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Identification of synthetic compounds active against VRE: the role of the lipidated aminoglucose and the structure of glycopeptide binding pocket

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Abstract—A modified vancomycin binding pocket (D–O–E ring) incorporating an α-hydroxy-β-amino acid at the AA4 position is designed and synthesized. Some of these compounds display potent bioactivities against both sensitive- and resistant-strains (8 μg/ml against VREF). Both the lipidated aminoglucose and the structure of the 16-membered macrocycle are found to be important for the anti-VRE activities. The polyamine appendage at the C-terminal, on the other hand, improved the activity against vancomycin-sensitive strains.
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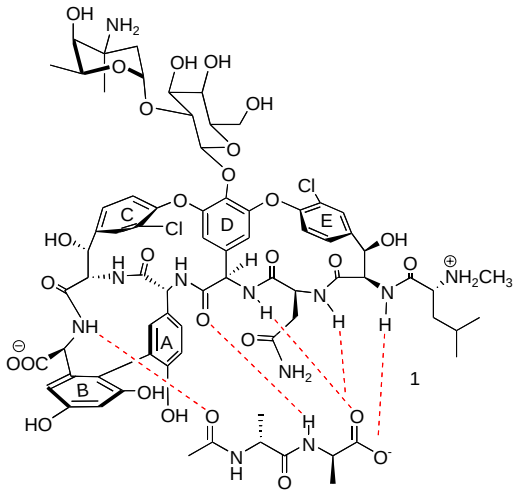
Vancomycin (**1**) and teicoplanin are two drugs used worldwide for the treatment of infections due to methicillin-resistant *Staphylococcus aureus* and other Gram-positive organisms in patients allergic to β-lactam antibiotics.[1] They act by binding to the D-Ala-D-Ala dipeptide of peptidoglycan precursors, preventing maturation of the bacterial cell-wall.[2] After more than 30 years of clinical use, resistance to drugs of the vancomycin family has been recognized in the late 1980s and the frequency of resistance has increased significantly over the past decade, reaching 30% among hospitalized patients in 2000 in the USA. Since vancomycin-resistant enterococci (VRE) also carry resistance to virtually all other known antibiotics, it represents thus a serious threat to public health. Reprogramming of the peptidoglycan termini from D-Ala-D-Ala dipeptide to D-Ala-D-Lac depsipeptide 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, due to one missing hydrogen bond and the ground state repulsion between the two oxygen lone-pairs in the former complex.[4,5] The reduced binding affinity translated into the 1000-fold reduced sensitivity of vancomycin-resistant bacteria to drug (Fig. 1).



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

Figure 1. Structure of vancomycin and its *N*-Ac-D-Ala-D-Ala complex.

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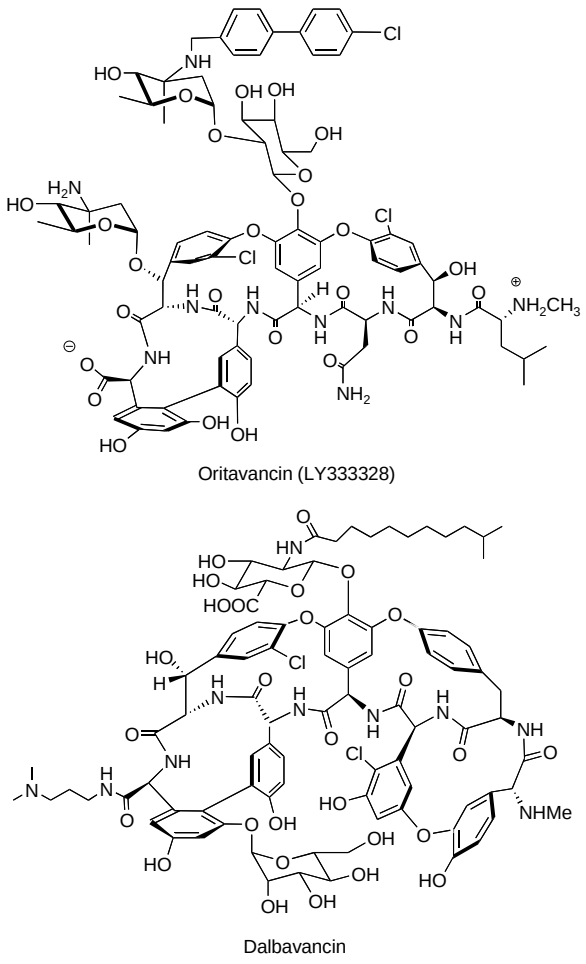


Figure 2. Second-generation semi-synthetic lipoglycopeptides in clinic development.

Extensive structure–activity relationship studies performed by both academic and industrial researchers indicated that the incorporation of a hydrophobic chain into the natural product is highly beneficial for activities against VRE. Indeed, two semi-synthetic analogues of glycopeptides: oritavancin (LY333328) and dalbavancin (Fig. 2) that entered into late-stage clinical trials contain a hydrophobic group.[6] However, given the architectural complexity of vancomycin family glycopeptide, the structural modification of the natural products is particularly challenging and to date most of the chemical transformations have been localized on the peripheral of the macrocycles relying mainly on the simple chemical reactions. Indeed, it would be extremely difficult, if it is not impossible, to reengineer the carboxylate-binding pocket (D–O–E ring) of natural glycopeptides to include new hydrogen bond contacts with the modified peptidoglycan termini.[7] Therefore, the minimum struc-

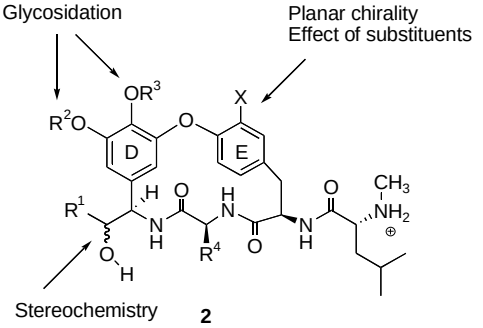
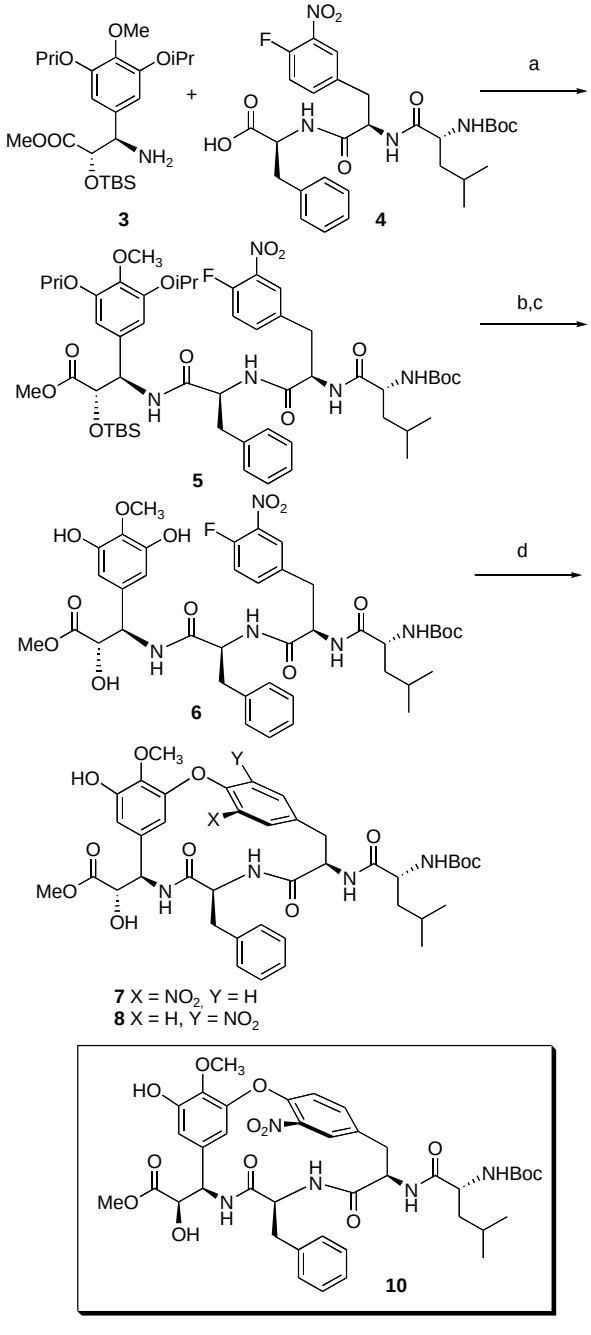


Figure 3. Generic structure of modified carboxylate-binding pocket of glycopeptides.



Scheme 1. Synthesis of 16-membered *meta*,*para*-cyclophane with an *endo* aryl–aryl ether bond. Reagents and conditions: (a) EDC, HOBT, CH_2Cl_2, room temperature, 89%; (b) BCl_3, CH_2Cl_2, 0 °C, then MeOH; (c) $NaHCO_3$, Boc_2O, dixoane–H_2O (1:1), room temperature, 81% (over two steps); (d) CsF, DMSO, room temperature, 72%. TBS = *tert*-butyldimethylsilyl, Boc = *tert*-butoxycarbonyl, EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, HOBT = 1-hydroxybenzotriazole, DMSO = dimethyl sulfoxide.

ture required to carry the hydrophobic substituent remained unknown.[8] A notable exception is the work of Ellman who has synthesized a combinatorial library of 16-member macrocycles having different tripeptide appendage at the C-terminal and identified synthetic receptors that bind to the *N*-Ac_2-L-Lys-D-Ala-D-Ala.[9] We have recently described the synthesis and biological activity evaluation of modified D–O–E ring bearing a NHCOR group at AA4 position.[10,11] As a logical extension of this work, we report herein an efficient synthesis of a modified carboxylate-binding pocket of vancomycin (Fig. 3, generic structure 2) in which the fourth amino acid was replaced by an α-hydroxy-β-amino acid and demonstrate that both the structure of the macrocycle and the presence of a lipidated aminoglucose are important for anti-VRE activity. We also document that compound **2f** can serve as a useful template, to a certain degree even more effectively than the entire glycopeptide framework, in searching for the active compounds against both vancomycin-sensitive strains and VRE.

Synthesis of the parent compounds **7**, **8** and **10** is summarized in Scheme 1. Coupling of α-hydroxy-β-amino acid (**3**)[12] with tripeptide **4** under standard conditions afforded tetrapeptide **5**. Treatment of **5** with BCl_3 caused deprotection of isopropyl ether, the *tert*-butyldimethylsilyl ether and the *N*-Boc function. Re-protection of the primary amine by *tert*-butoxycarbonlyation furnished the phenol **6** in 81% overall yield. The key S_NAr-based cycloetherification of **6** was performed in DMSO (concentration of substrate: 0.05 M) in the presence of CsF at room temperature to provide two separable atropisomers **7** and **8** in 87% overall yield (ratio **7/8** = 3/1).[13] The absolute configuration of the planar chirality of **7** and **8** was deduced by NOE studies.[14] Concurrently, starting from (2*R*,3*R*) methyl 2-*tert*-butyldimethylsilyoxy-3-amino-3-(3′,5′-diisopropyloxy-

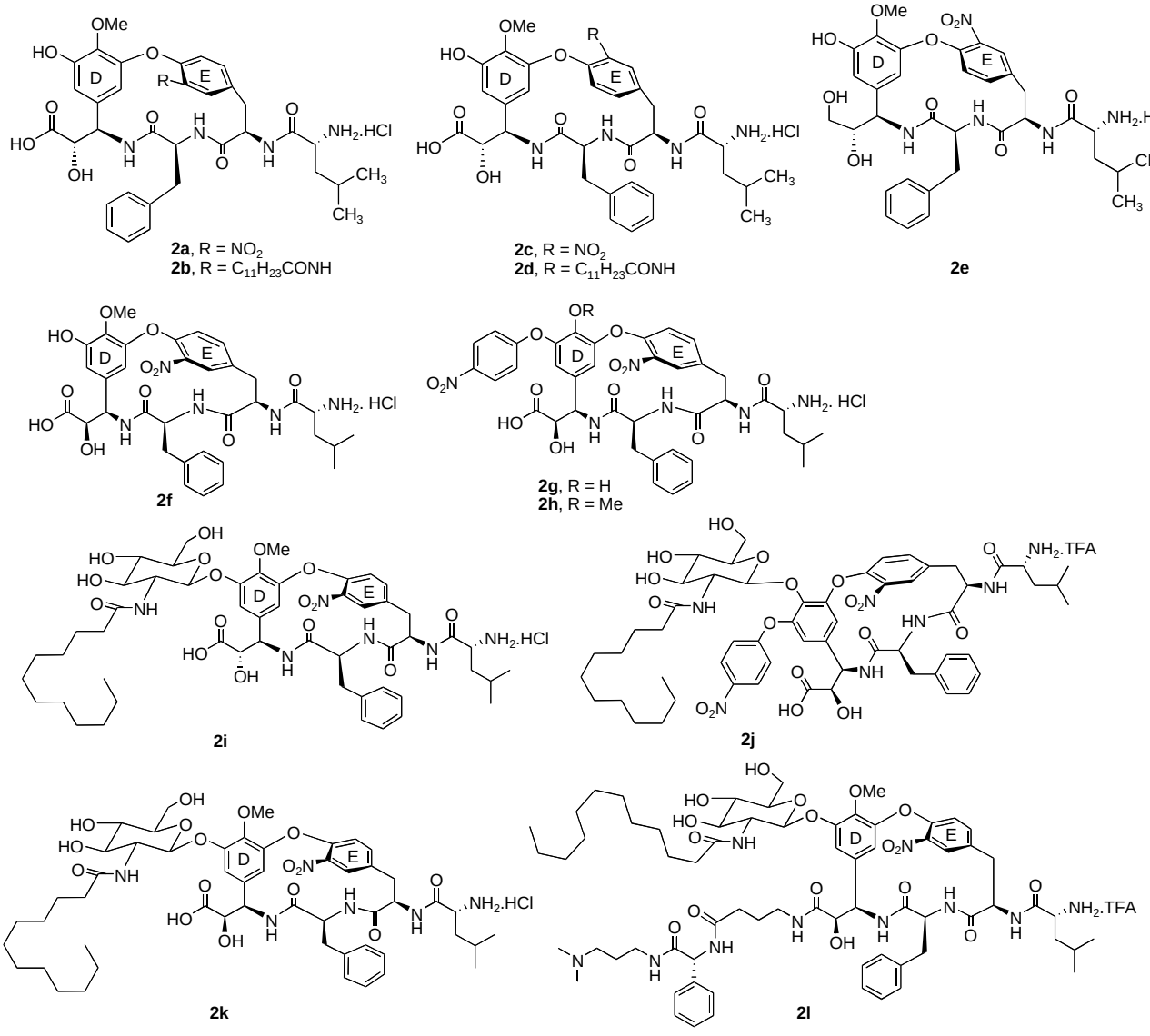
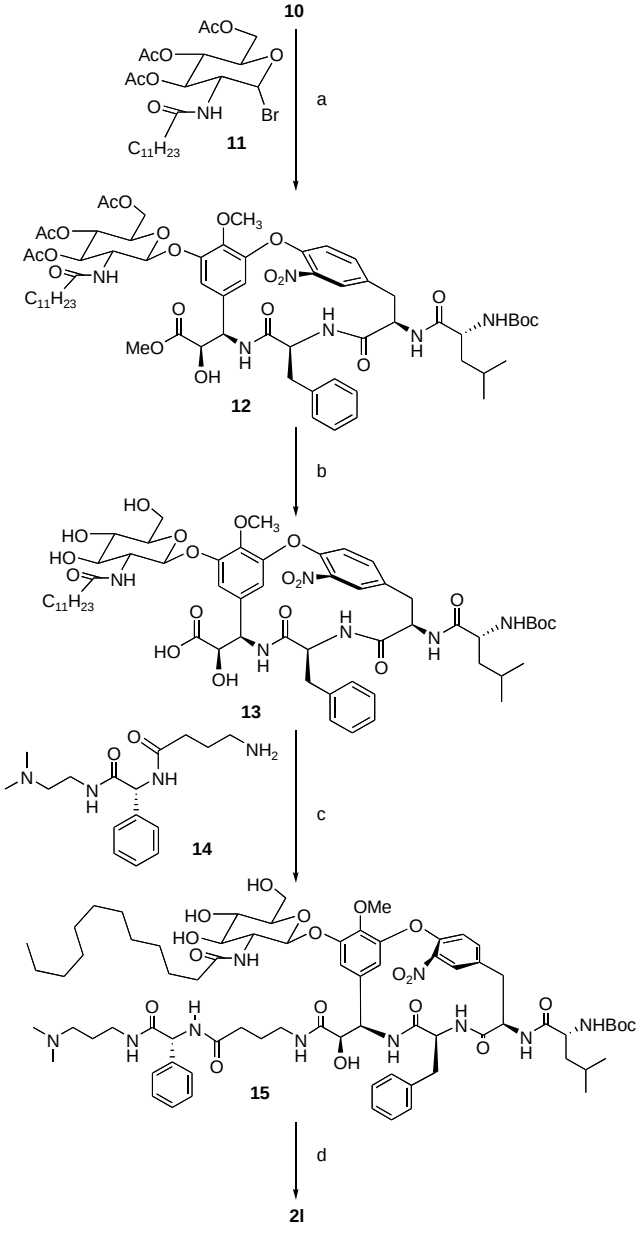


Figure 4. Structures of macrocycles.

4′-methoxyphenyl)propionate **9**, the macrocycle **10** was synthesized following the same synthetic scheme as detailed for **7** and **8**. From compounds **7**, **8** and **10**, a series of derivatives were synthesized and their structures are enlisted in Figure 4. While most of these compounds have been prepared without event, the access to compound **2l** was more demanding and was detailed in Scheme 2. Glycosylation of phenol was best realized with the freshly prepared *N*-acyl glucosaminyl bromide (**11**) under mild phase transfer conditions.[15] Saponification of **12** followed by coupling with polyamine (**14**) furnished **15**, which upon *N*-deprotection under mild acidic conditions provided **2l** in good overall yield. Compound **2l** was designed with the hope to introduce an additional H-bond following the vancomycin/*N*-Ac-D-Ala-D-Ala binding model.



Scheme 2. Synthesis of compound **2l**. Reagents and conditions: (a) **11**, 10% aqueous Na_2CO_3–CH_2Cl_2 (1:1), $(n\text{-}Bu)_4NHSO_4$, room temperature, 76%; (b) LiOH, THF–H_2O (3:1), 0 °C, 62%; (c) **14**, EDC, HOBT, CH_2Cl_2, room temperature, 25%; (d) CF_3CO_2H, CH_2Cl_2, 0 °C, 75%.

Minimum inhibitory concentrations for compounds **2a**–**l** as well as reference compounds: vancomycin and teicoplanin, are measured using a standard microdilution assay and part of these results are summarized in Table 1. Regardless of the absolute configuration of the secondary alcohol (**2a** vs **2f**), the planar chirality (**2a** vs **2c** and **2b** vs **2d**) of the cyclophane, macrocycles **2a**–**f** were inactive against both vancomycin-sensitive and vancomycin-resistant enterococci (VRE), with MICs being higher than 256 mg/mg (data not shown). The introduction of a hydrophobic chain at the E-ring did not improve the activities of these compounds neither (**2a** vs **2b** and **2c** vs **2d**). Upon arylation of the free phenol of the D-ring, weak antibiotic activities against VRE were observed for compounds **2g** and **h**, but remained insignificant. On the other hand, the *O*-glycosylated derivatives of **2a** and **f** displayed interesting and disparate results. While compound **2i** remained inactive, **2j** and **k** became active against VRE. Furthermore, compound **2l** having an elongated peptide chain at the C-terminal displayed a broad spectrum of activity against both vancomycin-sensitive (*S. aureus*) and resistant-strains.

From these preliminary structure–activity relationship studies, we assumed that both the structure of the macrocycle (carrier) and the hydrophobic substituent contributed to the activity of compounds **2k** and **2l**.[10] Glycosylation of **2a** with a lipidated aminoglucose did not provide the active compound. On the other hand, **2f** became active against VRE only upon attachment of the lipidated aminoglucose. Both phenoxy group can be served as anchoring points for the lipidated aminoglucose leading to active compounds (**2j** and **k**). Attaching a polyamide chain to the C-terminal of **2k** improved significantly its activity against vancomycin-sensitive strains, although its potency against VRE remained almost unchanged.

The very interesting anti-VRE activity of macrocycles **2k** and **l** is intriguing. Indeed in vitro, it is more active against VRE than most of the vancomycin and teicoplanin derivatives reported to date. The generic structure **2** was designed with the hope to restore the missing hydrogen bond with the D-Ala-D-lact depsipeptide by switching the amide carbonyl (hydrogen-bond acceptor) of vancomcyin's fourth amino acid into a hydroxy group (hydrogen-bond donor). However, attempt to measure the binding affinity between **2k** and *N*-Ac-D-Ala-D-Ala as well as **2k** and *N*-Ac-D-Ala-D-lactate by either UV absorption technique or by NMR titration (in DMSO) failed to provide any exploitable results due most probably to the low receptor–substrate affinities. Overall and in accord with Kahne and co-workers seminal contribution,[8,16,17] we hypothesized that compounds **2k** and **2l** might have a direct interaction with proteins critical for VRE cell-wall biosynthesis. The role of the lipidated aminoglucose may be attributed to its ability to anchor the molecule to membrane in the vicinity of both lipid II and the *trans*-glycosylases, blocking consequently more

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

Entry	Microorganism	2a	2f	2g	2h	2i	2j	2k	2l	Teico	Vanco
1	819 *S. aureus* Smith MSSA	>1024	>1024	>128	>128	>128	>128	512	256	1	1
2	613 *S. aureus* clin. isolate Met-R	>1024	>1024	>128	>128	>128	>128	128	32	1	1
3	3797 S.aureus clin. isolate VISA	>1024	>1024	64	16	>128	32	128	32	16	8
4	3798 *S. aureus* clin. isolate VISA	>1024	>1024	>128	>128	>128	>128	256	16	4	8
5	1400 *S. aureus* clin. isolate Met-R	>1024	>1024	>128	128	>128	>128	—	—	1	1
6	147 *S. epidermidis* ATCC 12228	>1024	>1024	>128	128	>128	>128	64	16	2	1
7	49 *S. pyogenes* C203	>1024	>1024	64	16	32	32	32	8	⩽0125	⩽0125
8	1139 *E. faecalis* teico–vanco-sensitive	>1024	>1024	>128	64	>128	32	32	32	⩽0.125	2
9	2981 *E. faecalis* clin. Isolate Van-A	>1024	>1024	—	—	>128	—	32	32	>128	>128
10	559 *E. faecalis* (isogenic of L 560)	>1024	>1024	64	16	>128	8	8	16	⩽0.125	1
11	560 *E. faecalis* Van-A	>1024	>1024	64	16	>128	8	8	8	128	>128
12	568 *E. faecium* (isogenic of L569)	>1024	>1024	>128	128	>128	64	64	16	⩽0.125	2
13	569 *E. faecium* clin. isolate Van-A	>1024	>1024	>128	128	>128	32	32	8	>128	>128
14	2215 *E. faecium* clin. isolate Van-A	>1024	>1024	—	—	>128	—	32	32	>128	>128

[a] MICs = minimum inhibitory concentrations.

efficiently the last stages of peptidoglycan assembly. Importantly, such a hydrophobic effect seems to be specific as no beneficial effect is observed when the same aliphatic chain was introduced to E-ring of the molecule (**2b** and **d**). The efficiency of the inhibitory interaction with *trans*-glycosylases will in turn depend on the structure of the carrier, in our case, the 16-membered macrocycle. The macrocycle **2f** with an external *R*-configured secondary alcohol being more active than **2a**, we surmised that either the hydroxy group was involved in the interaction with the *trans*-glycosylases or the stereochemistry of the secondary alcohol modified the overall conformation of the molecule making it more or less efficient in interacting with the enzymes.

In conclusion, we demonstrated in the present study that a combination of a modified binding pocket with a suitably positioned hydrophobic chain constitutes a viable approach in the searching for compound active against VRE and that these two structural elements contribute synergistically the activity of a given compound. While the positive hydrophobic effect is well known in the field, we demonstrated that a simple macrocycle (**2f**) can serve as a useful template for the development of agents active against VRE as well as against vancomycin-sensitive strains. We also documented that the stereochemistry of the peptide backbone can significantly influence the efficiency of the macrocycle as a carrier of the hydrophobic chain, which is understandable if the enzyme–substrate interaction was to be considered.

Acknowledgments

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