Dynamics and Entropy of Cyclohexane Rings Control pH-Responsive Reactivity

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ABSTRACT: Activation entropy (ΔS‡) is not normally considered the main factor in determining the reactivity of unimolecular reactions. Here, we report that the intramolecular degradation of six-membered ring compounds is mainly determined by the ΔS‡, which is strongly influenced by the ring-flipping motion and substituent geometry. Starting from the unique difference between the pH-dependent degradation kinetics of geometric isomers of 1,2-cyclohexanecarboxylic acid amide (1,2-CHCAA), where only the cis isomer can readily degrade under weakly acidic conditions (pH < 5.5), we found that the difference originated from the large difference in ΔS‡ of 16.02 cal·mol⁻¹·K⁻¹. While cis-1,2-CHCAA maintains a preference for the classical chair cyclohexane conformation, trans-1,2-CHCAA shows dynamic interconversion between the chair and twisted boat conformations, which was supported by both MD simulations and VT-NMR analysis. Steric repulsion between the bulky 1,2-substituents of the trans isomer is one of the main reasons for the reduced energy barrier between ring conformations that facilitates dynamic ring inversion motions. Consequently, the more dynamic trans isomer exhibits much a larger loss in entropy during the activation process due to the prepositioning of the reactant than the cis isomer, and the pH-dependent degradation of the trans isomer is effectively suppressed. When the ring inversion motion is inhibited by an additional methyl substituent on the cyclohexane ring, the pH degradability can be dramatically enhanced for even the trans isomer. This study shows a unique example in which spatial arrangement and dynamic properties can strongly influence molecular reactivity in unimolecular reactions, and it will be helpful for the future design of a reactive structure depending on dynamic conformational changes.

KEYWORDS: pH-Responsive reactivity, Cyclohexane, Ring inversion, Dynamics, Entropy, Amide degradation

INTRODUCTION

The chair form is classically known as the thermodynamically favored conformation among the various conformations of six-membered ring compounds. The chair conformation is very close to the ensemble average of a six-membered ring with a 5–10 kcal·mol⁻¹ lower energy than those of other conformations.¹,² Therefore, most reactions of six-membered-ring compounds are described using the chair conformation as the default state.³

However, the ring-flipping motions including interconversion between the chair, boat, and twist-boat conformations of six-membered rings as well as distortion from such major conformations must not be ignored since the energy barriers can be overcome by thermal energy.⁴,⁵ Moreover, when the energy barrier can be lowered by additional factors, the six-membered ring should no longer be considered to have the rather static chair conformation but instead should have a dynamic mixture of various conformations. Unlocking the structural degree of freedom may significantly influence the entropic factor of 6-membered ring compounds.

In this study, we found that the reactivity of the unimolecular degradation of a cyclohexane analogue, 1,2-cyclohexanecarboxylic acid amide (1,2-CHCAA), is strongly dependent upon the activation entropy. This strong dependence on the activation entropy seems to be very unique because unimolecular reactions are generally much more dependent upon activation enthalpy, as the entropy loss during the activation process of a single molecule is supposed to be insignificant compared to that of multimolecular activation.⁶,⁷ We revealed that the entropic dependence originates from unlocking the dynamic conformational interconversion of the cyclohexane ring, which is largely determined by the geometric arrangement between the ring substituents.
Our study shows that the reactivity of cyclohexane analogues, representative cyclic molecules, is determined not by a single predominant conformation but by the overall dynamic behavior. The ring dynamics can be strongly affected by geometric relationships such as trans–cis and equatorial/axial substituents. Interestingly, bulky substituents at equatorial positions, which are generally recognized as the thermodynamically more stable spatial arrangement compared with the axial positions of six-membered rings, significantly lower the barrier between ring conformations and make the rings more dynamic. This dramatic increase in the degree of freedom can effectively inhibit the reactivity of the cyclohexane analogue by increasing the entropy loss during the activation process.

We expect that this study will play an important role in understanding the motions and reactions of ring-containing molecules. Spatial arrangement and dynamic properties can strongly influence molecular reactivity even in unimolecular reactions. Although various linkers with selective degradability have been applied to develop smart materials, this study is the first report that describes the degradability according to the difference in dynamic behavior. Through a deeper understanding of the relationship between selective reactivity and geometric control of molecular motion, we could imagine novel chemical reactions and future smart materials based on the delicate control of the dynamic molecular structure and not a static structure.

RESULTS AND DISCUSSION

pH-Responsive Degradability of 1,2-CHCAA

A β-carboxylic acid amide with a fixed conformation presents unique pH-dependent degradation properties. Such a β-carboxylic acid amide is stable in neutral or basic pH environments, whereas it will be degraded to amine and dicarboxylic anhydride/amides in acidic environments.8,9 As representative β-carboxylic acid amides, maleic acid amide analogues with α,β-cis double bonds have been widely used as cleavable linkers for prodrugs and biomaterials with degradability that responds to biologically tolerable pH changes.10 The degradation mechanism by which the neighboring β-carboxylate group attacks the carbonyl group of the amide was proposed in the early days of bioorganic chemistry,11 and has been continuously supported by recent computational simulations.12,13

Since the conformational fixation of β-carboxylic acids is critical to increase the effective concentration for intramolecular attack, the introduction of a ring structure that prevents free rotation of the σ-bond of a cyclic β-carboxylic acid amide can also facilitate the pH-dependent degradation of the β-carboxylic acid amide. For instance, 1,2-CHCAA has been applied as a pH-responsive degradable linker,14,15 although a fundamental study of its degradation mechanism has not been identified thus far.

There are two geometrical isomers, cis and trans, that exist in 1,2-CHCAA derivatives (Figure 1a). Both cis- and trans-1,2-CHCAA possess a β-carboxylic acid group that may attack the neighboring amide carbonyl group to form a five-membered ring. Both CHCAA isomers can be synthesized via a one-step reaction between primary butyl amines (n-butylamine (1), sec-butylamine (2), and tert-butylamine (3)) and cis/trans-1,2-cyclohexanedicarboxylic anhydride (CHDCA) under anhydrous conditions (Figure S1). The degradation kinetics of each sample were analyzed at 310 K in weakly acidic buffers (pH 3.0 and 5.5). cis-1 readily decomposed into cis-1,2-CHDCA and n-butylamine in the pH 3.0 and 5.5 acidic solutions, showing degradation profiles similar to those of the maleic acid amide derivatives (Figure 1b and c).16 The resulting cis-1,2-CHDCA could be successively hydrolyzed into 1,2-cyclohexanedicarboxylic acid in this condition (Figure S2). However, trans-1 was hardly degraded under these conditions. Even under extremely acidic pH conditions (pH < 1.0) at ambient temperature, trans-1 degradation was not detected in the 1H NMR spectrum (Figure S3). Likewise, these phenomena were also observed for the degradation of cis/trans-2 and cis/trans-3 with the amides from bulkier sec-butylamine and tert-butylamine, respectively (Figure S4). Since the tertiary amide of a β-carboxylic acid amide more favorably degrades compared with the secondary amide of a β-carboxylic acid amide,17 we also measured the degradability of trans-4 and trans-5, which possess a tertiary amide, and found that the trans isomers were hardly degraded under acidic conditions (Figure S5).

Averaged Conformations of 1,2-CHCAA

In order to investigate the cause of this remarkable difference in reactivity between the cis- and trans-1,2-CHCAAs, we initially examined the averaged conformations of both geometric isomers, focusing especially on the spatial relationships of the amide and the β-carboxylic acid substituents, which may strongly contribute to intramolecular nucleophilic attack by nuclear magnetic resonance (NMR) spectroscopy. The dihedral angles associated with each substituent were obtained from the Karplus-type equation.18,19 The averaged conformation of cis-1 was found to be a chair conformation in which the carboxylic acid and amide groups are possibly located in the axial and equatorial positions, respectively.
trans-1 showed that both the carboxylic acid and amide groups stretched in the equatorial direction (Table S1b). The interproton distances calculated from the NOESY intensities also showed that the averaged conformation was close to a chair conformation with both groups in the equatorial direction (Table S2). These averaged conformations of cis-1 and trans-1 in the solution state were further supported by their structures in the solid-state as determined by X-ray diffraction (XRD, Figure S6 and Table S1). Based on the above structural analyses, we estimated that both the cis- and trans- isomers present reasonable preferences on chair conformations that the acid and amide substituents are located close enough to each other to allow intramolecular nucleophilic attack.

Activation Energy Barriers for 1,2-CHCAA Degradation

Since the carboxylic acid group of trans-1 is within a distance that allows it to access the amide group for nucleophilic attack to form the five-membered ring intermediate, we suspected that the determining factor for the reactivity difference between cis-1 and trans-1 may be the activation energy during the degradation reaction steps. The structural similarities between β-carboxylic acid amides with a fixed αβ-bond led us to hypothesize that the degradation of 1,2-CHCAA would follow a similar mechanism to that suggested for the degradation of maleic acid amides in the pioneering work of Kirby’s group.11,20 Starting from the degradation mechanism of maleic acid amide, we estimated the degradation routes and suitable conformations at each step by repeated geometry optimization and by calculation of the energies as global minima through density functional theory (DFT) quantum mechanical simulations using the B3LYP-GD3/6-31G(d,p) basis set and a PCM solvation model in water.21 For simplification, we calculated the energies of 1,2-CHCAA with a methyl amide group and not the butyl amide group.

Figure 2. Proposed mechanism for 1,2-CHCAA degradation and the thermodynamic parameters from the degradation kinetics. Proposed schematic route for 1,2-CHCAA breakdown (a). Arrhenius plots (b) and Eyring plots (c) for the measured degradation rates of cis-1 and trans-1. First-order Arrhenius plots and Eyring plots were obtained by measuring the degree of degradation by observing the 1H NMR spectra in a phosphate buffer at pH 1.2 at five points of temperature. Each data point is represented as the average value of three experiments (±standard deviation). Rate constants, activation energies and thermodynamic parameters at 310 K (d) were calculated from the Arrhenius equation and the Eyring equation for the breakdown of cis-1 and trans-1 at pH 1.2.
Figure 3. MD simulations for the conformational changes of 1,2-CHCAA. Distance ($r_{CO}$) between the nucleophile (carboxyl oxygen) and the electrophile (amide carbonyl carbon) and the distance ($r_{OH}$) between the carboxylic hydrogen and the amide oxygen (a). Probability distributions of $r_{CO}$ (b) and $r_{OH}$ (c) of 1,2-CHCAA in water at 300 K. Dihedral angles (d) between the $\alpha$-$\beta$-C=C bond and the substituents ($\psi_1$, $C2$-$C1$-$C$=O$_{COOH}$, $C1$-$C2$-$C$=O$_{CONH}$). Two-dimensional probability distributions of dihedral angles ($\psi_1$ and $\psi_2$) of cis- (e) and trans-1,2-CHCAA (f) in water at 300 K. Representative conformations of the cis and trans isomers are shown in the insets. Dihedral angles (g) of the cyclohexane ring ($\phi_1$, $C6$-$C1$-$C2$-$C3$; $\phi_2$, $C1$-$C2$-$C3$-$C4$). Two-dimensional probability distributions of dihedral angles ($\phi_1$ and $\phi_2$) of cis- (h) and trans-1,2-CHCAA (i) in water at 300 K.

Figure S7 shows the reaction profiles in the first and second phases, respectively, in which the calculated energy at each state in the mechanism is compared as a relative value from the energies of the reactant (Re) and the intermediate (Int), respectively. We found that the activation energy of the first phase (25–27 kcal mol$^{-1}$) is much higher than that of the second phase and that the rate-determining step (RDS) is the formation of the tetrahedral intermediate. More remarkably, the activation energy of the cis isomer is slightly higher (1.2–2.6 kcal mol$^{-1}$) than that of the trans isomer in both the first and second phases. The similar or even higher activation energy observed for the cis isomer was hardly expected since the cis isomer shows much faster degradation than the trans isomer under mildly acidic conditions (Figure 1). Therefore, why does the trans isomer with a lower activation energy show almost no degradability of the amide bond, unlike the cis isomer?

To investigate the reasons for the clear difference in degradability between cis- and trans-1,2-CHCAA, we returned to experiments to measure the degradation kinetics of cis-1 and trans-1 at varying temperatures. We conducted experiments under extreme conditions under which the trans molecules could be degraded, inducing reactions at the temperatures of 308, 313, 318, 323, and 328 K for cis-1 and 348, 353, 358, 363, and 368 K for trans-1 in extremely acidic phosphate buffer in D$_2$O (0.1 M, pH 1.2), and the degradation was measured by $^1$H NMR. While cis-1 favorably degraded, decomposition of trans-1 was barely observed after a long reaction time (48 h) at these high temperatures. The degradation rate constant ($k$) of both isomers was calculated from the changes in the reactant concentrations, assuming that the degradation follows first-order kinetics. The rate constants were used to complete the Arrhenius plots (Figure 2b) and corresponding Eyring plots (Figure 2c). The activation energy ($E_a$) and the pre-exponential Arrhenius coefficient ($A$) were obtained from the Arrhenius plot, and the enthalpy factor ($\Delta H^\ddagger$) and the entropy factor ($\Delta S^\ddagger$) of the activation free energy ($\Delta G^\ddagger$) were acquired from the Eyring plot (Figure 2d).

Similar to the results of the DFT calculations, the activation energy difference was insignificant between the cis and trans isomers. The large difference in the rate constant was due to the pre-exponential Arrhenius coefficient, which represents the steric and geometric factors involved in the reaction. On the other hand, the $\Delta G^\ddagger$ values obtained from the Eyring equation showed a significant difference (3.94 kcal mol$^{-1}$) between the two isomers. trans-1 had a significantly higher $\Delta G^\ddagger$ (+28.88 kcal mol$^{-1}$) than that of cis-1 (+24.94 kcal mol$^{-1}$). Interestingly, cis-1 required a slightly higher enthalpy ($\Delta H^\ddagger$) (+19.90 > 18.87 kcal mol$^{-1}$) but lost a lot of entropy ($\Delta S^\ddagger$) (−16.25 > −32.27 cal mol$^{-1}$ K$^{-1}$) during the activation process compared...
with trans-1. At 310 K, the enthalpic and entropic contributions to ΔG\text{\textdegree} of cis-1 were \(-1.03\) and \(+4.97\) kcal mol\(^{-1}\), respectively, resulting in the significantly higher ΔG\text{\textdegree} of trans-1 over cis-1. The much slower degradation kinetics of trans-1 were mainly due to a much larger loss in entropy during activation. Because the entropy loss from the unimolecular reaction largely originates from the loss of conformational freedom during activation, we anticipated that the dynamic behaviors of cis-1 and trans-1 may be significantly different from each other. Large differences in both the Arrhenius coefficient (A) and ΔS\text{\textdegree} indicated a larger loss of freedom in the dynamic motions of trans-1 than cis-1 during the activation process.

**Dynamic Properties of 1,2-CHCAA**

Since the DFT calculations focus on the energy of a specific conformation, i.e., a single conformation of a reactant or a single conformation of a transition state, the calculation results rarely reflect the entropy factor originating from the dynamic interconversion among various conformations of the reactant.\(^{24,25}\) Although the NMR and SC-XRD analyses (Tables S1, S2 and Figure S6) indicated that the averaged conformations of both isomers are close to chair conformations, they might dynamically interconvert between various conformations. Therefore, we conducted molecular dynamics (MD) simulations on 1,2-CHCAA in water in order to understand the much larger entropy loss during the activation of the trans isomer than the cis isomer. We started from the dynamic motions of the two reactive substituents, the amide and carboxylic acid groups (Figure 3a). We measured the internuclear distance (\(r_{CO}\)) during the MD simulation at 300 K because TS\(_1\) formation is initiated by access to the carboxyl oxygen and amide carbonyl carbon (Figure 3b). This distance varied significantly from 2.5 to 4.7 Å, indicating that the structure is not static but rather quite dynamic. During the dynamic conformational change, the two atoms in the cis isomer have a much higher probability of being close to each other (within 3 Å) than these two atoms in the trans isomer. We also analyzed the distance between the hydrogen of the carboxylic acid group and the oxygen of the amide group (\(r_{OH}\)) (Figure 3c). The probability of being below 2 Å, a typical hydrogen bond distance, is almost the same for both isomers, but the probability of being below 3 Å is much higher for the cis isomer. This larger shoulder probability of the cis isomer denotes that the carboxylic acid hydrogen exists near the amide group, although it does not form a hydrogen bond.

Next, we wanted to know the orientations of the two substituents of 1,2-CHCAA. We examined the distribution of dihedral angles between the αβ-C=C bond and the C=O bonds of the carboxylic acid (Ψ\(_1\)) and amide (Ψ\(_2\)) (Figure 3d). In the cis isomer, Ψ\(_1\) is mainly distributed from \(-90°\) to \(-180°\) and Ψ\(_2\) is almost fixed at approximately \(-110°\) (area A, Figure 3e), which indicates that the rotation of the amide substituent is much less favored than the rotation of the carboxylic acid substituent. A representative example of the conformations at approximately Ψ\(_1\) = \(-90°\) and Ψ\(_2\) = \(-110°\) is shown in Figure 3, in which a hydrogen bond forms between the carboxylic acid and the amide. On the other hand, the trans isomer shows a clear bimodal distribution with high probability densities at Ψ\(_1\) = \(-45°\) and Ψ\(_2\) = \(-10°\) (area A) and at Ψ\(_1\) = \(-160°\) and Ψ\(_2\) = \(-10°\) (area B, Figure 3f). In almost half of the trans isomer conformations (area A), the carboxylic acid hydrogen is nearly antiparallel to the amide carbonyl group with no probability of hydrogen bond formation between these groups. Moreover, Figure S8 shows that the amide group of the trans isomer rotates much more actively than the cis isomer. The orientation and dynamics of the reactive substituents also supports that the cis isomer has a higher probability of existing in easy-to-access conformations than the trans isomer.

Since these substituents are directly linked to the cyclohexane ring, the ring-flipping motion will definitely contribute to the critical dynamic behavior of the molecules, including the motion of the substituents. In order to observe the ring-flipping motion of 1,2-CHCAA, we conducted MD simulations. We plotted the conformational distribution of 1,2-CHCAA with the coordinates \(\phi_1\) and \(\phi_2\), which denote the C6–C1–C2–C4 and C1–C2–C3–C4 dihedral angles, respectively, because they are more suitable terms for describing the conformational changes of the cyclohexane ring (Figure 3g). Surprisingly, the two isomers showed very different dynamic behaviors in water as well as in the gas phase. The cis isomer retained conformations that were close to the classical chair conformation (\(\phi_1 = 60°\) and \(\phi_2 = -60°\)) (Figures 3b and S9a). However, the trans isomer actively transited from the initial conformation, which was close to the classical disequatorial (e,e) chair conformation, to other conformations, such as the twisted boat or even the diaxial (aa) chair conformation in the gas phase (Figure S9b). The diaxial chair conformation would hardly be expected by most organic chemists because it has been generally predicted to have a much higher energy than the other conformations due to the 1,3-diaxial interactions.\(^{26,27}\) As shown in Figure S9c and d, the cis isomer showed no ring inversion, but the trans isomer frequently interconverted between the twisted boat and (aa)/(ee) chair conformations during a longer simulation for 100 ns. The dynamic interconversion between the conformations of the trans isomer was also observed in a simulation in water. Although no (aa) chair conformation was observed, twisted boat-like conformations were frequently observed in the simulation (Figures 3i and S10).

Since it is generally believed that the trans isomer of 1,2-disubstituted six-membered ring compounds with two equatorial substituents is thermodynamically more stable than the cis isomer with an axial substituent, as shown by the thermodynamic stability of β-glucose compared to α-glucose, the MD simulation results showed that trans-1,2-CHCAA had much more dynamic behaviors than cis-1,2-CHCAA, which should provide new insight for understanding the structures and reactivities of these ring compounds. Next, we performed an NMR study to confirm whether these unexpected simulation results could be observed in an experiment.

Low-temperature peak separation can be used to estimate molecular dynamic properties by \(^1\)H NMR.\(^{28}\) The peaks from the α-hydrogen (H1) and β-hydrogen (H2) of cis-1 at 2.7 ppm and 298 K started to broaden when the temperature went below 263 K, and new separate peaks appeared at 2.3 and 3.1 ppm (Figure S11). On the other hand, the H1 and H2 peaks of trans-1 at 2.4 and 2.6 ppm and 298 K maintained their shape and position at 213 K. This result implies that the ring inversion of cis-1 is sufficiently prohibited at low temperatures and that the activation barrier of cis-1 may be higher than that of trans-1.

The ring-inversion rates were compared in more detail by using the \(^1\)C NMR spectra with varying temperature (Figures 4 and S12).\(^{30,31}\) The broadening and separation of axial and
equatorial carbon peaks could be used for the calculation of the inversion rates of six-membered rings. Through the comparison of the experimental data and dynamic NMR (DNMR) simulation with the peaks, which were assigned by $^{13}$C-DEPT and 2D HSQC NMR at 163 K (Figure S13), the ring inversion rate of cis-1 was calculated as $k_{ex} = 1.7 - 2.7 \times 10^4$ s$^{-1}$ at 298 K (Figure S14). On the other hand, only slight peak broadening without peak separation was observed in the spectra of trans-1 even at 143 K. It was expected that temperature lower than 90 K, which is below the low temperature limit of current variable temperature NMR (VT-
NMR) technique, might be required to observe the peak separation of trans-1 (Figure S15). Instead, we could estimate that the activation energy barrier of the ring inversion of trans-1 is below 4.5 kcal mol\(^{-1}\), the limit of the measurement of the current VT-NMR method, and that the inversion rate is well over 10\(^8\) s\(^{-1}\) at 298 K.\(^{34}\) The greater than 1000-times higher inversion rate of trans-1 over cis-1 quantitatively supported much faster interconversion between the conformations in trans-1 than cis-1. Also, we could explain why we could not observe conformational interconversion of cis-1 with milli-second order inversion rate but could observe frequent interconversion of trans-1 with the nanosecond-order inversion rate, during the 100 ns MD simulation (Figure S9).

The dynamic properties of 1,2-CHCAA give us an important clue for the origin of the \(\Delta S^*\) difference between the two isomers. Due to the more dynamic interconversion between various conformations of the trans isomer, the population of the trans isomer in the Re conformational state (Figure 2) may be much smaller than that of the cis isomer. A significantly large entropy loss would be expected through positioning at the Re conformation from various other conformations of the trans isomer, unlike the more prepositioned cis isomer.

**Origin of the Distinct Dynamic Behaviors of cis- and trans-1,2-CHCAA**

We further conducted MD simulations in order to find determining factors responsible for the dynamic properties of 1,2-CHCAA. The cyclohexane ring-inversion motion mainly proceeds through half-chair and boat conformations (Figure S16).\(^{35}\) Therefore, we calculated the potential of mean force (PMF) of 1,2-CHCAA according to the angle of the chair leg, \(\Omega\), as a collective variable for describing the energy barrier for the ring inversion (Figure 5a). PMF(\(\Omega\)) denotes the free energy required to change from the ground state conformation of 1,2-CHCAA to an angle \(\Omega\) (deg) when the ring inversion proceeds by the lifting the leg of the chair. Of course, while the PMF(\(\Omega\)) describes the free energy of the ring inversion motion of six-membered ring compounds, it should be mentioned that the PMF(\(\Omega\)) may not fully coincide with the actual free energy of the ring inversion since the ring inversion is not a single path reaction which cannot be completely described by a single collective variable. Therefore, it makes more sense to interpret it qualitatively by comparing the PMF(\(\Omega\)) between isomers or between similar molecules than to give great significance to the absolute PMF(\(\Omega\)) values.

The PMF(\(\Omega\)) values gradually increased with increasing \(\Omega\). Remarkably, cis-1,2-CHCAA showed a much higher PMF value than trans-1,2-CHCAA in the range between 130° and 180°, where conformational change from chair to boat proceeded. The maximum values, which could represent the energy barrier for the ring inversion, were calculated to be 10.49 and 6.26 kcal mol\(^{-1}\) for cis- and trans-1,2-CHCAA, respectively. The significantly smaller PMF(\(\Omega\)) value of trans-1,2-CHCAA could also support the easier ring inversion of trans-1,2-CHCAA than cis-1,2-CHCAA.

We compared the PMF(\(\Omega\)) value of 1,2-CHCAA with that of 1-methylcarbamoyl-2-isopropycyclohexane (AiPCH), possessing similar-sized 1,2-substituents with an isopropyl group instead of the hydrogen bond-donating carboxylic acid group, as well as the PMF(\(\Omega\)) value of 1,2-dimethylcyclohexane (DMCH) with smaller substituents than 1,2-CHCAA, to determine whether hydrogen bonding or steric repulsion is the main determining factor for the dynamic difference between the cis and trans isomers. cis-AiPCH showed a much higher PMF(\(\Omega\)) value than trans-AiPCH, similar to the case of 1,2-CHCAA. Clearly, hydrogen bonding showed small effect on the large difference in PMF(\(\Omega\)) between the two isomers (Figure 5b). On the other hand, the difference in the PMF(\(\Omega\)) values between cis-DMCH and trans-DMCH was only approximately 1 kcal mol\(^{-1}\), which is far less than the difference observed for the cis and trans isomers of 1,2-CHCAA (Figure 5c). Furthermore, trans-AiPCH exhibited much more dynamic behavior than cis-AiPCH, whereas both cis- and trans-DMCH were observed almost exclusively as fixed chair conformations in the MD simulations (Figures S17 and S18). All of these results strongly suggest that steric repulsion is the main factor for the decrease in the energy barriers among the conformations.

From another viewpoint, we calculated the PMF values for the inner dihedral angle (C6-C1-C2-C3) of 1,2-CHCAA (\(\theta\)) (Figure 5d) and provided Newman projections to describe the steric factors between the 1,2-substituents (Figure 5e). Ring inversion occurs between the two chair conformations at \(\theta = -60^\circ\) and 60°. In the graph of cis-1,2-CHCAA, the highest PMF(\(\theta\)) was observed as approximately 8 kcal mol\(^{-1}\) in the boat conformation at \(\theta = 0^\circ\). Two bulky substituents in the axial and equatorial positions of the cis isomer inevitably become closer to each other during the ring inversion process, eventually reaching the totally eclipsed position in the boat conformation. The large repulsive interaction between substituents in this position can elevate the energy barrier of the ring inversion. In contrast, such a strong energy barrier was not observed during the ring inversion of trans-1,2-CHCAA, and the overall PMF(\(\theta\)) change was only approximately 2 kcal mol\(^{-1}\). Rather, a few shallow energy minima were observed between \(\theta = -60^\circ\) and 60°, and even the boat conformation (\(\theta = 0^\circ\)) showed an energy level that was similar to that of the (aa)-chair conformation (\(\theta = -60^\circ\)). Of course, as mentioned before, the PMF(\(\theta\)) may also not fully coincide with the actual free energy of the ring inversion.

As shown in the Newman projections (Figure 5e), the ring inversion process can readily reduce the gauche interaction between the bulky substituents because they are apart from each other in the trans isomer. At \(\theta = 0^\circ\), the energy elevation of the trans isomer is not as high as that of the cis isomer because an eclipsed conformation occurs between a hydrogen and the substituent. This steric repulsion competes with the 1,3-diagonal interactions that prefer the diequatorial chair conformation of the trans isomer. When the size of the substituents is small (i.e., –CH\(_3\)), 1,3-diagonal interactions may become dominant to stabilize the diequatorial chair conformation. When the substituent is large enough (i.e., –CH(CH\(_3\))\(_2\)–COOH or –CONH\(_2\)), the gauche interaction factor may become important to decrease the ring inversion energy barrier and to induce dynamic interconversion among conformations.

**Control of Ring Dynamics to Tailor Degradability**

Now, we can understand the relationship between the ring dynamics and the degradability of 1,2-CHCAA. Six-membered ring compounds with small substituents maintain the preferred chair conformation with significantly reduced ring inversion irrespective of the cis or trans substituent arrangement. On the other hand, compounds with large substituents show different dynamic behaviors between these geometric isomers. Since only trans substituent arrangements can effectively reduce the
steric repulsion by the ring inversion motion, the trans isomers exhibit a dramatic enhancement in the dynamic interconversion among various conformations.

Figure 6a summarizes the relationship between the structure, dynamics and the reactivity of 1,2-CHCAA. Before the reaction, trans-1,2-CHCAA can adopt larger numbers of microstates in the available multiple conformations, i.e., twisted boat and chair conformations, compared to cis-1,2-CHCAA, at a certain temperature ($T$). Therefore, trans-1,2-CHCAA exhibits a much larger entropy loss or reduction of available microstate numbers during the activation process for prepositioning of the reactant into the ready-to-react Re conformation than cis-1,2-CHCAA, which has a higher probability of existing in the Re conformation. Then, we assume that both isomers in their own Re conformations may follow the reaction paths with the lowest activation barrier to be degraded. As a whole, even though both isomers have similar activation enthalpy ($\Delta H^\ddagger$), the enthalpy difference between Re and TS$_{\text{Ex}}$, the larger entropy loss of the trans isomer in the conformational space gives the larger negative $\Delta S^\ddagger$ for the trans isomer. Therefore, the $\Delta G^\ddagger$ for the trans isomer is significantly higher than that for the cis isomer, thus suppressing the pH-responsive degradation of the trans acid amide structure. Even during the unimolecular reaction where it is generally believed that $\Delta S^\ddagger$ is much less important than in multimolecular reactions, if the structure can dynamically change conformations under the reaction conditions, $\Delta S^\ddagger$ should be considered a significant determining factor for reactivity.

On the basis of this understanding, we envisioned that the reactivity of ring compounds could be tailored by controlling ring dynamics. For example, if the ring inversion motion of 1,2-CHCAA could be stabilized, the acidic degradability could be enhanced by the reduction in the entropy loss during the activation process. As a proof of concept, we designed 4-methyl-1,2-CHCAA, where the additional 4-methyl group may stabilize the chair conformation through the preference of two (cis-1,2-CHCAA) or three (trans-1,2-CHCAA) substituents at equatorial positions with minimal effect on the other dynamic factors. Comparison of the degradation kinetics between cis-1 and Me-cis-1 (c) and trans-1 and Me-trans-1 (d) at a concentration of 13 mM in pH 5.5 buffer at 310 K. The amount of each degradation product was measured by $^1$H NMR, and this procedure was repeated three times. Each data point is the average value ± standard deviation ($n = 3$). Figure 6b shows the degradation of 4-methyl-1,2-CHCAA and interconversion between chair conformations by ring inversion (b). Axial and equatorial substituents are illustrated in blue and red, respectively. Ch, twb, and TS$_{\text{Ex}}$ mean the chair and twisted boat conformations and transition state of exchange between the Re and twb conformations, respectively. Probability density distributions in Figure 3h and i are inserted as inlets for indicating the difference of the conformational freedom between the isomers and the location of each conformation in the probability distribution. Degradation of 4-methyl-1,2-CHCAA and interconversion between chair conformations by ring inversion (b). Axial and equatorial substituents are illustrated in blue and red, respectively. Comparison of the degradation kinetics between cis-1 and Me-cis-1 (c) and trans-1 and Me-trans-1 (d) at a concentration of 13 mM in pH 5.5 buffer at 310 K. The amount of each degradation product was measured by $^1$H NMR, and this procedure was repeated three times. Each data point is the average value ± standard deviation ($n = 3$).
(Me-cis-1) and 4-methyl-trans-1,2-CHCAA (Me-trans-1) were prepared from reaction between n-butylamine and 4-methyl-1,2-cis/trans-1,2-cyclohexanedicarboxylic anhydride, and their degradation kinetics were measured via 1H NMR of the mixture of Me-cis-1 and Me-trans-1 under acidic conditions (Figures 6c,d and S19). Remarkably, Me-trans-1 degraded rapidly at an even higher rate than cis-1 at pH 5.5 and ambient temperature. The barely degradable trans-1,2-CHCAA readily degraded under mildly acidic conditions through the addition of a single methyl group. Furthermore, the degradation rate of cis-1,2-CHCAA was more accelerated by the stabilization of the ring inversion. This is the first example showing that bond degradability could be regulated by controlling ring dynamics.

**CONCLUSIONS**

In this work, we disclose the reason why the pH-dependent degradability of cis- and trans-1,2-CHCAA is completely different. We found that the dynamic interconversion between various conformations of the cyclohexane ring determines amide degradability. In contrast to the common belief that bulky substituents at the 1- and 2-positions of six-membered ring compounds remain fixed in the diequatorial positions of a chair conformation, steric repulsion of the diequatorial positioning lowers the energy barrier of the ring inversion, causing the ring to interconvert between various conformations. The much larger entropy loss during activation due to dynamic behaviors determines the far slower degradation kinetics of trans-1,2-CHCAA than cis-1,2-CHCAA. The dynamic behavior and conformational entropy become more important for understanding reactivity of larger molecules such as proteins and catalysts. As shown in this study, they can also be essential for understanding reactivity of small molecules with dynamic conformational interconversion. Only a small geometric variation could be enough to induce the molecular dynamic behavior and, as a result, the dramatic change of the reactivity. We believe that this result will provide a basic understanding of the reactivity and kinetics of a variety of ring-shaped molecules. It may help the future design of reactive shaped molecules. It may help the future design of reactive

**ASSOCIATED CONTENT**

- Supporting Information
  - Experimental details of the synthesis, characterization, and calculations (PDF)
  - X-ray structure of cis-1 (CIF)
  - X-ray structure of trans-1 (CIF)

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**Author Contributions**

*S. Kang, C.N., and H.K. contributed equally to this work. S.L., N.-K.K., Y.J., and Y.L. jointly developed the idea of this project. Y.L. conceived, directed and oversaw the project. S.Kang designed and conducted the experiments with the help of S. Kim. C.N. conducted the MD simulations. H.K. carried out the computations of DFT. J.-Y.S., S.-Y.K., E.P., and H.K.S. performed the NMR spectroscopic experiments and data analysis. M.-G.S. and S.S. performed early DFT and MD simulation studies. S. Kang, C.N., Y.J., and Y.L. wrote the primary manuscript. All authors discussed the results, analyzed the data, and commented on the manuscript.

**Notes**

The authors declare no competing financial interest.

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