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Quantum Simulations of the Structure and Binding of Glycopeptide Antibiotic Aglycons to Cell Wall Analogues

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The recent rise of vancomycin-resistant enterococci (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) has given new impetus to the study of the binding between glycopeptide antibiotics and bacterial cell wall termini. Here, we report on an extensive first principles investigation of the binding of vancomycin, avoparcin, teicoplanin, and ristocetin aglycons with dipetides, Ac-D-Ala-X, where X = D-Lac and D-Ser (characteristic of VREs) and X = D-Ala, Gly (characteristic of non-VREs), and a model "methylated D-Ala" $CH_2CH(CH_3)COO^-$, in liquid as well as gas phase. The gas-phase ordering of the binding, from strongest to weakest, is Gly, D-Ala, D-Ser, $CH_2CH(CH_3)COO^-$, and D-Lac. Calculations show that the order of the Gly and D-Ala binding is reversed in solution. The results are in good agreement with recent experimental findings.

I. Introduction

Vancomycin is the archetypical member of the clinically important family of glycopeptide antibiotics (GAs), which currently includes some 200 known members.[1−3] First isolated from soil samples in 1956, vancomycin is produced by the *Amycolatopis orientalis* microorganism.[4] In clinical use since 1959, vancomycin is used with patients allergic to β-lactams, to fight infections during cancer chemotherapy, and as a last resort for the treatment of infections caused by gram-positive bacteria.[5] With its more frequent therapeutic use, along with that of the related GA avoparcin in animal feeds,[6] vancomycin-resistant gram-positive bacteria have emerged: vancomycin-resistant enterococci (VRE)[7] was first observed in 1987, while vancomycin-resistant *Staphylococcus aureus* (VRSA) was first observed as late as 1998.[8] These unfortunate developments represent a major, worldwide health risk and have given new urgency to understanding the detailed structure and function of GAs, with the aim of designing next-generation drugs to fight these evolving bacterial strains.

Vancomycin and other GAs act by inhibiting bacterial wall biosynthesis.[1,9,10] Bacterial cell walls are surrounded by a meshlike peptidoglycan layer, which provides mechanical support to the cell. This peptidoglycan layer is composed of linear polysaccharide chains, with alternate residues composed of the carbohydrates *N*-acetyl glucosamine and *N*-acetyl muramic acid.

These chains run parallel to each other and are connected to a peptide moiety consisting of five amino acids, L-Ala-D-Glu-L-Lys-D-Ala-D-Ala.[9] These chains cross-link to form a network, which imparts a tremendous amount of strength and stability to the bacterial cell wall. Without this support, the growing bacterial cell cannot resist the intercellar osmotic pressure and the result is bactericide by lysis. GAs take advantage of this as follows. Peptidoglycan synthesis involves two key steps: (i) the assembly of the polysaccharide subunits via the transglycosylases and (ii) the cross-linking of the pentapeptide chains by means of the transpeptidases. While penicillin antibiotics act by directly binding to the active sites of the transpeptidase enzymes,[11] GAs selectively bind to the peptidoglycan peptide precurser *N*-acyl-D-Ala-D-Ala, thereby preventing the crucial peptidoglycan cross-linking.[12−15] However, enterococci have developed a sophisticated strategy for neutralizing the action of the GAs through a modification of the antibiotic target: some of the D-Ala-D-Ala termini are substituted with D-Ala-D-Lac or, to a lesser extent, with D-Ala-D-Ser.[5] Altering this last peptide does not effect the quality of the peptidoglycan layer or its ability to cross-link the glycan strands. It does, however, reduce the affinity of the antibiotic for the peptidoglycan precursor by a factor of over 1000—at least for the D-Ala-D-Lac terminations—thereby de facto rendering the antibiotic ineffective.[13] Clearly, the binding between the antibiotic and the modified precursors is the key issue that needs understanding in order to overcome the VREs.

Given the therapeutic and industrial importance of these antibiotics, they have been the subject of considerable experimental and theoretical investigations over the past few decades. Recent work, however, has primarily focused on the GA structure,[16−18] their binding to cell wall analogues,[19−27] cooperative phenomena related to dimerization issues,[19,28−34] and the role of sugar residues on select GAs.[35−38] In a previous paper,[27] we reported on the binding of the most stable conformer of vancomycin and teicoplanin with Ac-D-Ala-D-Ala and Ac-D-Ala-D-Lac in the gas phase at zero temperature. In this paper, we present the results of an extensive quantum chemistry study of the binding of prototypical GAs to bacterial cell wall analogues in the liquid phase at room temperature, which complements existing studies based on classical potential methods.[24−26,33] Specifically, the bindings of the aglycon versions of the GAs to a series of dipeptides, Ac-D-Ala-X, where X is one of D-Ala, D-Lac, D-Ser, Gly, or "methylated D-Ala" ($CH_2(CH(CH_3)COO^-$), have all been considered. In agreement with previous experimental[16−18,20,22,24] and theoretical work, binding of the GAs is understood in terms of the number of hydrogen bonds, the conformations of the GAs and/or cell wall

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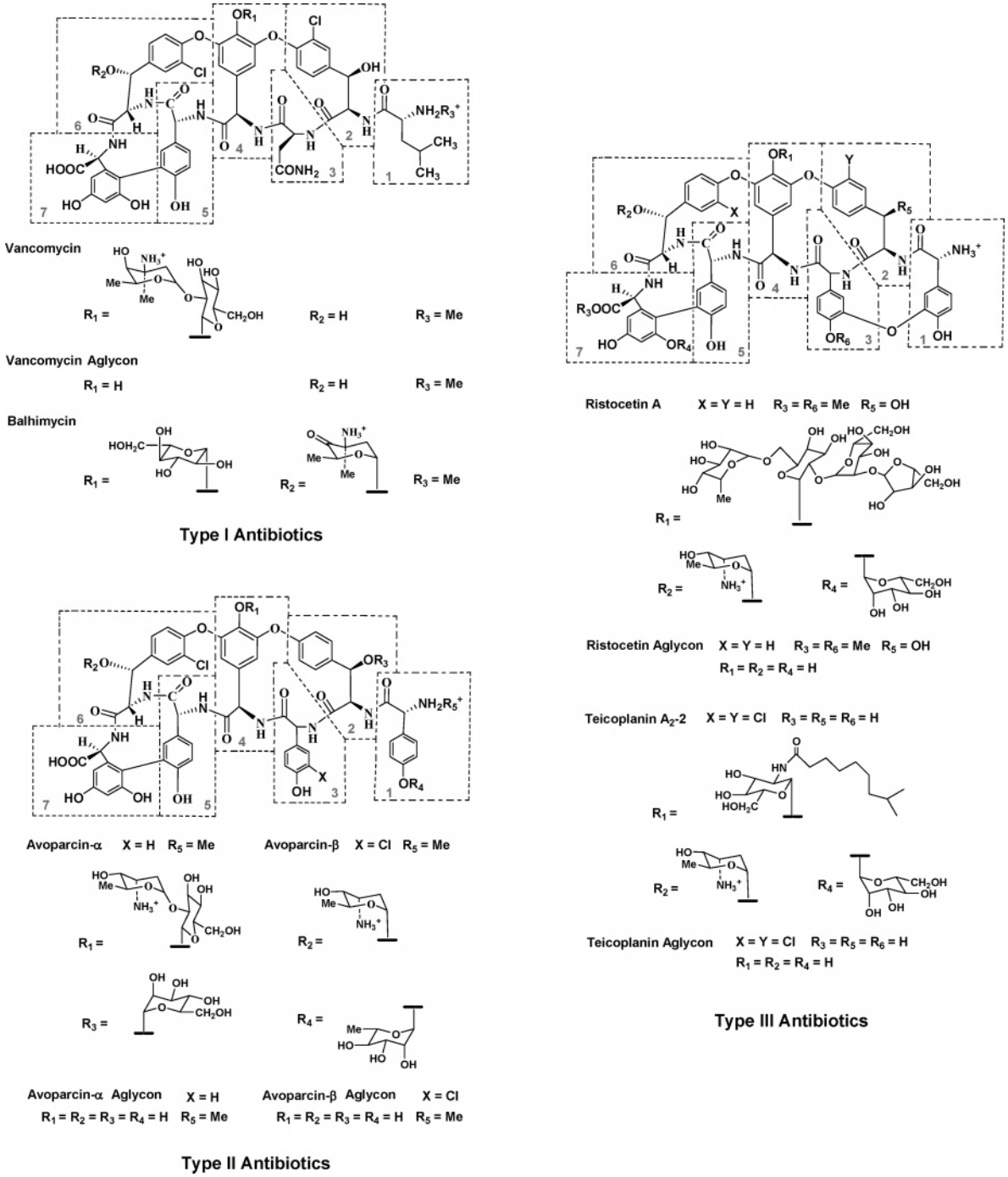

Figure 1. Chemical structure of prototypical glycopeptide antibiotics.

analogues, and solvent effects. These issues are all discussed in the following sections.

II. Calculations

Figure 1 shows a schematic of prototypical GAs. They are characterized by a heptapeptide core of seven amino acid residues, with the side chains of residues 2−4, 4−6, and 5−7 covalently joined. In addition, various residues carry a variety of sugar substitients. The amino acid residues for vancomycin consist of *N*-methyl-D-leucine at position 1, asparagines at position 3, substituted phenylglycines at positions 4, 5, and 7, and substituted phenylalanines at positions 2 and 6.

It is convenient to classify the different GAs into three different categories based on the kinds of residues located at positions 1 and 3.[3] Type I antibiotics such as vancomycin and balhimycin are characterized by aliphatic residues. Type II antibiotics such as α- and *β*-avoparcin are characterized by individual aromatic residues, while in type III, GAs such as teicoplanin and ristocetin, the aromatic residues are covalently joined. Vancomycin is the most commonly used GA for therapeutic applications, along with teicoplanin, which is mainly used in Europe. Ristocetin has also been used in clinical applications, until its side effects proved to be too toxic. Avoparcion is an industrial chemical that is added to animal feeds, to promote their growth.[6] Its extensive use is believed to have contributed to the rise of VREs, and so this application is currently being discouraged.

To investigate GA binding, we conducted first principles calculations based on density functional theory (DFT) simulations, augmented with Hartree−Fock (HF) calculations. The

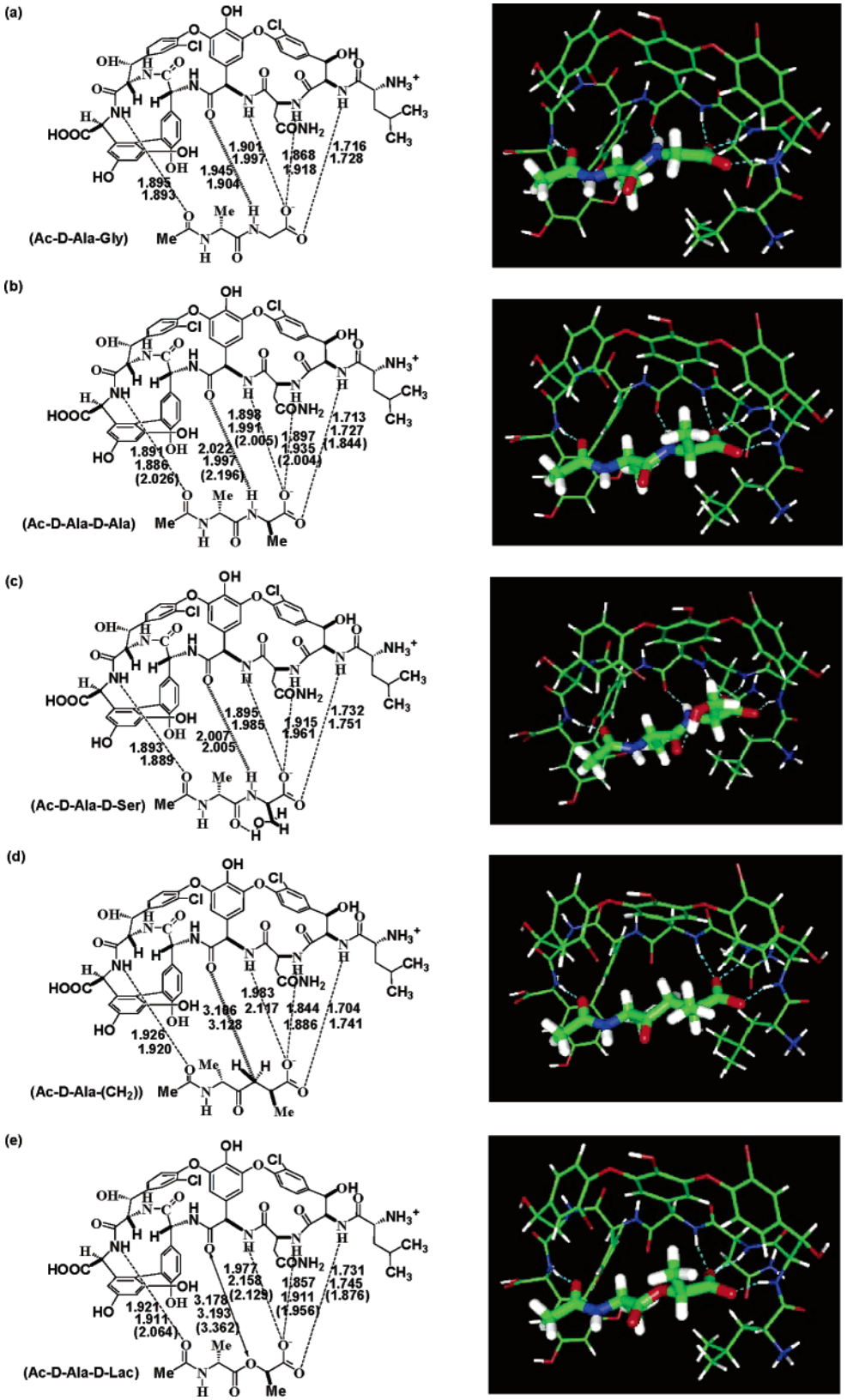

Figure 2. Exploded views (left panel) and full three-dimensional (right panel) views of the vancomycin−dipeptide complexes. Here, vancomycin is bound to (a) Ac-D-Ala-Gly, (b) Ac-D-Ala-D-Ala, (c) Ac-D-Ala-D-Ser with "OH" moiety pointing downward, (d) with "methylated D-Ala", i.e., Ac-D-Ala-C $H_2CH(CH_3)COO^-$, and (e) Ac-D-Ala-D-Lac. Dotted lines mark the crucial hydrogen bonds stabilizing these complexes, while an arrow marks the loss of binding for the corresponding hydrogen bond for Ac-D-Ala-D-Lac. The DFT-based hydrogen-bond distances in angstroms for the C_A (first) and C_B (second) conformers are directly marked. In addition, for D-Ala-D-Ala and D-Ala-D-Lac, the HF-based hydrogen-bond distances are marked in the brackets.

bindings of the aglycon forms of the antibiotics (i.e., vancomycin aglycon (VA) $(C_{52}H_{51}N_8O_{17}Cl_2)^+$, α-avoparcin aglycon (α-AV) $(C_{58}H_{49}N_7O_{18}Cl)^+$, *β*-avoparcin aglycon (*β*-AV) $(C_{58}H_{48}N_7O_{18}Cl_2)^+$, ristocetin aglycon (RA) $(C_{58}H_{48}N_7O_{19})^+$, and teicoplanin aglycon (TA) $(C_{58}H_{46}N_7O_{18}Cl_2)^+$ with dipeptides Ac-D-Ala-D-Ala $(C_8H_{13}N_2O_4)^-$, Ac-D-Ala-D-Lac $(C_8H_{12}NO_5)^-$, Ac-D-Ala-

Gly $(C_7H_{11}N_2O_4)^-$, and Ac-D-Ala-D-Ser $(C_8H_{13}N_2O_5)^-$ were studied with optimized (by means of the analytic gradient method) Gaussian03 calculations.[39] In addition, to allow for a direct comparison to recent experiments,[24] the binding of vancomycin to methylene-substituted D-Ala, i.e., $CH_2CH(CH_3)COO^-$ was also considered. The calculations employed both the 4-31G and 6-31G* basis sets, using between 960 and 1030 (4-31G) and 1500−1700 (6-31G*) basis functions. The DFT calculations also made use of the popular B3LYP gradient-corrected functional for the exchange and correlation energies.[40]

The simulations are subject to the following. First, because the sugar substituents do not play a direct role in the binding within the GA monomeric framework, the calculations were limited for the most part to the aglycon versions of the GAs. For example, the important relative binding energy differences between the monomeric vancomycin/D-Ala-D-Ala and vancomycin/D-Ala-D-Lac complex, both *with* and *without* sugars, were found to be 8.5 and 8.4 kcal/mol, respectively, using HF/4-31G calculations. This energy difference is relatively small, when compared to other factors such as the energy contained in a hydrogen bond. Hence, the presence of sugars is expected to make only a secondary contribution to the binding of the GA monomers. Second, we primarily report on GA complexes in the gas phase. Quantities such as the absolute values of the binding energies will be different in solution. To elucidate this, we have calculated the relative binding energies of the vancomycin complexes with the polarizable continuum model (PCM),[41] which is an implicit solvent model.[42] This also requires the computation of the gaseous free energies, which are now listed in Table 2. Finally, we note that it is well-known that some GAs such as vancomycin form "back-to-back" dimers in solution.[28−34] It is believed that this dimerization aids antibiotic binding to the cell wall precursors by limiting the dynamical conformations that the antibiotic can undergo. Here, in contrast to the monomeric forms, the sugars are expected to play an important role in enhancing the dimerization. While we have some evidence for this, we limit our studies here to the monomer forms of the antibiotic, with the aim of relegating dimerization and dynamical issues to the future.[43]

III. Results and Discussion

The main features of the GA binding are the following. All the GAs buckle in such a way as to form a pocket, which makes the important contact with the dipeptides, Ac-D-Ala-X. Binding takes place primarily through a series of five hydrogen bonds, as illustrated for VA in Figure 2. This is true for the dipeptides with X = D-Ala, Gly, and D-Ser. The X = D-Lac terminus is different insofar as it involves the substitution of a linking ester for an amide through the exchange of a single ligand (i.e., NH → O, for X = D-Ala). This change acts to decrease the binding of the antibiotic/D-Lac complex through the removal of a single hydrogen bond and through the repulsive interaction between the two oxygen lone pairs that have been created (second position from the left, as marked by an arrow in Figure 2e). In complete analogy with recent experimental work,[24] we have also investigated the binding of VA with X = $CH_2CH(CH_3)COO^-$ (which we denote by "X = CH_2" as a shorthand), which is obtained from D-Ala by means of the substitution NH → CH_2. Since the binding to this "methylated D-Ala" involves the loss of a single hydrogen bond but no oxygen-based lone-pair repulsion, this will enable us to quantify the relative importance of the two effects responsible for the loss of binding. For the VA/CH_2 complex, the average O−C distance is 3.117 Å, while the average O−O distance of the VA/D-Lac complex is a slightly larger 3.185 Å, which may be ascribed to the repulsive nature of the two oxygens. In addition, except for the second hydrogen bond from the right, all other hydrogen bonds are pushed apart by a small amount, when compared to the D-Ala bond distances. This further serves to reduce the binding of the D-Lac complex. These features are in agreement with previous models based on classical potentials and consistent with experimental results.[1−3,16−18,22]

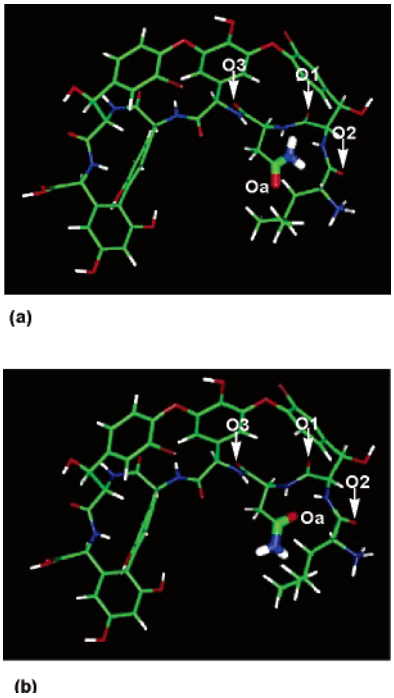

Figure 3. Details of the three-dimensional structure of the C_A and C_B conformations of vancomycin aglycon, based on the rotation of the highlighted "$CONH_2$" entity. The positions of the negatively charged oxygen atoms (i.e., O_1, O_2, O_3) with O_a are marked with arrows.

Our investigations of the GA complexes involved an examination of several different conformations. For VA, two were found to be particularly important. These conformations arise through a rotation of the amide side chain within residue 3, as shown in Figure 3. While there is experimental evidence for both conformations,[16−18,22] it appears experimentally that conformation A (C_A) is somewhat more common. In the gas phase, C_A is the most stable. This is because C_B involves the repulsive electrostatic interaction of three oxygen atoms in relatively close proximity on the back end of the vancomycin pocket. For C_A, the O_a-O_1, O_a-O_2, and O_a-O_3 oxygen−oxygen distances are 4.79, 4.34, and 5.58 Å, respectively. For C_B, these distances are somewhat shorter, with corresponding distances of 3.68, 4.71, and 4.85 Å. We speculate that the experimental presence of C_B is due to the additional hydrogen-bond stabilization provided by solvent water molecules or perhaps the presence of other dimers.[18] These conformations are characteristic of type I glycopeptides only, because of the presence of aromatic residues at position 3 for type II and III GAs. A second set of conformations involves the dipeptides with D-Ser terminations. Here, the hydroxyl group (CC−OH) on D-Ser can rotate in such a way as to form a hydrogen bond with the oxygen on the D-Ala (Figure 2c), which gives an additional stabilization to this conformation. A second, slightly less favorable conformation involves a rotation of the same hydroxyl group by about 180°. Hydrogen-bond lengths between the VA C_A (C_B) and the dipeptide Ac-D-Ala-D-Ser in this configuration (not shown), from left to right, are 1.904 (1.901), 1.977 (1.965), 1.908 (2.009), 1.873 (1.933), and 1.725 (1.734) Å, respectively.

TABLE 1: Relative Gas-Phase Binding Affinities $-\Delta\Delta E$ in kcal/mol for the Aglycon Forms of the Antibiotics with the Dipeptides Ac-D-Ala-X, Where X Is One of D-Ala, D-Lac, D-Ser, Gly, or CH_2[a]

X	Gly	D-Ala	D-Ser (up)	D-Ser (down)	D-Ser (av)	CH_2
VA C_A	6.9 (4.5)	6.3 (3.6)	7.9 (5.0)	−0.5 (−1.4)	3.7 (1.8)	4.5 (2.0)
VA C_B	6.2 (4.6)	5.3 (3.5)	7.7 (5.1)	−1.7 (−1.7)	3.0 (1.7)	3.1 (1.4)
VA (av)	6.5 (4.6)	5.8 (3.6)	7.8 (5.1)	−1.1 (−1.5)	3.4 (1.8)	3.9 (1.7)
α-AV	6.8 (4.4)	6.1 (3.8)	7.8 (4.9)	−0.3 (−0.8)	3.8 (2.1)	
β-AV	6.6 (4.4)	6.0 (3.7)	7.8 (4.9)	−0.4 (−0.9)	3.7 (2.0)	
RA	6.7 (4.4)	6.5 (3.7)	8.7 (4.7)	−0.3 (−1.7)	4.2 (1.5)	
TA	6.8 (4.4)	5.8 (3.6)	8.3 (5.0)	−0.1 (−1.7)	4.1 (1.7)	

[a] Antibiotics are indicated with the following notation: vancomycin (VA), α-avoparcin (α-AV), *β*-avoparcin (*β*-AV), ristocetin (RA), and teicoplanin (TA), respectively. Numbers of the 4-31G (6-31G*) results are measured with respect to the D-Ala-D-Lac results. Explicit results for the two conformations of vancomycin (C_A, C_B) and D-Ser (with "OH" up or down) and their average values (av) are given.

TABLE 2: Relative $T = 298°$ Gas-Phase Binding Affinities $-\Delta\Delta G$ in kcal/mol for the Aglycon Forms of the Antibiotics with the Dipeptides[a]

X	Gly	D-Ala	D-Ser (up)	D-Ser (down)	D-Ser (av)
VA C_A	6.3	5.1	6.1	−2.0	2.1
VA C_B	6.5	5.2	6.7	−2.0	2.4
VA (av)	6.4	5.2	6.4	−2.0	2.2
α-AV	6.6	5.9			
β-AV	5.3	5.8			
RA	6.1	4.9	7.0	−1.6	2.7
TA	5.6	5.0	5.9	−1.1	2.4

[a] Results are for 4-31G basis set, notation is as for Table 1, and differences are measured with respect to Ac-D-Ala-D-Lac results.

TABLE 3: Gas-Phase Binding $-\Delta E$ and $-\Delta G$ in kcal/mol for the Aglycon Forms of the Antibiotics with Ac-D-Ala-D-Lac[a]

	$-\Delta E$	$-\Delta G$
VA C_A	129.0 (119.1)	107.9
VA C_B	121.7 (112.9)	102.3
VA (av)	125.3 (122.5)	105.1
α-AV	127.3 (115.8)	106.4
β-AV	132.8 (117.6)	112.2
RA	127.1 (117.4)	107.8
TA	130.2 (119.8)	111.4

[a] This gives information as to the *relative* binding of the different types of antibiotics. For $-\Delta E$, results for 4-31G (6-31G*) are given.

TABLE 4: Liquid-Phase Binding Affinity $-\Delta\Delta G$ in kcal/mol for Vancomycin Aglycon with Dipeptides, as Obtained with a 4-31G Basis Set[a]

X	D-Ala	Gly	D-Ser (up)	D-Ser (down)	D-Ser (av)	CH_2
VA C_A	6.1	6.3	6.1	1.6	3.9	3.1
VA C_B	5.9	4.8	4.2	−0.7	1.8	1.9
VA (av)	6.0	5.5	5.1	0.5	2.8	2.4

[a] Notation is as in Table 1.

Table 1 gives the gas-phase binding energy differences $-\Delta\Delta E$ for the GA complexes, as measured with respect to the Ac-D-Ala-D-Lac binding. Results for both DFT/4-31G and 6-31G* calculations are given. In general, the more accurate 6-31G* binding energies are 1.5 to 2.5 kcal/mol less than those predicted by the 4-31G results. However, for the most part, the predicted ordering of the binding is same. From strongest to weakest, the ordering is Gly, D-Ala, D-Ser, CH_2, and D-Lac. The exception here is the binding of the VA/CH_2 complex: the 4-31G calculations predict this complex to be slightly stronger than the corresponding VA/D-Ser complex. Roughly speaking, glycine is the smallest amino acid, and as such, it can enter somewhat deeper into the antibiotic pocket. This is reflected in the shorter hydrogen-bond distances, especially for the second hydrogen bond from the left. By contrast, serine is the largest of the three amino acids and fits somewhat less well into the pocket. As may be expected, results for D-Ala are intermediate to these two cases. In a previous paper,[27] we carried out DFT/6-31G* and HF/6-31G* calculations of vancomycin (C_A) and teicoplanin with Ac-D-Ala-D-Ala and Ac-D-Ala-D-Lac. The HF results are somewhat larger than the DFT-based results. For VA/D-Ala, the HF (DFT) binding energy difference is 4.9 (3.6) kcal/mol, while for TA, it is 5.1 (3.6) kcal/mol. These numbers bracket the most recent experimental estimate of 4.4 kcal/mol.[24,25] At room temperature, one can therefore expect that the binding to Ac-D-Ala-D-Ala is stronger by a factor of about 400−5000 over Ac-D-Ala-D-Lac.[24] These results also enable one to quantify the relative importance of the gas-phase hydrogen-bond loss and the oxygen−oxygen repulsion in reducing the D-Lac vs D-Ala binding. From the CH_2 results given in Table 1, it is clear that approximately *half* (i.e., ≈1.9 kcal/mol) of the binding energy is associated with the former and ≈1.7 kcal/mol with the oxygen−oxygen repulsion.

In terms of the two VA configurations, the results all show the same trends. Because of its slightly more favorable orientation, the right-most three hydrogen bonds assciated with the carboxyl group are slightly shorter for C_A as compared to the C_B configuration. This is a reflection of the proximity of this group to the amide moiety. By contrast, the bond lengths of the left-most hydrogen bonds—which are relatively far away—are virtually the same for both configurations. Turning to GA

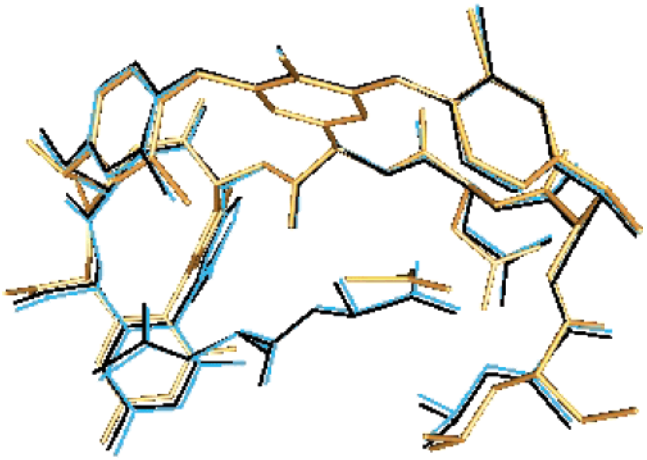

Figure 4. Wire mesh overlay comparing the experimental (orange) with the theoretical DFT/4-31G (blue) and DFT/6-31G* (black) three-dimensional structures of the VA complex.

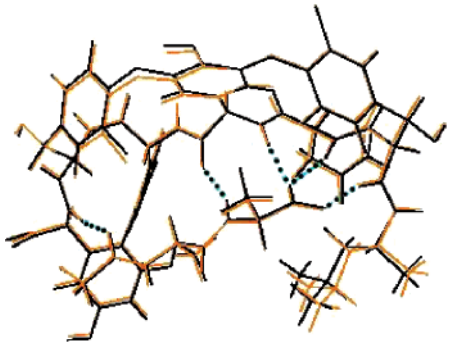

Figure 5. Wire mesh overlay comparing the three-dimensional structure of the VA/D-Ala complex, as calculated with HF/6-31G* (light) and DFT/6-31G* (dark) calculations.

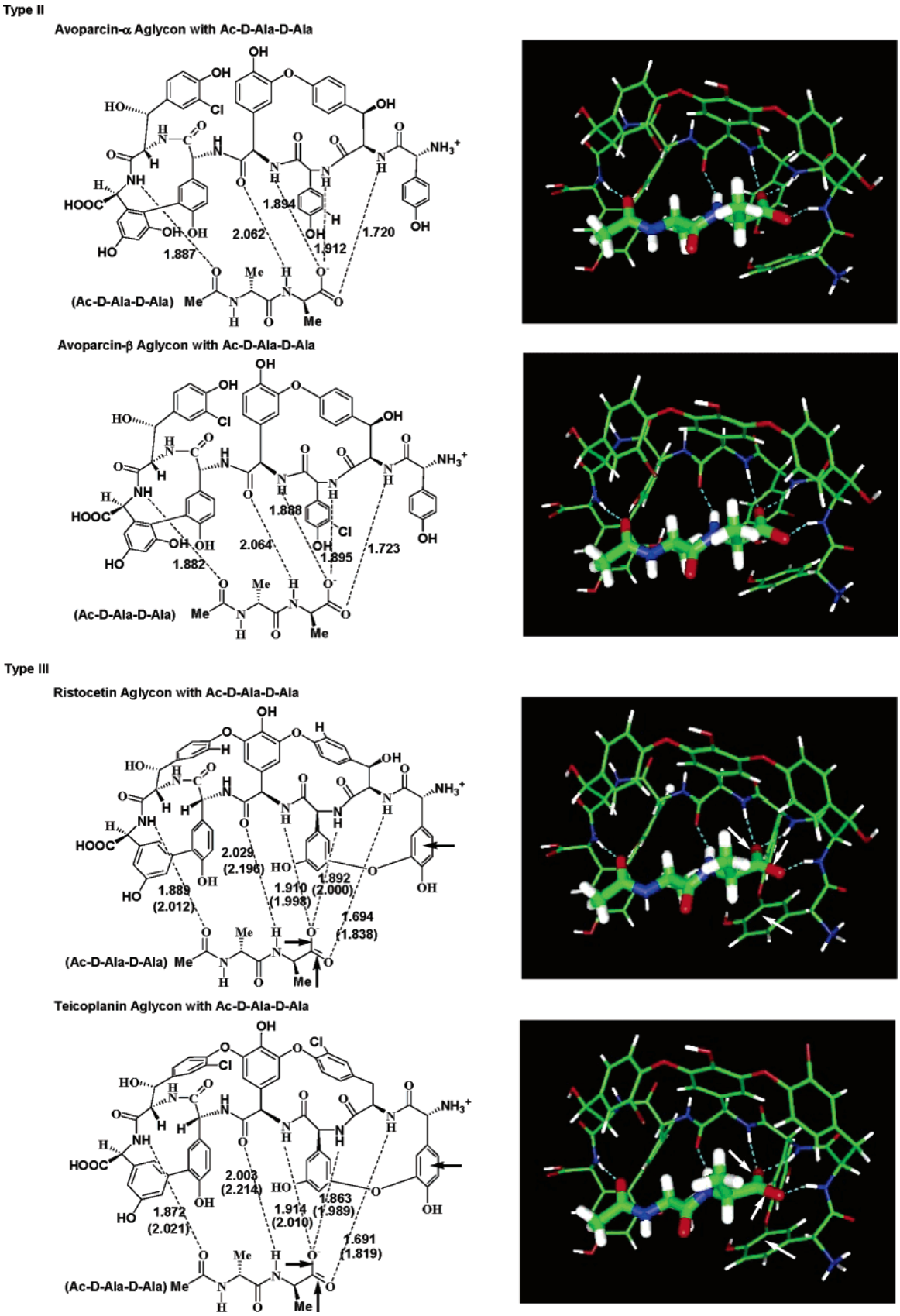

Figure 6. Exploded views (left panels) and full three-dimensional structures (right panels) of the binding of type II and type III antibiotics with D-Ala-D-Ala. The five significant hydrogen bonds stabilizing the complexes are marked with dotted lines, and the DFT-based bond distances in angstroms are also given. In addition, for ristocetin and teicoplanin, HF-based bond distances are also given in brackets. For these type III antibiotics, the small arrows on the left panels mark the weak interaction between the resonating glycopeptide aromatic bonds and the D-Ala terminations as discussed in the text.

binding to D-Ser, we find that the binding is quite sensitive to the structure of the dipeptide. When the hydroxyl group (CC−OH) on D-Ser is rotated upward, the structure of the complex resembles that of the D-Ala-Gly complex insofar as the

hydrogen-bond distances are concerned. By contrast, when the "OH" group of the D-Ser points downward, such that an additional hydrogen bond is formed between the D-Ala and D-Ser groups of the dipeptide, binding is considerably reduced. This appears to be odd because the D-Ser (OH down) complex is more stable (by ~4kcal/mol) than the D-Ser (OH up) complex. However, the isolated D-Ser (OH down) turned out to be much more stable (by ~13kcal/mol) than the isolated D-Ser (OH up) conformer. As a result, the binding of the D-Ser (OH down) complex is decreased. As a whole, the average binding of the D-Ser is weaker than that of the D-Ala or Gly complexes. The type II and III glycopeptide antibiotics examined all display the same trends with respect to the binding of the D-Ser configurations.

Table 2 gives the room-temperature gas-phase free energy affinities, $-\Delta\Delta G$, measured with respect to the D-Ala-D-Lac free energy. Here, trends are very much the case as in Table 1, with the calculated free energy differences being somewhat lower than the $-\Delta\Delta E$ values, typically by about 0.5 kcal/mol. Table 3 presents a comparison of the absolute values for GA binding to Ac-D-Ala-D-Lac. Roughly speaking, the free energies are uniformly lower than the $-\Delta\Delta E$ values by about 20 kcal/mol.

On the whole, the gas-phase results are in good agreement with the experimental results,[16,17,24,28] with the exception of the Gly-terminated dipeptide. Here, we believe that solvation effects play an important role. To test this, we have recomputed the binding free energy affinites for VA with the PCM solvent model.[41] Results for these calculations are given in Table 4. These results indicate, in agreement with experimental results and previous work based on classical potential modeling,[25] that the order of the binding between Gly and D-Ala is now reversed, with binding to D-Ala being the strongest. This reversal may be traced back to the contributions made by the cavity and dispersion energies, which contribute to the nonelectrostatic parts of the binding. For Gly, this part is 1.8 kcal/mol lower than the corresponding value for D-Ala. As a result, the relative binding affinity of vancomycin with D-Ala is estimated to be 0.5 kcal/mol larger than the binding with Gly. This estimate is in good agreement with the experimental result of 0.7 kcal/mol.[22] In terms of partitioning the loss of binding of D-Lac over D-Ala, these results predict about 2.4 kcal/mol associated with the oxgyen−oxygen repulsion. This is in good agreement with the most recent experimental estimate of 2.6 kcal/mol.[24] It is also noted that the VA binding to D-Ser turned out to be much more stable (by 2.8 kcal/mol) than that to D-Lac, which is again close to the experimental estimate of 3.0 kcal/mol.[20]

How do the structures compare with the experimental results? Figure 4 gives the direct visual comparison between the experimental[16,17] and theoretical VA structures, based on 79 "backbone" atoms (sugars, hydrogens, and dipeptide excluded). The agreement is good, with most deviations being centered on the floppy residue 1. Quantitatively, the root-mean-square deviations (rmsd's) are 0.42 Å (experiments with an acetate anion for D-Ala[16,17] vs DFT/6-31G*), 0.45 Å (experiments with an acetate anion for D-Ala[16,17] vs DFT/4-31G), 0.37 Å (experiments with a D-Lac[22] vs DFT/6-31G*), and 0.07 Å (DFT/4-31G versus DFT/6-31G* calculations). Figure 5 shows a similar comparison between the DFT and HF 6-31G* calculations, with the dipeptide atoms now included. The agreement between the structures is also quite good, with a rmsd of 0.23 Å.

Having discussed the relative binding of the GA complexes with the bacterial cell wall analogues, we turn to the differences in strengths between the different GA types. For instance, Figure 6 gives a comparison between type II and type III binding to Ac-D-Ala-D-Ala. As expected, the binding here is remarkably the same, reflecting the strong similarities between the different binding pockets. Focusing on avoparcin, the main difference between the two versions is that one of the hydrogens on the aromatic ring in residue 3 is replaced by a chlorine atom. The effect of this change is to decrease two out of the three, rightmost hydrogen-bond distances. This in turn stabilizes the β-avoparcin dipeptide complexes over the corresponding α-avoparcin complexes by about 2 kcal/mol, as given in Table 3. This is in qualitative agreement with the experimental results, which show that β-avoparcin has the higher affinity constant.[21] Another feature is that the binding of the type III antibiotic complexes is slightly stronger than that for type I (Table 3). This appears to be *partially* due to a weak $\pi-\pi$ bonding associated with the stabilization of the "O−C═O" resonance at the end of the dipeptide terminus and the phenyl sidegroups of both ristocetin and teicoplanin. Figure 7 specifically illustrates the overlap of the molecular orbitals, while small arrows in Figure 6 mark the specific sites of the $\pi-\pi$ overlap on the exploded and three-dimensional views. Similarly, a π-stacking interaction between the *m*-dihydroxylated benzene ring of residue 7 or ristocetin A and ristocetin-Ψ (same as ristocetin-A but with $R_1 = R_4 = H$) and the acetyl group of Ac-Gly-D-Ala was noted experimentally.[44] Here, the lack of a sugar group at R_4 played a role in increasing the binding of ristocetin-Ψ over ristocetin-A due to a lack of favorable π-stacking interaction. Finally, between the two type III GAs, we note that teicoplanin binding is slightly stronger than that of ristocetin, which again is consistent with the experimental results for the monomers.[19] As with all the other antibiotics considered, this is reflected in the slightly shorter hydrogen-bond distances.

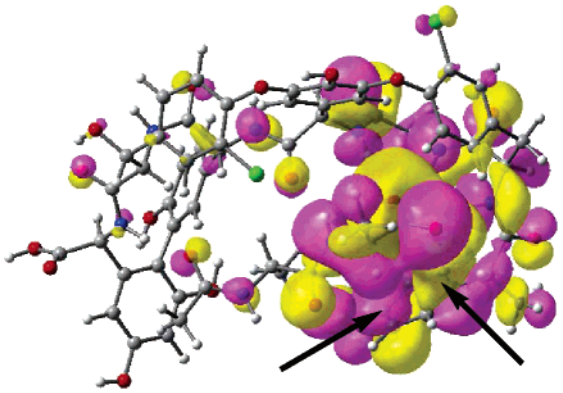

Figure 7. One of the valence molecular orbitals of teicoplanin with Ac-D-Ala-D-Ala illustrating the overlap between the π-orbitals of the carboxyl group of the D-Ala termination and those of the aromatic ring of residue 1, as marked by arrows.

To get a sense of the nonbonded electrostatic interaction of the GA complexes, we have examined the electrostatic potential and the excess partial charges as calculated by the Merz−Singh−Kollman scheme.[45] In isolation, each of the GAs carries a positive charge, the cell wall analogues are negatively charged, and the combined complex is neutral. Electrostatic potential energy surfaces for VA with Ac-D-Ala-D-Ala and Ac-D-Ala-D-Lac are shown in Figure 8. It is evident that most of the negative charges associated with the pocket come from the oxygen atoms. This is particularly noticeable for the D-Lac case, where this is readily visible. Table 5 lists the excess charges associated with each of the atoms comprising the five hydrogen bonds important for the vancomycin complexes. While there is variation between C_A and C_B, the trends on the partial charges are mostly similar with the oxygens carrying negative excess charges (units

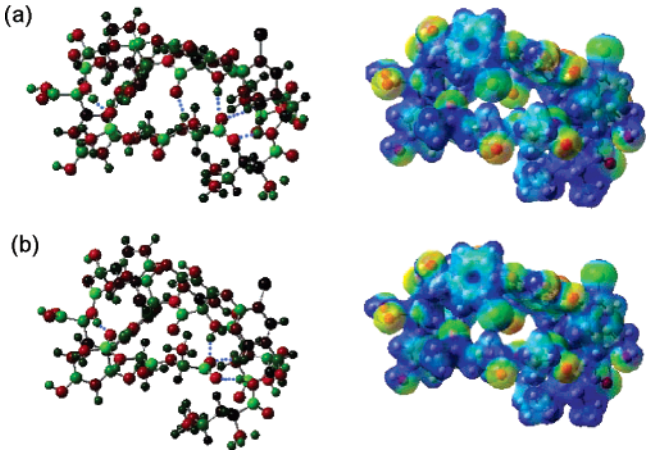

Figure 8. Two VA complexes: (a) with Ac-D-Ala-D-Ala and (b) with Ac-D-Ala-D-Lac showing the charges on the atoms, with red (green) corresponding to excess negative (positive) charges (left panel) and the electrostatic potential as projected onto the charge density (right panel). Here, red (blue) represents the regions of negative (positive) charges, respectively.

TABLE 5: Excess ESP Charges (units e) on Atoms Forming the Hydrogen Bonds of the VA Complexes

	Gly	D-Ala	D-Ser (up)	D-Ser (down)	D-Lac
O_1[a]	−0.701[b]	−0.671[b]	−0.665[b]	−0.658[b]	−0.683[b]
	−0.682	−0.703	−0.736	−0.682	−0.695
	−0.735	−0.723	−0.742	−0.690	−0.710
O_2[a]	−0.689[b]	−0.664[b]	−0.648[b]	−0.649[b]	−0.684[b]
	−0.541	−0.587	−0.540	−0.477	−0.533
	−0.611	−0.608	−0.639	−0.604	−0.608
H_1[a]	0.253[b]	0.302[b]	0.296[b]	0.263[b]	
	0.225	0.291	0.342	0.284	
	0.182	0.333	0.334	0.291	
O_3[a]	−0.579[b]	−0.583[b]	−0.586[b]	−0.574[b]	−0.578[b]
	−0.608	−0.626	−0.615	−0.604	−0.644
	−0.619	−0.607	−0.619	−0.591	−0.655
H_1[c]	0.327[b]	0.327[b]	0.327[b]	0.327[b]	0.370
	0.375	0.381	0.533	0.445	0.375
	0.481	0.432	0.523	0.374	0.408
H_2[c]	0.214[b]	0.214[b]	0.214[b]	0.214[b]	0.214[b]
	0.155	0.214	0.271	0.212	0.105
	0.262	0.353	0.316	0.374	0.278
H_3[c]	0.188[b]	0.188[b]	0.188[b]	0.188[b]	0.188[b]
	0.305	0.284	0.190	0.215	0.270
	0.293	0.286	0.189	0.264	0.306
O_1[c]	−0.536[b]	−0.536[b]	−0.536[b]	−0.536[b]	−0.536[b]
	−0.468	−0.498	−0.490	−0.492	−0.500
	−0.455	−0.500	−0.509	−0.505	−0.538
H_4[c]	0.405[b]	0.405[b]	0.405[b]	0.405[b]	0.405[b]
	0.369	0.375	0.374	0.384	0.384
	0.374	0.361	0.385	0.358	0.374

[a] The first four atoms listed correspond to atoms on the bacterial wall analogues, listed left to right as in Figure 2. [b] Represents the charges on the atom for the isolated fragments, while charges listed below those labeled "*b*" correspond to configurations C_A and C_B, respectively. For D-Lac, the O^b corresponds to the oxgen atoms that otherwise substitues for the "NH" ligand on D-Ala. [c] Below the triple line, the subsequent atoms correspond to those on the VA pocket, again listed left to right as marked in Figure 2.

electron charge e) in the 0.5−0.7 range and the hydrogens carrying positive excess charges in the 0.2−0.3 range.

IV. Summary

We have investigated the binding of prototypical GAs to bacterial cell wall analogues with quantum chemistry methods. Specifically, the binding of the aglycon forms of vancomycin, avoparcin, ristocetin, and teicoplanin to the dipeptides, Ac-D-Ala-X, with X = D-Ala, D-Lac, D-Ser, Gly, and "methylated D-Ala", was investigated with DFT-based methods. Except for the D-Lac and CH_2 cases, binding takes place through a series of five hydrogen bonds. For the case of D-Lac, bonding is decreased not only because of the loss of a single hydrogen bond but also because of an additional oxygen−oxygen lone-pair repulsion. For the case of D-Ser, despite the five hydrogen bonds, binding is decreased because of the extra stable conformation of the isolated D-Ser dipeptide (with OH down). It is interesting to note that the order of the gas binding (from strongest to weakest) is Gly, D-Ala, D-Ser, CH_2, and D-Lac. Using the PCM implicit solvent model, we find that the Gly and D-Ala ordering is reversed in solution, in agreement with experimental results. Having understood the loss and gain of binding for these complexes, efforts are currently underway to theoretically reengineer the GAs, so that these recover their antibacterial fighting power.

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Supporting Information Available: Coordinates and structural information as to the binding of vancomycin aglycon to Ac-D-Ala-D-Ala, Ac-D-Ala-D-Lac, Ac-D-Ala-D-Ser, and Ac-D-Ala-Gly, along with a complete ref 39. This material is available free of charge via the Internet at http://pubs.acs.org.

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