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Vancomycin resistance: Modeling backbone variants with D-Ala-D-Ala and D-Ala-D-Lac peptides

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This paper is dedicated to Professor E.J. Corey with best wishes on the occasion of his 80th birthday.

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ABSTRACT

To seek vancomycin analogs with broader antibacterial activity, effects of backbone modifications for the aglycon **2** on binding with D-Ala-D-Ala- and D-Ala-D-Lac-containing peptides were investigated by Monte Carlo/free energy perturbation (MC/FEP) calculations. The experimental trend in binding affinities for **2** with three tripeptides was well reproduced. Possible modifications of the peptide bond between residues 4 and 5 were then considered, specifically for conversion of the O=C—NH linkage to $CH_2NH_2^+$ (**6**), FC=CH (**7**), HC=CH (**8**), and HN—C=O (**9**). The MC/FEP results did not yield binding improvements for **7**, **8**, and **9**, though the fluorovinyl replacement is relatively benign. The previously reported analog **6** remains as the only variant that exhibits improved affinity for the D-Ala-D-Lac sequence and acceptable affinity for the D-Ala-D-Ala sequence.

Vancomycin (**1**) is a potent glycopeptide antibiotic that is a treatment of last resort for infections caused by methicillin resistant *Staphylococcus aureus* (MRSA).[1,2] The mechanism of action of **1** is to halt cell-wall biosynthesis of Gram-positive bacteria by binding to the terminal D-Ala-D-Ala sequence of the peptidoglycan cell-wall precursors.[3,4] In the common strains of vancomycin-resistant enterococci, VanA and VanB, the terminal residues are reprogrammed to the depsipeptide sequence, D-Ala-D-Lac.[3] This replacement of the terminal peptide bond by an ester linkage decreases the antibiotic activity by a factor of 1000.[4]

To elucidate the Ala → Lac modification, Boger and co-workers studied the binding of **1** and vancomycin aglycon (VA, **2**) to a series of tripeptide cell-wall precursor mimics, including Ac_2-L-Lys-D-Ala-D-Ala (**3**), Ac_2-L-Lys-D-Ala-D-Lac (**4**), and Ac_2-L-Lys-D-Ala-D-Ket (**5**) (Fig. 1).[1] For both **1** and **2**, removal of the ligand's hydrogen-bond donor (**3** → **5**, amide to ketone) leads to a 10-fold decrease in binding affinity, and addition of the lone pair repulsion (**5** → **4**, ketone to ester) further decreases the binding 100-fold.[1] Motivated by the strength of the latter effect, Crowley and Boger synthesized the amine analog **6**, which has the C=O of residue 4 replaced by a methylene group (Fig. 1).[5] Relative to **2**, **6** yields a 40-fold increase in binding affinity for **4** but a 35-fold decrease in binding affinity for **3**; it has similar affinities for both peptides.

The NH → O ligand modification converts a hydrogen-bond (H-bond) donor to a weak H-bond acceptor at a critical site for binding. Substitutions that aim to accommodate the lactate linkage are likely not to be optimal for the D-Ala-D-Ala sequence. Nevertheless, following the success of Crowley and Boger,[5] the present computational studies were undertaken to seek additional productive backbone modifications and also to provide a quantitative understanding of the effects of the modifications at the molecular level. The computations focused on four VA analogs with the O=C—NH linkage between residues 4 and 5 replaced by $CH_2NH_2^+$ (**6**), *trans*-FC=CH (**7**), *trans*-HC=CH (**8**), and HN—C=O (**9**), respectively, the amine, fluorovinyl, vinyl, and retropeptide analogs. The first three alternatives remove the H-bond accepting carbonyl group, while the retropeptide modification inverts the H-bond character to potentially allow the amide NH to H-bond with the ester oxygen of the D-Lac residue. The effects of these modifications are evaluated in terms of relative free energies of binding, $\Delta\Delta G_{Binding}$, for peptides **3** and **4** as computed by Monte Carlo/free energy perturbation (MC/FEP) calculations.[6–10]

Computational details. The binding of tripeptide ligands **3**, **4**, and **5** to **2** was first modeled as a check before examination of the backbone variants. The thermodynamic cycles for the MC/FEP calculations are shown in Figure 2. For the study of the tripeptide ligands, two alchemical perturbations were performed (Fig. 2a): first from D-Ala (**3**, X = NH) to D-Lac (**4**, X = O) and then from D-Lac to D-Ket (**5**, X = CH_2). Using equations 1 and 2, the relative

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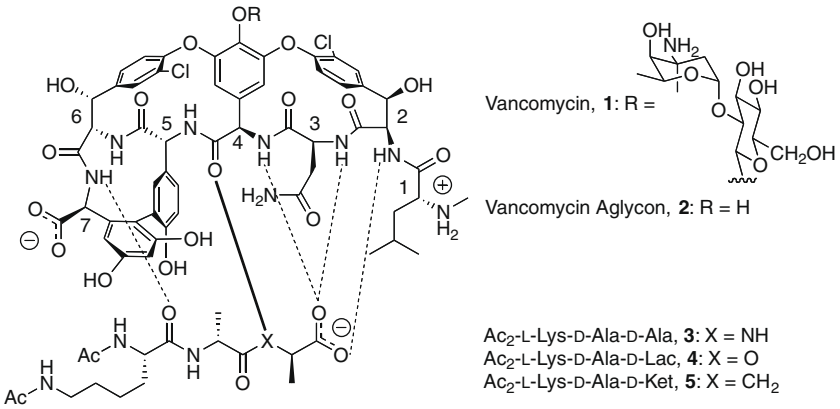


Figure 1. Vancomycin (**1**), vancomycin aglycon (**2**), and the tripeptide ligands as cell wall precursor mimics (**3**–**5**).

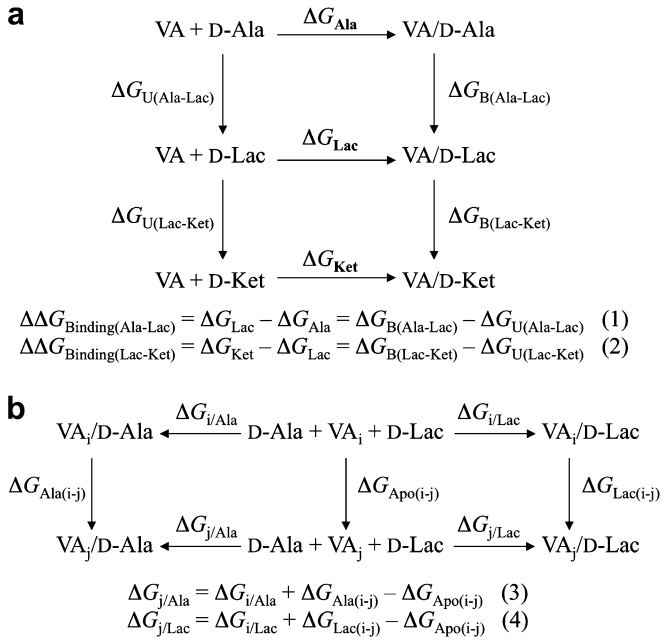


Figure 2. Thermodynamic cycles for MC/FEP calculations. VA, D-Ala, D-Lac and D-Ket represent **2**, **3**, **4**, and **5**, respectively. (a) Thermodynamic cycle for the tripeptide ligands. (b) Thermodynamic cycle for a pair of VA analogs, VA_i and VA_j.

free energy of binding, $\Delta\Delta G_{Binding}$, for each pair of ligands was computed from the FEP simulations for the bound and unbound ligands. For study of the backbone variants (Fig. 2b), **2** was perturbed to the fluorovinyl (**7**), vinyl (**8**), and retropeptide (**9**) analogs. The relative binding affinities of ligands **3** and **4** were evaluated for each analog using equations 3 and 4. For the amine analog, the perturbation from **2** was not carried out as this involves a change in net charge ($CONH \rightarrow CH_2NH_2^+$), which can lead to electrostatic artifacts. Instead, the D-Ala→D-Lac perturbation of the ligand (**3** → **4**) was again performed.

The initial coordinates of apo **2** and the **2**/**3** complex were based on the crystal structures of apo **1** (PDB:1AA5) and the **1**/**3** complex (PDB:1FVM). The sugar residues were replaced with a hydroxyl group to produce the aglycon structures. These structures were relaxed via MC simulations covering ca. 1 billion configurations in a periodic water box containing ca. 1170 TIP4P water molecules.[11] The subsequent MC/FEP calculations involved 11 windows of simple overlap sampling, single-topology perturbations, and 9-Å residue-based cutoffs.[12] All computations were performed with *MCPRO* and the OPLS–AA force field.[13,14] For the FEP calculations, the solutes were re-hydrated using a 25-Å radius cap containing ca. 2000 TIP4P water molecules. Each MC/FEP window was composed of 20 million configurations of solvent-only equilibration, 25–75 million configurations of full system equilibration, and 50 million configurations of averaging. Attempted moves are made for one amino acid residue or water molecule at a time. All degrees of freedom were sampled except for the TIP4P water molecules, which are internally rigid.[11]

*Results for **2** with **3**–**5**.* The computed $\Delta\Delta G_{Binding}$ values for the tripeptide ligands agree well with the trend in the experimental binding affinities, as summarized in Table 1. The binding of **3** is computed to be most favorable, whereas the binding of **4** is computed to be weakest. For **5**, the ligand neither forms an H-bond nor receives electrostatic repulsion from the backbone carbonyl of **2**, so the binding affinity is intermediate. Representative illustrations of the bound complexes are shown in Figure 3. An interesting observation is that, in addition to the characteristic backbone–backbone H-bonds, all three complexes display another side-chain-side-chain H-bond between a phenolic OH of residue 7 in **2** and the terminal amide C=O from the acetylated Lys of the ligands. In Figure 3, this additional H-bond between the solutes appears to be coupled to an intermolecular clustering of methyl groups, which involves two methyl groups from the Leu1 side chain of **2** and two methyl groups from the side chains of the Ac-Lys and D-Ala of the ligand. The hydrophobic clustering is expected to enhance binding, providing an explanation for why the acetylation of the Lys side chain of α-Ac-L-Lys-D-Ala-D-Ala improves its binding to **1** by a factor of 3.[15]

Results for vancomycin analogs. The subsequent modeling of the backbone variants of **2** provided the MC/FEP results in Table 2. The computed $\Delta G_{Binding}$ values for the D-Ala-D-Ala (**3**) and D-Ala-D-Lac (**4**) peptides are relative to the **2**/**3** complex. For binding of **3**, a negative $\Delta\Delta G_{Binding}$ would indicate an improvement in affinity over that for VA **2**. For the binding of **4**, an improvement is indicated by $\Delta\Delta G_{Binding}$ <4.4 kcal/mol.

Table 1
Calculated and experimental $\Delta\Delta G_{Binding}$ values (kcal/mol) for ligands **3**, **4**, and **5** with vancomycin aglycon, **2**

Perturbation	ΔG_B	ΔG_U	$\Delta\Delta G_{Binding}$ (Calc)[a]	$\Delta\Delta G_{Binding}$ (Expt)[b]
3 → **4** (X = NH → O)	30.5	24.2	6.3 ± 0.1	4.4
4 → **5** (X = O → CH2)	−18.7	−16.2	−2.5 ± 0.2	−2.6

[a] Uncertainties (±1σ) from separate averages over batches of 2.5 million configurations. See Ref. 11 for a review.
[b] Ref. 1.

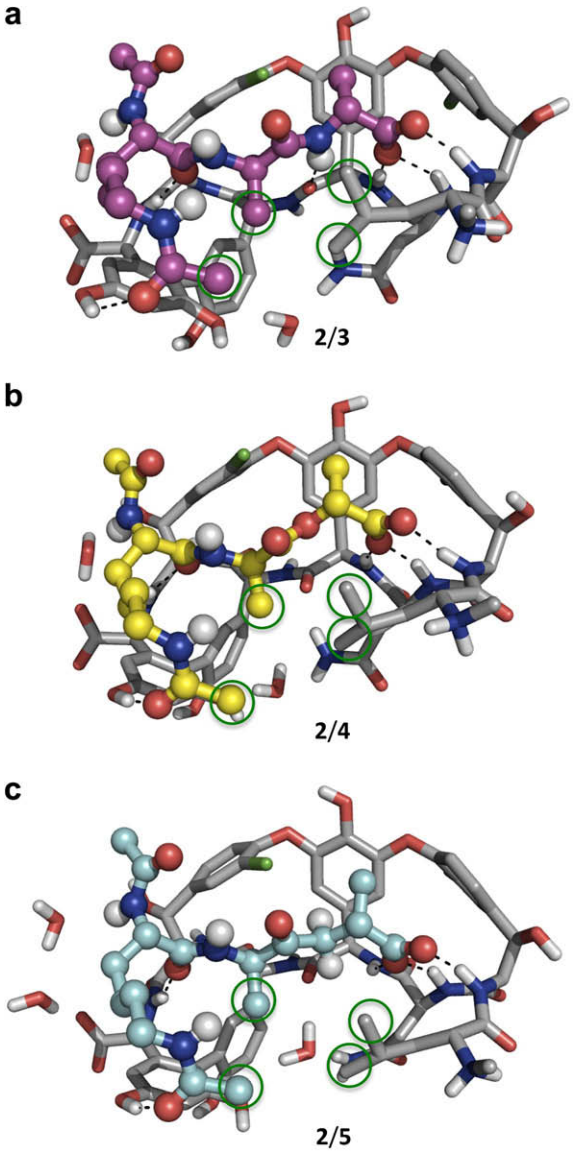
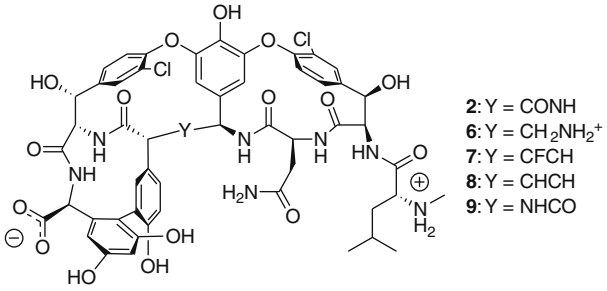


Figure 3. Representative structures of the bound complexes of **2** from MC/FEP calculations. **2** is shaded in grey and is represented in sticks; the tripeptide ligands are shown in ball-and-stick representations. The intermolecular H-bonds are marked by dashed lines, and the clustering methyl groups are circled in green. Structures are rendered using PyMOL.[16] (a) Complex **2**/**3** with **3** in purple. (b) Complex **2**/**4** with **4** in yellow. (c) Complex **2**/**5** with **5** in cyan. Hydrogens on carbon are hidden except for in the keto methylene group of **5**.

None of the analogs improves the binding for the D-Ala-D-Ala peptide **3**, while the amine analog **6** is the only one that improves binding for the D-Ala-D-Lac peptide **4**. As observed,[5] the computed results for **6** find its affinity for **3** and **4** to be almost the same. The binding for both **3** and **4** with **6** benefits from the charge–charge interaction between the protonated amine and the terminal carboxylate of the ligands.

The fluorovinyl (**7**) and vinyl (**8**) modifications explore alternative modulation of the non-bonded interactions, e.g., with F as a weak H-bond acceptor. From OPLS–AA optimizations, the geometries for *N*-methylacetamide (NMA) dimer and the NMA-2-fluoro-but-2-ene complex are very similar, though the interaction energy weakens from −8.8 to −5.1 kcal/mol (Fig. 4). Thus, the C=O to F change does reduce the affinity for **3** with **7**, but just to a similar level as for **3** with **6**. However, no improvement is computed for the D-Ala-D-Lac sequence despite the expected reduction

Table 2
Calculated and experimental relative $\Delta G_{Binding}$ values (kcal/mol) for peptides **3** and **4** with the backbone-modified vancomycin analogs



Analog	Relative $\Delta G_{Binding}$	
	3 (D-Ala-D-Ala)	**4** (D-Ala-D-Lac)
2 (Y = CONH)[a]	(0.0)	(4.4)
6 (Y = $CH_2NH_2^+$)[b]	(2.1)	2.3 ± 0.1 (2.0)
7 (Y = CFCH)	2.6 ± 0.2	6.0 ± 0.2
8 (Y = CHCH)	5.0 ± 0.2	4.8 ± 0.2
9 (Y = NHCO)	10.9 ± 0.5	10.3 ± 0.4

[a] Ref. 1. Experimental data in parentheses.
[b] Ref. 5.

in electrostatic repulsion between **4** and the fluorine rather than oxygen. To investigate further, a FEP calculation was performed using the amide geometry, but perturbing to the fluorovinyl charges; this does improve the binding with **4** by 0.8 kcal/mol. Thus, the change in geometry and van der Waals (vdW) interactions are unfavorable. The longer C—F bond length (1.35 Å) than C=O (1.23 Å) is noted. The vinyl analog **8**, suggested by Crowley and Boger,[5] was hoped also to bind better with **4**. However, the computed affinity for **4** with **8** shows little change from **2**, while the binding of **3** by **8** is poorer than with **2** or **7**. Diminished vdW and H-bond interactions for **3** with **8** are implicated.

With **9**, the retropeptide linkage might invert the usual binding preference by acting as a H-bond donor for the D-Ala-D-Lac peptide (**4**) and by promoting an unfavorable NH. . .HN interaction with the D-Ala-D-Ala sequence (**3**). However, the computed relative binding affinities are high (10 kcal/mol), predicting negligible binding of both **3** and **4** by **9**. As shown in Figure 5, the orientation of the retropeptide linkage turns out to be poor for intermolecular H-bonding, probably owing to avoidance of unfavorable intramolecular interactions with the NH of residue 4 and C=O of residue 5. The

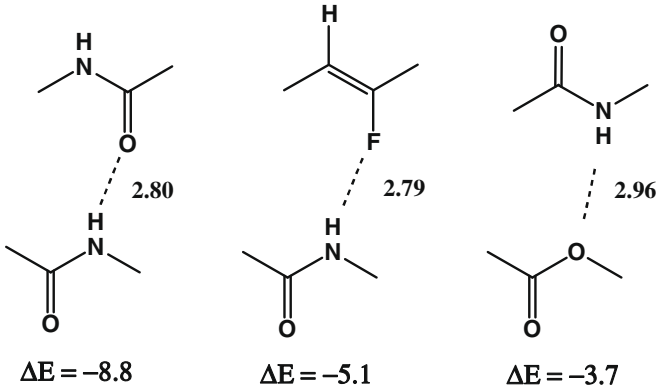


Figure 4. OPLS–AA interaction energies (kcal/mol) and N···O or N···F distances (Å) for gas-phase complexes with *N*-methylacetamide (NMA). DFT (B3LYP/6-31G(d)) optimizations for the corresponding complexes of acetamide with NMA, 2-fluoro-but-2-ene, and methylacetate yield $\Delta E = -8.5$, −5.1, and −4.3 kcal/mol, respectively. The molecular planes are roughly at right angles.

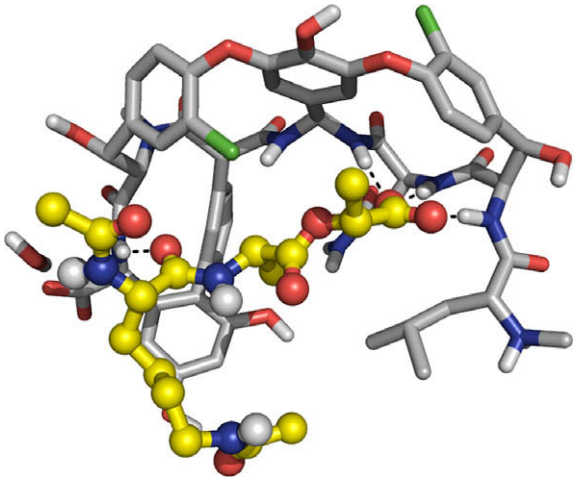


Figure 5. Representative structure from the MC simulations for the complex of **4** with the retropeptide analog **9**; the poor NH...OC=O geometry caused by twisting of the retropeptide linkage is illustrated. Hydrogens on carbon are not shown for clarity.

average N—O distance of 4.4 Å for the amide–ester interaction from the **4/9** simulation is beyond the limit for H-bonds. Even when optimally oriented, such amide–ester H-bonds are weak. From OPLS–AA optimization of the NMA–methyl acetate complex with the alkoxy oxygen of the ester as the acceptor, the interaction energy is only −3.7 kcal/mol (Fig. 4).

Summary. MC/FEP calculations were shown to reproduce the experimental trend in binding affinities for vancomycin aglycon **2** with peptides **3–5**. The binding of four backbone variants of **2** to the D-Ala-D-Ala (**3**) and D-Ala-D-Lac (**4**) peptides was then modeled to seek modifications that might improve binding of both sequences. The results indicate that the most promising design remains the previously reported amine analog, **6**, which benefits from favorable electrostatic interactions between the ammonium group and the carboxylate terminus of the peptides. Isosteric replacement of the residue 4–5 peptide bond with a fluorovinyl group in **7** was moderately disruptive of binding with **3**, but did not provide the desired improvement with **4**. The vinyl alternative **8** was more damaging for the interaction with **3** in view of the complete loss of the NH···O or NH···F H-bond. Finally, **9** did not deliver a H-bond with the ester oxygen owing to geometrical mismatch (Fig. 5) and intrinsic weakness of such amide–ester H-bonds. Overall, the simulations shed light on the challenges of re-engineering the well-evolved binding site of vancomycin. Exploration of vancomycin analogs continues with emphasis on side-chain variants as a route to improved antibacterial agents.

Acknowledgments

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