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Design and synthesis of macrocycles active against vancomycin-resistant enterococci (VRE): the interplay between D-Ala-D-Lac binding and hydrophobic effect

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Abstract—A modified vancomycin binding pocket (D–O–E ring) incorporating a *CHNHCOR* function at the AA4 position is designed and synthesized. Potent bioactivities against both sensitive- and resistant-strain are found for some of these compounds (MIC 4 μg/mL against VREF). From this preliminary SAR studies, it was speculated that the D-Ala-D-Ala binding was required for this series of compounds since the corresponding des-leucine derivative is inactive. The presence of long aliphatic chain was important for the desired activities and such hydrophobic effect is specific as no beneficial effect is observed when the same aliphatic chain was attached to the other part of the molecule.

Vancomycin and teicoplanin are the drugs of the last resort for the treatment of infections due to methicillin-resistant *Staphylococcus aureus*.[1] The antibacterial activity of this family of glycopeptides arises from specific binding of these drugs to bacterial cell wall precursor terminating in the sequence D-Ala-D-Ala.[2] After more than 30 years of clinic use, resistance to drugs of the vancomycin family has been recognized at the late 1980's. Biosynthesis of a D-Ala-D-Lac depsipeptide and its incorporation as the terminal peptidoglycan of resistant bacteria has been proposed as the principal mechanism of resistance.[3] In fact, in vitro binding studies have shown that the affinity of vancomycin for *N*-Ac-D-Ala-D-Lac is about 1000 times lesser than its affinity for *N*-Ac-D-Ala-D-Ala, paralleling the 1000-fold reduced sensitivity of vancomycin-resistant bacteria to drug. Since vancomycin-resistant enterococci (VRE) also carry resistance to virtually all other known antibiotics, the prognosis for patients with such refractory infections is grim. This growing problem of resistance has rekindled multidisciplinary interest in this field resulting in the development of more active molecules[4] and new mechanistic insights regarding how to combat the VRE infections[5–7] (Fig. 1).

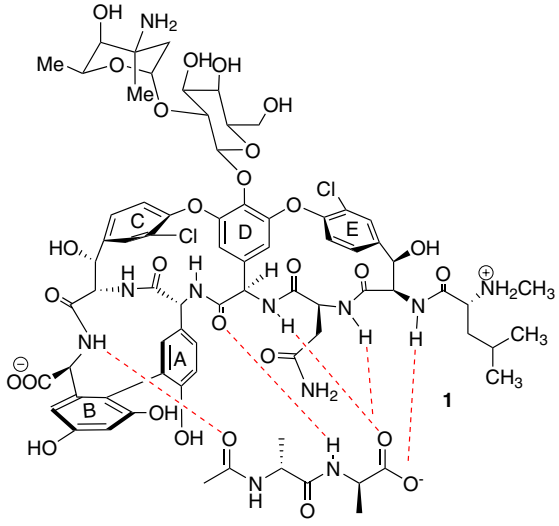

Figure 1. Vancomycin (**1**) and D-Ala-D-Ala complex.

Keywords: Antibiotic; Biaryl ether; Intramolecular S_NAr reaction; Macrocycle; Vancomycin type glycopeptide; Vancomycin-resistant enterococci (VRE).
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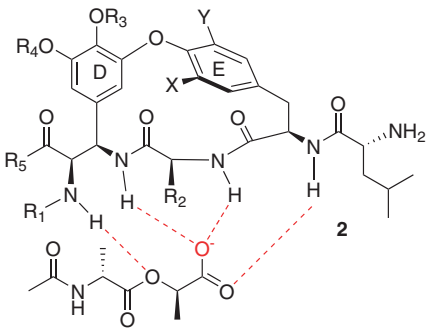
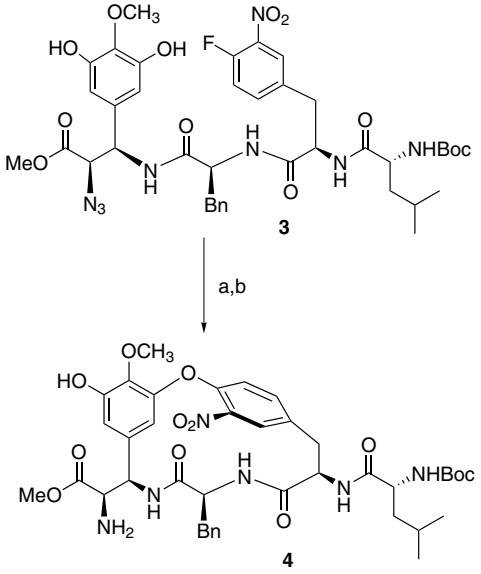


Figure 2. Generic structure of compound **2** and expected interaction with D-Ala-D-Lac.

In the search for synthetic analogs having enhanced affinity toward the D-Ala-D-Lac, we designed molecules of general structure **2** (Fig. 2) in which the carboxyl binding pocket of glycopeptide antibiotics is modified. We hypothesized that replacing the carbonyl group of the AA4 of vancomycin by a CH*NH*COR function can, a priori, lead to a compound with increased affinity toward *N*-Ac-D-Ala-D-Lac by restoring the missing hydrogen bond and by avoiding the unfavorable electronic repulsion found in the vancomycin and D-Ala-D-Lac complex. We describe in this communication synthesis and bioactivity evaluation of macrocycles **2** that led to the identification of compounds active against VRE. From the preliminary SAR studies, we postulate that combination of a modified binding pocket with a suitably positioned hydrophobic chain could well be a useful design principle in the search of compounds active against VRE.

Scheme 1 illustrates the key steps for the synthesis of the parent compound **4**. Reduction of azide (**3**) with triphenylphosphine followed by an intramolecular S_NAr reaction[8] led to the formation of 16-membered biaryl ether containing macrocycle **4** in over 65% yield as a single isolable atropisomer whose axial chirality (*P*) was determined by NOE studies.[9]



Scheme 1. (a) Ph_3P, THF–H_2O; (b) CsF, DMSO, 0.01 M, room temperature.

From compound **4**, a series of macrocycles **2a**–**g** and **2i**,**j** were synthesized (Fig. 3). Compound **2e** was obtained by thermal atropisomerization of **2d** (150 °C, DMSO, 1/1 ratio). Glycosylation of phenol with *N*-acyl aminoglucose was found to be more difficult than with the glucose derivative.[10] The desired transformation was finally realized in good yield with the freshly prepared *N*-acyl glucosaminyl bromide under mild phase transfer conditions (Scheme 2). Compound **2h** and the desleucyl derivative **2l** were synthesized by similar cycloetherification strategy.

Minimum inhibitory concentrations for compounds **2a**–**l** are measured using a standard microdilution assay and the results are summarized in Table 1. As can be seen, the macrocycles **2a** and **2b** with two amino functions

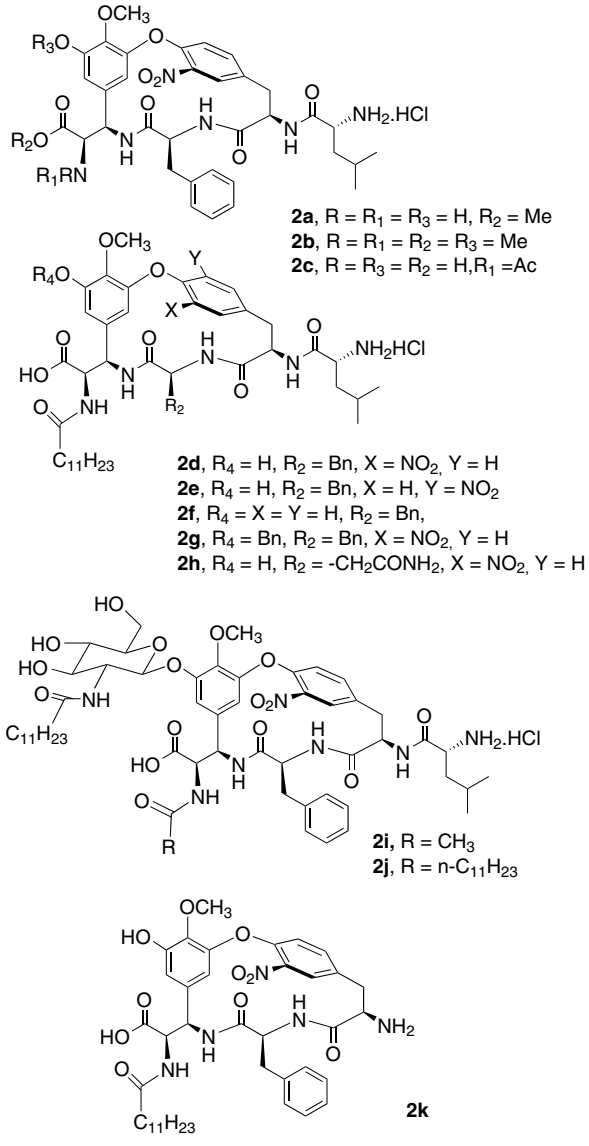
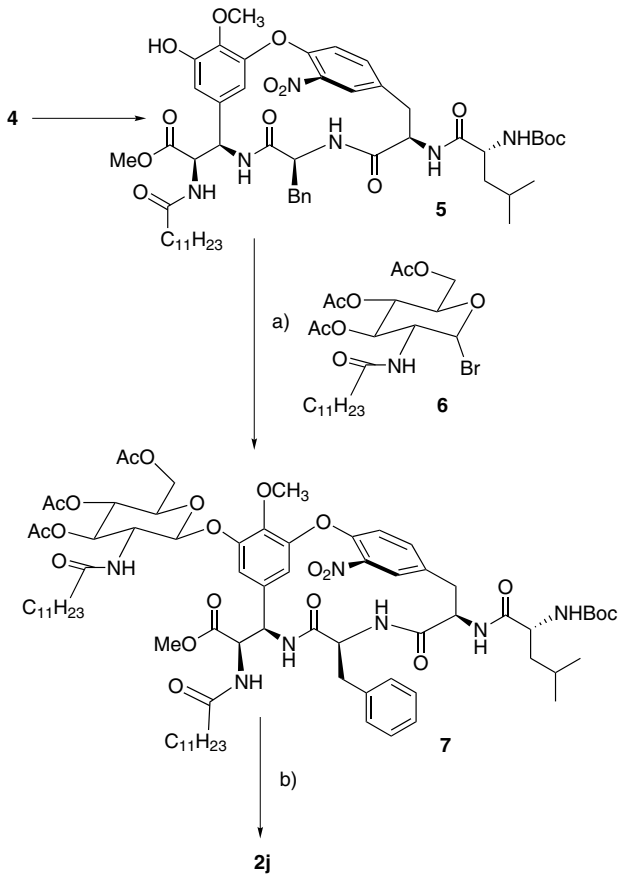


Figure 3. Structure of macrocycles.



Scheme 2. (a) 10% Na_2CO_3, $^nBu_4NHSO_4$, CH_2Cl_2–H_2O, 75%; (b) LiOH, THF–H_2O, then 10% conc HCl in CH_3CN, 85%.

were inactive against both sensitive and resistant bacterial strains, neither was the *N*-acetylated derivative **2c**. However, the lauroyl amide (dodecanoyl) **2d** was active against VRE indicating the important role of hydrophobic interaction for the anti-VRE activity (entry 4). Compound **2h** having an asparagine unit, instead of phenylalanine as the third AA residue, was slightly less active than **2d**. Based on these results, further structural ramification was performed on **2d**. It was observed that the planar chirality (**2d** vs **2e**) played only minor effect on the bioactivity (entries 4, 5). However, the presence of the nitro group at the E ring is beneficial since its removal leads to an inactive compound **2f** (entry 6). The activity against VRE remained, within experimental error, essentially unchanged upon benzylation (**2g**) and glucosylation of the phenol function (**2j**, entry 7, 10). Des-leucyl derivative **2k** was inactive (entry 12) indicating the important role played by the binding pocket for the activity of **2d**.

The very interesting anti-VRE activity of macrocycle **2d** is intriguing. Indeed in vitro, it is more active against VRE than most of the vancomycin and teicoplanin derivatives reported to date and it is almost as active as Synercid®, a clinically used drug for combating VRE. The fact that **2k** with a damaged binding pocket is inactive suggested that D-Ala-D-Lac binding may be important for the observed activity of **2d**. The role of the hydrophobic chain may be attributed to its ability to anchor the molecule to membrane in the vicinity of both lipid II and the transglycosylase and transpeptidase enzymes, blocking consequently more efficiently the last stages of peptidoglycan assembly.[11] Importantly, such a hydrophobic effect seems to be specific as no beneficial effect is observed when the same aliphatic chain was introduced to the other part of the molecule (**2i**). Further investigations are required in order to elucidate the exact mode of action of this series of compounds.

The principal mechanism of vancomycin resistance has been fully decoded and compounds active against VRE have been synthesized. However the mode of action of these active compounds is still unclear and a second mechanism, besides steric blockage by substrate

Table 1. MICs (μg/mL) of macrocycles **2a**–**l** and reference compounds[a]

Entry	Compd	*E. Faecium*		*E. Faecalis*		*Staph. Aureus*[f]
		Sensitive[b]	Resistant[c]	Sensitive[d]	Resistant[e]	
1	**2a**	>1024	>1024	512	512	>1024
2	**2b**	>128	>128	128	128	>128
3	**2c**	>1024	>1024	>1024	>1024	>1024
4	**2d**	**128**	**8**	**4**	**4**	**64**
5	**2e**	>128	16	8	8	>128
6	**2f**	>128	>128	128	128	>128
7	**2g**	256	16	8	4	128
8	**2h**	128	>128	16	8	64
9	**2i**	>1024	1024	64	64	>1024
10	**2j**	256	32	8	8	>256
11	**2k**	128	>128	128	>128	>128
12	Vanco	2	>128	1	>128	1
13	Teico	0.5	>128	0.125	64	1
14	Synercid	4	4	4	8	1

[a] MICs = minimum inhibitory concentrations.
[b] Bacterial strain L568 (isogenic of L569).
[c] Bacterial strain L2215 clin. isolate Van-A.
[d] Bacterial strain L559 (isogenic of L560).
[e] Bacterial strain L560.
[f] Bacterial strain L613 clin. isolate Met-R.

binding, involving a direct interaction with proteins critical for VRE cell-wall biosynthesis has been advanced. Indeed, the importance of substrate binding (D-Ala-D-Ala) for reversing VRE has been questioned since the original contribution of Kahn and his co-workers. The fact that certain vancomycin and teicoplanin derivatives with a damaged binding pocket (des-leucine derivative) are still active against VRE supports this late hypothesis.[12] Previously, introduction of suitably functionalized disaccharide into the natural products, dimerization or polymerization of vancomycin and its simplified analogs have been successfully applied for the identification of anti-VRE compounds. We demonstrated in the present study that a combination of a modified binding pocket with a suitably positioned hydrophobic chain could be an alternative working direction. As a proof of concept, it is demonstrated that the modified D–O–E ring of vancomycin can serve as a template, to a certain degree even more effectively than the entire glycopeptide framework, for the development of active compounds against VRE. Binding studies as well as further structural optimization are in progress.

Acknowledgements

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12. However, this statement cannot be generalized. Depending on the structure of sugar and the position of the hydrophobic chain, the presence of an intact peptide binding pocket is required in order for the compound to display the desired anti-VRE activity, see Ref. 11a.