by oxidative elimination lead exclusively to the bicyclo[3.3.1] regioisomer **15** as a mixture of nitrile epimers. As precedented by work in the shikimate series,[13] this material undergoes epoxidation with a peracid from the exo face and furnishes the desired allylic alcohol **16** on rearrangement via the bromohydrin silyl ether. Alkaline hydrolysis of **16** gives a 6:1 mixture of the exo and endo isomers **8** and **10**. In addition to the expected predominance of the more stable exo isomer **8**, the stereostructure of these compounds was readily assigned on the basis of the NMR coupling pattern of the hydrogens at the 3-position: exo isomer **8**, dd, J = 3.2, 12.2 Hz; endo isomer **10**, dd, J = 3.0, 7.2 Hz. An additional compound, the unsaturated derivative **9**, was obtained on hydrolysis of a side product from a related synthesis.[14]

Compounds **8**–**10** as well as adamantane-1-phosphonic acid (**7**) were evaluated as inhibitors against the chorismate mutase/prephenate dehydrogenase from *E. coli*. The assays were performed at pH 7.5 using conditions similar to those reported by SampathKumar and Morrison.[15] The results detailed in Table II indicate that the exo and unsaturated derivatives **8** and **9** are not significantly better as inhibitors than their saturated carbocyclic analogue **6**. In contrast, the endo isomer **10** is bound some 100-fold more tightly, with an I_{50} value of 1.5×10^{-7} M at pH 7.5. The true K_i value could therefore be as low as 4×10^{-8} M for the active enantiomer. At this pH adamantane-1-phosphonate is a considerably weaker inhibitor than **10**. The crucial element in the efficacy of the endo isomer **10** is its chair conformation and the resulting orientation of the bridge-carboxylate moiety over the unsaturated ring. In contrast to the endo isomer of the saturated carbocycle **4**,[6] which adopts the chair–boat conformation for steric reasons, ^{1}H NMR analysis indicates that the tetrahydropyran ring of **10** is in the chair conformation as shown.[16] Although the binding enhancement observed with **10** falls short of that expected for a "perfect" transition-state analogue, these results confirm the supposition that orientational effects are critical for a chorismate mutase inhibitor and point the way for future improvements.

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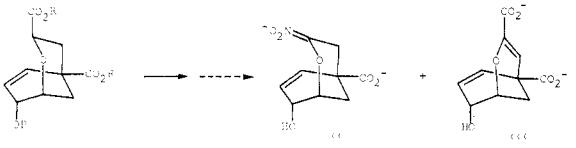
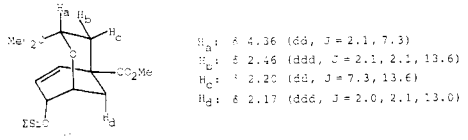
Supplementary Material Available: Experimental details of the synthesis of **8** and **10** and their enzymatic evaluation (8 pages). Ordering information is given on any current masthead page.

(11) Evans, D. A.; Truesdale, L. K. *J. Chem. Soc. Chem. Commun.* **1973**, 55–56.
(12) Nicolaou, K. C. *Tetrahedron* **1981**, *37*, 4097–4109.
(13) Bartlett, P. A.; McQuaid, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 7854–7860.
(14) Nitration of the enolate of i was undertaken in an attempt to produce ultimately the nitronate analogue ii. The unsaturated compound iii was



derived from a side product of this reaction.
(15) SampathKumar, P.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *702*, 212–219.
(16) The NMR datum most indicative of the chair conformation is the long-range W coupling of 2.1 Hz between H_b and H_d observed for the derivative iv



(Dr. Y. Nakagawa, unpublished results). A similar albeit less well-resolved pattern is seen for the corresponding hydrogens of the dianion **10**.

Hydration of Chloride and Bromide Anions: Determination of Relative Free Energy by Computer Simulation

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Computer-simulation techniques that can reliably predict relative free energies of reactions have great potential usefulness in chemistry, biochemistry, and pharmacology. Such techniques could be used to calculate relative solubilities, relative free energies of binding for ligand–receptor complexes, and relative free energies of activation (i.e., relative reaction rates). In particular, the ability to calculate relative free energies of solvation is of special interest. For example, relative free energies of solvation (or more precisely, relative free energies of *desolvation*) often play a major role in determining the relative binding affinity of two ligands at a common receptor site.

One simulation technique used to compute the free energy of reaction is the umbrella sampling technique.[1-4] In this approach, one uses molecular dynamics or Monte Carlo simulations to compute the free energy change as a function of reaction advancement along some predefined reaction coordinate. This method has been used to study molecular association complexes[1-3] and a chemical reaction[4] in water. In principle, this method could be used to predict relative free energies of solvation for two molecules L and M by, e.g., gradually immersing the molecules

$$\mathrm{L(g)} \rightarrow \mathrm{L(aq)} \qquad \Delta A_1 \tag{1}$$

$$\mathrm{M(g)} \rightarrow \mathrm{M(aq)} \qquad \Delta A_2 \tag{2}$$

and computing ΔA_1 and ΔA_2 in separate simulations. The relative free energy of solvation, $\Delta\Delta A = \Delta A_2 - \Delta A_1$, would then be computed as the difference of the two simulation results, ΔA_1 and ΔA_2. The umbrella sampling technique possesses several shortcomings, however, which limit its usefulness in relative free energy calculations.[5]

An alternative simulation approach applies perturbation theory techniques to a set of reactions forming a closed thermodynamic cycle in order to compute relative free energies of reaction.[5] The type of free energy obtained (e.g., Helmholtz free energy A, or Gibbs free energy G) depends on the type of ensemble used in the simulation (see below). In the perturbation–thermodynamic cycle approach, two hypothetical reactions would be defined:

$$\mathrm{L(g)} \rightarrow \mathrm{M(g)} \qquad \Delta A_3 \tag{3}$$

$$\mathrm{L(aq)} \rightarrow \mathrm{M(aq)} \qquad \Delta A_4 \tag{4}$$

Reactions 1–4 form a closed thermodynamic cycle; thus, $\Delta\Delta A = \Delta A_2 - \Delta A_1 = \Delta A_4 - \Delta A_3$, since A is a thermodynamic state function. A perturbation technique[5-7] is used to compute ΔA_4 and, if necessary, ΔA_3. Potential energy functions V_L for the L/solvent system, V_M for the M/solvent system, and V_λ for a "hybrid" system are defined, where

$$V_\lambda = \lambda V_M + (1 - \lambda) V_L \tag{5}$$

Molecular dynamics or Monte Carlo simulations based on one or more of these potential functions are then carried out. For each simulation, the free energy for values of λ about λ_i is obtained from the perturbation result

(1) Pangali, C.; Rao, M.; Berne, B. J. *J. Chem. Phys.* **1979**, *71*, 2975.
(2) Ravishanker, G.; Mezei, M.; Beveridge, D. L. *Faraday Symp. Chem. Soc.* **1982**, *17*, 79.
(3) Berkowitz, M.; Karim, O. A.; McCammon, J. A.; Rossky, P. J. *Chem. Phys. Lett.* **1984**, *105*, 577.
(4) Chandrasekhar, J.; Smith, S. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1985**, *107*, 154.
(5) Tembe, B. L.; McCammon, J. A. *Comput. Chem.* **1984**, *8*, 281.
(6) McQuarrie, D. A. "Statistical Mechanics"; Harper and Row: New York, 1976.
(7) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; Hermans, J. In "Intermolecular Forces"; B. Pullman, Ed.; Reidel: Holland, 1981.

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$$A_4(\lambda) - A_4(\lambda_i) = -\beta^{-1} \ln \langle \exp[-\beta(V_\lambda - V_{\lambda_i})] \rangle_{\lambda_i} \quad (6)$$

where $\beta^{-1} = kT$ (k = Boltzmann's constant, T = temperature in Kelvin) and $\langle \rangle_{\lambda_i}$ is a simulation average for V_{λ_i}. The free energy thus obtained is of the Helmholtz type if the canonical ensemble (constant T, V, N) is used for the simulation and is of the Gibbs type if the isothermal–isobaric ensemble is used (constant T, P, N). The free energy change for the reaction is $\Delta A_4 = A_4(\lambda = 1) - A_4(\lambda = 0)$. A single simulation may be sufficient to span this range of λ if ΔA_4 is not more than a few times kT; otherwise, ΔA_4 can be computed by piecing together the results of simulations based on different values of λ_i. ΔA_3 can be computed by similar procedures in general. In the present application, it is a good approximation to assume that any physical effects that would contribute to ΔA_3 would contribute equally to ΔA_4; by consistently omitting such effects, one obtains the relative free energy of solvation as $\Delta\Delta A = \Delta A_4 - \Delta A_3 = \Delta A_4$.

In this study, we have examined the thermodynamic cycle

$$\begin{array}{ccc} Cl^-(aq) & \xrightarrow{\Delta A_4} & Br^-(aq) \\ \Delta A_1 \uparrow & & \uparrow \Delta A_2 \\ Cl^-(g) & \xrightarrow[\Delta A_3]{} & Br^-(g) \end{array} \quad (7)$$

As stated previously, $\Delta A_3 = 0$. Therefore, $\Delta\Delta A$ can be determined directly by computing ΔA_4. The simulations employed the canonical ensemble (constant T, V, N) at 300 K. The system contained an anion (Cl^- or Br^-) and 214 water molecules in a cubic box of edge length 18.6216 Å with minimal image periodic boundary conditions.

The SPC potential functions were used for water[7] and Cl^-.[8] Lennard–Jones parameters for Br^- were derived to reproduce experimental relative interaction energies for $Cl^-(H_2O)_1$ and $Br^-(H_2O)_1$ complexes.[9] The Br^- parameters are $r^* = 2.5950$ Å and $\epsilon = 0.0900$ kcal/mol. The Cl^- parameters are $r^* = 2.4954$ Å and $\epsilon = 0.1070$ kcal/mol. Constant-temperature molecular dynamics simulations[10] employing the Verlet algorithm were used to generate configurations of the anion–water systems. The SHAKE procedure was used to constrain all covalent bonds at equilibrium lengths.[11] Hydrogen masses were set to 10 amu; this slowed librational motions of the water molecules and allowed use of a large dynamics time step, $\Delta t = 4$ fs. The increased hydrogen mass leads to a more efficient sampling of configurations without affecting the equilibrium properties of the system.[12] The simulations were performed using the software package AMBER.[13] After extensive equilibration, simulations of the Cl^-/H_2O and Br^-/H_2O systems were run for 30 ps, with configurations saved every 0.1 ps. These configurations were then used in the perturbation method outlined above to compute ΔA_4.

From the perturbation of $Cl^- \rightarrow Br^-$ (and that of $Br^- \rightarrow Cl^-$), the relative free energy of solvation $\Delta\Delta A$ is estimated to be 3.35 ± 0.15 kcal/mol. Although the full range $\lambda = 0$–1 can be spanned in a single simulation, additional simulations were performed on a hybrid anionic species with Lennard–Jones parameters intermediate to those of Cl^- and Br^-. The perturbation calculations were now performed in two steps, first perturbing the Cl^- into the hybrid anion and then perturbing the hybrid anion to Br^-. The $\Delta\Delta A$ value obtained by piecing the two steps together was essentially identical with the above results. The estimated relative free energy of solvation is in excellent agreement with the experimental value ($\Delta\Delta A_{hydr} \approx \Delta\Delta G_{hydr} = 3.3$ kcal/mol).[14] Subsequent to submission of this manuscript, the assumption that $\Delta\Delta A \approx \Delta\Delta G$ for the present system has been confirmed by repeating the calculations using an isobaric–isothermal ensemble; in this case, one obtains $\Delta\Delta G = 3.15 \pm 0.15$ kcal/mol.

It should be noted that this result is part of a larger simulation to compute the relative free energy of binding for Cl^- and Br^- to the macropolycyclic ionophore, SC-24.[15] The computed relative free energy of solvation, along with the calculated relative free energy of interaction for Cl^- and Br^- with SC-24, predict a relative free energy of binding for Cl^- vs. Br^- to SC-24 of -4.15 ± 0.35 kcal/mol. This value is also in good agreement with experiment. The results of the work with SC-24, and a complete analysis of structural and thermodynamic aspects of the present simulation, will be presented elsewhere.[16] These results, together with those of other perturbation approach studies,[17,18] indicate the power and potential utility of this method for many problems in chemistry and related fields.

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(8) van Gunsteren, W. F., The GROMOS molecular modeling program.
(9) Arshadi, M.; Yamdagni, R.; Kebarle, P. *J. Phys. Chem.* **1970**, *74*, 1475.
(10) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684.
(11) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.
(12) Wood, D. W. In "Water: A Comprehensive Treatise"; Franks, F., Ed.; Plenum: New York, 1979; Vol. 6.
(13) Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1984**, *5*, 129.
(14) Friedman, H. L.; Krishnan, C. V. In "Water: A Comprehensive Treatise"; Franks, F., Ed.; Plenum: New York, 1972; Vol. 3.
(15) Graf, E.; Lehn, J. M. *J. Am. Chem. Soc.* **1976**, *98*, 6403.
(16) Lybrand, T. P.; McCammon, J. A.; Wipff, G. *Proc. Natl. Acad. Sci. U.S.A.*, in press. Lybrand, T. P.; Mazor, M.; McCammon, J. A., unpublished results.
(17) Jorgensen, W. L.; Ravimohan, C. *J. Chem. Phys.* **1985**, *83*, 3050.
(18) van Gunstern, W. F., et al., unpublished results.