Supporting Information

Thermodynamics of Interactions of Vancomycin and Synthetic Surrogates of Bacterial Cell Wall

Mikhail Rekharsky,[†] Dusan Hesek,[§] Mijoon Lee,[§] Samy O. Meroueh,[§] Yoshihisa Inoue[†]* and Shahriar Mobashery[§]*

[†]*Entropy Control Project, ICORP, JST, 4-6-3 Kamishinden, Toyonaka 560-0085, Japan*

[§]*Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA*

Microcalorimetric titration experiments. Microcalorimetric experiments were performed using isothermal titration calorimeter VP-ITC (MicroCal, USA). Each experiment consisted of 25-55 consecutive injections (5-10 μL) of peptidoglycans solution in aqueous 0.1 M sodium-acetate buffer (pH 4.7) into microcalorimetric reaction cell (1.4 mL) charged with solution of vancomycin in the same buffer solution. Heat of reaction was corrected for the heat of dilution of peptidoglycans solution determined in separate experiments. Concentration of vancomycin was selected below 10^{-4} M level to avoid self-dimerization. All solutions were degassed prior titration experiment according to procedures provided by MicroCal, Inc.

Computer simulations (curve fitting) were performed using ORIGIN 7.0 software adapted for ITC data analysis. In the case of complexation of vancomycin with $(Ac)_2$KAA and compounds **1**-**3**, the Single Set of Identical Sites model was applied. More complex interaction between vancomycin and compound **4** was treated using Sequential Binding Sites model in its deconvolution mode. Equations that describe the binding models used in this study are discussed in more details elsewhere.[1]

Representative examples of microcalorimetric titration experiments and curve fitting are shown at Figure 1-3.

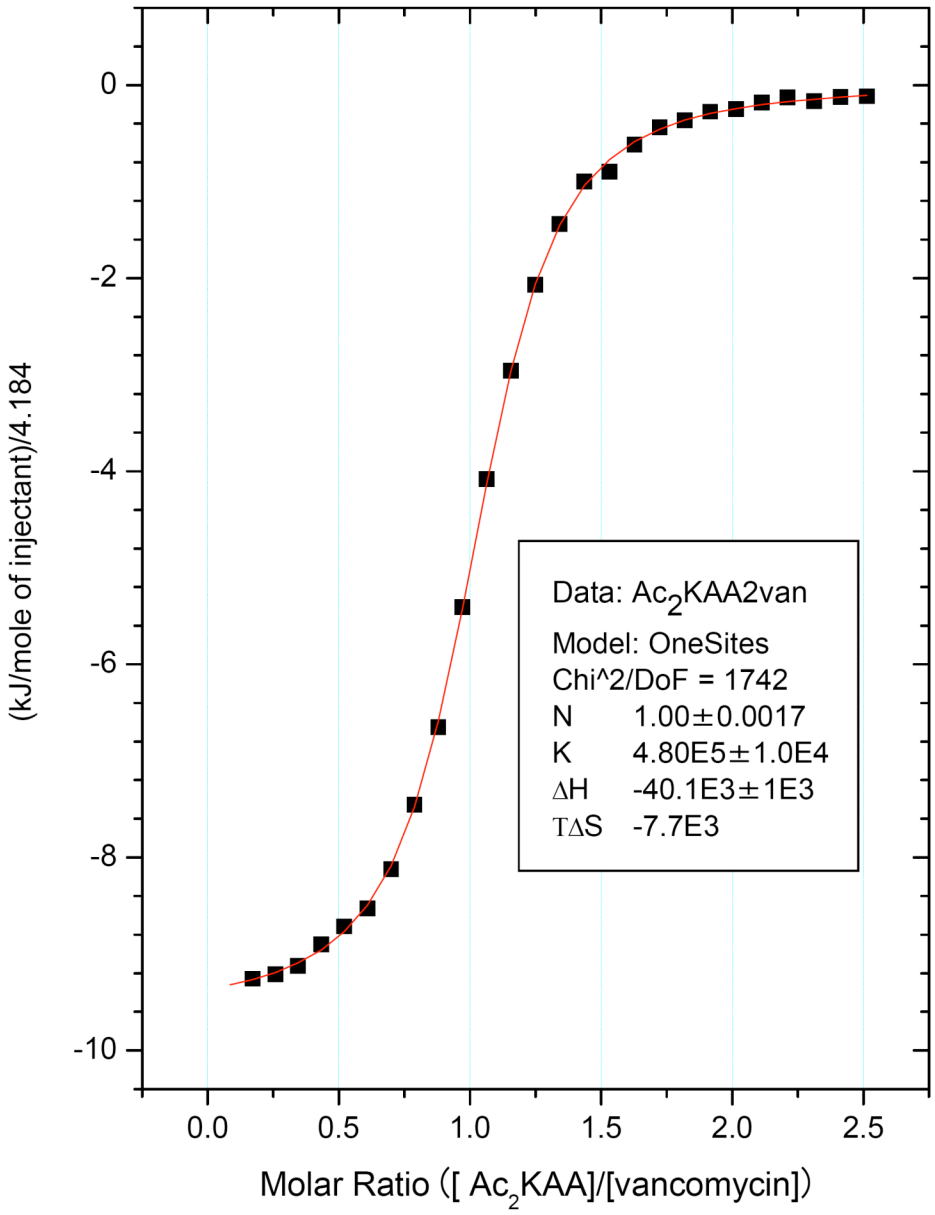

Figure 1. Computer simulation of experimental titration curve upon interaction of vancomycin with $(Ac)_2KAA$. Concentration of vancomycin was 0.081 mM to assure that vancomycin exists in the solution in the monomeric form.[2,3] Heat of reaction was measured in the presence of varying concentration of $(Ac)_2KAA$ (0.014 to 0.20 mM) in 0.1 M sodium-acetate buffer (pH 4.7). ■ represents experimentally determined heat effect of each separate injection (kJ/mol of injectant); ── represents computer simulation of titration curve; Chi^2/DoF represents magnitude of scattering (Chi^2) of experimental data points (■) over computer simulated titration curve (──) normalized by degree of freedom (DoF).

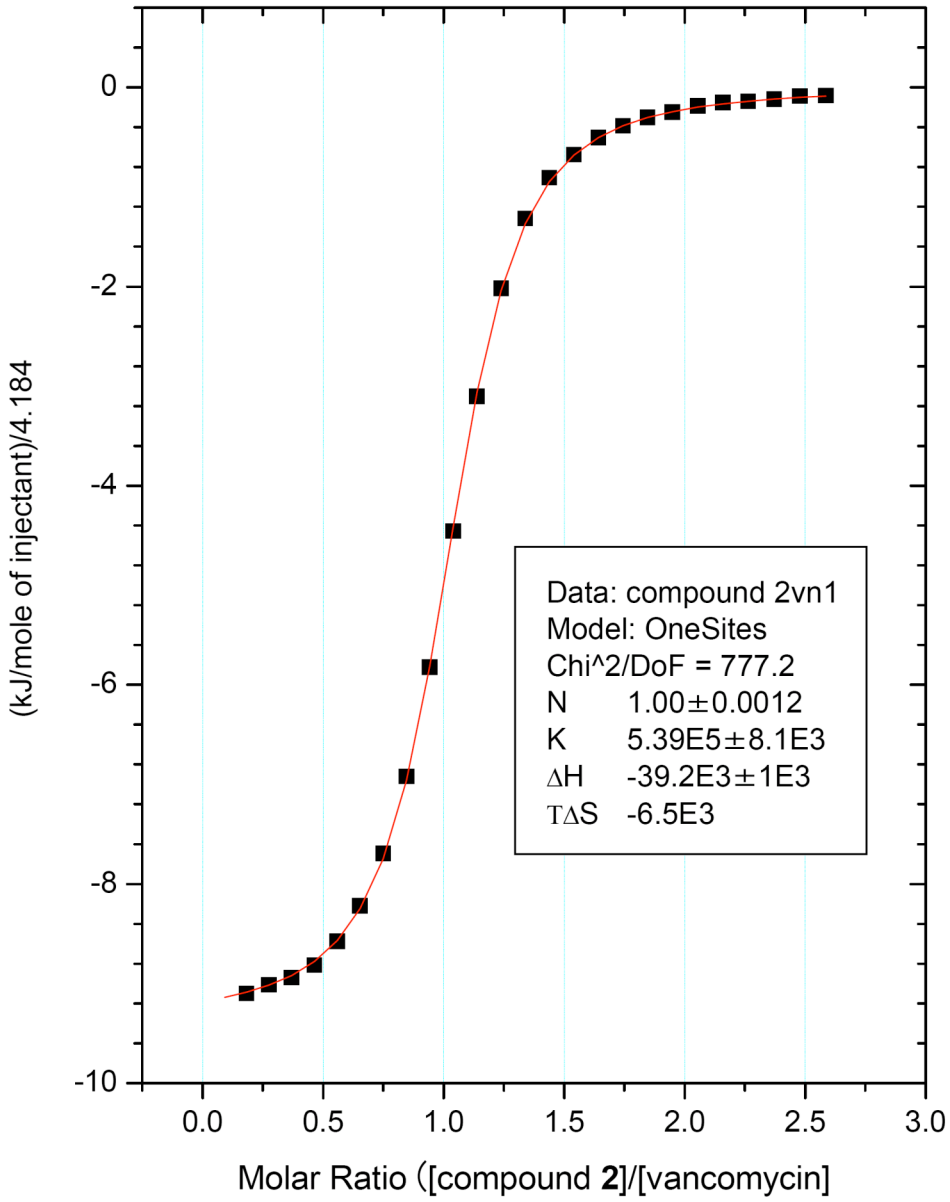

Figure 2. Computer simulation of experimental titration curve upon interaction of vancomycin with compound **2**. Concentration of vancomycin was 0.081 mM to assure that vancomycin exists in the solution in monomeric form.[2,3] Heat of reaction was measured in the presence of varying concentration of compound **2** (0.015 to 0.21 mM) in 0.1 M sodium-acetate buffer (pH 4.7). ■ represents experimentally determined heat effect of each separate injection (kJ/mole of injectant); ── represents computer simulation of titration curve; Chi^2/DoF represents magnitude of scattering (Chi^2) of experimental data points (■) over computer simulated titration curve (──) normalized by degree of freedom (DoF).

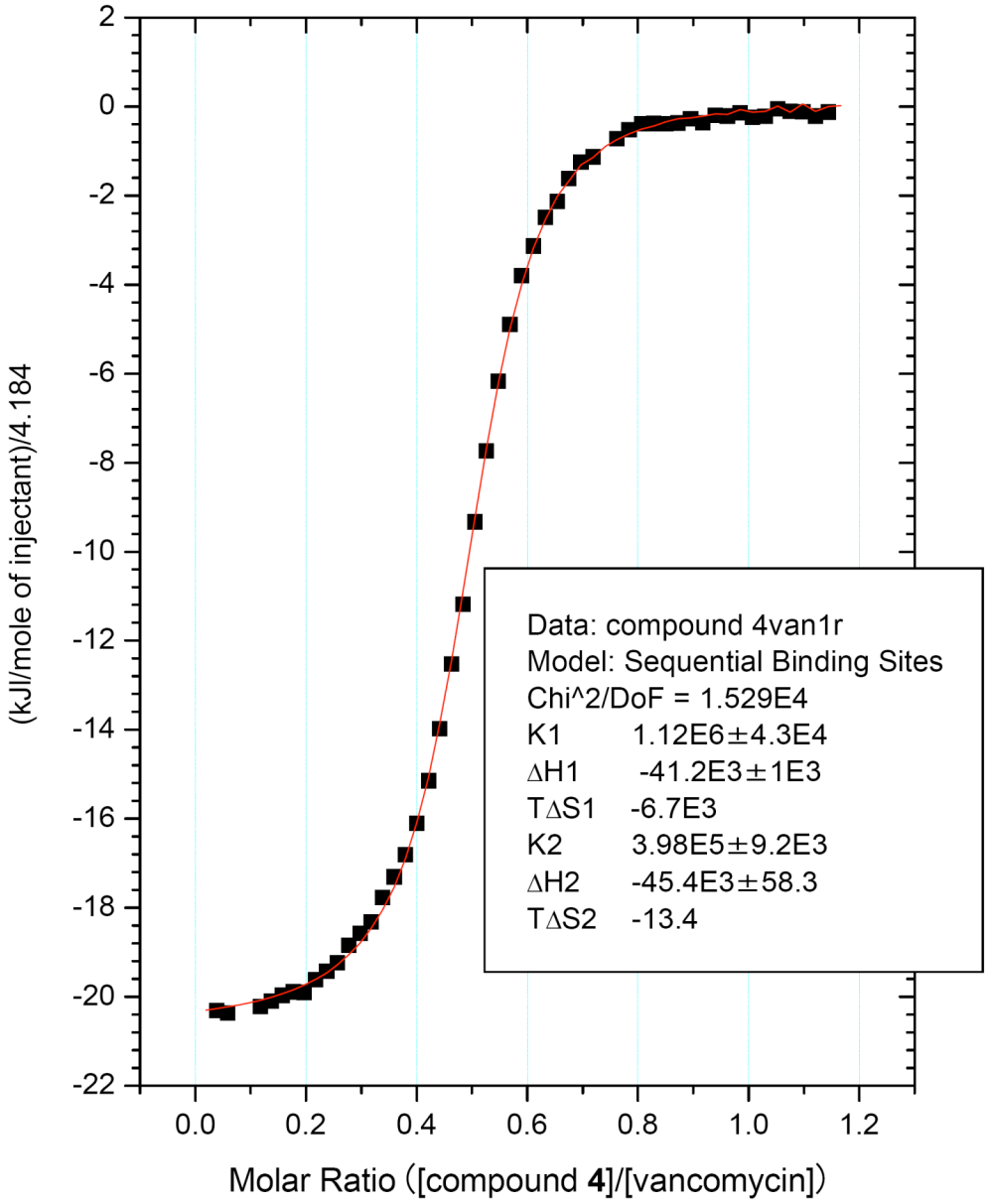

Figure 3. Computer simulation of experimental titration curve upon interaction of vancomycin with compound **4**. Concentration of vancomycin was 0.081 mM to assure that vancomycin exists in the solution in monomeric form.[2,3] Heat of reaction was measured in the presence of varying concentration of compound **4** (0.0028 to 0.092mM) in 0.1 M sodium-acetate buffer (pH 4.7). ■ represents experimentally determined heat effect of each separate injection (kJ/mole of injectant); ── represents computer simulation of titration curve; Chi^2/DoF represents magnitude of scattering (Chi^2) of experimental data points (■) over computer simulated titration curve (──) normalized by degree of freedom (DoF).

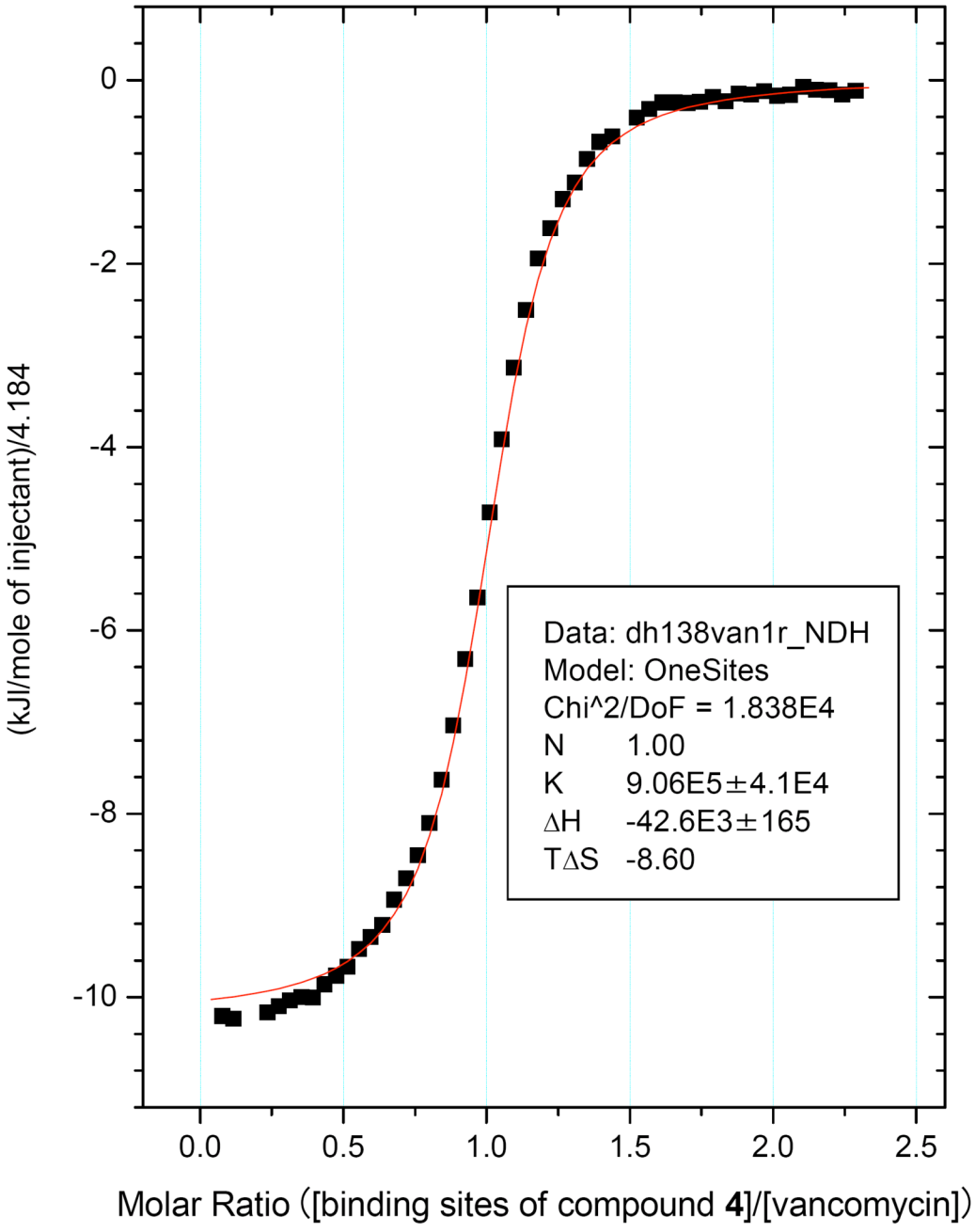

Figure 4. Computer simulation of experimental titration curve upon interaction of vancomycin with compound **4** (the same experimental data and the same experimental conditions as indicated for **Figure 3**) with the assumption that both binding sites of compound **4** are identical and they do not interact upon formation of the 1:2 complex (compound **4** + 2 vancomycin). ■ represents experimentally determined heat effect of each separate injection (kJ/mole of injectant); ⎯ represents computer simulation of titration curve; Chi^2/DoF represents magnitude of scattering (Chi^2) of experimental data points (■) over computer simulated titration curve (⎯) normalized by degree of freedom (DoF).

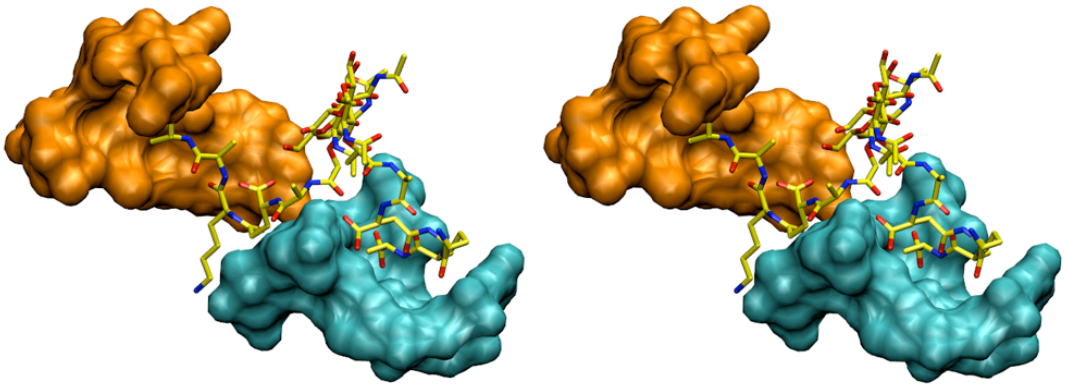

Figure 5. Another perspective of the complex of compound 4 and two vancomycin molecules (shown as Figure 1 of the main text).

Computational procedures. The three-dimensional structure of compound **4** was recently solved by NMR, providing Cartesian coordinates for the construction of the complex with vancomcyin. The coordinates for vancomycin were obtained from its crystal structure in complex with diacetyl-L-Lys-D-Ala-D-Ala, which was downloaded from the RCSB database (http://www.rcsb.org; PDB ID 1FVM). The program Sybyl 7.0 was then used to construct a three-dimensional model of compound **4** with a vancomycin molecule bound at each of its peptide stem, based on the NMR structure of peptidoglycan and the X-ray structure for the complex of vancomycin and the peptide. This was carried out by first superimposing the L-Lys-D-Ala-D-Ala portion of the peptide stem from compound **4** to that of L-Lys-D-Ala-D-Ala of the vancomycin/diacetyl-L-Lys-D-Ala-D-Ala crystal structure along carbon, nitrogen and oxygen atoms. The backbone dihedral angles of the diacetyl-L-Lys-D-Ala-D-Ala portion of compound **4** were set to conform to those observed in the vancomycin/diacetyl-L-Lys-D-Ala-D-Ala. The resulting complex was then prepared for molecular dynamics simulations with the AMBER 8 package.[4] Atomic charges for compound **4** were determined earlier,[5] while atomic charges for vancomycin were determined using the RESP methodology.[6] We have computed the charge for the entire molecule. This consisted of subjecting vancomycin to a HF/6-31G* geometry optimization using the Gaussian03 suite of programs.[7] The program Antechamber was then used to create a Mol2 formatted file that contained gaff atom types and the RESP charges, using an overall charge of 1, and multiplicity of 1. The program Xleap was then used to create a topology and coordinate files based on the coordinates of the complex of compound **4** with a vancomycin molecule bound to each of its peptide (from the Sybyl 7.0 modeling step). This consisted of first loading the Mol2 formatted files for each of the vancomycin molecules, a Prep file containing the Cartesian coordinates and atomic charges of compound **4** from a previous study,[2] and a text file that contained all the forcefield information for compound **4** as reported earlier. This was followed by the creation of a TIP3P[9] water box, so that the complex of compound **4** with two vancomycin molecules was completely submerged by water, such that no atom in the complex was less than 12 Å from any side of the box. This resulted in a system of 20,823 atoms. The resulting topology and coordinate files contained all necessary information to carry out the molecular dynamics simulation with the AMBER package. The program Sander from the AMBER 8 suite of programs was using in a multiprocessor mode, using 16 processors to propagate the trajectory. Periodic boundary conditions are used during the simulation, and the SHAKE algorithm was turned on, affording the use of a 2 fs time step. The particle mesh Ewald methodology (PME) was used to treat long-range electrostatics, and the system is automatically neutralized by the method.[10] The

equilibration protocol consisted of the following steps. The system was first energy minimized for 50,000 conjugate gradient steps. Subsequently, the complex was restrained, using Cartesian restraints with a force constant of 500 kcal/mol·Å, and a 40 ps molecular dynamics run was carried out, to allow water molecules to equilibrate around the system. Harmonic restraints were used during the simulations to maintain the integrity of the 5 hydrogen bonding interactions that are observed in the vancomycin/diacetyl-L-Lys-D-Ala-D-Ala crystal structure. A series of 6 energy minimizations were subsequently conducted using 500, 100, 50, 10, 2, and then 0 kcal/mol·Å restraints, respectively. Each of these energy minimizations was carried out for 1000 steepest descent steps. Subsequently, the system was slowly heated to 100 K over 12 ps, followed by 16 ps of dynamics at 100 K. Then the system was heated to 200 K over 12 ps, followed by 16 ps of dynamics. Finally, the system was heated to 300 ps over 12 ps, and the resulting complex was used to carry out production dynamics. The simulation was carried out for a total of 12 ns. Analysis of the trajectory was carried out with the Ptraj module of the AMBER 8 software package. Snapshots at 10 ps intervals are collected and water is stripped from the trajectory. The snapshots are root-mean-squared fitted using Ptraj along the carbon and oxygen atoms of the polysaccharide backbone of compound **4**. The program VMD is then used to visualize the trajectory, which is ultimately rendered into an MPEG animation and made available at the http://pubs.acs.org Web site.

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