Entropic factors provide unusual reactivity and selectivity in epoxide-opening reactions promoted by water

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Entropic factors provide unusual reactivity and selectivity in epoxide-opening reactions promoted by water

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Edited by Larry E. Overman, University of California, Irvine, CA, and approved August 21, 2013 (received for review June 14, 2013)

Despite the myriad of selective enzymatic reactions that occur in water, chemists have rarely capitalized on the unique properties of this medium to govern selectivity in reactions. Here we report detailed mechanistic investigations of a water-promoted reaction that displays high selectivity for what is generally a disfavored product. A combination of structural and kinetic data indicates not only that synergy between substrate and water suppresses undesired pathways but also that water promotes the desired pathway by stabilizing charge in the transition state, facilitating proton transfer, doubly activating the substrate for reaction, and perhaps most remarkably, reorganizing the substrate into a reactive conformation that leads to the observed product. This approach serves as an outline for a general strategy of exploiting solvent-solute interactions to achieve unusual reactivity in chemical reactions. These findings may also have implications in the biosynthesis of the ladder polyether natural products, such as the brevetoxins and ciguatoxins.

The authors declare no conflict of interest.

Author contributions: J.A.B. and T.F.J. designed research; J.A.B. performed research; J.A.B. and T.F.J. analyzed data; and J.A.B. and T.F.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Author contributions: J.A.B. and T.F.J. designed research; J.A.B. performed research; J.A.B. and T.F.J. analyzed data; and J.A.B. and T.F.J. wrote the paper.

The authors declare no conflict of interest.

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Results and Discussion

It is clear from the data in Table 1 that selectivity in the cyclization reactions under neutral aqueous conditions is very sensitive to the identity of the template and the size of the ring being formed (10–12). We were particularly surprised to find that a 1:1 mixture of products resulted from the seemingly minor change from 1a to 1b, which differ only by the replacement of an oxygen atom with a CH₂ group in the template (entries 1 and 2). Unfortunately, the combination of water and cyclic ether template did not allow for the incorporation of larger rings either for substrate 1c or for substrate 1d, the latter containing a seven-membered oxepane ring as the purported template (entries 3 and 4, respectively). Nevertheless, some modifications of the template did lead to enhanced selectivity. For example, 1e, possessing a second fused tetrahydropyran ring, afforded the highest levels of endo selectivity reported to date (entry 5) (24).

Our earlier mechanistic experiments (25) revealed that the cyclization reactions are kinetically controlled, occur in water (rather than on its surface or in micelles), and at pH 7.0 occur in a regime where neutral or “water-catalyzed” mechanisms prevail (26), instead of acid or base-catalyzed mechanisms that are more commonly invoked. Interestingly, kinetic studies revealed that the carbocyclic analog 1b cyclized nearly 10 times faster (as well as 10 times less selectively) than the original epoxy alcohol 1a. The kinetic behavior of reactions in dimethyl sulfoxide/water mixtures was most consistent with two additional water molecules required for the cyclization of 1a, but only one additional water molecule being requisite for cyclization of 1b.

Uncovering the Specific Role of the Template. We hypothesized that the additional water molecule required for 1a facilitates cyclization by forming a hydrogen-bonding network bridging the oxygen atom within the cyclic template and the oxygen atom of the hydroxyl group attached to the template (25). This interaction encourages reorganization of the epoxy alcohol from an energetically favorable chair conformation into a higher energy twist conformation (Fig. 3). This structural change in turn alters the trajectory of the nucleophile toward the epoxide electrophile, a factor that computations suggest is most important for selectivity in epoxide-opening reactions (27, 28). Because the template of 1b cannot participate in similar hydrogen-bonding interactions, an unselective reaction proceeds with the template in the more stable chair conformation.

However, we recognized that an alternative explanation for the differences between 1a and 1b is that the electron-withdrawing element oxygen within its template encourages endo cyclization of 1a by inductively disfavoring exo cyclization. Although this rationale accounts for the increased reaction rate and decreased selectivity observed for 1b compared with 1a, it does not explain why exo cyclization is observed for epoxy alcohol 1d, which has a different ring structure (oxepane) but retains the oxygen atom in the same position from the epoxide moiety. This would suggest that the different reactivity is due to another major difference between 1a and 1d, such as the greater conformational flexibility of the seven-membered oxepane ring.

Table 1. Endo:exo selectivity (2:3) for epoxy alcohol cyclizations in water at 20 °C and pH 7.0 (0.1 M potassium phosphate buffer)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Label</th>
<th>Structure</th>
<th>2:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>MeO</td>
<td>10:1</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>1:1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>MeO</td>
<td>&lt;1:20</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>1:3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>MeO</td>
<td>19:1</td>
</tr>
</tbody>
</table>

Fig. 1. Endo selective cyclization of epoxy alcohols templated by a tetrahydropyran ring(s) and promoted by water.

Fig. 2. Biosynthetic proposal for the formation of the ladder polyether natural product brevetoxin B, a potent neurotoxin.
Two additional pieces of evidence supported the importance of substrate conformation upon selectivity in these reactions and uncovered a unique role of the template. Determination of the activation parameters for cyclizations of 1a and 1b from Eyring plots revealed large and negative entropies of activation ($\Delta S^\ddagger$) in both cases (Table 2). This striking feature was somewhat surprising considering that the cyclization reactions are ostensibly unimolecular, which is typically an entropically favorable situation characterized by small entropies of activation (SI Text). Moreover, 1a displayed considerably more negative entropies of activation compared with 1b, despite a seemingly minor structural difference between the template rings—O vs. CH$_2$ (Table 2). These findings are completely consistent with cyclization being assisted by additional water molecules and with the higher kinetic order in water measured for 1a compared with 1b.

Comparing the activation parameters obtained for exo versus endo cyclization for 1a and 1b led to the important realization that entropy, not enthalpy, was most important in dictating selectivity in the cyclization of 1a—that is, $|T\Delta S^\ddagger| > |\Delta H^\ddagger|$ (Fig. 4). Hypothetically, if the reaction were governed entirely by enthalpy (i.e., $\Delta H^\ddagger$), then cyclization of 1a would exhibit slight selectivity for the exo product rather than the 10:1 endo selectivity observed. Substitution of an oxygen atom in the template ring apparently incurs a significant entropic penalty for exo cyclization of 1a that is completely absent in the case of 1b. This finding is more consistent with the hypothesis that explains the differences between 1a and 1b based on conformational differences rather than electronic differences because the former is largely entropic (i.e., affecting the organization of the transition state), whereas the latter is largely enthalpic (i.e., affecting bond making/breaking in the transition state).

The second piece of evidence that supported the importance of conformation to selectivity came from comparing the kinetic behavior for a series of epoxy alcohols: 1a, 1d, and 1e. These three epoxy alcohols contain templates that are virtually identical electronically yet are conformationally very different. As Table 1 demonstrates, selectivity for cyclization of these substrates increased with the rigidity of the template (i.e., 1d > 1a > 1e). Comparing the elementary rate constants for endo cyclization ($k_{endo}$) to exo cyclization ($k_{exo}$) revealed that $k_{endo}$ was similar for all three substrates, whereas $k_{exo}$ increased by a factor of 50 from

![Fig. 3. Proposed transition state for the cyclization of 1a aided by two water molecules in excess of those required for solvation.](image)

**Table 2.** Activation parameters ($\Delta X^\ddagger$) for the cyclizations of 1a and 1b in D$_2$O (pD 7.0, 0.1 M KPi buffer)

<table>
<thead>
<tr>
<th>$\Delta X^\ddagger$</th>
<th>1a</th>
<th>1b</th>
<th>1a</th>
<th>1b</th>
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<tbody>
<tr>
<td>$\Delta H^\ddagger$</td>
<td>16.4 ± 0.7</td>
<td>16.2 ± 0.8</td>
<td>16.3 ± 0.2</td>
<td>15.4 ± 0.2</td>
</tr>
<tr>
<td>$\Delta S^\ddagger$</td>
<td>-27.2 ± 2.1</td>
<td>-32.5 ± 2.5</td>
<td>-23.4 ± 1</td>
<td>-25.9 ± 1</td>
</tr>
<tr>
<td>$\Delta G^\ddagger$</td>
<td>24.5</td>
<td>25.9</td>
<td>23.3</td>
<td>23.1</td>
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$\Delta H^\ddagger$ in kcal/mol, $\Delta S^\ddagger$ in cal/mol·K, and $\Delta G^\ddagger$ in kcal/mol at 298 K. Error is represented as average error.

In addition to solvent pK$_a$, virtually all other solvent properties, such as dielectric constant and polarizability, were poorly correlated to reaction rate. However, a good correlation was obtained with $\alpha$, the Taft parameter that describes the hydrogen-bond–donating ability of a solvent (Fig. 4). In contrast, a poor correlation was observed with $\beta$, the Taft parameter for hydrogen-bond–accepting ability of the solvent. An important step in the reaction mechanism for these cyclization reactions is a proton transfer event that occurs before or in the rate-determining step of the reaction, which is impeded by the small normal solvent isotope effect observed for the cyclization of 1a (25). That hydrogen-bond–accepting ability is better correlated than the least (1e) to the most (1d) flexible epoxy alcohol (Fig. 5). These data suggest that an important role of the template is to restrict conformational motion so that reaction pathways to the normally favored exo cyclization products are limited. With limited access to exo cyclization pathways, the reaction occurs via a normally disfavored pathway to form endo products selectively.

**The Role of the Solvent (Water).** Up to this point, the importance of the template structure for selective endo cyclizations has been emphasized, yet as noted above, water was also implicated (10). Further investigation has revealed that selectivity is much less sensitive to the identity of the solvent than is the reaction rate (Fig. 5B). The selectivities ($k_{endo}/k_{exo}$) measured for the cyclization of 1a in various hydroxyllic solvents at room temperature are the same within experimental uncertainty. In sharp contrast, however, the absolute rate constants ($k_{endo}$ and $k_{exo}$) decrease by more than two orders of magnitude among the solvents studied in the order: 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) > water > 2,2,2-trifluoroethanol (TFE) > methanol > ethanol. With the exception of water, this order is roughly correlated to the acidity of the solvent as indicated by solvent pK$_a$, which increases in the order: HFIP > TFE > methanol > water > ethanol. Even if reactions in water are ignored, the correlation between solvent acidity and cyclization are not consistent with typical acid/base catalysis (Supporting Information). Nevertheless, it is noteworthy that reaction rates in water are similar to solvents with acid dissociation constants that are over six orders of magnitude greater than water at room temperature.

![Fig. 4. Comparison of activation parameters determined for the cyclization of epoxy alcohols 1a and 1b (T = 298 K).](image)
hydrogen-bond–donating ability is consistent with the proton transfer step being asynchronous for most solvents. In other words, proton donation from the solvent is kinetically more important than proton accepting. Once again, water is the exception to this trend. Reactions in water displayed unusually fast reaction rates for a solvent with its hydrogen-bond–donating ability (Fig. 6A). This anomaly suggests that water, unlike any other solvent, promotes a more synchronous proton transfer step (29).

A trend that includes all solvents (including water) is the linear correlation observed between reaction rate and $Y_{OTS}$, the Grumwald–Winstein parameter for solvent ionizing power (Fig. 6B). That this correlation is the best of all solvent properties evaluated indicates that the primary role of the solvent is to stabilize charged intermediates and transition states. Highlighting the importance of this solvent property is the relatively large slope (0.37) observed in Fig. 6B. A reaction that displays equal bond making and bond breaking in the transition state (e.g., purely $S_N^2$ behavior) involves relatively small charge buildup. Consequently, such reactions typically display smaller slopes of log($k$) vs. $Y_{OTS}$ plots (m = 0.20–0.35) (30). The slopes observed in Fig. 6B are at the upper limit of this range, which suggest that there is significant C–O$_{epox}$ bond breaking in the transition state. Importantly, water is the solvent that is best at stabilizing charge according to the $Y_{OTS}$ scale. It is likely that it is the ability of water to stabilize charge coupled with its ability to donate and accept hydrogen bonds that leads to the anomalously fast cyclization rates observed in water.

**Implications for the Biogenesis of the Ladder Polyether Natural Products.** In addition to uncovering the specific details about epoxy alcohol cyclization reactions, these mechanistic studies are relevant to the biosynthetic proposal (18) for the formation of the ladder polyether natural products. The very slow reaction rates observed for 1a and even slower rates for cascade reactions of multiple epoxides, such as 4 and 6 (12, 24), are problematic for a biosynthetic proposal involving consecutive or concurrent epoxy alcohol cyclizations promoted by neutral water. The data presented above suggest rate constants for endo cyclizations to be on the order of $10^{-5}$ s$^{-1}$ at 300 K, which translates to a half-life of 16.6 h. These rates seem unlikely under physiological conditions where metabolism or extrusion of intermediates during a cascade reaction would compete. No such cascade intermediate has ever been isolated along with the ladder polyether natural product.

Nevertheless, our results do not rule out the possibility that the ladder polyether natural products are formed by enzyme-catalyzed cyclizations of epoxy alcohols under conditions that are similar to those in neutral water. This possibility stems from the fact that the cyclization reactions in neutral water appear to suffer severe entropic penalties with only modest enthalpic barriers to overcome. If the primary role of this hypothetical enzyme is to organize the epoxy alcohol appropriately for cyclization or alternatively organize the solvent molecules appropriately for efficient proton transfer, then the entropic penalty incurred for cyclization under neutral aqueous conditions would be significantly decreased. Our data show that if entropy is ignored, endo cyclization rates would be on the order of 10 s$^{-1}$ at 300 K or a half-life of 70 ms. This translates into a theoretical reaction rate that is seven orders of magnitude greater for reactions catalyzed by enzymes whose primary function is to organize the substrate (or solvent) appropriately for cyclization.

Supporting this notion is the seminal work from Janda, Shevlin, and Lerner, who have developed a catalytic antibody that catalyzes 6-endo and 7-endo cyclizations of simple epoxy alcohols (i.e., without an appended tetrahydropyran ring) (27, 31, 32). Catalytic rate constants measured for cyclizations leading to the 6-endo product displayed half lives of 45 s ($k_{cat} = 1.5 \times 10^{-2}$ s$^{-1}$). If the rate for 6-endo cyclization presented herein is similar to the “uncatalyzed” rate for cyclization of the simple epoxy alcohols, then a thousand-fold rate enhancement was observed as a result of the catalytic antibody. Presumably, the rate increase observed
is due in large part to entropic confinement of the epoxy alcohol that occurs upon binding to the antibody.

Perhaps more biologically relevant is the epoxide hydrolase enzyme Lsd19 that has recently been reported to catalyze a 6-endo cyclization in the biosynthesis of lasolocid A (33). Although the class of natural products and the species that the enzyme originate from are different in this case compared with the ladder polyether natural products, there is nonetheless a striking parallelism between the role of the enzyme and the role that the combination of water and substrate play in the reactions discussed above. We suggest that the enzyme, like the tetrahydropyran template, serves to organize the substrate appropriately to limit the otherwise favorable 5-exo cyclization, thereby allowing the less favored 6-endo cyclization reaction to occur.

Noteworthy is that the crystal structure of Lsd19 contains a 5-exo cyclization substrate in one of its catalytically active sites rather than the expected 6-endo cyclization product (34). We speculate that this cyclization product was observed due to the conditions (pH < 4) that were necessary to provide X-ray quality crystals of the enzyme. This implies that the neutral conditions used in the catalytic reactions plays a role in dictating selectivity for this enzyme. Although highly speculative, it is interesting to consider the possibility that the enzyme works in concert with neutral water to achieve the highly selective cyclization reaction observed in this enzyme and for enzymes that may lead to the ladder polyether natural products.

**Conclusions**

The mechanistic studies presented above have uncovered several key features of epoxy alcohol cyclizations in neutral water (Fig. 7). First, an important role of the cyclic template is to prevent the epoxy alcohol from undergoing exo cyclization rather than promote endo cyclization. High levels of selectivity were also observed only for substrates that can interact with water through hydrogen bonding. These interactions likely cause conformational reorganization that alters the trajectory and consequently selectivity of the chemical reaction. In addition to facilitating reorganization of the template, a critical role of the solvent is to stabilize charge in the transition state and/or intermediates during the reaction. A secondary role for the solvent is to act as a proton shuttle, which activates the epoxy alcohol for cyclization. This process appears to be asynchronous for reactions in neutral water (Fig. 6).

![Fig. 6](image_url)

**Fig. 6.** (A) Sensitivity of the rate of cyclization of 1a to the hydrogen-bond–donating ability of the solvent ($\alpha$) at 25 °C. Linear regression displayed does not include reactions in neutral water. (B) Sensitivity of the rate of cyclization of 1a to the ionizing power of the solvent ($Y_{OTs}$) at 25 °C. Error is represented as average error.

![Fig. 7](image_url)

**Fig. 7.** Venn diagram illustrating the features of water, epoxy alcohol 1a, and their synergism to enhance endo selectivity in epoxy alcohol cyclization reactions.
most solvents except water, where water behavior is more consistent with a synchronous proton transfer. Just as the equal number of hydrogen-bond donors and acceptors present in a water molecule engenders some of its unique properties, we surmise that a similar bivalency is a very important feature of the promoter of these reactions. As it can provide both Bronsted acids and Lewis bases, water exerts upon the substrate multiple reinforcing interactions, some that orient it appropriately and others that initiate the selective chemical transformation.

In closing, our investigations into the water-promoted epoxide-opening reactions discussed here provide a vivid reminder of the importance of entropic (rather than enthalpic) considerations in catalytic reactions. It has been known for decades that substrate organization is one of the major modes of assistance that enzymes provide in accelerating selective catalysis. More recently similar phenomena have been convincingly documented in reactions catalyzed by synthetic organic molecules (35). Herein we have shown that one of the at least five important roles of an even smaller molecule, water, is organization of a substrate much larger than itself. This effect is ultimately the source of selectivity in these reactions, but it comes at a high entropic cost. Do these results suggest that the proposed biogenesis of the ladder polyethers (epoxide-opening cascades) is indeed operative? Were the cascades originally catalyzed by water? As intimated above, has evolution subsequently provided enzymes to help catalyze these reactions? The answers to these questions remain to be seen and may never be known. However, in the meantime, a powerful and general approach to catalysis by small molecules strongly suggested by our findings bears a striking resemblance to enzymatic catalysis: To maximize both rate and selectivity, use water to its maximum enthalpic advantage and develop small organic molecules to supplement its entropic role. This strategy is under current investigation, and we will report our results in due course.

Materials and Methods

Kinetic measurements were made using 1H NMR spectroscopy on a Varian Inova 500 MHz spectrometer equipped with an inverse broadband gradient probe (gH). Efficient reactions ($t_{1/2} \leq 6\ h$) were carried out in the spectrometer, which was brought to the desired reaction temperature and calibrated using an ethylene glycol external standard. Less efficient reactions ($t_{1/2} \geq 6\ h$) were carried out in NMR tubes that were thermostatted in an oil bath. Presaturation measurement of an NMR spectrum was carried out at regular intervals at ambient temperatures. The pD of the buffer was measured at the reaction temperature using a Symphony Posi-pHilo Ag/AgCl pH glass electrode calibrated at the reaction temperature with standard solutions. For reactions in D2O, a correction for the solvent isotope effect that is typical for glass electrodes was applied (pD = pHwater + 0.4) (36). Synthetic procedures and specific details about kinetic analysis appear in SI Text.

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36. Byers and Jamison