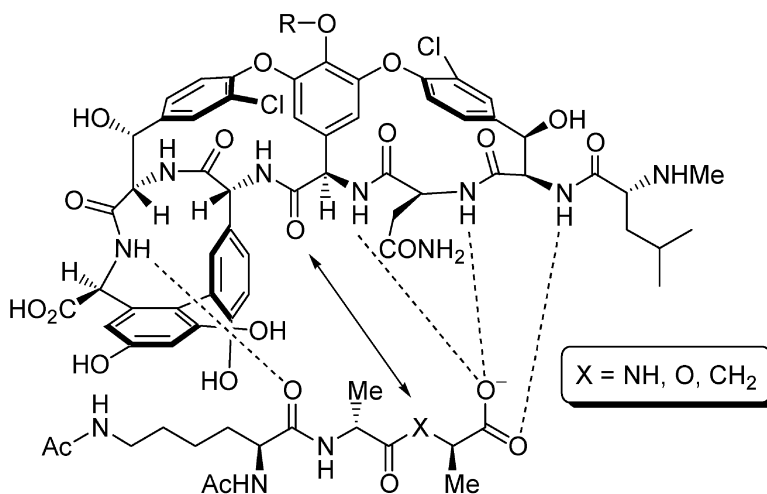


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## Partitioning the Loss in Vancomycin Binding Affinity for D-Ala-D-Lac into Lost H-Bond and Repulsive Lone Pair Contributions

Casey C. McComas, Brendan M. Crowley, and Dale L. Boger\*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute,  
10550 North Torrey Pines Road, La Jolla, California 92037

Received May 1, 2003; E-mail: boger@scripps.edu

Vancomycin (**1**) is the parent member of the class of clinically important glycopeptide antibiotics (Figure 1).<sup>1</sup> It is used with patients allergic to  $\beta$ -lactam antibiotics, and it is now a frontline therapy for endocarditis and for many bacterial infections in patients undergoing cycles of cancer chemotherapy.<sup>2</sup> Most importantly, it has become the drug of last resort for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA).<sup>3</sup>

Following studies which indicated that the glycopeptide antibiotics inhibit bacterial cell wall biosynthesis,<sup>4</sup> Perkins demonstrated that vancomycin selectively binds the precursor peptidoglycan peptide terminus *N*-acyl-D-Ala-D-Ala, preventing bacterial cell wall maturation.<sup>5</sup> Shortly after the disclosure of the structure of vancomycin,<sup>6</sup> Williams provided the structure of the *N*-acyl-D-Ala-D-Ala complex with the antibiotic which was found to be stabilized by an extensive array of van der Waals contacts (hydrophobic contacts) within the vancomycin binding pocket and five key H-bonds lining the pocket (Figure 1A).<sup>7,8</sup>

With its more frequent use, vancomycin-resistant Gram-positive bacteria have emerged, including vancomycin-resistant enterococci (VRE).<sup>2</sup> The elucidation of the origin of the VanA and VanB bacterial resistance and its structural basis were described by Walsh, Courvalin, and co-workers.<sup>9,10</sup> It entails the reprogramming of the peptidoglycan termini from D-Ala-D-Ala to D-Ala-D-Lac. This simple substitution of a linking ester for an amide with the exchange of a single atom (NH $\rightarrow$ O) reduces the binding to vancomycin 1000-fold and accounts fully for the 3 orders of magnitude higher MICs seen in VRE clinical isolates.<sup>9</sup> The complex of vancomycin with *N*-acyl-D-Ala-D-Lac lacks the central H-bond characteristic of the D-Ala-D-Ala complex and has been suspected to suffer from a repulsive lone pair interaction between the vancomycin residue 4 carbonyl and the D-Ala-D-Lac ester oxygen (Figure 1B). Herein, we provide the first experimental estimation of the magnitude of these two effects which suggests that it is the repulsive lone pair interactions (2.6 kcal/mol), not the H-bond loss (1.5 kcal/mol), that is responsible for the larger share of the reduced binding affinity (4.1 kcal/mol).

The estimation is derived from a comparison of the vancomycin and vancomycin aglycon binding affinity for **4**<sup>11</sup> (Scheme 1) versus that for Ac<sub>2</sub>-L-Lys-D-Ala-D-Ala (**3**) and Ac<sub>2</sub>-L-Lys-D-Ala-D-Lac (**5**) measured by titration of the antibiotic hosts with the ligand (UV), Figure 2.<sup>12</sup> The structure of **4** incorporates a methylene (CH<sub>2</sub>) in place of the amide NH of **3** and the ester O of **5**. Thus, **4** lacks the capabilities for forming the H-bond of **3** and does not suffer the repulsive lone pair/lone pair destabilization of **5**. To a first approximation, the comparison of **4** with **3** provides an estimation of the H-bond contribution to binding, whereas its comparison with **5** provides an estimation of the repulsive lone pair binding destabilization of **5**. In the case of both vancomycin (**1**) and the vancomycin aglycon (**2**), the affinity for **4** was roughly 10-fold less than that of Ac<sub>2</sub>-L-Lys-D-Ala-D-Ala (**3**), but 100-fold greater than

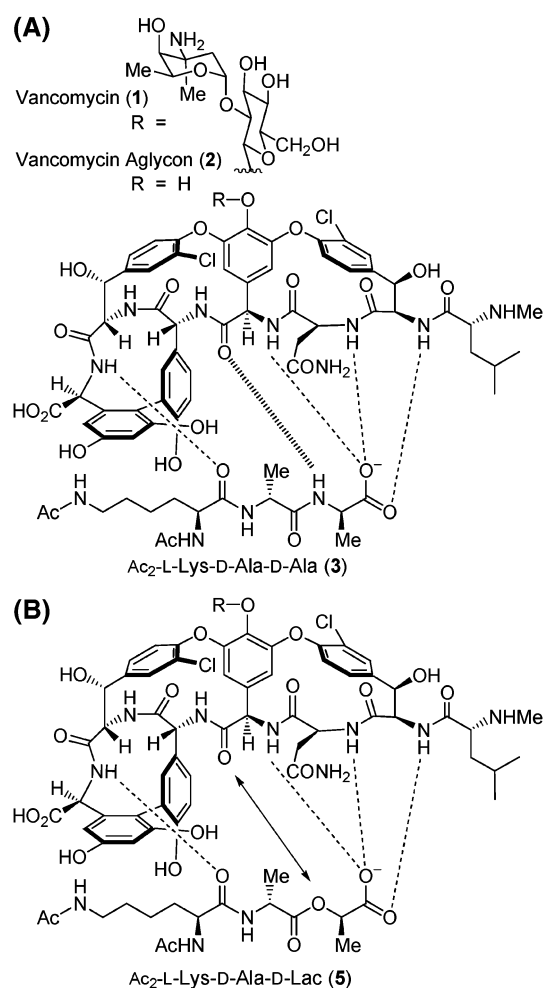


Figure 1.

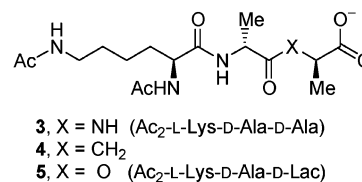


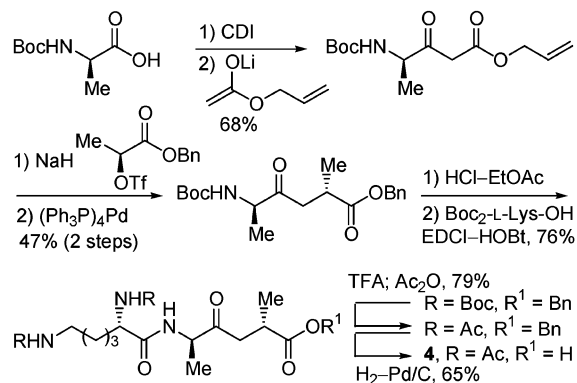
Figure 2.

that of Ac<sub>2</sub>-L-Lys-D-Ala-D-Lac (**5**), Table 1. In the absence of compensating or detrimental effects of the methylene substitution in **4**, this suggests that the reduced binding affinity of **5** (4.1 kcal/mol) may be attributed to both the loss of the H-bond of **3** (1.5–1.8 kcal/mol) and the destabilizing lone pair/lone pair interaction introduced with **5** (2.6 kcal/mol) with the latter, not the lost H-bond, being responsible for the largest share of the reduction.

**Table 1.** Association Constants ( $K$ ) and Binding Free Energy ( $-\Delta G^\circ$ , 25 °C)<sup>a</sup>

ligand	vancomycin (1) $K$ , $M^{-1}$ ( $-\Delta G^\circ$ , kcal mol <sup>-1</sup> )	vancomycin aglycon (2) $K$ , $M^{-1}$ ( $-\Delta G^\circ$ , kcal mol <sup>-1</sup> )
3 (X = NH)	$4.4 \times 10^5$ (7.7)	$5.8 \times 10^5$ (7.8)
4 (X = CH <sub>2</sub> )	$3.3 \times 10^4$ (6.2)	$2.5 \times 10^4$ (6.0)
5 (X = O)	$4.3 \times 10^2$ (3.6)	$3.1 \times 10^2$ (3.4)

<sup>a</sup> 25 °C, 0.00011 M vancomycin in 0.02 M sodium citrate, pH 5.1, observed at 279 nm, ref 12.

**Scheme 1**

Notably, this substitution in **4** can have several effects that would impact binding beyond those which we purport to be estimating. In addition to removing the repulsive lone pair destabilization of the ester O of **5**, it increases the hydrophobic character of the ligand favoring binding in a hydrophobic pocket and alters the solvation characteristics of the ligand in a way that may favor binding relative to **5**. In this case, the difference in the **4** versus **5** binding would overestimate the lone pair electrostatic destabilization with **5**. In addition to removing the H-bond of **3**, the methylene substitution in **4** increases its conformational flexibility and reduces its conformational preference relative to both **3** and **5** (rigidity: **3** (trans amide) > **5** (syn eclipsed ester) > **4**) and could introduce unfavorable steric interactions,<sup>13</sup> both of which would disfavor binding relative to **3**. This would result in the comparison of the **4** versus **3** binding overestimating the H-bond contribution to the binding of **3**. Moreover, the simplistic partitioning of the effects into a lost H-bond versus introduction of repulsive lone pair interactions does not take into account cooperative enthalpic or entropic binding enhancements attributable to adjacent binding interactions.<sup>14</sup> Nonetheless, the estimates are consistent with intuitive expectations resulting from the stabilizing differential binding

energy<sup>15</sup> of an amide H-bond (0.0–1.5 kcal/mol) and that resulting from a destabilizing lone pair/lone pair interaction (1.6–2.7 kcal/mol).<sup>15</sup>

More importantly and independent of the origin of the effects, these observations have significant ramifications in the reengineering of the vancomycin structure to bind D-Ala-D-Lac, suggesting that the design could focus principally on removing the destabilizing lone pair interaction rather than reintroduction of a H-bond and that this may be sufficient to compensate for 2 of the 3 orders of magnitude in binding affinity lost with D-Ala-D-Lac. Such efforts are underway and will be reported in due course.

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**Supporting Information Available:** Experimental details and characterization data for the synthesis of **4** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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