Combinatorial Library Approach for the Identification of Synthetic Receptors Targeting Vancomycin-Resistant Bacteria

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The glycopeptide antibiotic vancomycin is active against Gram-positive bacteria and is the drug of choice for the treatment of serious infections due to many methicillin-resistant strains including *Staphylococcus aureus* strains and multiply resistant strains of *Streptococcus pneumoniae*.[1] The emergence of global clinical resistance to vancomycin has clear, serious clinical consequences.[1] Vancomycin exerts its main bactericidal effect through inhibition of cell wall peptidoglycan cross-linking by binding to the terminal L-Lys-D-Ala-D-Ala[2] of the mucopeptide precursor units at the crucial site of attachment. Resistance is effected by the biosynthesis of an altered cell wall precursor ending in L-Lys-D-Ala-D-Lac.[3] Replacement of the terminal D-alanine with D-lactate introduces a repulsive electrostatic interaction in place of a hydrogen bond resulting in a ~1000-fold reduction in binding affinity (Figure 1).[4]

The identification of synthetic receptors that bind with high affinity to L-Lys-D-Ala-D-Lac could provide a powerful strategy for overcoming vancomycin resistance.[5] However, the design of synthetic receptors that bind molecules in *aqueous* solution is a daunting task, and only minimal success has previously been achieved.[6] Herein, we report synthetic receptors that bind L-Lys-D-Ala-D-Lac greater than 5-fold more tightly than vancomycin in aqueous solution. This work not only has resulted in the most potent synthetic receptor toward this target but also represents the first successful application of receptor library synthesis and screening for the identification of synthetic receptors that bind small molecules in aqueous solution.

In our receptor design strategy we chose to preserve the right-hand carboxylate binding pocket of vancomycin in order to retain a significant element of the hydrogen-bonding network and hydrophobic interactions that are common to the D-Ala-D-Lac and D-Ala-D-Ala complexes.[7] The left-hand side of the molecule is replaced with a variable tripeptide unit (Figure 2). Because the tripeptide is acyclic, free rotation should eliminate the unfavorable electrostatic interaction observed between the phenylglycine carbonyl of vancomycin and the oxygen of the lactate group. The amino acid side chains and stereochemistries in the tripeptide unit would then be combinatorially selected for their ability to enhance binding to L-Lys-D-Ala-D-Lac and L-Lys-D-Ala-D-Ala.

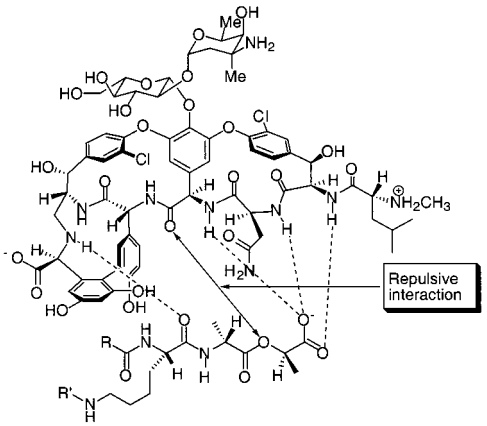

Figure 1. Repulsive electrostatic interaction in vancomycin binding to L-Lys-D-Ala-D-Lac.

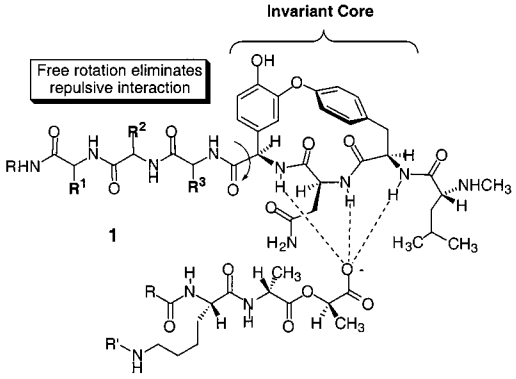

Figure 2. Design of synthetic receptor **1**.

The vancomycin E-ring chlorine and sugar residues are not incorporated into **1** in order to expedite receptor library synthesis. Binding studies on vancomycin derivatives lacking these groups have established that the E-ring chlorine[8] and the sugar residues[9] each contribute less than 2- to 3-fold to the binding of vancomycin to *N*-Ac_2-L-Lys-D-Ala-D-Ala. The invariant core macrocycle is employed as a fixed building block in the receptor library synthesis and therefore is prepared in solution (Scheme 1). We selected the thallium(III) trinitrate (TTN)-mediated oxidative cyclization strategy to synthesize the key biaryl ether linkage because of the success demonstrated by Yamamura[10] and Evans[11] and more importantly, because of the ready availability of the amino acid starting materials. Indeed, tripeptide cyclization precursor **2** can be prepared from commercially available material in five linear steps and 60% overall yield on tens of gram scale

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(1) Tomasz, A. *N. Engl. J. Med.* **1994**, *330*, 1247. (b) Leclercq R.; Courvalin P. *Clin. Infect. Dis.* **1997**, *24*, 545.
(2) Barne, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* **1984**, *38*, 339.
(3) Walsh, C. T.; Fisher, S. L.; Park, I. S.; Prahalad, M. *Chem. Biol.* **1996**, *3*, 21.
(4) For related electrostatic repulsive interactions, see: (a) Morgan, B. P.; Scholtz, J. M.; Ballinger, M. D.; Zipkin, I. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991**, *113*, 297.
(5) The total synthesis of vancomycin aglycon has recently been achieved. For leading references, see: (a) Evans, D. A.; Dinsmore, C. J.; Watson, P. S.; Wood, M. R.; Richardson, T. I.; Trotter, B. W.; Katz, J. L. *Angew. Chem., Int. Ed.* **1998**, *37*, 2704. (b) Nicolaou, K. C.; Takayanagi, M.; Jain, N. F.; Natarajan, S.; Koumbis, A. E.; Bando, T.; Ramanjulu, J. M. A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2717.
(6) (a) Hinzen, B.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **1996**, *79*, 942. (b) Nesloney, C. L.; Kelly, J. W *J. Am. Chem. Soc.* **1996**, *118*, 5836. (c) Peczuh, M. W.; Hamilton, A. D.; Sanchez-Quesada, J.; de Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327. (d) Torneiro, M.; Still, W. C. *Tetrahedron* **1997**, *53*, 8739. (e) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208. (f) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536.
(7) Williamson, M. P.; Williams, D. H.; Hammond, S. J. *Tetrahedron* **1984**, *40*, 569.
(8) Harris, C. M.; Kannan, R.; Kopicka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1985**, *107*, 6652.
(9) Kannan, R.; Harris, C. M.; Harris, T. M.; Waltho, J. P.; Skelton, N. J.; Williams, D. H. *J. Am. Chem. Soc.* **1988**, *110*, 2946. Upon identification of active receptors, the sugars could be reattached for cell targeting and interactions with the bacterial cell wall.
(10) Suzuki, Y.; Nishiyama, S.; Yamamura, S. *Tetrahedron Lett.* **1990**, *31*, 4053.
(11) Evans, D. A.; Ellman, J. A.; DeVries, K. M. *J. Am. Chem. Soc.* **1989**, *111*, 8912.

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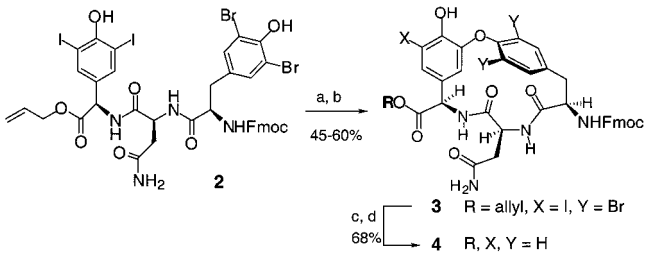
Scheme 1[a]



[a] (a) TTN, pyridine, MeOH, dioxane; (b) Zn; (c) $Pd(PPh_3)_4$, Bu_3SnH; (d) $NaBH_4$, $Ni(OAc)_2$.

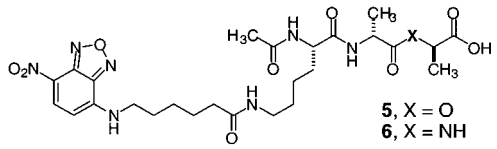


Figure 3. Fluorophore-labeled ligands.

without chromatography. Oxidative cyclization of **2** followed by in situ reduction with zinc metal provides macrocycle **3** in moderate overall yields. Removal of the allyl ester of **3** under standard conditions[12] followed by hydrodehalogenation using nickel(II) acetate and sodium borohydride[13] provides the suitably protected core macrocycle **4** in 68% overall yield.

A library of 39 304 theoretical members was prepared by split synthesis with 34 amino acid inputs used to introduce diversity at the R^1, R^2, and R^3 positions. The amino acids were selected largely on the basis of the side chain display found in the proteinogenic amino acids; however they also included a number of constrained amino acids to enhance the rigidity of the receptors.[14] An "on bead" assay[15] was used to detect the most active library members by incubation of the support-bound library with fluorophore labeled depsipeptide **5** or tripeptide **6** (Figure 3). The nitrobenzodioxazole (NBD) label was selected over other fluorophores due to its small size and relatively hydrophilic nature to minimize binding interactions with the support-bound receptors.[16] The label was attached to the Lys side chains to place the label as distantly as possible from the binding site. To determine the structures of the most active receptors, the stained beads were decoded using the matrix-assisted laser desorption ionization mass spectrometry sequencing method of Youngquist.[17]

Library screening was performed with 5 mM vancomycin as a competing receptor and with 0.2 mM **5** or **6**. Under these conditions only 0.1% of the beads were stained in screening experiments against **5** and **6**, with 59 and 106 beads picked and decoded, respectively.[18] Remarkable selectivity was observed. Of the 59 beads selected against ligand **5**, L-Tpi was observed at the first amino acid position for 39 beads (66%) and L-*N*-Me-Phe was observed for 12 beads (20%). High double combination consensus sequences were also observed (Table 1, entries 1 and 2). It is significant that screening against **6** also provided similar high consensus at the first amino acid position with L-Tpi observed for 61 out of 106 beads (57%). The double combination L-Tpi at amino acid 1 and L-His at amino acid 2 also had the highest frequency of occurrence (entry 3).

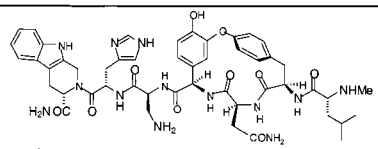
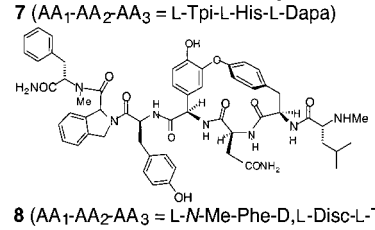
Microcalorimetry[19] was used to determine the binding affinity of the selected receptors to *N*-Ac_2-L-Lys-D-Ala-D-Lac and *N*-Ac_2-L-Lys-D-Ala-D-Ala (Table 2).[20] Receptor **7** is based upon the consensus sequence obtained for both ligands **5** and **6**, while receptor **8** was observed for **5**. Receptor **7** exhibits binding to tripeptide *N*-Ac_2-L-Lys-D-Ala-D-Ala that is only 6-fold less than that of vancomycin. These numbers are remarkable since the receptors are much less structurally complex than vancomycin, yet still retain most of vancomycin's binding affinity. *More significantly, each receptor showed significantly increased binding to N-Ac_2-L-Lys-D-Ala-D-Lac when compared to vancomycin.* Other synthetic receptors showed no binding by microcalorimetry (data not shown), thereby demonstrating that the tripeptide portion contributes considerably to the binding interaction.

Table 1. Sequences Selected by Ligands **5** and **6** in the Assay of the Synthetic Receptor Library[a]

					occ freq (%)	
entry	ligand	AA1	AA2	AA3	obsd	theor[b]
1	**5**	L-Tpi	L-His	X	29	0.087
2	**5**	L-*N*-Me-Phe	Disc	X	20	0.087
3	**6**	L-Tpi	L-His	X	15	0.087
4	**6**	L-Tpi	L-Lys	X	9	0.087
5	**6**	L-Tpi	L-*N*-Me-Gly	X	12	0.087
6	**6**	L-Dapa	L-Tpi	X	13	0.087
7	**6**	X	L-Tpi	L-Lys	9	0.087
8	**6**	X	L-Tpi	L-His	6	0.087
9	**6**	X	L-Tpi	L-Dapa	8	0.087

[a] For amino acid structures see Table 2 and Supporting Information. [b] Percent probability that a library bead will contain the indicated amino acid combination.

Table 2. Binding Constants, K_a (M^{-1}) of Synthetic Receptors Determined in Water by Microcalorimetry (ITC)

synthetic receptor	ligand binding constant (M^{-1})
 **7** (AA_1-AA_2-AA_3 = L-Tpi-L-His-L-Dapa)	*N*-Ac_2-L-Lys-D-Ala-D-Ala (K_a = 75,400 ± 9,000) *N*-Ac_2-L-Lys-D-Ala-D-Lac (K_a = 17,300 ± 3,400)
 **8** (AA_1-AA_2-AA_3 = L-*N*-Me-Phe-D,L-Disc-L-Tyr)	*N*-Ac_2-L-Lys-D-Ala-D-Ala (K_a = 102,400 ± 41,000) *N*-Ac_2-L-Lys-D-Ala-D-Lac (K_a = 31,900 ± 15,000)
Vancomycin	*N*-Ac_2-L-Lys-D-Ala-D-Ala (K_a = 550,000 ± 12,000) *N*-Ac_2-L-Lys-D-Ala-D-Lac (K_a = 6,300 ± 1,400)

Evaluation of the synthetic receptors for activity against vancomycin-resistant bacteria is in progress. The molecular basis of recognition will be explored as will additional rounds of library synthesis to identify even more potent receptors.

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Supporting Information Available: Full experimental procedures for the preparation of **4** and for library synthesis and screening (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Four, P.; Guibe, F. *Tetrahedron Lett.* **1982**, *23*, 1825.
(13) Stiles, M. *J. Org. Chem.* **1994**, *59*, 5381.
(14) See Supporting Information for the complete list of amino acids.
(15) For a review, see: Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155.
(16) Wennemers, H.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 6413.
(17) Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900.
(18) The sequences for all of the selected beads are included in the Supporting Information. Also included in the Supporting Information is the list of sequences for 50 beads selected at random. No consensus was observed.
(19) McPhail, D.; Cooper, A. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 2283 and references therein.
(20) Characterization of the binding potency of the selected receptors to *N*-Ac_2-L-Lys-D-Ala-D-Lac is necessary to establish that binding occurs with receptors in solution against unlabeled ligand.