Monte Carlo Simulations on Atropisomerism of Thienotriazolodiazepines Applicable to Slow Transition Phenomena Using Potential Energy Surfaces by \textit{ab initio} Molecular Orbital Calculations

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Compounds with a medium-sized flexible ring often show atropisomerism that is caused by the high-energy barriers between long-lived conformers that can be isolated and often have different biological properties to each other. In this study, the frequency of the transition between the two stable conformers, aS and aR, of thienotriazolodiazepine compounds with flexible 7-membered rings was estimated computationally by Monte Carlo (MC) simulations and validated experimentally by NMR experiments. To estimate the energy barriers for transitions as precisely as possible, the potential energy (PE) surfaces used in the MC simulations were calculated by molecular orbital (MO) methods. To accomplish the MC simulations with the MO-based PE surfaces in a practical central processing unit (CPU) time, the MO-based PE of each conformer was pre-calculated and stored before the MC simulations, and then only referred to during the MC simulations. The activation energies for transitions calculated by the MC simulations agreed well with the experimental changes determined by the NMR experiments. The analysis of the transition trajectories of the MC simulations revealed that the transition occurred not only through the transition states, but also through many different transition paths. Our computational methods gave us quantitative estimates of atropisomerism of the thienotriazolodiazepine compounds in a practical period of time, and the method could be applicable for other slow-dynamics phenomena that cannot be investigated by other atomistic simulations.

Key words atropisomerism; Monte Carlo simulation; molecular orbital method; diazepine

Stereoisomers often show different biological activity, pharmacokinetics and toxicity profiles because a ligand is three-dimensionally recognized by biological receptors. Therefore regulatory guidelines, such as Food and Drug Administration (FDA) guidelines,1) often request information on the biological properties of each stereoisomer in the drug development process. In addition to classical stereoisomers with chiral centers, much attention has been paid recently to the atropisomer.2–4) A compound which has axial chiralities caused by hindered rotations about rotatable bonds shows atropisomerism. Compounds showing atropisomerism have long-lived (1000 s or longer) conformers that can be isolated,5) and, like classical stereoisomers, each long-lived conformer often shows different biological activities.2,3)

Atropisomerism is caused by the high energy barrier between long-lived conformers due to the steric and/or electronic repulsions and is often observed in compounds with bulky substituents in the ortho positions. Half lives \(t_{1/2}\) of the atropisomers are dependent on the energy barriers, thus the degree of atropisomerism is time-dependent.2–5) For compounds with an energy barrier of 120 kJ/mol or higher, the atropoisomers have a \(t_{1/2}\) of several years at room temperature and behave like classical stereoisomers, but when the energy barrier is lower than 80 kJ/mol, the \(t_{1/2}\) is less than 10 s and compounds behave as mixtures or are simply flexible compounds. Compounds with a moderate energy barrier of ca. 100 kJ/mol have several hours of \(t_{1/2}\) at room temperature. From the viewpoint of quality control of pharmaceutical products and evaluating their biological properties, compounds with a moderate energy barrier are troublesome because racemization can occur within several hours at room temperature.

Estimating the energy barrier between atropisomers in the early stage of drug discovery and development is preferred because it will significantly affect the overall process of drug development. To evaluate the energy barriers around one acyclic rotational bond, LaPlante \textit{et al.} applied a molecular orbital (MO) method and obtained satisfactory results.4) In addition to an atropisomerism caused by the rotational barrier of an acyclic bond, another kind of atropisomerism exists in the case of compounds with a medium-sized flexible ring. For such compounds, flexibility of the ring (ring inversion) causes atropisomers. Calculating the energy barrier along the transition paths for one acyclic chemical bond, in the manner of LaPlante \textit{et al.}, is straightforward and can be done by changing the torsion angle of the acyclic bond systematically and searching for the transition state (TS) of the compound. However, as LaPlante \textit{et al.} pointed out, it is difficult to apply their method to estimate the energy barrier of compounds with a medium-sized flexible ring, where several torsion angles of rotatable bonds vary simultaneously and in concert.

Gilman \textit{et al.} reported estimating the energy barriers of benzodiazepine analogs, which have a \(7\)-membered ring.6) In their study, two torsion angles of the 7-membered ring of benzodiazepine were considered to define a single transition path between the atropisomers and the energy barrier along the single transition path was calculated using the MM2 force field potential. In other studies not limited to compounds with a medium-sized flexible ring, the energy barrier between atropisomers was estimated by calculating the energy difference between the lowest energy conformer and the transition conformer by assuming a single transition path or a single transition conformer.7–10) In order to estimate the energy barriers between atropisomers with a medium-sized ring, which have some freedom of motion, it is preferable to consider all of the flexible torsion angles and the multiple transition paths across the energy barrier between the atropisomers, because the

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shape of the high dimensional potential energy (PE) surface for ring inversion is complicated and there may be more than one transition path to get across the energy barrier.

In this study, a computational method to estimate the energy barriers of transitions between atropisomers with a medium-sized flexible ring was developed and was applied to two thienotriazolodiazepine (TTDZ) analogs (Fig. 1), which have a 7-membered ring (all of the flexible torsion angles and the multiple transition paths were considered). In case that a single transition path of one acyclic rotatable bond is examined, a transition state of the compound can be identified straightforwardly as LaPlante reported. But, in case of multiple transition paths of the high dimensional PE surfaces with complicated shape such as a PE surface of medium-sized flexible, applying computational methods, such as Monte Carlo (MC) simulations or molecular dynamics (MD) simulations is required to seek many possible paths or trajectories in the high dimensional PE surfaces. In this study, the MC simulation method was selected so as to seek multiple transition paths, and the activation energies for transitions were directly estimated from the temperature dependency of the number of transitions observed during the MC simulations based on the Arrhenius equation. In order to make an MC simulation with a large number of MC steps (e.g. $10^{11}$ steps) possible, the PE surface, which controls transition dynamics between atropisomers, was calculated before the MC simulations, not during it, and the energy value of the pre-calculated PE surface for each conformer was simply referred to at each step of the MC simulation. A grid sampling of the whole PE surface using four torsion angles was performed and the potential energy for each grid point, which corresponds to each conformer, was calculated before the MC simulations, not during it, and the energy value of the pre-calculated PE surface for each conformer was simply referred to at each step of the MC simulation. A grid sampling of the whole PE surface using four torsion angles was performed and the potential energy for each grid point, which corresponds to each conformer, was calculated before the MC simulations, not during it, and the energy value of the pre-calculated PE surface for each conformer was simply referred to at each step of the MC simulation.

**Experimental**

In this study, the atropisomerism of two TTDZ analogs, as shown Fig. 1, was evaluated computationally and experimentally as described below. These compounds possess a flexible 7-membered ring, thus they were considered to have a possibility of atropisomerism caused by the ring inversion of the 7-membered ring like other compounds with 7-membered rings.6,7,10–12

**Potential Energy Surfaces** As shown in Fig. 1, four rotatable torsion angles ($\phi_1$, $\phi_2$, $\phi_3$, and $\phi_4$) that cause the ring inversion of the 7-membered ring of TTDZ should be considered when evaluating the atropisomerism. As shown in Fig. 2, the lowest energy conformers and the PE surface for each compound were obtained by a grid search using the four torsion angles as follows. In the first step of the grid search for evaluating the PE surfaces, the initial conformers were generated in such a way as to rotate all four of the torsion angles by increments of 60°, and the energy value of the pre-calculated PE surface for each conformer was simply referred to at each step of the MC simulation.

Monte Carlo Simulations One MC step of the MC simu
lation consisted of two procedures, a selection of a new conformer and a judgment to accept or reject this new conformer based on the potential energy of the new conformer. In the process of selecting a new conformer, a new conformer was randomly chosen from the neighboring conformers of the current conformer in the conformation space defined by the four torsions ($\phi_1, \phi_2, \phi_3, \phi_4$), as described below. In this conformation space, the distance ($\Delta \phi_{ij}$) between the conformers $i$ and $j$ were defined by the following equation (Eq. 1).

$$
\Delta \phi_{ij} = \sum (\phi_{ki}^{(i)} - \phi_{kj}^{(j)})^2
$$

where $\phi_{ki}^{(i)}$ was the $k$-th torsion angle ($k=1, 2, 3, 4$) of the $i$-th conformer. The neighboring conformers were defined as the conformers $i$ and $j$ with a distance $\Delta \phi_{ij}$ less than a constant value of $\Delta \phi_c$. In this study, $\Delta \phi_c$ was set at 21.2 degrees which means that the conformational change at one MC step is limited to the change of only one or two torsion angles of 15°. Under these conditions, each conformer had an average of 25 neighboring conformers, and about 20% of the conformers had 32 neighboring conformers, which is the maximum number of neighboring conformers for one conformer.

To accept or reject a new conformer at one MC step simply obeyed the Metropolis method. If the $\Delta E$ of a new conformer was less than or equal to that of the current conformer, the new conformer was accepted. If the $\Delta E$ of the new conformer was greater than that of the current conformer, the new conformer was accepted if $e^{-\Delta \Delta E / RT}$ was greater than a uniform random number between 0 and 1, where $\Delta \Delta E$ was the $\Delta E$ of the new conformer minus the $\Delta E$ of the current conformer ($\Delta \Delta E = \Delta E_{\text{new}} - \Delta E_{\text{current}}$). $R$ was a gas constant and $T$ was the absolute temperature. To find the $\Delta E$ for new and current conformers, the program simply referred to the $\Delta E$ value stored in the pre-calculated PE surface. These MC procedures were done using in-house programs.

Assuming the number of transitions between atropisomers observed during the MC simulations with the constant MC steps is proportional to the rate constant $k$, the activation energy, $E_a$, which corresponds to the energy barrier between atropisomers, could be calculated from the Arrhenius equations (Eqs. 2, 3),

$$
k = A e^{-E_a / RT}
$$

$$
n_{\text{trans}} = A' e^{-E_a / RT}
$$

where $k$ is the rate constant of the transitions between atropisomers, $n_{\text{trans}}$ is the number of transitions between atropisomers during the MC simulations, $R$ is a gas constant, $T$ is an absolute temperature and $A$ and $A'$ are arbitrary values. Therefore, the number of the transitions between atropisomers during the MC simulation was counted up. MC simulations for each compound at nine different temperatures ranging from 500 to 900K in 50K increments were executed to estimate the temperature dependency of the number of transitions. At each of the nine temperatures, 10 MC simulations with different random seeds and different starting atropisomers were
performed. One MC simulation consisted of $10^{11}$ MC steps, which was thought to be long enough to cover a slow transition between atropisomers.

**NMR Experiments**  NMR spectra of compounds 1 and 2 were measured in dimethyl sulfoxide (DMSO) solution at different temperatures to determine the coalescence temperatures ($T_c$) and the chemical shift differences in a slow exchange ($\Delta \nu_{ab}$) of the methylene protons at position 6 (Fig. 1). At the coalescence temperature, the rate constant ($k$) of exchange between the atropisomers is defined as an Eq. 4.

$$ k = \frac{\pi \Delta \nu_{ab}}{\sqrt{2}} $$

The Gibbs free energy ($\Delta G$) of exchange between the atropisomers is calculated by the Eyring–Polanyi equation (Eq. 5),

$$ \Delta G = -RT \ln(hk / k_b T_c) $$

where $R$ is a gas constant, $h$ is a Planck’s constant and $k_b$ is a Boltzmann constant.

**Results and Discussion**

**Potential Energy Surfaces**  For each compound, the two lowest energy conformers, aS_min and aR_min, were identified after the sequence of full optimizations at the AM1 level starting from 162 initial conformers followed by the full optimizations at the HF/6-31G(d) level starting from low energy conformers at the AM1 level, according to the process shown in Fig. 2. Three dimensional (3D) structures of aS_min and aR_min for each compound are shown in Figs. 3a and b.

The lowest energy conformer aS_min was a complete mirror image of aR_min in both compounds 1 and 2. The crystal structure of compound 1 determined in-house superimposed well with the structure of the lowest energy conformer aS_min of compound 1 at the HF/6-31G(d) level, as shown in Fig. 3c. The starting conformers of the MC simulation, aS_MC and aR_MC, were defined as the conformers with four fixed torsion angles ($\phi_1$, $\phi_2$, $\phi_3$, and $\phi_4$) that were closest to those of the lowest energy conformers aS_min and aR_min. The details of aS_MC and aR_MC are listed in Table 1. The number of the transitions between aS_MC and aR_MC observed during the MC simulations was counted up.

The PE surfaces at the HF/6-31G(d) level for compounds 1 and 2 were calculated as described in the Experimental section. Rough images of the shape of these PE surfaces are shown in Fig. 4. To create these two-dimensional PE surface maps, the dimensionality of the PE surface, defined by the four torsion angle values ($\phi_1$, $\phi_2$, $\phi_3$, and $\phi_4$) and one potential energy value $\Delta E$, was reduced through two reduction steps. Firstly, the information of the $\phi_4$ angle was reduced because $\phi_4$ is the torsion angle that defines the rotation of the thiophene ring attached at 4 position of TTDZ core and the conformations of the 7-membered ring of TTDZ core itself can only be defined by the torsion angles $\phi_1$, $\phi_2$, and $\phi_3$. Among the set of conformers that have the same combination of $\phi_1$, $\phi_2$, and $\phi_3$ angles, but have a different $\phi_4$ angle, the lowest energy conformer was selected as a representative conformer for each combination of $\phi_1$, $\phi_2$, and $\phi_4$. Secondly, the information of the three torsion angles $\phi_1$, $\phi_2$, and $\phi_4$ was reduced to two dimensions by using principal component analysis (PCA). The conformers selected in the previous step were projected onto the two-dimensional map using the first and second principal components (PCs) derived from the PCA of the three torsion angles $\phi_1$, $\phi_2$, and $\phi_4$ (Fig. 4). In the two-dimensional PE surface map, the $\Delta E$ was represented by the color. The contribution ratios of the first and second PCs were 0.59 and 0.32 for compound 1 and 0.60 and 0.32 for compound 2, respectively. The high contribution ratios of the first two PCs suggest that there mainly exist only one or two conformational freedoms for the 3D structure of 7-membered rings of compounds 1 and 2 and they probably stem from the geometrical restrictions of 7-membered ring formation and three planar non-rotatable bonds (one double bond and two aromatic bonds) in 7-membered rings.

The existence of many red points, which mean high PE conformers, in Fig. 4a clearly show that the energy barriers between aS and aR for compound 1 were much higher than those for compound 2. In addition to the number of high energy conformers, Fig. 4b suggests that a transition between aS and aR without visiting any red-colored points might be possible in the case of compound 2, but in the case of compound 1, the transition without passing through high PE conformers in red might be difficult because most of the regions between aS and aR were covered by red points and aS and aR were separated by the conformers colored in red (Fig. 4a). The high energy barriers of compound 1 shown in Fig. 4a were caused by the steric hindrance between the methyl group at position 3 of the TTDZ core and the thiophene ring at 4 position. The steric effect of the thiophene ring at 4 position was supported by the results of our MC simulations which showed that in the 4H-TTDZ compound, which is a TTDZ analog with the hydrogen at 4 position instead of the thiophene group, the difference of the estimated $E_b$ with methyl group at 3 position and without the methyl group becomes much smaller compared with that between compounds 1 and 2 (data not shown).

In order to confirm that the selection of the conformers

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**Table 1.** Torsion Angles, $\Delta E$ and Principal Component (PC) Values of aS_min, aR_min, aS_MC, and aR_MC

| Compound   | $\phi_1$ | $\phi_2$ | $\phi_3$ | $\phi_4$ | $\Delta E$ (kJ/mol) | PC-1 | PC-2 |
|------------|----------|----------|----------|----------|---------------------|------|------|
| **Compound 1** |          |          |          |          |                     |      |      |
| aS_min     | 47       | -66      | 63       | -159     | 0                   | 93   | 23   |
| aS_MC      | 45       | -60      | 60       | -165     | 2.2                 | 93   | 23   |
| aR_min     | -47      | 66       | -63      | 159      | 0                   |      |      |
| aR_MC      | -45      | 60       | -60      | 165      | 2.2                 | -93  | -23  |
| **Compound 2** |          |          |          |          |                     |      |      |
| aS_min     | 42       | -65      | 63       | -162     | 0                   |      |      |
| aS_MC      | 45       | -60      | 60       | -165     | 1.2                 | 93   | 24   |
| aR_min     | -42      | 65       | -63      | 162      | 0                   |      |      |
| aR_MC      | -45      | 60       | -60      | 165      | 1.2                 | -93  | -24  |
in the first step by the AM1 level calculations for further calculation of the PE surfaces at the HF/6-31G(d) level (Fig. 2) is valid, we compared the $\Delta E$s (obtained when generating the PE surfaces) between the conformers with four identical torsion angles at two different levels. The equations, $\Delta E(\text{HF/6-31G(d)})=1.96 \Delta E(\text{AM1}) (r^2=0.75)$ for compound 1 and $\Delta E(\text{HF/6-31G(d)})=1.84 \Delta E(\text{AM1}) (r^2=0.64)$ for compound 2, were obtained from simple linear regression analyses of
the ΔEs at both levels. These regression models suggested that the ΔE at the HF/6-31G(d) and at the AM1 levels were linearly correlated and that the selection of the conformers by the AM1 level calculations in the first step was valid. These regression models also suggested that the absolute value of the ΔE at the HF/6-31G(d) level was about 2 times greater than the ΔE at the AM1 level, meaning that the PE surface at the AM1 level might underestimate the energy barriers. For this reason, there existed conformers with ΔE at the HF/6-31G(d) level greater than 80 kJ/mol (magenta or red squares) in the two-dimensional PE surface maps (Fig. 4), even though only conformers with ΔE less than 80 kJ/mol at the AM1 level had been selected for optimization at the HF/6-31G(d) level when generating the PE surfaces.

Monte Carlo Simulations

The Arrhenius plots of the number of the transitions between aS_MC and aR_MC observed during the MC simulations with $10^{11}$ MC steps are shown in Fig. 5. The number of transitions observed during the ten MC simulations for each temperature was averaged, and the relation between the inverse of the temperature and the natural logarithm of the averaged number of the transitions was plotted. In the case of compound 1, the number of transitions at 500 K and 550 K were excluded from the analysis because the transitions occurred rarely (0–5 times at 500 K and 6–12 times at 550 K) during the MC simulations with $10^{11}$ steps and, from the statistical point of view, the number of transitions estimated from these rare observations was less reliable. The activation energy $E_a$ for transition between atropisomers was estimated by the Eq. 3, $n_{trans} = A e^{-E_a/RT}$ because a strong linearity between the natural logarithm of the number of transitions and the inverse of the absolute temperature ($r^2 \sim 1$) in both compounds 1 and 2 was observed, as shown in Fig. 5. The $E_a$ values of compounds 1 and 2 were estimated to be 89 kJ/mol and 60 kJ/mol, respectively.

When the MC sampling details such as the Δϕ value were changed, the estimated $E_a$ values were almost identical, and strong linearity ($r^2 \sim 1$) between the natural logarithm of the number of transitions during the MC simulations and the inverse of the absolute temperature (Arrhenius type relation) were constantly observed. For example, in the case of compound 2, the estimated $E_a$ value was 61 kJ/mol when Δϕ was changed to 15, which meant only one torsion angle was allowed to change in one MC step, and the $E_a$ was estimated to be 60 kJ/mol when Δϕ was changed to 26, which meant up to three torsion angles were allowed to change in one MC step, while the $E_a$ was 60 kJ/mol when Δϕ was 21.2, as described in the Experimental section.

To identify the transition state (TS) conformers of both compounds, optimizations to find TS at the HF/6-31G(d) level were performed. The initial conformers for TS optimization were selected as follows: firstly, from among the trajectories at 600 K in ten runs of MC simulation, each transition trajectory between aS_MC and aR_MC was extracted. Secondly, for each transition trajectory, the highest energy conformer was selected as the initial conformer for TS optimization. For compound 1, the initial conformer for TS optimization was the conformer with the highest energy in the trajectory at 600 K. For compound 2, the initial conformer for TS optimization was the conformer with the highest energy in the trajectory at 600 K.

The TS conformers for each compound were mirror images of each other. The torsion angles (ϕ₁, ϕ₂, ϕ₃, ϕ₄) of TSs of compound 1 are (−2, −6, 9, 104) and (2, 6, −9, −104), and those of compound 2 are (−2, −9, 12, 123) and (2, 9, −12, −123). 3D coordinates of these TS conformers are listed in the supplemental material.
the trajectory was selected. These highest energy conformers from the transition trajectories were collected and were used as initial conformers for TS optimization at the HF/6-31G(d) level. As shown in Fig. 6, two TS conformers for aS – aR transition with an almost planar 7-membered ring \((\phi_1, \phi_2, \phi_3, \phi_4, \phi_5, \phi_6)\) were obtained for both compounds 1 and 2. It was confirmed that both TS conformers satisfied the necessary condition of a TS in having only one imaginary frequency that can be calculated by a normal mode analysis. The \(\Delta E\) of these TS conformers were 94.4 kJ/mol for compound 1 and 65.1 kJ/mol for compound 2. These TS conformers are located at almost the center of the two-dimensional PE surface maps because the first and second PC values of these TS conformers were almost 0.

In order to examine whether the aS – aR transitions pass through the TS conformers or not, all of the trajectories of the transition between aS_MC and aR_MC observed during the MC simulations at 600 K and 800 K were traced. Transitions that did not pass through the TS conformers and their neighboring conformers (as defined in the Experimental section) were observed in about 40% of successful transitions of the MC simulation at 600 K and about 50% at 800 K of all of the transition trajectories for both compounds. This suggested that there exist paths for getting across the energy barriers between the atropisomers aS and aR without passing through the TS conformers.

To gain more information about the existence of the multiple transition paths, all of the transition trajectories observed in 10 MC simulations of compound 1 at 600 K were examined further. Among the 715 transition trajectories between aS_MC and aR_MC at 600 K, three representative trajectories are shown in Fig. 7. In case of the trajectory colored in red, compound 1 passed through the farthest point from the center, which corresponded to the TS conformers, of the two-dimensional map, and one other trajectory with a similar transition pattern was found from among the 715 transition trajectories. In the case of the trajectory in orange, compound 1 passed through the second farthest point from the center of the two-dimensional map, and three other trajectories with a similar transition pattern were found. In the case of the blue trajectory, compound 1 passed through the center of the map, where the TS conformer is located, with the smallest number of MC steps. Our method using the grid-based PE surfaces suggests that there are multiple transition paths between atropisomers, including ones not passing through TS conformers, at least at high temperatures.

**Required CPU Time for Calculations** The required CPU time for calculating the \(\Delta E\) at the HF/6-31G(d) level per conformer was about two hours on average using one core of a Xeon X5640 CPU (2.93 GHz). Therefore, a total of about 200000 \((=2 \times 100000)\) conformers per compound is needed to calculate the PE surface at the HF/6-31G(d) level if one core of Xeon CPU is used. About 11000 \((=200000 \times 18)\) cores, \(ca. 46 d\) would be needed to calculate the PE surface for one compound if 18 CPU cores were fully used in parallel. One of the advantages of our \(\Delta E\) calculations is that the \(\Delta E\) calculation for each conformer is independent of others and so can be easily parallelized for the available number of CPU cores, without any special implementation. The required CPU time could be halved because the PE surfaces of compounds 1 and 2 had two-fold symmetry, which means that all of the conformers except two conformers \(((\phi_1, \phi_2, \phi_3, \phi_4) = (0, 0, 0, 0), (\phi_1, \phi_2, \phi_3, \phi_4) = (0, 0, 180))\) had mirror-imaged conformers and thus had identical \(\Delta E\)s.

The MC simulation with \(10^{11}\) steps took about 20 h using one core of a Xeon CPU. A total of 1800 h \((=20 h \times 90000)\) MC simulations, \(ca. 75 d\) would be required to estimate the \(E_a\) of one compound if only one core of Xeon CPU is used. About 100 h \((=1800 h / 18\) cores, \(ca. 4 d)\) would be taken to calculate the \(E_a\) for one compound if 18 CPU cores were fully used in parallel. In the same way as the \(\Delta E\) calculations, the MC simulations are also independent of each other and can be easily parallelized. During the MC simulation by our method, the CPU was mostly occupied with generating the random number because no \(\Delta E\) calculation is required at each MC step, since the pre-calculated \(\Delta E\) value of the PE surface was simply referred to. If the energy of the conformer is evaluated at each step (as is usually done in MC simulations) and if the energy for each step is evaluated by a single point MO calculation at the HF/6-31G(d) level and if a single point MO calculation takes \(0.004 h\) (\(=0.004 h \times 10^{11}\) steps, \(46000\) years) for one MC simulation of \(10^{11}\) steps. In fact, \(0.07 h\) was required for the single point MO calculations at the HF/6-31G(d) level for the compounds in this study in one CPU core and, therefore, we expect that ideally \(0.004 (\approx 0.07 / 18) h\) hours per single point MO calculation would be required if 18 CPU cores were fully used in parallel. The difference between the duration needed for the MC simulation by our method and the estimated time required for MC simulations with energy calculations at each MC step demonstrates the power of the using the pre-calculated PE surface.

**NMR Experiments** In Fig. 8, the NMR spectra of the methylene protons at 6 position of the TTDZ core of compounds 1 and 2 are shown. For compound 2, the slow exchange state was observed at 288 K and the chemical shift difference in slow exchange \(\Delta \nu_{6a}\) was determined to be 526 Hz. As the temperature was raised, an intermediate state appeared at 333 K and a coalescence state was observed at 338 K (Fig. 8b) and the coalescence temperature \(T_c\) of compound 2 was determined to be 338 K. At 343 K, compound 2 reached the fast exchange state. Based on the Eq. 4 and 5 and the values of 526 Hz of \(\Delta \nu_{6a}\) and 338 K of \(T_c\), the \(\Delta G\) of exchange between the atropisomers for compound 2 was estimated to be 63 kJ/mol. For compound 1, the slow exchange state was observed at 295 K and the \(\Delta \nu_{6a}\) value was observed to be 410 Hz. At 423 K, compound 1 was in the intermediate state but could not reach the coalescence state. Further elevation of the temperature was impossible because of a limitation in the hardware of the NMR device. Thus, the \(\Delta G\) of compound 1 was estimated to be higher than 81 kJ/mol, based on the values of 410 Hz of \(\Delta \nu_{6a}\) at 295 K and \(>423 K\) of \(T_c\).

**Comparison of Calculated \(E_a\) and Experimentally Determined \(\Delta G\)** As written in many text books, the following relationship between \(E_a\) and \(\Delta G\) exists. From the Arrhenius equation, rate constant \((k)\) is written as the Eq. 2.

\[
k = Ae^{-E_a/k_BRT}
\]

From the Eyring–Polanyi equation, \(k\) is also written as

\[
k = (k_bT / h)e^{-\Delta G / RT}
\]
where $k_b$ is Boltzmann constant, $h$ is Planck's constant, $R$ is gas constant and $T$ is absolute temperature. Taking a natural logarithm of these Eqs. 2 and 6

$$\ln A - \left( \frac{E_a}{RT} \right) = \ln (k_b T / h) - (\Delta G / RT)$$

(7)

Since $\Delta G = \Delta H - T \Delta S$

$$\ln A - \left( \frac{E_a}{RT} \right) = \ln (k_b T / h) - (\Delta H / RT) + (\Delta S / R)$$

(8)

Differentiation by $T$

$$\frac{E_a}{RT^2} = \frac{1}{T} + (\Delta H / RT^2)$$

(9)

Then $E_a$ is obtained as

$$E_a = \Delta G + T \Delta S + RT$$

(10)

Assuming the $T \Delta S$ and the $RT$ are small compared with the $\Delta G$ and are negligible, a comparison between the $E_a$ calculated by the MC simulations and the experimentally determined $\Delta G$ of exchange between the atropisomers is possible. For compound 2, the activation energy $E_a$ for the transition between atropisomers calculated by the MC simulations was 60 kJ/mol, which was almost equal to the experimentally determined $\Delta G$, 63 kJ/mol. For compound 1, the calculated $E_a$ of 89 kJ/mol was consistent with the experimental $\Delta G$ estimated to be greater than 81 kJ/mol.

Half-lives ($t_{1/2}$) of atropisomers can be estimated from an equation written below

$$t_{1/2} = \frac{\ln 2}{k}$$

(11)

where $k$ is the rate constant, which can be estimated by the Eyring–Polanyi equation (Eq. 6). The half-life of atropisomer $t_{1/2}$ of compound 2 at 300 K was 0.01 s from the experimentally determined $\Delta G$ and 0.003 s based on the calculated $E_a$. These calculated and experimental $t_{1/2}$ values suggested that the exchange of compound 2 between the atropisomers aS and aR is very fast and compound 2 will not display atropisomerism if the criterion for atropisomerism of having a $t_{1/2}$ of more than 1000 s is applied.\(^{5}\) For compound 1, $t_{1/2}$ at 300 K was experimentally determined to be greater than 14 s and $t_{1/2}$ at 300 K was computationally estimated to be 350 s. The computationally estimated $t_{1/2}$ of 350 s was used to judge the atropisomerism of compound 1 because the experimentally determined value, >14 s, of $t_{1/2}$ includes both ranges of less than and more than the criterion, <1000 s and >1000 s respectively. It was suggested that compound 1 with $t_{1/2}$ of 350 s will not exhibit atropisomerism, although the value of 350 s of compound 1 is numerically very close to the criterion, 1000 s.

It was interesting that the values of the potential energy $\Delta E$ of the TS conformers of compound 1 (94 kJ/mol) and 2 (65 kJ/mol) obtained from the analysis of the transition state were relatively close to the values of the activation energy $E_a$ of compound 1 (89 kJ/mol) and 2 (60 kJ/mol) obtained from the MC simulations respectively. Although an analysis of the MC transition trajectories suggested the existence (at least, at high temperatures) of multiple transition paths and of transition paths that do not pass through the TS conformers, the relatively small difference between the $\Delta E$ of the TS conformers and the $E_a$ derived from the MC simulations suggested that, at low temperatures, the contributions to the activation energy $E_a$ of the transition paths that were far from the path along the TS conformers might not be significant. In other words, based on the MC simulations on grid-based PE surfaces in this study, there is a possibility that, at low temperatures, the compounds in this study get across the energy barriers between the atropisomers mainly at conformers near the TS conformers, even though there exist multiple transition paths at high temperatures. To verify the hypothesis described above, MC simulations at low temperatures with larger steps than $10^{11}$ might be needed, because the number of transitions between the atropisomers dramatically decreased at low temperatures.

**Conclusion**

In this study, a simple computational method of calculating $E_a$ of transitions between atropisomers of a flexible 7-mem-

![Fig. 8. NMR Spectra of the Methylene Protons at 6 Position of a) Compound 1 and b) Compound 2](image-url)
bered ring was proposed and was applied to the two TTDZ analogs. In our method, the $\Delta E$ of all of the possible conformers related to the transition between atropisomers was calculated at the HF/6-31G(d) level before the Monte Carlo (MC) simulations. Instead of calculating the $\Delta E$ at each MC step, as is often done for MC simulations, $\Delta E$ of the conformer at each step of the MC simulation was simply referred to the value of the pre-calculated PE surface. This procedure enabled an MC simulation with MC steps large enough to be applied to a slow transition phenomenon, such as an atropisomerism of a 7-membered ring with a high energy barrier, and $10^{11}$ MC steps were assigned for one run of the MC simulation in our case. Based on the Arrhenius equation, the $E_a$ required for the transition between the atropisomers was estimated by the temperature dependency of the number of the transitions between atropisomers observed during the MC simulations. The calculated $E_a$ for compound 2 was 60 kJ/mol, and this value agreed well with the $\Delta G$ for the transition between atropisomers, 63 kJ/mol, as determined by an NMR experiment. For compound 1, the experimental $\Delta G$ was estimated to be a range of value, >81 kJ/mol, rather than a specific value, because of a limitation of the NMR experiment. The $E_a$ of compound 1 calculated by the MC simulations was 89 kJ/mol and was within the range of the experimental $\Delta G$. The half-lives ($t_{1/2}$) of the exchange between the atropisomers for compounds 1 and 2 were estimated to be 0.003 s and 350 s, respectively, based on the $E_a$ values calculated by the MC simulations. It was suggested that neither compound will exhibit atropisomerism, based on the criterion that a $t_{1/2}$ longer than 1000 s indicates atropisomerism. The analysis of the transition trajectories between atropisomers observed during the MC simulations revealed that, especially at high temperatures, the transitions occurred through multiple paths and there existed transition paths that did not pass through the transition state conformers. About 46 d of computation time were taken to calculate the PE surface of one compound at the HF/6-31G(d) level, which corresponds to about 10000 optimizations, with 18 cores of Xeon CPU used in parallel. It took about 4 d for the MC simulations of one compound, which corresponds to the 90 MC simulations with $10^{11}$ steps, using 18 cores in parallel. These computation times were very much shorter than the 46000 years that are estimated as required for one run of MC simulation at the HF/6-31G(d) level of $10^{11}$ steps when the potential energy is calculated at each MC step. Our method enabled MC simulations with an ab initio molecular orbital method-based PE surface and with MC steps large enough for slow transition phenomena to be run within a realistic computation time and the results agreed well with the experimental results. Further applications of this method to other phenomena with slow dynamics are expected.

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