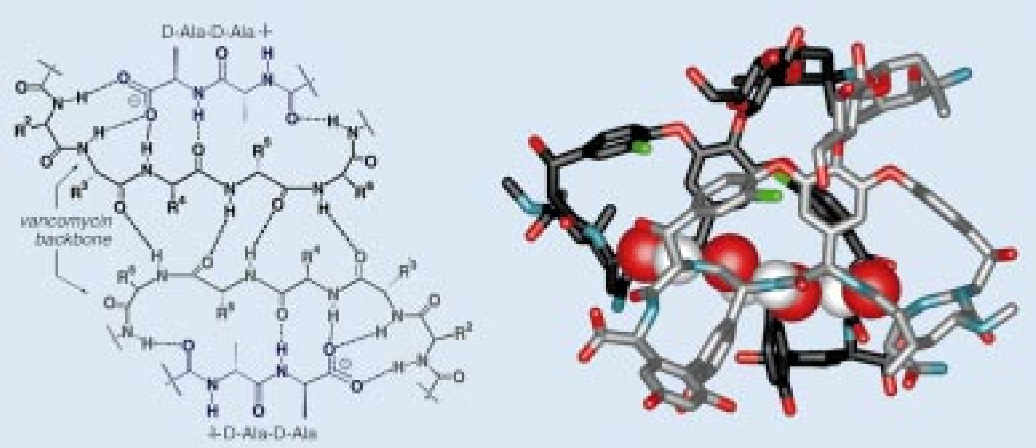

Figure 2. Hydrogen-bond network of vancomycin head-to-tail, back-to-back dimer (left) and its wire frame representation (right, one vancomycin unit is gray and the other is black).[7]

Figure 3. Types of previously synthesized vancomycin dimers: a) Griffin[13] and Whitesides[14] (head-to-head); b) Williams[15] (head-to-tail), c) Abbott[16] and Eli Lilly[17] groups (back-to-back).

intermediate resistant, and vancomycin-resistant bacteria for these disulfide dimers were determined and are presented in Tables 1 and 2. For the non-substituted dimers **3a**–**d** (Table 1) and **3n**–**o** (Table 2) the activity against vancomycin-resistant strains is far superior to that exhibited by vancomycin. Indeed, compounds **3a** and **3b** (Table 1) showed MIC values against VRE strain L4001 of 1 $\mu g\,mL^{-1}$ and 2 $\mu g\,mL^{-1}$, respectively. Also, apparent from these data is a dependence on tether length of antibacterial activity. This trend is summarized in Figure 4 and reflects a requirement of a fifteen to twenty atom bridge between the vancosamine nitrogens of the two joined vancomycin units for optimal activity. Efforts to further enhance the activity of such vancomycin dimers through introduction of certain lipophilic groups as membrane anchoring devices, as in compounds **3f**–**m** (Table 1), failed to produce the desired higher potency. Not only did such groups fail to endow the dimers with enhanced biological activity, but in fact, as the lipophilicity of the side chain increased the activity of the compounds decreased. For instance, **3h** and **3i** were found to be less active than **3f** and **3g**; and **3l** and **3m** exhibited decreased activity as compared to **3j** and **3k**. In further studies, some of the most active

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Scheme 1. Dimerization of vancomycin analogues **2** to vancomycin dimers **3** through disulfide bond formation. NaOH (10.0 equiv), H_2O, 23 °C, 48 h, 50–90%. See Tables 1 and 2 for definitions of R and *n*. Ac = acetate.

compounds were screened against additional strains of vancomycin-resistant organisms (Table 3). We were pleased to find that some of these compounds (**3b–d**, **n**, **o**, **q**) exhibited uniformly excellent activity against this broader range of resistant bacterial strains.

The ease by which disulfide dimers could be formed encouraged us to further consider such constructs. A heterodimer with one vancomycin unit capable of binding the natural D-Ala-D-Ala segment of the cell-wall and the other vancomycin moiety poised to bind its mutant counterpart (D-Ala-D-Lac) was contemplated as an attractive proposition for the development of uniquely active and broad spectrum agents effective against drug-resistant pathogens. In order to explore this possibility, a number of heterodimers with the optimum tether length was designed and targeted for synthesis. Thus, as shown in Scheme 2, vancomycin-derived thioacetate **2a** was converted, in 80% yield, to the mixed disulfide **4** by the action of NaOMe and pyS-Spy in MeOH. Treatment of a 1:1 mixture of this mixed disulfide **4** and a binding pocket modified thioacetate (**5**, R = various amino acid residues, see Table 4) synthesized as previously described[3] with NaOMe in MeOH gave smoothly, and in 65–90% yield, the desired heterodimers **6**. The latter compounds **6** were screened against a panel of vancomycin-resistant bacteria revealing a number of interesting data (Table 4). Thus, several of the compounds synthesized rival the "natural" homodimer (entry **3b**, **3c**, **3n**; Table 3) in activity. However, it appears that the D configuration of amino acid 1 endows the dimer with higher biological activity than the corresponding L-amino acid does. For example, compound **6-(D-Ala)** exhibited excellent antibacterial activity while **6-(L-Ile)** and **6-(L-Val)** showed less antibiotic activity.[20] Interestingly, compound **6-(H)** (lacking an amino acid) proved to be still reasonably active. Additionally, amino acid substitutions bearing a heteroatom such as in **6-Ser** and **6-Thr** resulted in improved activity.

Olefinic dimers: Encouraged by the success of the disulfide dimers we sought to develop the second ligation method that was based on the olefin metathesis[21] and by which olefinic (mixture of *cis* and *trans*) dimers were expected to be formed. As this work was in progress Arimoto and co-workers[18] reported the application of the ring opening metathesis polymerization (ROMP) reaction in methanolic solution to the construction of vancomycin-based oligomers. However, our circumstances dictated the requirement of conducting the dimerization in aqueous media. Initial experiments using the emulsion (H_2O/CH_2Cl_2) conditions reported by Grubbs[22] were encouraging as we were able to obtain dimeric compounds. As applied to the formation of vancomycin dimers, however, this protocol required heating to 50 °C and the amount of CH_2Cl_2 was too high for our purposes. After

Table 1. Antibacterial activity (MIC: μg mL^{-1}) of vancomycin-derived disulfide dimers (**3a–m**) against vancomycin-susceptible, vancomycin-intermediate resistant and vancomycin-resistant bacteria.

Compound	R^1	R^2	Sa8250[a]	Sp670[a]	27266[b]	4002[b]	27261[b]	48N[c]	25701[c]	LO3[c]	133[c]	MU50[d]	4001[e]
1	**vancomycin**		0.5	0.25	2	0.5	2	1	1	0.5	0.25	4	>16
3a	O(⌒)$_2$S–S(⌒)$_2$O	H	<0.03	<0.03	1	1	1	8	8	8	1	8	1
3b	O(⌒)$_3$S–S(⌒)$_3$O	H	<0.03	<0.03	2	1	1	4	8	8	1	8	2
3c	O(⌒)$_4$S–S(⌒)$_4$O	H	0.125	0.06	4	2	2	8	8	16	2	8	2
3d	O(⌒)$_5$S–S(⌒)$_5$O	H	0.5	1	8	8	8	16	16	16	8	16	8
3e	O(⌒)$_9$S–S(⌒)$_9$O	H	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
3f	O(⌒)$_2$S–S(⌒)$_2$O	O-butyl	2	2	4	4	8	8	8	4	8	8	4
3g	O(⌒)$_3$S–S(⌒)$_3$O	O-butyl	2	4	8	4	8	8	8	8	8	8	4
3h	O(⌒)$_2$S–S(⌒)$_2$O	O(⌒)$_3$⌒⌒	16	8	16	16	16	>16	>16	16	16	>16	>16
3i	O(⌒)$_3$S–S(⌒)$_3$O	O(⌒)$_3$⌒⌒	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
3j	O(⌒)$_2$S–S(⌒)$_2$O	O⌒Ph	4	2	8	4	8	8	8	8	8	16	4
3k	O(⌒)$_3$S–S(⌒)$_3$O	O⌒Ph	4	4	8	4	8	16	>16	>16	16	>16	4
3l	O(⌒)$_2$S–S(⌒)$_2$O	O⌒Ph*p*Cl	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
3m	O(⌒)$_3$S–S(⌒)$_3$O	O⌒Ph*p*Cl	>16	16	>16	>16	>16	>16	>16	>16	>16	>16	>16

[a] Vancomycin-susceptible strains of *Streptococcus pneumoniae.* [b] Vancomycin-susceptible strains of *Enterococcus faecalis.* [c] Vancomycin-susceptible strains of *Staphylococcus aureus.* [d] Vancomycin-intermediate resistant *Staphylococcus aureus.* [e] Vancomycin-resistant (van A) *Enterococcus faecium.*

considerable experimentation, we found that by employing thoroughly degassed H_2O and a phase transfer catalyst ($C_{12}H_{25}NMe_3Br$) and a trace of CH_2Cl_2 (<5%), the reaction proceeded smoothly at 23°C. Furthermore, dimeric vancomycin derivatives were cleanly obtained as the only product under these new conditions. Indeed, the fact that this reaction proceeds so well in H_2O with such polyfunctional substrates as vancomycin derivatives is a testament to the robust nature of the Grubb's catalyst [$(PCy_3)_2Ru(CHPh)Cl_2$] system. Incidentally, the recently reported water soluble ruthenium-based catalyst[23] was also assayed but failed to yield significant amounts of product. The successful olefin metathesis based ligation reaction is summarized in Scheme 3. Thus, monomeric olefins **7** were subjected to the olefin metathesis conditions {[$(PCy_3)_2Ru(CHPh)Cl_2$] (0.2 equiv), $C_{12}H_{25}NMe_3Br$ (2.2 equiv), H_2O/CH_2Cl_2 (>95:5), 23°C] to give dimers **8** in good yields. The dimers shown in Table 5 and Table 6 were synthesized by this method and tested for antibacterial activity against a variety of bacterial strains. As with the disulfides, a strong relationship between the length of the tether and the antibacterial action was noted (Figure 4). The dependence of activity on tether length is more pronounced for the olefins than for the disulfides, with the optimal length of sixteen atoms between the nitrogens of the vancosamine moieties. Furthermore, activity decreased when additional lipophilic branching was introduced to the dimers (compounds **8g** and **8h**, as compared to **8c** and **8d**, respectively, Table 5). However, this effect is less dramatic than in the case of the disulfides. In addition, we have synthesized eleven dimeric olefins with modified binding pockets (amino acid 1 variations, see Scheme 4). The antibacterial activity of these compounds against a range of bacterial strains is summarized in Table 7. Several of these compounds (the most potent being **10c**, **10e**, **10h**) rival the activity of natural vancomycin-derived homodimers (see Table 5 and Table 6). Interestingly, the inclusion of a C-terminal valine (compound **10k**, Table 7) reduced the activity of the dimers by some two to four-fold over the

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Table 2. Antibacterial activity (MIC: $\mu g\,mL^{-1}$) of vancomycin-derived disulfide dimers (**3n–s**) against vancomycin-susceptible, vancomycin-intermediate resistant and vancomycin-resistant bacteria.

3

Compound	R	Sa8250[a]	Sp670[a]	27266[b]	4002[b]	27261[b]	48N[C]	25701[C]	LO3[C]	133[c]	MU50[d]	4001[e]
1	**vancomycin**	0.5	0.25	2	0.5	2	1	1	0.5	0.25	4	>16
3n	$O(\,)_2S{-}S(\,)_2O$	<0.03	<0.03	2	1	1	4	8	16	1	8	2
3o	$O(\,)_3S{-}S(\,)_3O$	0.5	0.25	8	4	4	8	8	>16	4	8	4
3q	$O(\,)_4S{-}S(\,)_4O$	8	4	16	16	16	>16	>16	>16	>16	>16	>16
3r	$O(\,)_5S{-}S(\,)_5O$	8	4	16	16	16	>16	>16	>16	16	>16	>16
3s	$O(\,)_9S{-}S(\,)_9O$	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16

[a] Vancomycin-susceptible strains of *Streptococcus pneumoniae*. [b] Vancomycin-susceptible strains of *Enterococcus faecalis.* [c] Vancomycin-susceptible strains of *Staphylococcus aureus.* [d] Vancomycin-intermediate resistant *Staphylococcus aureus.* [e] Vancomycin-resistant (van A) *Enterococcus faecium*.

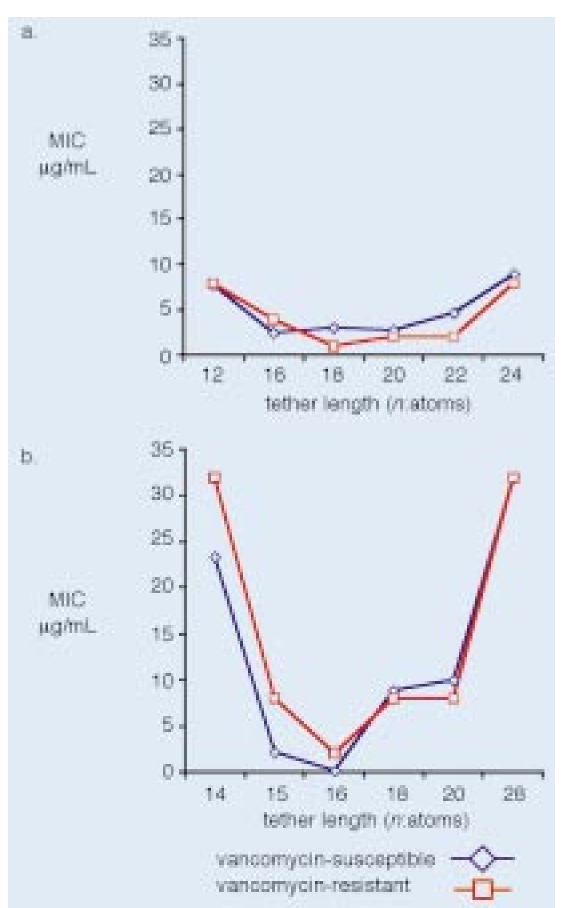

Figure 4. Correlation of tether length and antibacterial activity against vancomycin-susceptible and vancomycin-resistant strains of disulfide dimers (a) and vancomycin olefin dimers (b). Tether length is the number of atoms between neighboring vancosamine nitrogens. The vancomycin-susceptible data is an average of nine strains [*Streptococcus pneumoniea* (Sa8250 and Sp670), *Enterococcus faecalis* (27266, 4002, 27261), *Staphylococcus aureus* (48N, 25701, LO3, 133)]. See Table 2 and Scheme 1 for structures. The disulfide dimers with tether lengths of 12 and 16 atoms were prepared from thioacetates bearing a 2-(CH_2CH_2SAc) or a 4-(CH_2CH_2SAc) substituted benzyl appendage to the vancosamine nitrogen, respectively.

unsubstituted variety (compound **8c**, Table 5). In reflecting on the above findings and considering the potential metabolic instability of the disulfides, we opted to further pursue only the olefinic dimers.

Target-accelerated combinatorial synthesis of vancomycin dimers: In order to more rapidly access potent vancomycin dimers than possible by the parallel synthesis described above we contemplated a combinatorial approach to such systems. At the onset of our studies we were aware of the work of Lehn and colleagues[9] who have advanced the concepts of dynamic combinatorial libraries. Similar concepts have also been reported by Sanders,[10] Benner,[11] and others.[12, 24] This strategy, whereby the building blocks of a potential combinatorial library are allowed to pre-organize and react in the presence of a target (or a host) (see Figure 5), should generate, preferentially, products with the highest affinity for the target out of all "virtually" possible library members. The vancomycin D-Ala-D-Ala system appeared to us an ideal

Scheme 2. Formation of the vancomycin-derived heterodimers **6**. a) NaOMe (10.0 equiv), pyS-Spy (5.0 equiv), MeOH, 23 °C, 30 min, 80 %; b) **4** (1.0 equiv), **5** (1.1 equiv), NaOMe (10.0 equiv), MeOH, 23 °C, 45 min, 55 – 90 %. See Table 4 for definitions of R. Compounds are designated and referred to as **6-(xxx)** where **xxx** is the three letter code for the amino acid represented by R. py = pyridine.

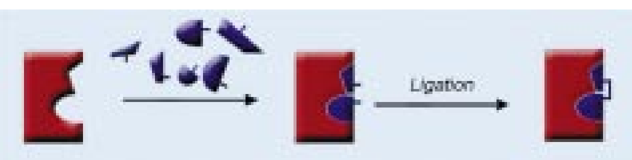

Figure 5. Schematic representation of target-accelerated combinatorial synthesis. A target (red) is incubated with a library of building blocks (blue). The assembly with the highest affinity to the target should be formed preferentially upon ligation under dynamic conditions.

case to apply such a strategy. Specifically, we hypothesized that a target (D-Ala-D-Ala)-accelerated combinatorial synthesis (TACS) would facilitate the generation of dimeric vancomycin derivatives with optimized biological properties, since both binding to D-Ala-D-Ala and the non-covalent dimerization were implicated in the mechanism of action of this antibiotic. According to this plan, a library of vancomycin monomers bearing appropriately reactive functionality would be allowed to pre-organize themselves onto D-Ala-D-Ala. Supramolecular complexes that adopt the most stable bonding arrangement should be selectively ligated to form covalent dimers. As mentioned, vancomycin has a higher dimerization constant in the presence of its target and, as such, we reasoned that TACS would be able to select for both the proper tether

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Table 3. Antibacterial activity (MIC: μg mL^{-1}) of selected vancomycin-derived disulfide dimers (**3b–d**, **n**, **o**, and **q**) against vancomycin-susceptible, vancomycin-intermediate resistant, and vancomycin-resistant bacteria. See Tables 1 and 2 for structures.

Compound	MU50[a]	133[a]	4002[b]	1528[c]	2689[c]	2741[c]	2781[C]	2805[C]	4001[d]	1669[e]	2671[e]	2823[e]	1803[f]	1924[f]	1944[f]
1 vancomycin	3.13	0.39	0.39	>100	50	>100	100	25	>100	100	50	100	50	25	50
3b	8	1	1	1	1	1	2	0.25	2	0.5	0.5	1	0.5	0.25	0.5
3c	8	2	2	1	1	2	2	2	2	0.5	1	1	1	0.125	1
3d	16	8	4	8	8	8	8	4	8	8	4	4	4	1	4
3n	16	2	1	1	1	1	1	0.5	2	0.125	0.25	0.5	0.13	0.06	0.25
3o	8	4	2	2	2	4	2	>16	2	1	2	1	2	0.5	1
3q	16	8	8	4	8	8	8	8	8	2	2	4	4	2	4

[a] Vancomycin-intermediate resistant *Staphylococcus aureus*. [b] Vancomycin-susceptible *Enterococcus faecalis*. [c] Vancomycin-resistant *Enterococcus faecalis*. [d] Vancomycin-resistant *Enterococcus faecium*. [e] Vancomycin-resistant (van A) and Synercid-resistant (sat G) *Enterococcus faecium*. [f] Vancomycin-resistant (van A) and Synercid-resistant (sat A) *Enterococcus faecium*.

length and for optimum amino acid 1 substitution which is postulated to perturb vancomycin's binding pocket (Figure 6).

In order to determine whether or not the dimerization of vancomycin monomers would be effected with accelerated rates in the presence of vancomycin's target (Ac-D-Ala-D-Ala or Ac_2-L-Lys-D-Ala-D-Ala), we subjected olefinic monomers **7-(LeuNMe)C_2** and **7-(LeuNMe)C_4** separately to the olefin metathesis conditions {olefin (550 μM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 μM), H_2O/CH_2Cl_2 (>95:5), 23 °C} in the presence of 110 μM Ac-D-Ala-D-Ala or Ac_2-L-Lys-D-Ala-D-Ala. The reaction progress was monitored by HPLC analysis of aliquots taken at regular time intervals. As shown in Figure 7, a pronounced rate enhancement for dimerization was observed, particularly in the case of Ac_2-L-Lys-D-Ala-D-Ala as expected since this peptide binds to vancomycin more tightly[25] and induces its dimerization to a greater extent[9] than Ac-D-Ala-D-Ala.

Having demonstrated the validity of the target-accelerated synthesis of vancomycin dimers from individual monomers, we then proceeded to investigate a combinatorial version of the dimerization process. To this end, three vancomycin analogues **7-(LeuNMe)C_2**, **7-(LeuNMe)C_3**, and **7-(LeuN-**

Scheme 3. Dimerization of vancomycin analogues **7** to vancomycin dimers **8** through olefin metathesis. $[(PCy_3)_2Ru(CHPh)Cl_2]$ (0.2 equiv), $C_{12}H_{25}NMe_3Br$ (2.2 equiv), H_2O/CH_2Cl_2 (>95:5), 23 °C, 30–80%. See Tables 5 and 6 for definitions of R^1, R^2 and n. Cy = cyclohexyl.

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Table 4. Antibacterial activity (MIC: μg mL^{-1}) of vancomycin-derived disulfide heterodimers (**6**) against vancomycin-susceptible, vancomycin-intermediate resistant, and vancomycin-resistant bacteria.

Compound	R	MU50[a]	133[a]	4002[b]	1528[c]	2689[c]	2741[c]	2781[C]	2805[C]	4001[d]	1669[e]	2671[e]	2823[e]	1803[f]	1924[f]	1944[f]
1	**vancomycin**	3.13	0.39	0.39	>100	50	>100	100	25	>100	100	50	100	50	25	50
6-(H)	H	>16	4	2	4	8	2	16	8	16	8	4	4	2	1	4
6-(Gly)	Gly	>16	4	1	4	8	1	8	8	16	4	2	4	0.5	1	2
6-(D-Ala)	L-Ala	>16	4	2	2	8	2	8	4	8	8	4	2	1	1	1
6-(β-Ala)	β-Ala	>16	4	2	4	8	2	16	8	16	8	8	4	1	2	4
6-(Sar)[g]	Sar	16	2	1	2	4	1	4	2	8	4	2	2	0.5	1	1
6-(γ-Abu)	γ-Abu	>16	8	2	4	>16	1	>16	8	>16	8	4	4	1	0.5	4
6-(ε-Ahx)[g]	ε-Ahx	>16	4	2	4	8	2	8	2	16	4	2	2	0.5	1	2
6-(Ile)	L-Ile	>16	16	8	8	16	8	16	16	16	16	8	8	4	4	4
6-(Val)	L-Val	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
6-(Cha)[g]	L-Cha	>16	8	4	4	4	2	8	8	8	4	4	2	2	1	2
6-(Leu)	L-Leu	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
6-(Ser)	L-Ser	16	2	1	2	4	2	8	2	8	4	2	2	0.5	0.25	2
6-(Thr)	L-Thr	>16	8	2	4	8	1	8	4	16	8	4	2	1	0.5	2
6-(Met)	L-Met	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
6-(Phe)	L-Phe	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
6-(Tyr)	L-Tyr	>16	8	4	4	4	2	8	4	8	4	1	2	1	1	1
6-(Thi)	L-Thi	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
6-(Orn)[g]	L-Orn	16	8	4	8	16	2	16	8	16	8	8	4	2	2	4
6-(Lys)	L-Lys	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
6-(Cit)[g]	L-Cit	>16	8	2	4	16	1	16	16	16	8	4	8	2	1	4
6-(Asp(O*t*Bu))	R[h]	>16	16	16	8	8	4	16	8	>16	8	4	4	4	1	4
6-(Glu-(O*t*Bu))	R[i]	>16	16	16	8	8	4	16	16	16	8	4	4	4	2	4

[a] Vancomycin-intermediate resistant *Staphylococcus aureus.* [b] Vancomycin-susceptible *Enterococcus faecalis.* [c] Vancomycin-resistant (van A) *Enterococcus faecalis.* [d] Vancomycin-resistant (van A) *Enterococcus faecium*. [e] Vancomycin-resistant (van A) and Synercid-resistant (sat G) *Enterococcus faecium*. [f] Vancomycin-resistant (van A) and Synercid-resistant (sat A) *Enterococcus faecium*. [g] Sar is sarcosine; ε-Ahx is 6-aminocaproic acid; Cha is β-cyclohexylalanine; Orn is ornithine; Cit is citrulline. [h] R = Asp(O*t*Bu). [i] R = Glu(O*t*Bu).

Me)C$_4$ bearing tethers of differing length were mixed and subjected to target-accelerated covalent dimerization by olefin metathesis (Scheme 5). The experiment was monitored by mass spectrometric analysis performed, periodically, directly on the reaction mixture. In the absence of the target (Figure 8),[26] the expected statistical mixture (1:2:3:2:1; two permutations are degenerate) of the six possible dimers [**8-(LeuNMe)C$_2$-(LeuNMe)C$_2$**, **8-(LeuNMe)C$_2$-(LeuNMe)C$_3$**, **8-(LeuNMe)C$_2$-(LeuNMe)C$_4$**, **8-(LeuNMe)C$_3$-(LeuNMe)C$_3$**, **8-(LeuNMe)C$_3$-(LeuNMe)C$_4$**, **8-(LeuNMe)C$_4$-(LeuNMe)C$_4$**] was observed, whereas in the presence of the target (Ac$_2$-L-Lys-D-Ala-D-Ala) a clear preference for the dimers with shorter tethers [**8-(LeuNMe)C$_2$-(LeuNMe)C$_2$**, **8-(LeuNMe)C$_2$-(LeuNMe)C$_3$**] was evident (Figure 8). From evaluating the biological activity of the pure dimers (Table 5), it was apparent that a strong correlation between target-induced

Table 5. Antibacterial activity (MIC: μg mL^{-1}) of vancomycin-derived olefinic dimers (**8a–w**) against vancomycin-susceptible, vancomycin-intermediate resistant and vancomycin-resistant bacteria. Unbranched compounds (**8a–f** and **8o–w**) are referred to in a short-hand to convey tether length and position one amino acid substitution. Thus, compound **8w** is designated as **8-(H)C$_4$-(H)C$_4$**. This designation implies that **8-(H)C$_4$-(H)C$_4$** was formed by joining two **7-(H)C$_4$** molecules (see Scheme 3). The olefin bridge is ≈1:1 mixture of *cis*:*trans* isomers.

Compound	R[1]	R[2]	R[3]	Sa8250[a]	Sp670[a]	27266[b]	4002[b]	27261[b]	48N[C]	25701[c]	LO3[c]	133[c]	MU50[d]	4001[e]
1	**vancomycin**			0.5	0.25	2	0.5	2	1	1	0.5	0.25	4	>16
8a	O O	H	D-NMeLeu	1	1	16	>16	>16	>16	>16	>16	>16	>16	>16
8b	O O	H	D-NMeLeu	0.06	<0.03	2	2	2	4	4	4	2	8	8
8c	O O	H	D-NMeLeu	<0.03	<0.03	0.125	0.25	0.25	0.25	0.25	0.25	0.25	1	2
8d	O ( )$_2$ ( )$_2$ O	H	D-NMeLeu	<0.03	<0.03	1	1	1	4	4	2	1	8	4
8e	O ( )$_3$ ( )$_3$ O	H	D-NMeLeu	4	8	>16	16	16	16	>16	16	16	>16	>16
8f	O ( )$_7$ ( )$_7$ O	H	D-NMeLeu	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
8g	O O	O	D-NMeLeu	1	0.5	8	4	8	8	8	8	8	16	8
8h	O ( )$_2$ ( )$_2$ O	O	D-NMeLeu	4	2	16	8	16	16	16	16	16	16	16
8i	O O	O ( )$_3$	D-NMeLeu	4	4	8	8	16	16	16	8	8	16	8
8j	O ( )$_2$ ( )$_2$ O	O ( )$_3$	D-NMeLeu	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
8k	O O	O Ph	D-NMeLeu	2	0.5	4	2	4	4	4	4	16	8	4
8l	O ( )$_2$ ( )$_2$ O	O Ph	D-NMeLeu	4	2	8	8	16	8	8	8	8	16	8
8m	O O	O Ph*p*Cl	D-NMeLeu	4	4	8	8	16	>16	>16	>16	16	>16	16
8n	O ( )$_2$ ( )$_2$ O	O Ph*p*Cl	D-NMeLeu	4	8	8	8	>16	>16	>16	>16	>16	>16	>16
8o	O O	H	L-Asn	2	4	16	16	16	>16	16	16	8	>16	>16
8p	O ( )$_2$ ( )$_2$ O	H	L-Asn	4	8	16	8	16	16	>16	16	16	>16	>16
8q	O O	H	β-Ala	0.25	0.25	1	0.25	1	2	16	8	>16	4	>16
8r	O ( )$_2$ ( )$_2$ O	H	β-Ala	1	0.5	4	2	4	2	>16	16	2	4	8
8s	O O	H	γ-Abu	0.125	0.25	1	0.5	8	8	8	0.5	1	8	>16
8t	O O	H	L-Phe	0.5	1	4	1	2	16	4	1	2	16	>16
8u	O O	H	L-Arg	0.5	1	1	0.5	1	16	2	2	1	16	>16
8v	O O	H	H	8	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
8w	O ( )$_2$ ( )$_2$ O	H	H	8	4	16	8	16	16	16	16	8	>16	>16

[a] Vancomycin-susceptible strains of *Streptococcus pneumoniae.* [b] Vancomycin-susceptible strains of *Enterococcus faecalis.* [c] Vancomycin-susceptible strains of *Staphylococcus aureus.* [d] Vancomycin-intermediate resistant *Staphylococcus aureus.* [e] Vancomycin-resistant (van A) *Enterococcus faecium*.

Table 6. Antibacterial activity (MIC: μg mL^{-1}) of vancomycin-derived olefinic dimers (**8x–z**) against vancomycin-susceptible, vancomycin-intermediate resistant and vancomycin-resistant bacteria. The olefin bridge is ≈1:1 mixture of *cis*:*trans* isomers.

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Compound	R	Sa8250[a]	Sp670[a]	27266[b]	4002[b]	27261[b]	48N[c]	25701[c]	LO3[c]	133[c]	MU50[d]	4001[e]
1	**vancomycin**	0.5	0.25	2	0.5	2	1	1	0.5	0.25	4	>16
8x	O( )$_2$∼( )$_2$O	0.125	0.125	2	1	2	2	2	2	1	8	4
8y	O( )$_3$∼( )$_3$O	0.125	0.125	1	0.5	1	2	2	1	0.5	4	2
8z	O( )$_7$∼( )$_7$O	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16

[a] Vancomycin-susceptible strains of *Streptococcus pneumoniae*. [b] Vancomycin-susceptible strains of *Enterococcus faecalis*. [c] Vancomycin-susceptible strains of *Staphylococcus aureus*. [d] Vancomycin-intermediate resistant *Staphylococcus aureus*. [e] Vancomycin-resistant (van A) *Enterococcus faecium*.

Table 7. Antibacterial activity (MIC: μg mL^{-1}) of vancomycin derived olefinic dimers (**10a–k**) against vancomycin-susceptible, vancomycin-intermediate resistant and vancomycin-resistant bacteria. The olefin bridge is ca. 1:1 mixture of *cis*:*trans* isomers.

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Compound	R^1	R^2	MU50[a]	133[a]	4002[b]	1528[c]	2689[c]	2741[c]	2781[c]	2805[c]	4001[d]	1669[e]	2671[e]	2823[e]	1803[f]	1924[f]	1944[f]
1	**vancomycin**		3.13	0.39	0.39	>100	50	>100	100	25	>100	100	50	100	50	25	50
10a	Gly	H	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
10b	Gly	L-Met	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
10c	Gly	L-Ser	2	0.125	1	1	2	>16	4	1	0.25	4	>16	0.5	>16	0.5	0.25
10d	Gly	L-Cha[g]	>16	>16	>16	>16	>16	1	>16	>16	>16	>16	0.5	>16	1	>16	>16
10e	Gly	L-Lys	2	0.25	1	2	4	1	4	1	0.25	8	0.5	0.5	2	0.5	0.25
10f	Gly	L-Phe	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
10g	Gly	L-Val	>16	8	4	4	>16	2	>16	16	>16	>16	>16	>16	>16	>16	>16
10h	Gly	L-Thi[g]	2	0.25	1	2	2	1	4	2	0.5	8	0.5	2	2	1	0.25
10i	Gly	L-Ile	>16	8	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
10j	L-Val	L-Cha[g]	>16	>16	16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
10k	L-Val	D-NMeLeu	16	2	2	2	16	2	8	4	8	8	4	4	>16	1	4

[a] Vancomycin-intermediate resistant *Staphylococcus aureus*. [b] Vancomycin-susceptible *Enterococcus faecalis*. [c] Vancomycin-resistant (van A) *Enterococcus faecalis*. [d] Vancomycin-resistant (van A) *Enterococcus faecium*. [e] Vancomycin-resistant (van A) and Synercid-resistant (sat G) *Enterococcus faecium*. [f] Vancomycin-resistant (van A) and Synercid-resistant (sat A) *Enterococcus faecium*. [g] Cha is β-cyclohexylalanine; Thi is β-(2-thienyl)alanine.

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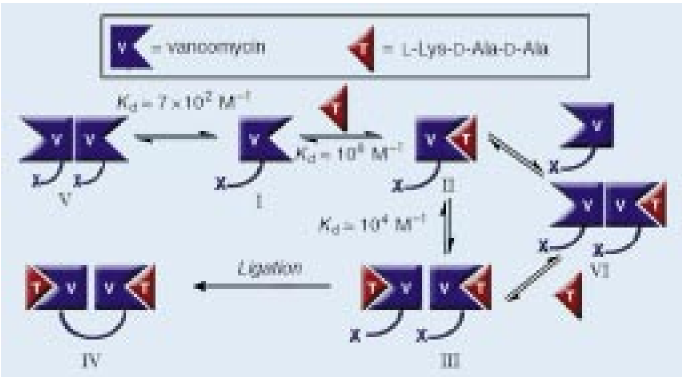


Figure 6. Schematic representation of dynamic target-accelerated synthesis of vancomycin dimers. A library of vancomycin analogues (V, blue) are incubated with their target, Ac_2-L-Lys-D-Ala-D-Ala (T, red). Since the dimerization constant of vancomycin ($K_d \approx 7 \times 10^2$ M^{-1}) is greater in the presence of its target ($K_d \approx 10^4$ M^{-1}), it is expected that the target-bound dimeric assemblies (III) should ligate preferentially over their non target-bound counterparts.

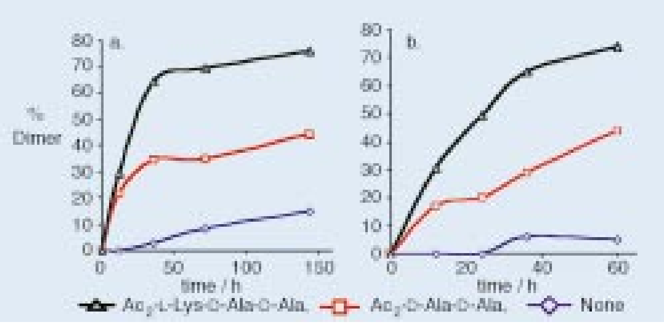
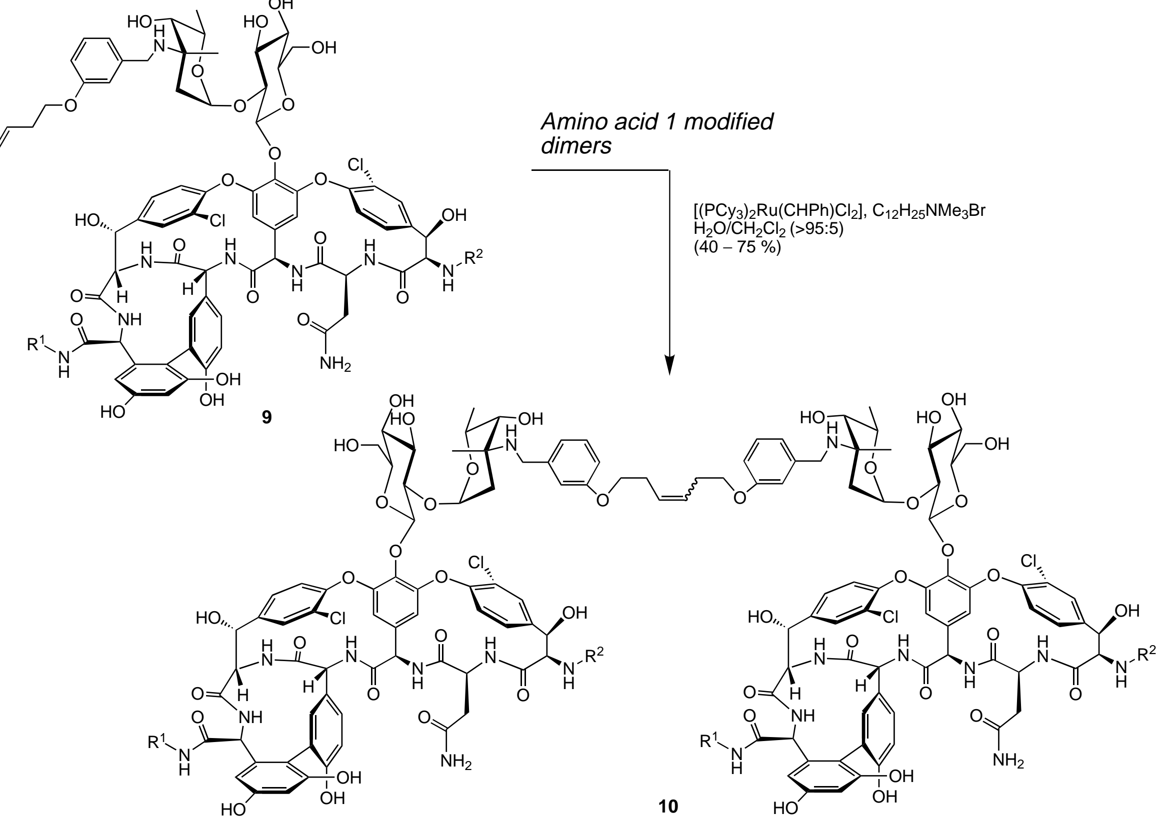


Figure 7. Proof of principle of target-accelerated combinatorial synthesis of covalently linked vancomycin dimers through the olefin metathesis reaction in the absence and presence of the target. a) **7-(LeuNMe)C$_2$ → 8-(LeuNMe)C$_2$-(LeuNMe)C$_2$**; b) **7-(LeuNMe)C$_4$ → 8-(LeuNMe)C$_4$-(LeuNMe)C$_4$**. Vancomycin analogue (550 mM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), target (110 mM), $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 mM), H_2O/CH_2Cl_2 (>95:5), 23 °C (see Table 5 for structures of compounds).

rate acceleration and biological potency (Figure 4) does exist. Most significantly, dimers from both the olefinic and disulfide classes with optimal tether length (16–18 atoms between the two nitrogens), predicted by the TACS experiment described above, exhibited potent antibacterial activities, particularly against VRE.

With the optimum length of the tethering bridge determined, we then turned our attention to the influence of the binding affinity of the target (Ac_2-L-Lys-D-Ala-D-Ala) to the ligand (vancomycin scaffold) on the rate of dimerization. A most direct and potentially more productive way of modulating this affinity is to vary the first amino acid residue of vancomycin. Thus, a mixture of two vancomycin analogues [**7-(LeuNMe)C$_2$** and **7-(β-Ala)C$_2$**] was treated under the olefin metathesis conditions described above in the absence and presence of the Ac_2-L-Lys-D-Ala-D-Ala (Scheme 6), and product formation was again monitored by mass spectroscopy (see Figrue 9).

It was interesting to note that, in the absence of the target, the homodimer of the β-Ala-substituted vancomycin [**8-(β-**



Scheme 4. Dimerization of vancomycin-derived olefins **9**, with modifications at the amino acid 1-position, to vancomycin dimers **10**. $[(PCy_3)_2Ru(CHPh)Cl_2]$ (0.2 equiv), $C_{12}H_{25}NMe_3Br$ (2.2 equiv), H_2O/CH_2Cl_2 (> 95:5), 23 °C, 40–75 %. See Table 7 for definitions of R^1 and R^2.

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$n = 2$: **7-(LeuNMe)C$_2$** ; $n = 3$: **7-(LeuNMe)C$_3$** ; $n = 4$: **7-(LeuNMe)C$_4$**

Scheme 5. Target-accelerated combinatorial synthesis (TACS) of vancomycin dimers: the effect of tether length. An equimolar mixture of **7-(LeuNMe)C$_2$**, **7-(LeuNMe)C$_3$**, and **7-(LeuNMe)C$_4$** (200 μM each) was treated with the metathesis catalyst, $[(PCy_3)_2Ru(CHPh)Cl_2]$ (120 μM), in the presence of $C_{12}H_{25}NMe_3Br$ (2.75 mM) in H_2O/CH_2Cl_2 (> 95:5) at 23 °C, in the presence and absence of the target (Ac$_2$-L-Lys-D-Ala-D-Ala, 120 μM).

Figure 8. Mass spectrometic analysis of the TACS experiment described in Scheme 5. In the absence of the target (a) a statistical mixture [1:2:3:2:1] of products is observed whereas in the presence of the target (b) the shorter tether dimers **8-(LeuNMe)C$_2$-(LeuNMe)C$_2$** and **8-(LeuNMe)C$_2$-(LeuNMe)C$_3$** are preferentially formed.

Ala)C$_2$-(β-Ala)C$_2$] was formed preferentially over the parent vancomycin homodimer [**8-(LeuNMe)C$_2$-(LeuNMe)C$_2$**]. This outcome may be attributed to a higher tendency of the β-Ala-substituted vancomycin monomer to dimerize in the absence of the target. In the presence of the target, however, the parent vancomycin homodimer [**8-(LeuNMe)C$_2$-(LeuNMe)C$_2$**] was obtained preferentially as expected. The preferences observed in this experiment are again consistent with the potencies exhibited by these compounds. The results were also consistent with the notion that stronger affinity for the target translates into higher dimerization rates through monomer selection. The observed enhancement results also established that the background reaction is relatively inconsequential. Table 8 summarizes the results of additional two-component target-accelerated dimerization experiments. In all cases the results of the TACS experiments faithfully predict the trend in biological potencies.

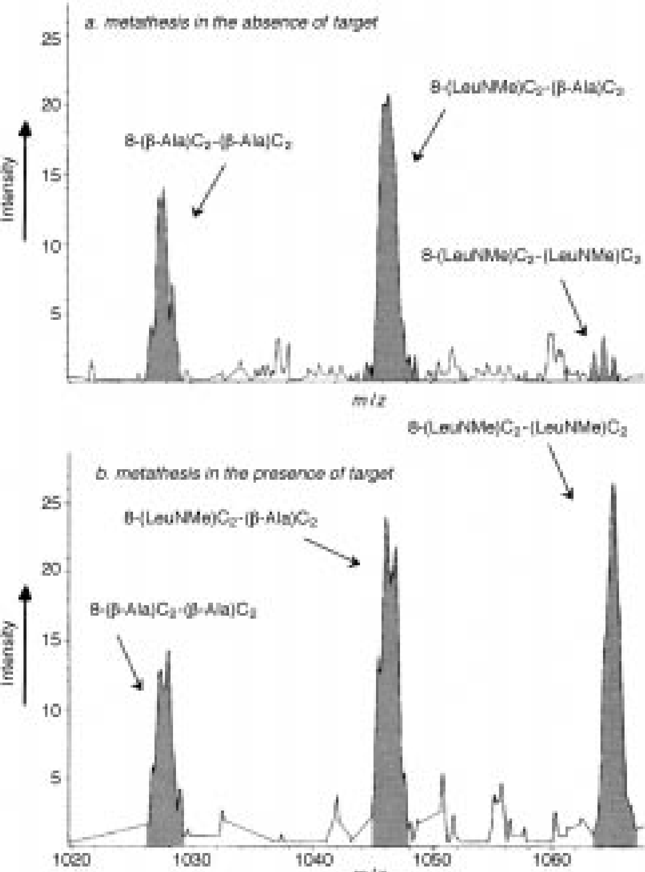


Figure 9. Mass spectrometric analysis of the vancomycin dimer mixture formed as described in Scheme 6. Note that in the absence of the target (a) less of the dimer containing the (LeuNMe) is formed. However, in the presence of the target (b), **8-(LeuNMe)C$_2$-(LeuNMe)C$_2$** is formed in preference to **8-(β-Ala)C$_2$-(β-Ala)C$_2$**.

Olefin metathesis driven TACS

$[(PCy_3)_2Ru(CHPh)Cl_2]$, $C_{12}H_{25}NMe_3Br$
H_2O/CH_2Cl_2 (>95:5)

with and without
Ac_2-L-Lys-D-Ala-D-Ala

R = D-NMeLeu, β-Ala

2 Monomers → 3 Dimers

R = LeuNMe : **7-(LeuNMe)C_2** ; R = β-Ala : **7-(β-Ala)C_2**

Scheme 6. Target-accelerated combinatorial synthesis (TACS) of vancomycin dimers: the effect of modulating binding affinity. An equimolar mixture of **7-(LeuNMe)C_2** and **7-(*β*-Ala)C_2** (275 µM each) was treated with the metathesis catalyst, $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 µM), in the presence of $C_{12}H_{25}NMe_3Br$ (2.75 mM) in H_2O/CH_2Cl_2 (>95:5) at 23 °C, in the presence and absence of the target (Ac_2-L-Lys-D-Ala-D-Ala, 110 µM).

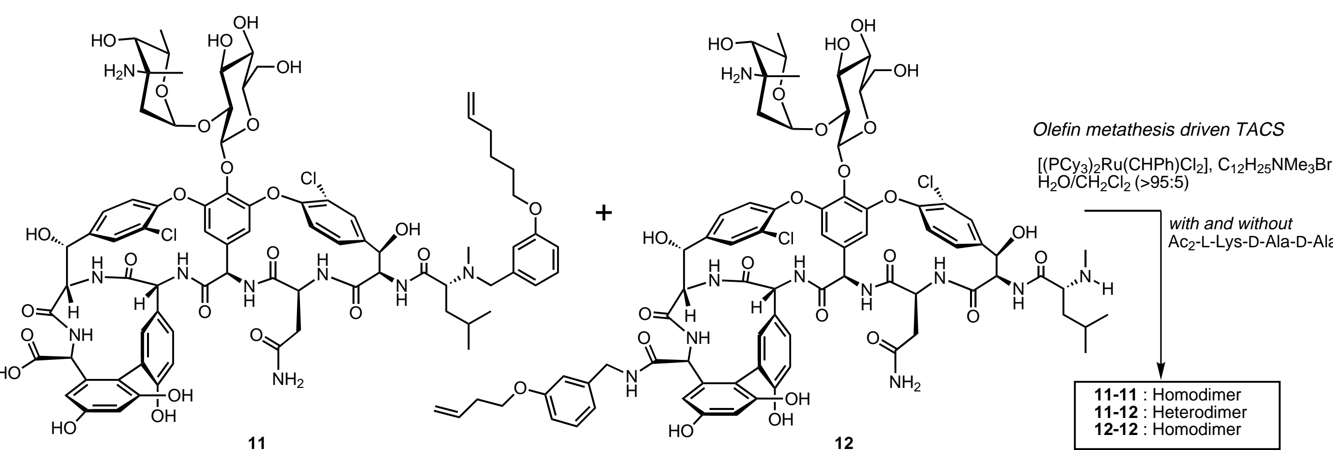
In an effort to examine the preferred orientation of vancomycin monomers during dimerization, we designed and synthesized olefin derivatives **11** and **12**, which are functionalized at the N-terminus and C-terminus, respectively. In combination these derivatives were subjected to TACS under the olefin metathesis conditions in the absence and presence of Ac_2-L-Lys-D-Ala-D-Ala as shown in Scheme 7.

In both instances, mass spectroscopic analysis (Figure 10) revealed the heterodimer **11-12** as the major product; but in the presence of the target, the heterodimer strongly predominated in the ratio of 1:4.7:1.1 (**11-11**:**11-12**:**12-12**) as opposed to the essentially statistical distribution of dimers observed in the absence of the target [ratio of 1:2.6:1.2 (**11-11**:**11-12**:**12-12**)]. These observations are in line with the proposition, as supported by modeling, that only the heterodimer (**11-12**) can adopt a head-to-tail, back-to-back orientation, and thus enjoy the maximum benefit of the hydrogen-bonding network associated with such an arrangement.

Table 8. Binary dimerization experiments. Reactions were conducted under the same conditions as indicated in the legend of Scheme 6.

Entry	Time [h]	Reactants	Additive	Product Ratio[a]
1	91	**7-(H)C_4 + 7-(D-LeuNMe)C_4**	**Ac_2-L-Lys-D-Ala-D-Ala**	1.0 : 6.1 : 3.3
2	91	**7-(H)C_4 + 7-(D-LeuNMe)C_4**	None	1.0 : 1.7 : 2.1
3	120	**7-(H)C_2 + 7-(D-LeuNMe)C_2**	**Ac_2-L-Lys-D-Ala-D-Ala**	1.0 : 6.0 : 4.3
4	120	**7-(H)C_2 + 7-(D-LeuNMe)C_2**	None	1.0 : 7.5 : 2.7
5	91	**7-(β-Ala)C_4 + 7-(D-LeuNMe)C_4**	**Ac_2-L-Lys-D-Ala-D-Ala**	1.0 : 2.1 : 1.2
6	91	**7-(β-Ala)C_4 + 7-(D-LeuNMe)C_4**	None	3.8 : 4.5 : 1.0
7	72	**7-(β-Ala)C_2 + 7-(D-LeuNMe)C_2**	**Ac_2-L-Lys-D-Ala-D-Ala**	1.0 : 2.5 : 1.4
8	72	**7-(β-Ala)C_2 + 7-(D-LeuNMe)C_2**	None	3.7 : 7.5 : 1.0
9	91	**7-(Asn)C_4 + 7-(D-LeuNMe)C_4**	**Ac_2-L-Lys-D-Ala-D-Ala**	1.0 : 6.0 : 4.8
10	91	**7-(Asn)C_4 + 7-(D-LeuNMe)C_4**	None	1.0 : 1.8 : 1.1

[a] The product ratio is listed as **8-(AA)C_x-(AA)C_x**:**8-(AA)C_x-(D-NMeLeu)C_x**:**8-(D-NMeLeu)C_x-(D-NMeLeu)C_x** as determined by mass spectroscopic analysis of the indicated mixture at the appropriate time. The antibacterial activities of the homodimers formed in the above reactions are reported in Table 5 [**7(H)C_4** → **8w**, **7(H)C_2** → **8v**, **7(D-LeuNMe)C_4** → **8d**, **7(D-LeuNMe)C_2** → **8c**, **7(*β*-Ala)C_4** → **8r**, **7(*β*-Ala)C_2** → **8q**, **7(Asn)C_4** → **8p**, and **7(*β*-Ala)C_2** → **8o**].



Scheme 7. Target-accelerated combinatorial synthesis (TACS) of vancomycin dimers: Examining the effects of orientation. An equimolar mixture of **11** and **12** (275 µM each) was treated with the metathesis catalyst, $(PCy_3)_2Ru(CHPh)Cl_2$ (110 µM), in the presence of $C_{12}H_{25}NMe_3Br$ (2.75 mM) in H_2O/CH_2Cl_2 (>95:5) at 23 °C, in the presence and absence of the target (Ac_2-L-Lys-D-Ala-D-Ala, 110 µM). Compound **12-12** is the dimer formed from the union, through olefin metathesis, of two molecules of **12**. Likewise, **11-11**, is the dimer formed from two units of **11** and **11-12** is the corresponding heterodimer.

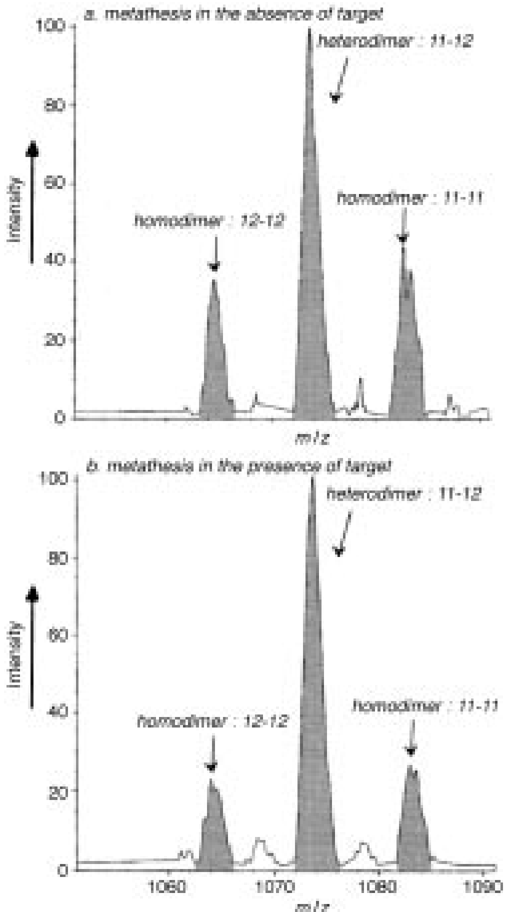


Figure 10. Mass spectrometric analysis of the experiment depicted in Scheme 7. Note that in the presence of the target (b) there is a greater preference for the heterodimer **11-12** which can, apparently, adopt a head-to-tail, back-to-back orientation. Furthermore this mode of dimerization appears to be enhanced in the presence of the target (b).

An additional experiment performed with vancomycin monomers **7-(LeuNMe)C$_4$** and **13** in the absence and presence of the target Ac$_2$-L-Lys-D-Ala-D-Ala (Scheme 8) was also revealing. Thus, in the absence of the target, mass spectroscopic analysis (Figure 11) showed a ratio of 0:2.7:1 for **13-13**:**13-7-(LeuNMe)C$_4$**:**8-LeuNMe)C$_4$-LeuNMe)C$_4$** as opposed to a ratio of 0.1:1:1.1 for this same reaction mixture in the

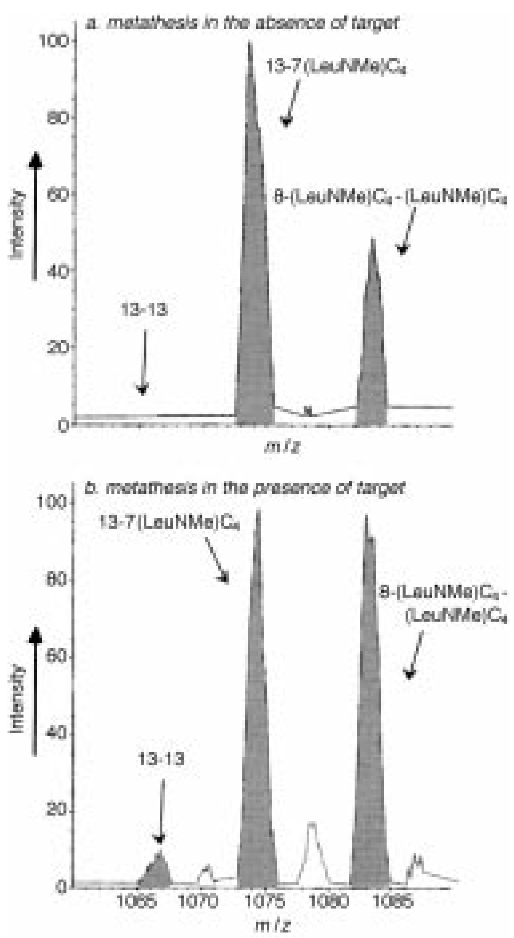
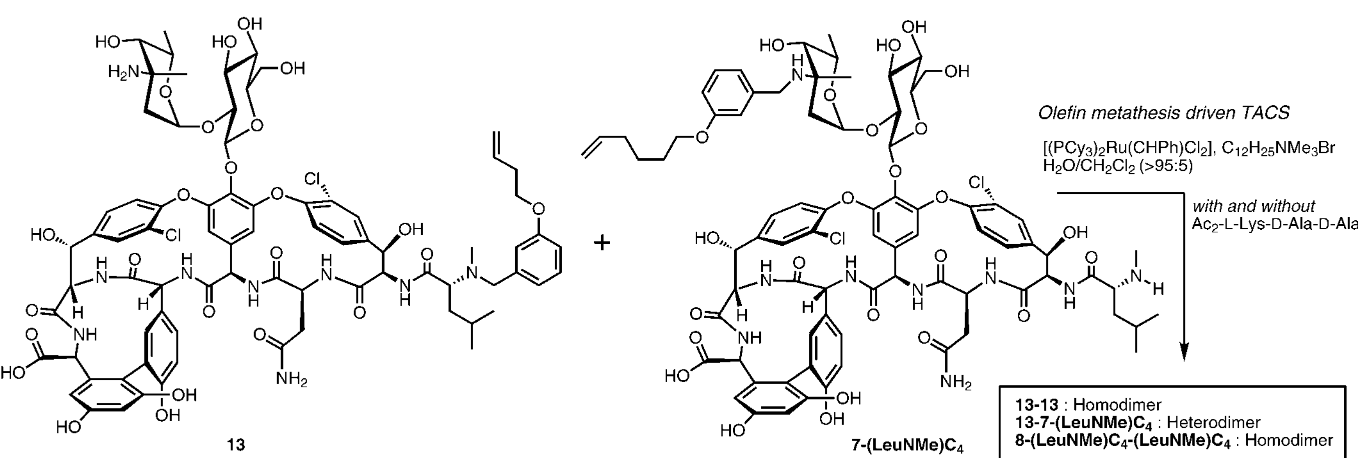


Figure 11. Mass spectrometric analysis of the experiment depicted in Scheme 8. Note that in the presence of the target (b) homodimer **7-(LeuNMe)C$_4$-(LeuNMe)C$_4$** is favored, apparently, due to its enhanced ability to adopt head-to-tail, back-to-back orientation.



Scheme 8. Target-accelerated combinatorial synthesis (TACS) of vancomycin dimers: Examining the effects of orientation. An equimolar mixture of **13** and **7-(LeuNMe)C$_4$** (275 μM each) was treated with the metathesis catalyst, [(PCy$_3$)$_2$Ru(CHPh)Cl$_2$], (110 μM), in the presence of $C_{12}H_{25}NMe_3Br$ (2.75 mM) in H_2O/CH_2Cl_2 (> 95:5) at 23 °C, with or without the target (Ac$_2$-L-Lys-D-Ala-D-Ala, 110 μM). Compound **13-13** is the dimer formed from the union through olefin metathesis of two molecules of **13**. Likewise, **8-(LeuNMe)C$_4$-(LeuNMe)C$_4$**, is the dimer formed from two units of **7-(LeuNMe)C$_4$** and **13-7-(LeuNMe)C$_4$** is the corresponding heterodimer.

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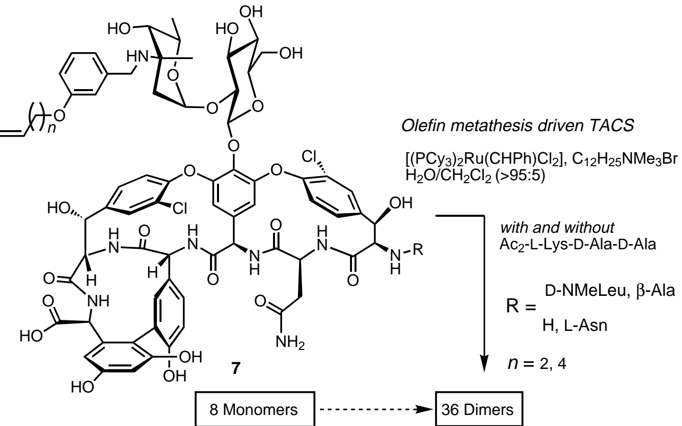
presence of Ac_2-L-Lys-D-Ala-D-Ala. The increased abundance of **8-(LeuNMe)C_4-(LeuNMe)C_4** observed, relative to the other dimers, in the presence of vancomycin's target is not surprising in view of the fact that this is the only dimer from this set that can readily adopt the head-to-tail, back-to-back orientation. Thus, these TACS experiments suggest the preferred supramolecular structure, in solution, of the dimeric vancomycin-derived complexes.[26]

Having demonstrated that the target-accelerated dimerization of vancomycin analogues selects for both the adequacy of the tether and the affinity for the target, we proceeded to perform an eight-component [**7-(LeuNMe)C_2**, **7-(LeuNMe)C_4**, **7-(Asn)C_2**, **7-(Asn)C_4**, **7-(β-Ala)C_2**, **7-(β-Ala)C_4**, **7-(H)C_2**, and **7-(H)C_4**] target-accelerated (Ac_2-L-Lys-D-Ala-D-Ala) combinatorial synthesis experiment employing the olefin metathesis reaction as a means of ligation (Scheme 9). From the thirty-six members expected, only thirty could be observed as distinct peaks by mass spectrometry as a consequence of the degeneracy of the dimers. The vertical bars in Figure 12 reflect the observed relative abundance of each of the thirty distinct (with regard to their mass) vancomycin dimers after adjustment to account for the expected statistical occurrence[28] (average values for three experiments). Figure 13 graphically exhibits the antibacterial activities of ten



Scheme 9. Target-accelerated combinatorial synthesis (TACS) of 36 vancomycin dimers: simultaneously selecting for binding affinity and tether length.

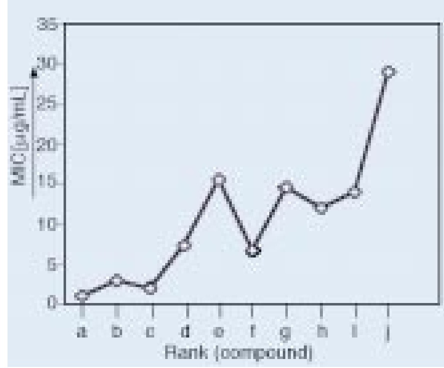


Figure 13. Correlation of relative abundance of vancomycin dimers (**a**–**j**, Figure 12 and Table 5), with antibacterial activity (MIC, average of the vancomycin susceptible strains listed in Table 5).

individually prepared compounds. Gratifyingly, the target-accelerated dimerization strategy predicted quite reliably the overall trend of the observed biological potencies of the library members, with only relatively minor deviations. The identification and correct ranking of compounds [a: **8-(LeuNMe)C_2-(LeuNMe)C_2**, b: **8-(LeuNMe)C_2-(β-Ala)C_2**, c: **8-(LeuNMe)C_2-(LeuNMe)C_4**] as highly potent antibiotics effective against both vancomycin-susceptible and vancomycin-resistant strains is highly significant. Since six out of the seven most abundant compounds contain LeuNMe, the results also underscore the importance of the amino acid residue at position one for strong binding and presumed (observed in some cases) biological activity.

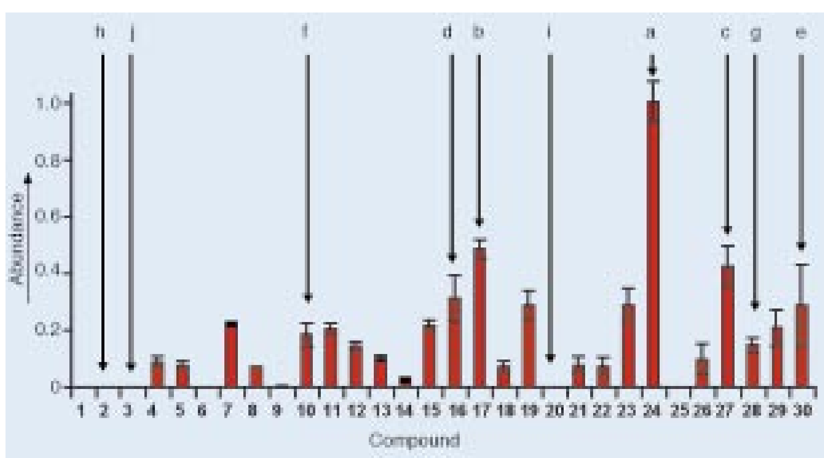


Figure 12. Target-accelerated combinatorial synthesis of a 36-member vancomycin dimer library. The vertical bars represent the relative abundance of vancomycin dimers as compared to that of a): **8-(LeuNMe)C_2-(LeuNMe)C_2** after the appropriate statistical adjustment. The data above represent the average of three experiments. Compounds are labeled as follows: 1: **8-(H)C_2-(H)C_2**, 2: **8-(H)C_2-(H)C_4**, 3: **8-(H)C_4-(H)C_4**, 4: **8-(H)C_2-(β-Ala)C_2**, 5: **8-(β-Ala)C_2-(H)C_4**, 6: **8-(H)C_2-(Asn)C_2**, 7: **8-(H)C_2-(LeuNMe)C_2**, 8: **8-(H)C_4-(β-Ala)C_4**, 9: **8-(Asn)C_2-(H)C_4**, 10: **8-(β-Ala)C_2-(β-Ala)C_2**, 11: **8-(LeuNMe)C_2-(H)C_4**, 12: **8-(β-Ala)C_2-(β-Ala)C_4**, 13: **8-(Asn)C_4-(H)C_4**, 14: **8-(β-Ala)C_2-(Asn)C_2**, 15: **8-(LeuNMe)C_4-(H)C_4**, 16: **8-(β-Ala)C_4-(β-Ala)C_4**, 17: **8-(LeuNMe)C_2-(β-Ala)C_2**, 18: **8-(β-Ala)C_2-(Asn)C_4**, 19: **8-(β-Ala)C_2-(LeuNMe)C_4**, 20: **8-(Asn)C_2-(Asn)C_2**, 21: **8-(Asn)C_2-(β-Ala)C_4**, 22: **8-(LeuNMe)C_2-(Asn)C_2**, 23: **8-(β-Ala)C_4-(LeuNMe)C_4**, 24: **8-(LeuNMe)C_2-(LeuNMe)C_2**, 25: **8-(Asn)C_2-(Asn)C_4**, 26: **8-(Asn)C_2-(LeuNMe)C_4**, 27: **8-(LeuNMe)C_2-(LeuNMe)C_4**, 28: **8-(Asn)C_4-(Asn)C_4**, 29: **8-(Asn)C_4-(LeuNMe)C_4**, 30: **8-(LeuNMe)C_4-(LeuNMe)C_4** (see Table 5 for structures of compounds).

Conclusion

In this and the preceding paper we have synthesized a series of vancomycin derivatives starting from vancomycin itself and subjected a number of them to target-accelerated combinatorial synthesis, a strategy that facilitated the rapid discovery of potent dimeric vancomycin-derived antibiotics. Active against vancomycin-susceptible and vancomycin-resistant bacteria, some of these compounds

Table 9. Antibacterial activity (MIC: $\mu g mL^{-1}$) of selected vancomycin-derived disulfide and olefinic dimers (**3b**, **3c**, **3n**, **10c**, **10e**, and **10h**) against vancomycin-susceptible, vancomycin-intermediate resistant, and vancomycin-resistant bacteria. See Tables 1, 2, and 7 for structures.

Compound	MU50[a]	133[a]	4002[b]	1528[c]	2689[c]	2741[c]	2781[c]	2805[c]	4001[d]	1669[e]	2671[e]	2823[e]	1803[f]	1924[f]	1944[f]
1 vancomycin	3.13	0.39	0.39	>100	50	>100	100	25	>100	100	50	100	50	25	50
3b	8	1	1	1	1	1	2	0.25	2	0.5	0.5	1	0.5	0.25	0.5
3c	8	2	2	1	1	2	2	2	2	0.5	1	1	1	0.125	1
3n	16	2	1	1	1	1	1	0.5	2	0.125	0.25	0.5	0.13	0.06	0.25
10c	2	0.125	1	1	2	>16	4	1	0.25	4	>16	0.5	>16	0.5	0.25
10e	2	0.25	1	2	4	1	4	1	0.25	8	0.5	0.5	2	0.5	0.25
10h	2	0.25	1	2	2	1	4	2	0.5	8	0.5	2	2	1	0.25

[a] Vancomycin-intermediate resistant *Staphylococcus aureus*. [b] Vancomycin-susceptible *Enterococcus faecalis*. [c] Vancomycin-resistant *Enterococcus faecalis*. [d] Vancomycin-resistant *Enterococcus faecium*. [e] Vancomycin-resistant (van A) and Synercid-resistant (sat G) *Enterococcus faecium*. [f] Vancomycin-resistant (van A) and Synercid-resistant (sat A) *Enterococcus faecium*.

rival (see Table 9) or exceed, in potency, the most active antibacterial agents known today. The concept of target-accelerated combinatorial synthesis (dynamic combinatorial synthesis) has been validated in a sophisticated system and in a practical way leading to the discovery of potential drug candidates. Further application of the principles involved and the ligation reactions used in this study (disulfide bond formation and olefin metathesis) in other situations are envisioned. These may include RNA and DNA binding constructs as well as protein and saccharide-type receptor ligand systems.

Experimental Section

General: See paper one in this sequence.[3]

General procedure for the conversion of thioacetates 2 to disulfides 3 (Scheme 1): A solution of thioacetate **2** (10 mg, ≈6.2 μmol) in water (1.0 mL) was treated with NaOH (2.5 mg, 10.0 equiv, 62.5 μmol) and stirred for 24–48 h at ambient temperature. Purification by reverse-phase HPLC (VYDAC C18, 25 mm × 250 mm, flow rate 6.5 mL min^{-1}, 0 → 100% CH_3CN (0.05% TFA) in H_2O (0.05% TFA) over 30 min) provided the desired disulfide **3** in yields ranging from 50–90% (analytical HPLC given below for LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100% CH_3CN (0.05% TFA) in H_2O (0.05% TFA) over the time indicated).

Representative ^{1}H NMR spectral data for compounds 3

3a: ^{1}H NMR (500 MHz, CD_3OD, 330 K): δ = 7.76–7.71 (m, 8H), 7.40–7.38 (m, 8H), 7.15–7.09 (m, 10H), 6.54–6.51 (m, 4H), 5.57–5.37 (m, 10H), 4.85–4.80 (m, 6H), 4.25–3.87 (m, 30H), 3.13–3.11 (m, 2H), 2.96–2.90 (m, 10H), 2.88–2.84 (m, 5H), 2.38–2.22 (m, 2H), 2.11–2.09 (m, 5H), 1.81–1.74 (m, 2H), 1.62–1.50 (m, 6H), 1.39–1.34 (m, 6H), 1.05–1.03 (m, 6H).

3a: t_R = 7.0 min (gradient over 10 min); LCMS (ES): calcd for $C_{152}H_{172}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1628.5, found 1628.8; $[M+3H]^+$: 1086.0, found 1086.1.

3b: t_R = 6.8 min (gradient over 8 min); LCMS (ES): calcd for $C_{154}H_{176}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1643.5, found 1643.2; $[M+3H]^+$: 1096.2, found 1095.8.

3c: t_R = 7.2 min (gradient over 10 min); LCMS (ES): calcd for $C_{156}H_{180}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1657.7, found 1657.2; $[M+3H]^+$: 1105.4, found 1105.2.

3d: t_R = 9.0 min (gradient over 10 min); LCMS (ES): calcd for $C_{158}H_{184}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1671.5, found 1672.4; $[M+3H]^+$: 1114.3, found 1114.4.

3e: t_R = 8.6 min (gradient over 8 min); LCMS (ES): calcd for $C_{166}H_{200}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1727.8, found 1727.7; $[M+3H]^+$: 1151.2, found 1151.7.

3f: t_R = 4.0 min (gradient over 7 min); LCMS (ES): calcd for $C_{160}H_{188}Cl_4N_{18}O_{52}S_2$ $[M+2H]^+$: 1702.0, found 1702.8; $[M+3H]^+$: 1134.5, found 1134.4.

3g: t_R = 4.2 min (gradient over 7 min); LCMS (ES): calcd for $C_{162}H_{192}Cl_4N_{18}O_{52}S_2$ $[M+2H]^+$: 1715.5, found 1715.5; $[M+3H]^+$: 1144.2, found 1144.2.

3h: t_R = 4.4 min (gradient over 7 min); LCMS (ES): calcd for $C_{166}H_{200}Cl_4N_{18}O_{51}S_2$ $[M+2H]^+$: 1743.2, found 1744.5; $[M+3H]^+$: 1162.5, found 1162.1.

3i: t_R = 4.5 min (gradient over 7 min); LCMS (ES): calcd for $C_{168}H_{204}Cl_4N_{18}O_{51}S_2$ $[M+2H]^+$: 1757.7, found 1758.2; $[M+3H]^+$: 1172.1, found 1172.8.

3j: t_R = 7.6 min (gradient over 8 min); LCMS (ES): calcd for $C_{166}H_{184}Cl_4N_{18}O_{52}S_2$ $[M+2H]^+$: 1735.8, found 1735.6; $[M+3H]^+$: 1157.2, found 1157.4.

3k: t_R = 4.1 min (gradient over 7 min); LCMS (ES): calcd for $C_{168}H_{188}Cl_4N_{18}O_{52}S_2$ $[M+2H]^+$: 1749.5, found 1750.7; $[M+3H]^+$: 1166.2, found 1166.5.

3l: t_R = 4.3 min (gradient over 7 min); LCMS (ES): calcd for $C_{166}H_{182}Cl_6N_{18}O_{52}S_2$ $[M+2H]^+$: 1770.2, found 1770.7; $[M+3H]^+$: 1180.9, found 1180.4.

3m: t_R = 4.3 min (gradient over 7 min); LCMS (ES): calcd for $C_{168}H_{186}Cl_6N_{18}O_{52}S_2$ $[M+2H]^+$: 1785.2, found 1785.7; $[M+3H]^+$: 1190.2, found 1190.6.

3n: t_R = 6.9 min (gradient over 10 min); LCMS (ES): calcd for $C_{152}H_{172}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1628.5, found 1628.7; $[M+3H]^+$: 1086.0, found 1086.1.

3o: t_R = 6.9 min (gradient over 8 min); LCMS (ES): calcd for $C_{154}H_{176}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1643.5, found 1643.5; $[M+3H]^+$: 1096.2, found 1096.2.

3p: t_R = 7.5 min (gradient over 8 min); LCMS (ES): calcd for $C_{156}H_{180}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1657.7, found 1657.2; $[M+3H]^+$: 1105.4, found 1105.0.

3q: t_R = 8.0 min (gradient over 8 min); LCMS (ES): calcd for $C_{158}H_{184}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1671.5, found 1671.7; $[M+3H]^+$: 1114.3, found 1114.4.

3r: t_R = 8.5 min (gradient over 8 min); LCMS (ES): calcd for $C_{166}H_{200}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1727.8, found 1727.9; $[M+3H]^+$: 1151.2, found 1151.9.

3s: t_R = 8.7 min (gradient over 8 min); LCMS (ES): calcd for $C_{166}H_{200}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1727.8, found 1727.9; $[M+3H]^+$: 1151.2, found 1151.1.

Preparation of thiopyridyl vancomycin derivative 4: A mixture of thioacetate **2a** (120 mg, 72 μmol) and dipyridyldisulfide (160 mg, 10.0 equiv, 720 μmol) was dissolved in MeOH (5.0 mL). To this mixture was added a solution of NaOH (28 mg, 10.0 equiv, 720 μmol in 3.5 mL MeOH). After 30 min, the reaction mixture was purified by reverse-phase HPLC (VYDAC C18, 25 mm × 250 mm, flow rate 6.5 mL min^{-1}, 0 → 100%

CH_3CN (0.05 % TFA) in H_2O (0.05 % TFA) over 30 min) to give **4** (124 mg, 80 %). Analytical HPLC: $t_R = 8.2$ min (LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100 % CH_3CN (0.05 % TFA) in H_2O (0.05 % TFA) over 10 min); ^{1}H NMR (500 MHz, CD_3OD, 330 K): $\delta = 7.88-7.71$ (m, 5H), 7.54–7.45 (m, 4H), 7.26–7.20 (m, 2H), 7.14–7.05 (s, 2H), 6.78 (br s, 2H), 6.63 (s, 1H), 6.50 (m, 2H), 6.08 (br s, 2H), 5.67 (d, $J = 7.6$ Hz, 1H), 5.58 (d, $J = 4.4$ Hz, 1H), 5.46 (d, $J = 13$ Hz, 2H), 5.35 (d, $J = 3.9$ Hz, 1H), 4.90 (m, 1H), 4.82 (s, 1H), 4.69 (s, 1H), 4.36 (d, $J = 8.8$ Hz, 1H), 4.20 (s, 1H), 4.14 (t, $J = 6.3$ Hz, 2H), 4.04–3.88 (m, 4H), 3.70 (d, $J = 7.9$ Hz, 2H), 3.49 (m, 1H), 3.03–3.00 (m, 3H), 2.41 (s, 3H), 2.17–2.13(m, 4H), 2.01 (d, $J =$ 13.5 Hz, 1H), 1.83–1.74 (m, 6H), 1.36 (m, 2H), 1.20 (d, $J = 6.3$ Hz, 3H), 1.03–1.00 (m, 6H); LCMS (ES): calcd for $C_{81}H_{91}Cl_2N_{10}O_{25}S_2$ $[M+H]^+$: 1739.5, found 1739.3.

General procedure for the synthesis of disulfide heterodimers 6 (Scheme 2): A 1:1 mixture (≈2 mg, 1.2 µmol) of thiopyridyl vancomycin derivative **4** and thioacetate **5** was dissolved in MeOH (0.8 mL) and NaOH (1.6 mg, 10.0 equiv, 12.0 µmol) was added. The reaction mixture was stirred for 45 min at ambient temperature and the product was purified by reverse-phase HPLC (VYDAC C18, 10 mm × 250 mm, flow rate 3.5 mL min^{-1}, 0 → 100 % CH_3CN (0.05 % TFA) in H_2O (0.05 % TFA) over 20 min) to give pure heterodimer **6** in yields ranging from 55–80 % (analytical HPLC data given below for LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100 % CH_3CN (0.05 % TFA) in H_2O (0.05 % TFA) over 10 min).

6-H: $t_R = 7.7$ min; LCMS (ES): calcd for $C_{147}H_{162}Cl_4N_{18}O_{50}S_2$ $[M+2H]^+$: 1594.8, found 1594.1; $[M+3H]^+$: 1063.5, found 1063.6.

6-Gly: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{149}H_{165}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1622.8, found 1621.4; $[M+3H]^+$: 1082.5, found 1082.8.

6-Ala: $t_R = 7.9$ min; LCMS (ES): calcd for $C_{150}H_{167}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1629.5, found 1629.0; $[M+3H]^+$: 1086.7, found 1086.4.

6-β-Ala: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{150}H_{167}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1629.5, found 1629.5; $[M+3H]^+$: 1086.7, found 1086.8.

6-Sar: $t_R = 7.9$ min; LCMS (ES): calcd for $C_{150}H_{167}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1629.5, found 1629.8; $[M+3H]^+$: 1087.5, found 1087.1.

6-γ-Abu: $t_R = 7.7$ min; LCMS (ES): calcd for $C_{151}H_{169}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1636.5, found 1636.2; $[M+3H]^+$: 1091.3, found 1091.8.

6-ε-Ahx: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{153}H_{173}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1650.2, found 1650.1; $[M+3H]^+$: 1100.5, found 1100.0.

6-Ile: $t_R = 8.0$ min; LCMS (ES): calcd for $C_{153}H_{173}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1650.3, found 1649.3; $[M+3H]^+$: 1101.5, found 1101.0.

6-Val: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{152}H_{171}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1643.5, found 1643.1; $[M+3H]^+$: 1096.3, found 1096.4.

6-Cha: $t_R = 8.2$ min; LCMS (ES): calcd for $C_{156}H_{177}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1670.0, found 1669.8; $[M+3H]^+$: 1113.5, found 1113.2.

6-Leu: $t_R = 7.9$ min; LCMS (ES): calcd for $C_{153}H_{173}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1650.3, found 1651.5; $[M+3H]^+$: 1101.5, found 1100.4.

6-Ser: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{150}H_{167}Cl_4N_{19}O_{52}S_2$ $[M+2H]^+$: 1637.5, found 1637.5; $[M+3H]^+$: 1091.8, found 1092.2.

6-Thr: $t_R = 7.9$ min; LCMS (ES): calcd for $C_{151}H_{169}Cl_4N_{19}O_{52}S_2$ $[M+2H]^+$: 1645.2, found 1644.8; $[M+3H]^+$: 1097.5, found 1097.5.

6-Met: $t_R = 8.00$ min; LCMS (ES): calcd for $C_{152}H_{171}Cl_4N_{19}O_{51}S_3$ $[M+2H]^+$: 1659.8, found 1659.3; $[M+3H]^+$: 1107.5, found 1106.7.

6-Phe: $t_R = 8.1$ min; LCMS (ES): calcd for $C_{156}H_{171}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1668.6, found 1667.2; $[M+3H]^+$: 1112.5, found 1112.8.

6-Tyr: $t_R = 8.1$ min; LCMS (ES): calcd for $C_{156}H_{171}Cl_4N_{19}O_{52}S_2$ $[M+2H]^+$: 1676.3, found 1675.4; $[M+3H]^+$: 1117.5, found 1117.2.

6-Thi: $t_R = 8.0$ min; LCMS (ES): calcd for $C_{154}H_{169}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1671.5, found 1671.4; $[M+3H]^+$: 1114.2, found 1114.5.

6-Orn: $t_R = 7.9$ min; LCMS (ES): calcd for $C_{152}H_{172}Cl_4N_{20}O_{51}S_2$ $[M+2H]^+$: 1651.2, found 1651.6; $[M+3H]^+$: 1100.2, found 1100.4.

6-Lys: $t_R = 7.7$ min; LCMS (ES): calcd for $C_{153}H_{174}Cl_4N_{20}O_{51}S_2$ $[M+2H]^+$: 1658.2, found 1658.3; $[M+3H]^+$: 1105.2, found 1105.6.

6-Cit: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{153}H_{173}Cl_4N_{21}O_{52}S_2$ $[M+2H]^+$: 1672.2, found 1672.2; $[M+3H]^+$: 1115.3, found 1115.0.

6-Asp(O*t*Bu): $t_R = 8.8$ min; LCMS (ES): calcd for $C_{155}H_{167}Cl_4N_{19}O_{51}S_2$ $[M+2H]^+$: 1679.2, found 1679.6; $[M+3H]^+$: 1120.2, found 1120.5.

6-Glu(O*t*Bu): $t_R = 8.1$ min; LCMS (ES): calcd for $C_{156}H_{177}Cl_4N_{19}O_{53}S_2$ $[M+2H]^+$: 1687.5, found 1686.8; $[M+3H]^+$: 1124.2, found 1122.8.

General procedure for dimerization of 7 to dimers 8 through olefin metathesis (Scheme 3): $C_{12}H_{25}NMe_3Br$ (4.2 mg, 14.0 equiv, 86.0 µmol) was added under argon to a solution of olefin **7** (10 mg, ≈6.25 µmol) in degassed water (1.0 mL). To this vigorously stirred solution (ambient temperature) was added, dropwise, a solution of Grubb's catalyst $[(Cy_3P)_2Ru(CHPh)Cl_2]$ (1.0 mg, 0.2 equiv, 1.25 µmol) in CH_2Cl_2 (200 µL). After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). After stirring vigorously for 48–96 h at ambient temperature, the desired olefinic dimer **8** was isolated, in pure form, after purification by reverse-phase HPLC (VYDAC C18, 25 mm × 250 mm, flow rate 6.5 mL min^{-1}, 0 → 100 % CH_3CN (0.05 % TFA) in H_2O (0.05 % TFA) over 30 min). Analytical HPLC given below for LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100 % CH_3CN (0.05 % TFA) in H_2O (0.05 % TFA) over 10 min.

Representative ^{1}H NMR spectroscopic data for compounds 8

8c: ^{1}H NMR (500 MHz, CD_3OD, 330 K): $\delta = 7.80-7.70$ (m, 10H), 7.45–7.35 (m, 7H), 7.13–7.06 (m, 10H), 6.6–6.51 (m, 6H), 5.55–5.35 (m, 12H), 4.83–4.81 (m, 8H), 4.26–3.82 (m, 25H), 3.23–3.21 (m, 4H), 2.96–2.89 (m, 11H), 2.88–2.83 (m, 6H), 2.13–2.09 (m, 5H), 1.80–1.74 (m, 2H), 1.65–1.55 (m, 6H), 1.37–1.32 (m, 6H), 1.07–1.05 (m, 6H).

8a: $t_R = 7.6$ min; LCMS (ES): calcd for $C_{150}H_{166}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1581.2, found 1581.3; $[M+3H]^+$: 1055.2, found 1055.2.

8b: $t_R = 7.7$ min; LCMS (ES): calcd for $C_{152}H_{170}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1596.3, found 1595.8; $[M+3H]^+$: 1064.2, found 1064.2.

8c: $t_R = 8.9$ min; LCMS (ES): calcd for $C_{154}H_{174}Cl_4N_{18}O_{5o}$ $[M+2H]^+$: 1610.0, found 1610.3; $[M+3H]^+$: 1073.6, found 1073.8.

8d: $t_R = 8.4$ min; LCMS (ES): calcd for $C_{156}H_{178}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1623.2, found 1624.3; $[M+3H]^+$: 1083.5, found 1083.7.

8e: $t_R = 11.5$ min; LCMS (ES): calcd for $C_{164}H_{1194}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1679.5, found 1679.8; $[M+3H]^+$: 1119.6, found 1119.5.

8f: $t_R = 11.5$ min; LCMS (ES): calcd for $C_{164}H_{194}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1679.5, found 1679.3; $[M+3H]^+$: 1119.6, found 1120.1.

8g: $t_R = 9.2$ min; LCMS (ES): calcd for $C_{160}H_{194}Cl_4N_{18}O_{52}$ $[M+2H]^+$: 1668.5, found 1668.6; $[M+3H]^+$: 1112.6, found 1112.4.

8h: $t_R = 9.5$ min; LCMS (ES): calcd for $C_{162}H_{190}Cl_4N_{18}O_{52}$ $[M+2H]^+$: 1682.5, found 1682.4; $[M+3H]^+$: 1122.2, found 1121.4.

8i: $t_R = 10.3$ min; LCMS (ES): calcd for $C_{166}H_{198}Cl_4N_{18}O_{52}$ $[M+2H]^+$: 1710.5, found 1710.2; $[M+3H]^+$: 1122.2, found 1122.7.

8j: $t_R = 11.0$ min; LCMS (ES): calcd for $C_{168}H_{202}Cl_4N_{18}O_{52}$ $[M+2H]^+$: 1724.5, found 1724.8; $[M+3H]^+$: 1150.2, found 1150.1.

8k: $t_R = 7.8$ min (gradient over 8 min); LCMS (ES): calcd for $C_{166}H_{182}Cl_4N_{18}O_{52}$ $[M+2H]^+$: 1702.5, found 1701.8; $[M+3H]^+$: 1135.3, found 1135.2.

8l: $t_R = 9.5$ min; LCMS (ES): calcd for $C_{168}H_{186}Cl_4N_{18}O_{52}$ $[M+2H]^+$: 1716.5, found 1716.2; $[M+3H]^+$: 1144.6, found 1144.4.

8m: $t_R = 9.8$ min; LCMS (ES): calcd for $C_{166}H_{180}Cl_6N_{18}O_{52}$ $[M+2H]^+$: 1737.1, found 1737.2; $[M+3H]^+$: 1158.3, found 1158.6.

8n: $t_R = 10.2$ min (gradient over 8 min); LCMS (ES): calcd for $C_{168}H_{184}Cl_6N_{18}O_{52}$ $[M+2H]^+$: 1751.0, found 1750.6; $[M+3H]^+$: 1167.7, found 1167.7.

8o: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{146}H_{156}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1582.4, found 1582.4; $[M+3H]^+$: 1056.4, found 1056.3.

8p: $t_R = 8.2$ min; LCMS (ES): calcd for $C_{150}H_{164}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1611.4, found 1611.4; $[M+3H]^+$: 1074.6, found 1074.6.

8q: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{144}H_{154}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1540.2, found 1540.3; $[M+3H]^+$: 1027.8, found 1027.9.

8r: $t_R = 8.3$ min; LCMS (ES): calcd for $C_{148}H_{162}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1568.3, found 1569.0; $[M+3H]^+$: 1045.9, found 1046.8.

8s: $t_R = 6.8$ min; LCMS (ES): calcd for $C_{146}H_{158}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1554.2, found 1554.2; $[M+3H]^+$: 1036.3, found 1036.6.

8t: $t_R = 7.5$ min (gradient over 8 min); LCMS (ES): calcd for $C_{156}H_{162}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1616.4, found 1616.2; $[M+3H]^+$: 1078.8, found 1077.8.

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8u: $t_R = 6.7$ min (gradient over 8 min); LCMS (ES): calcd for $C_{150}H_{168}Cl_4N_{24}O_{50}$ $[M+2H]^+$: 1625.4, found 1625.4; $[M+3H]^+$: 1083.9, found 1083.9.

8v: $t_R = 7.5$ min; LCMS (ES): calcd for $C_{138}H_{144}Cl_4N_{16}O_{48}$ $[M+2H]^+$: 1469.2, found 1469.2, $[M+3H]^+$: 979.2, found 979.3.

8w: $t_R = 8.31$ min; LCMS (ES): calcd for $C_{142}H_{154}Cl_4N_{16}O_{48}$ $[M+2H]^+$: 1497.3, found 1498.4; $[M+3H]^+$: 998.5, found 999.3.

8x: $t_R = 8.64$ min; LCMS (ES): calcd for $C_{154}H_{174}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1609.7, found 1610.3; $[M+3H]^+$: 1073.6, found 1073.8.

8y: $t_R = 9.7$ min; LCMS (ES): calcd for $C_{156}H_{178}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1623.2, found 1623.9; $[M+3H]^+$: 1083.5, found 1082.9.

8z: $t_R = 11.4$ min; LCMS (ES): calcd for $C_{164}H_{194}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1679.5, found 1679.9; $[M+3H]^+$: 1119.6, found 1120.4.

Dimerization of vancomycin derivatives 9 to dimers 10 through olefin metathesis (Scheme 4): The dimerization of compound **9**[3] to dimeric vancomycin derivative **10** (Scheme 4) through olefin metathesis was carried out in the same manner as described above for the conversion of compounds **7** to **8** (analytical HPLC given below for LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100% CH_3CN (0.05% TFA) in H_2O (0.05% TFA) over 10 min).

10a: $t_R = 7.1$ min; LCMS (ES): calcd for $C_{142}H_{150}Cl_4N_{18}O_{50}$ $[M+2H]^+$: 1526.3, found 1526.4; $[M+3H]^+$: 1017.8, found 1017.0.

10b: $t_R = 7.3$ min; LCMS (ES): calcd for $C_{160}H_{184}Cl_4N_{20}O_{54}S_2$ $[M+2H]^+$: 1729.6, found 1729.6; $[M+3H]^+$: 1153.4, found 1153.9.

10c: $t_R = 7.1$ min; LCMS (ES): calcd for $C_{156}H_{176}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1685.4, found 1685.4; $[M+3H]^+$: 1123.9, found 1123.8.

10d: $t_R = 8.0$ min; LCMS (ES): calcd for $C_{160}H_{180}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1679.5, found 1680.2; $[M+3H]^+$: 1120.0, found 1119.3.

10e: $t_R = 6.9$ min; LCMS (ES): calcd for $C_{154}H_{174}Cl_4N_{22}O_{52}$ $[M+3H]^+$: 1103.3, found 1103.2.

10f: $t_R = 7.3$ min; LCMS (ES): calcd for $C_{150}H_{168}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1673.4, found 1673.6; $[M+3H]^+$: 1115.9, found 1116.1.

10g: $t_R = 7.1$ min; LCMS (ES): calcd for $C_{152}H_{168}Cl_4N_{20}O_{52}$ $[M+3H]^+$: 1083.9, found 1083.8.

10h: $t_R = 7.3$ min; LCMS (ES): calcd for $C_{164}H_{180}Cl_4N_{20}O_{54}S_2$ $[M+2H]^+$: 1751.6, found 1751.7; $[M+3H]^+$: 1167.0, found 1167.2.

10i: $t_R = 6.8$ min; LCMS (ES): calcd for $C_{154}H_{172}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1639.4, found 1638.3; $[M+3H]^+$: 1093.3, found 1093.3.

10j: $t_R = 8.0$ min; LCMS (ES): calcd for $C_{166}H_{192}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1721.6, found 1720.8; $[M+3H]^+$: 1148.0, found 1148.2.

10k: $t_R = 7.8$ min; LCMS (ES): calcd for $C_{160}H_{184}Cl_4N_{20}O_{52}$ $[M+2H]^+$: 1681.5, found 1681.7; $[M+3H]^+$: 1121.3, found 1121.5.

Kinetics of dimerization through olefin metathesis in the presence and absence of ligand (Figure 7): Olefinic vancomycin analogue **7-(LeuNMe)C$_2$** (6.0 mg, 3.72 μmol) was dissolved in degassed H_2O (6.0 mL). To this solution was added $C_{12}H_{25}NMe_3Br$ (5.5 mg, 18.2 μmol). This solution was split into three equal parts (2.0 mL each) and to each solution was added either H_2O (200 μL), a solution of Ac-D-Ala-D-Ala (49 μg in 200 μL of H_2O), or a solution of Ac_2-L-Lys-D-Ala-D-Ala (90 μg in 200 μL of H_2O). To these individual, vigorously stirred solutions was added, dropwise, a solution of Grubbs' catalyst $[(PCy_3)_2Ru(CHPh)Cl_2]$ (181 μg) in CH_2Cl_2 (200 μL) at ambient temperature. After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). The final concentrations of the components in the reaction mixture were as follows: **7-(LeuNMe)C$_2$** (550 μM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), ligand (110 μM), and $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 μM). The reactions were monitored by HPLC (LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100% CH_3CN (0.05% TFA) in H_2O (0.05% TFA) over 10 min, with detection at 254 nm) at the following time intervals: 12, 36, 72, and 144 h. The percentage of dimer formed was plotted against time as shown in Figure 7. The kinetic experiment with vancomycin analogue **7-(LeuNMe)C$_4$** (Figure 7b) was performed under identical conditions and the analysis performed at the following time intervals: 12, 24, 36, and 60 h.

Target-accelerated combinatorial synthesis (length selective dimerization, Scheme 5): An equimolar mixture of three vancomycin analogues **7-(LeuNMe)C$_2$** (644 μg, 0.4 μmol), **7-(LeuNMe)C$_3$** (650 μg, 0.4 μmol), and **7-(LeuNMe)C$_4$** (655 μg, 0.4 μmol) was dissolved in degassed H_2O (1.8 mL). To this solution was added $C_{12}H_{25}NMe_3Br$ (1.7 mg, 5.56 μmol). This reaction mixture was split into two equal parts (900 μL each). To one part was added H_2O (100 μL) and to the other was added a solution of Ac_2-L-Lys-D-Ala-D-Ala (45 μg in 100 μL of H_2O). To these individual, vigorously stirred solutions was added, dropwise, a solution of Grubbs' catalyst $[(PCy_3)_2Ru(CHPh)Cl_2]$ (98 μg) in CH_2Cl_2 (200 μL) at ambient temperature. After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). The final concentrations of the components in the reaction mixture were as follows: **7-(LeuNMe)C$_2$** (200 μM), **7-(LeuNMe)C$_3$** (200 μM), **7-(LeuNMe)C$_4$** (200 μM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), ligand (120 μM), and $[(PCy_3)_2Ru(CHPh)Cl_2]$ (120 μM). After stirring for 24 h, the reaction mixtures were examined by MS (Hewlett Packard 1100 Series, electrospray). The z^{+3} region of the mass spectrum is depicted in Figure 8.

Target-accelerated combinatorial synthesis (binding selective dimerization, Scheme 6): An equimolar mixture of two vancomycin analogues **7-(LeuNMe)C$_2$** (866 μg, 0.55 μmol) and **7-(β-Ala)C$_2$** (855 μg, 0.55 μmol) was dissolved in degassed H_2O (1.8 mL). To this solution was added $C_{12}H_{25}NMe_3Br$ (1.7 mg, 5.56 μmol). This reaction mixture was split into two equal parts (900 μL each). To one part was added H_2O (100 μL) and to the other was added a solution of Ac_2-L-Lys-D-Ala-D-Ala (41 μg in 100 μL of H_2O). To these individual, vigorously stirred solutions was added, dropwise, a solution of Grubbs' catalyst $[(PCy_3)_2Ru(CHPh)Cl_2]$ (90 μg) in CH_2Cl_2 (200 μL) at ambient temperature. After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). The final concentrations of the components in the reaction mixture were **7-(LeuNMe)C$_2$** (275 μM), **7-(β-Ala)C$_2$** (275 μM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), ligand (110 μM), and $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 μM). After stirring for 24 h, the reaction mixtures were examined by MS (Hewlett Packard 1100 Series, electrospray). The z^{+3} region of the mass spectrum is depicted in Figure 9. The experiments summarized in Table 8 were conducted under identical conditions.

Target-accelerated combinatorial synthesis (orientation selective dimerization, Scheme 7): An equimolar mixture of the two vancomycin analogues **11** (901 μg, 0.55 μmol) and **12** (885 μg, 0.55 μmol) was dissolved in degassed H_2O (1.8 mL). To this solution was added $C_{12}H_{25}NMe_3Br$ (1.7 mg, 5.56 μmol). This reaction mixture was split into two equal parts (900 μL each). To one part was added H_2O (100 μL) and to the other was added a solution of Ac_2-L-Lys-D-Ala-D-Ala (41 μg in 100 μL H_2O). To these individual, vigorously stirred solutions was added, dropwise, a solution of Grubbs' catalyst $[(PCy_3)_2Ru(CHPh)Cl_2]$ (90 μg) in CH_2Cl_2 (200 μL) at ambient temperature. After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). The final concentrations of the components in the reaction mixture were as follows: **11** (275 μM), **12** (275 μM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), ligand (110 μM), and $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 μM). After stirring for 24 h, the reaction mixtures were examined by MS (Hewlett Packard 1100 Series, electrospray). The z^{+3} region of the mass spectrum is depicted in Figure 10.

Target-accelerated combinatorial synthesis (orientation selective dimerization, Scheme 8): An equimolar mixture of the two vancomycin analogues **13** (885 μg, 0.55 μmol) and **7-(LeuNMe)C$_4$** (901 μg, 0.55 μmol) was dissolved in degassed H_2O (1.8 mL). To this solution was added $C_{12}H_{25}NMe_3Br$ (1.7 mg, 5.56 μmol). This reaction mixture was split into two equal parts (900 μL each). To one part was added H_2O (100 μL) and to the other was added a solution of Ac_2-L-Lys-D-Ala-D-Ala (41 μg in 100 μL of H_2O). To these individual, vigorously stirred solutions was added, dropwise, a solution of Grubbs' catalyst $[(PCy_3)_2Ru(CHPh)Cl_2]$ (90 μg) in CH_2Cl_2 (200 μL) at ambient temperature. After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). The final concentrations of the components in the reaction mixture were as follows: **13** (275 μM), **7-(LeuNMe)C$_4$** (275 μM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), ligand (110 μM), and $[(PCy_3)_2Ru(CHPh)Cl_2]$ (110 μM). After stirring for 24 h, the reaction mixtures were examined by MS (Hewlett Packard 1100 Series, electrospray). The z^{+3} region of the mass spectrum is depicted in Figure 11.

Target-accelerated combinatorial synthesis (eight component competition, Scheme 9): An equimolar mixture of eight vancomycin analogues [**7-(LeuNMe)C$_2$** (708 μg, 0.44 μmol), **7-(LeuNMe)C$_4$** (721 μg, 0.44 μmol), **7-(β-Ala)C$_2$** (684 μg, 0.44 μmol), **7-(β-Ala)C$_4$** (696 μg, 0.44 μmol), **7-(Asn)C$_2$** (703 μg, 0.44 μmol), **7-(Asn)C$_4$** (715 μg, 0.44 μmol), **7-(H)C$_2$** (652 μg,

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0.44 µmol), and **7-(H)C$_4$** (664 µg, 0.44 µmol)] was dissolved in degassed H_2O (3.6 mL). To this solution was added $C_{12}H_{25}NMe_3Br$ (3.3 mg, 10.9 µmol). This reaction mixture was split into two equal parts (1.8 mL each). To one part was added H_2O (200 µL) and to the other was added a solution of Ac_2-L-Lys-D-Ala-D-Ala (261 µg in 200 µL H_2O). To these individual, vigorously stirred solutions was added, dropwise, a solution of Grubbs' catalyst [$(PCy_3)_2Ru(CHPh)Cl_2$] (289 µg) in CH_2Cl_2 (200 µL) at ambient temperature. After complete addition, argon was purged through the system until only a trace of CH_2Cl_2 remained (≈20 min). The final concentrations of the components in the reaction mixture were as follows: **7-(LeuNMe)C$_2$** (110 µM), **7-(LeuNMe)C$_4$** (110 µM), **7-(β-Ala)C$_2$** (110 µM), **7-(β-Ala)C$_4$** (110 µM), **7-(Asn)C$_2$** (110 µM), **7-(Asn)C$_4$** (110 µM), **7-(H)C$_2$** (110 µM), and **7-(H)C$_4$** (110 µM), $C_{12}H_{25}NMe_3Br$ (2.75 mM), ligand (176 µM), and [$(PCy_3)_2Ru(CHPh)Cl_2$] (176 µM). After stirring for 24 h, the reaction mixtures were examined by LCMS [LiChrospher C18, 6 mm × 250 mm, flow rate 1.0 mL min^{-1}, 0 → 100% CH_3CN (0.05% TFA) in H_2O (0.05% TFA) over 10 min, with detection at 254 nm] specifically observing the z^{+3} region (Hewlett Packard 1100 Series, electrospray). From these data, the relative amounts of each dimer were determined taking into account the expected statistical abundance for each compound [i.e., the abundance of heterodimers was halved due to the occurrence of two equivalent combinations for each, e.g. **8-(LeuNMe)C$_2$-(LeuNMe)C$_4$** is equivalent in mass to **8-(LeuNMe)C$_4$-(LeuNMe)C$_2$**]. The average result of three experiments are plotted in Figure 12.

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