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BRIEF COMMUNICATION

Computational study on formation of 15-membered azalactone by double reductive amination using molecular mechanics and density functional theory calculations

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Abstract

Formation of 15-membered azalactone by double reductive amination was analyzed using molecular mechanics and density functional theory calculations for simplified model compounds. As a result, the following aspects were clarified. When methylamine attacks a linear bis-aldehyde in the first step, there are possibilities that two regioisomers are formed. However, one of them exhibited remarkably stable energy level compared with the other. The stable isomer indicated a short distance between a methylamine moiety and an unreacted aldehyde. This short distance, about 2.3 Å, could be explained by hydrogen bonding, which implied relatively easy cyclization in the second step. Moreover, this cyclization process was supposed to be exothermic according to comparison of energy levels before and after cyclization.

Macrocyclic natural products [1] often display remarkable biological activities and many of these compounds and their derivatives are used as prescription medicines. Natural products [1] commonly contain a medium- to large-sized ring system, e.g., a 12-membered ring to a 20-membered ring or a bigger-sized ring can be often observed. These are more frequently encountered in natural than in synthetic drugs. Although a variety of useful methodologies [2-4] to construct a small-sized ring system had been reported (Scheme 1S (supple)), we focused on approaches for preparing a medium-sized ring system.

A variety of synthetic strategies for construction of a medium-sized cyclic molecule has been also reported (Fig. 1), although it is not easy to optimize a chemical structure of a precursor and reaction conditions for cyclization. Focusing on syntheses of 14- to 18-membered macrolactones, macrolactonization was practically applied as pioneer works by Tatsuta et al. [5] and Woodward et al., [6] and those reactions were precisely investigated. Based on their research, Woodward et al. [6] and Yonemitsu and colleagues [7-9] emphasized that certain structural feature and cyclic protecting groups or favorable/suitable conformations in the seco acid were very important for efficient macrolactonization. Other than a lactonization approach, Horner–Wadsworth–Emmonms reaction/Wittig–Horner reaction was reported as an alternative efficient macrocyclization by Tatsuta et al. [10] and Nakajima et al. [11] Stille-type coupling cyclization [12] and an olefin metathesis approach [13] were also reported as novel strategies by Nicolaou et al. Synthetic methodologies toward macrocycles were comprehensively introduced by Yu and Sun. [14] As a characteristic example, macrocyclization [15] by intramolecular Ullmann reaction has been reported in synthetic studies of engelhardione.

As a variety of cyclization strategies for small-sized to medium-sized ring constructions had been already reported, we focused on an alternative approach, i.e., reductive

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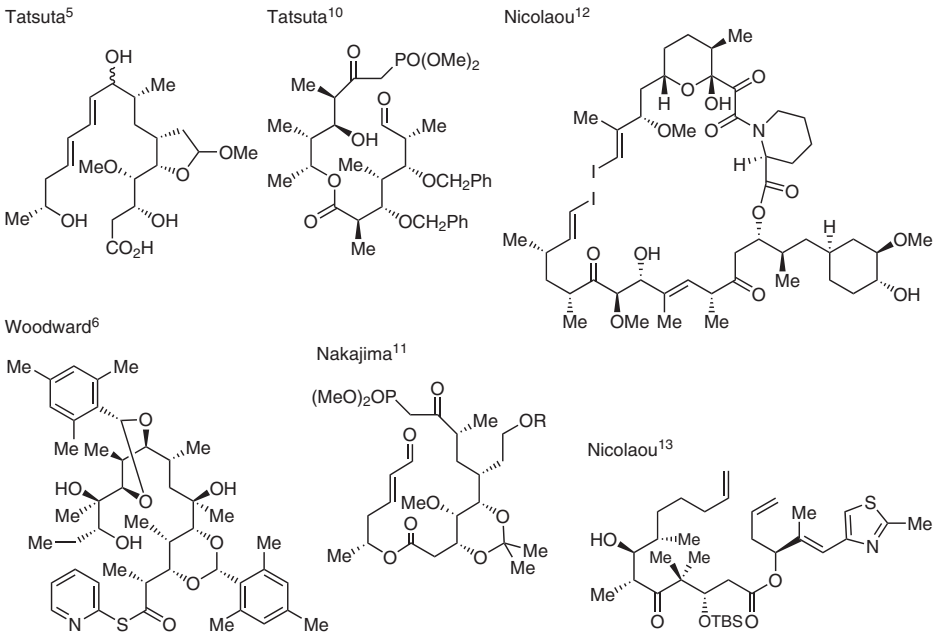
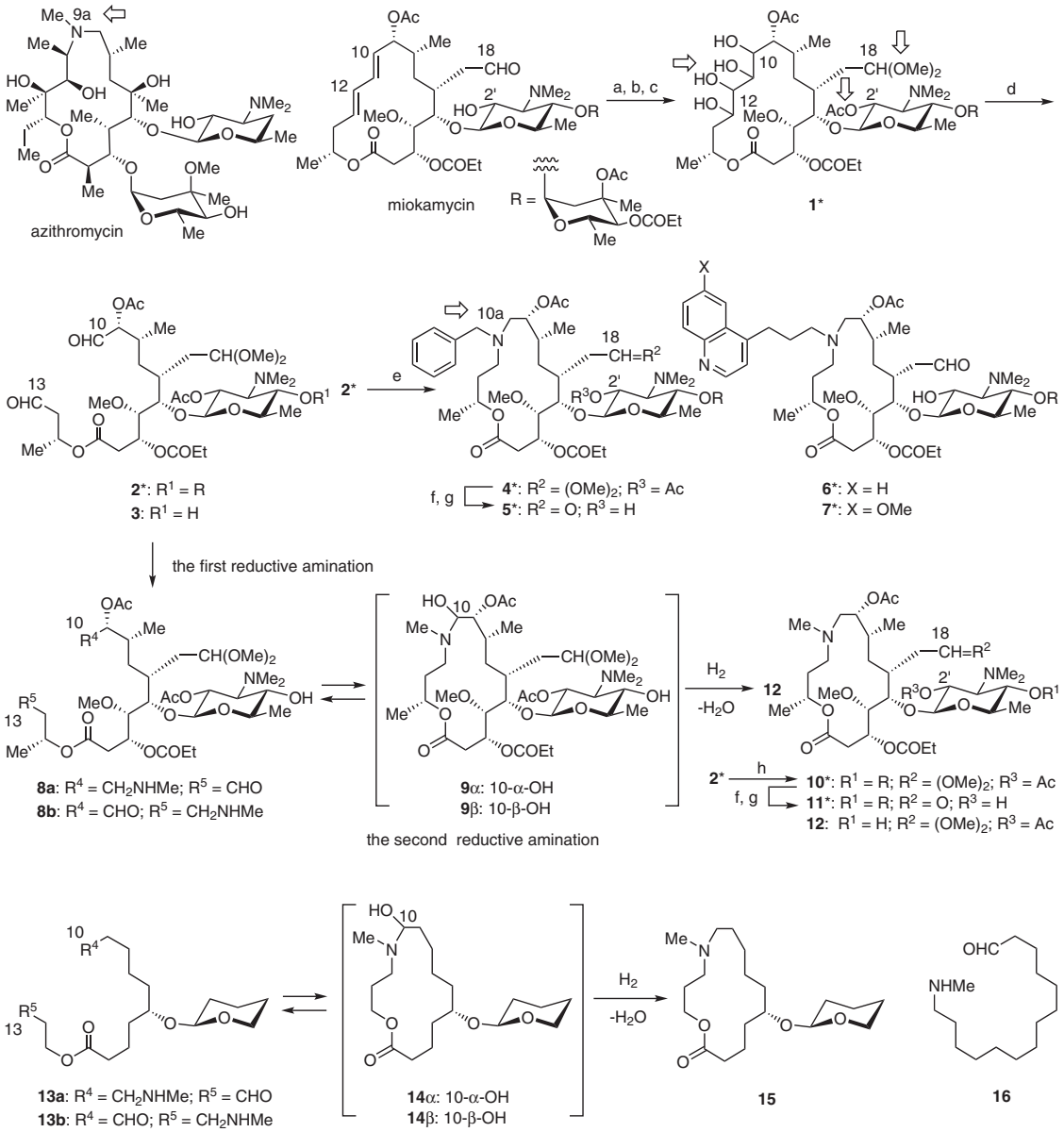


Fig. 1 Structures of representative precursors for macrocyclization reactions

amination (reductive alkylation) for novel medicinal chemistry. This method is supposed to be useful, because its reaction condition is relatively mild and there is no need to protect hydroxyl groups. Although intramolecular reductive amination in application of a linear amino aldehyde can construct a cyclic molecule, double reductive amination between a substituted amine and a linear bis-aldehyde also makes it possible to directly prepare a cyclic molecule *via* one-step. Syntheses of seven-membered molecules [16-19] by this method, double reductive amination, had been reported (Scheme 2S (supple)), but construction of a medium-sized ring by this method had not been reported yet when we started an azalide research [20, 21] program. Accordingly, we planned to pursue macrolide antibiotic drug discovery research in application of medium-sized aza-macrocycles. As azithromycin [22] possessing an aza-lactone (Scheme 1) is widely used in clinical sites as one of major class of macrolide antibiotics, application of an aza-lactone for macrolide drug discovery was thought to be appropriate. Incidentally, synthesis of aza-macrocycles [23] by nucleophilic ring closure without reductive amination was reported in 1984.

In order to generate a novel macrolide containing a medium-sized azalactone, we designed a linear bis-aldehyde, compound **2**, as a precursor for double reductive amination, because the 14- to 16-membered macrolide antibiotics are widely used in clinical sites. The precursor (**2**) [24] was easily prepared from tetraol **1** which was synthesized from miokamycin in three steps as shown in Scheme 1 (**R** = a neutral sugar). Our precursor possessed a carbohydrate moiety, because a sugar unit could not be easily introduced during the synthesis of macrolides. [25, 26] Several energy-related calculation results [27, 28] in formation or ring strain of medium-sized rings have already been reported. For example, lactone formation (Figure 1aS (supple)) and ring strain (Figure 1cS (supple)) of 14- to 16-membered rings exhibit stable energy levels. In other words, it might be difficult to form 8- to 10-membered lactones (Figure 1aS (supple)) and higher strains are estimated to be in 8- to 11-membered cyclanes (Figure 1cS (supple)). On the other hand, cyclic ether formation (Figure 1bS (supple)) of 14- and 16-membered rings implies synthetic difficulties. However, difficulties in cyclization of medium-sized ring by reductive amination had not been reported yet. Thus, we were interested in the possibilities for cyclization of medium-sized ring, especially 15-membered ring, by this method.

We previously reported that we prepared a linear bis-aldehyde (**2**) and isolated it as a pure single molecule, [24] which was cyclized with benzylamine under optimized conditions to afford desired 15-membered azalactone (**4**) in 10% yield (*via* 2 steps) as shown in Scheme 1. Synthesis of a 15-membered azalactone by double reductive amination was the first example. Deprotection of **4** afforded azalide (**5**) possessing comparable antibacterial activities to those of miokamycin. Partially optimized 15-membered azalides, [24] compounds **6** and **7**, exhibited 8 times stronger activities compared with miokamycin against susceptible *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Compound **7** has ≥ 32 times stronger activities compared with miokamycin against resistant *S. pneumoniae* with *erm* gene (induced methylase type) as previously reported. As described above, we clarified that these 15-membered azalides were important information for further medicinal



Scheme 1 Azithromycin, synthetic scheme of 15-membered azalides[a] and intermediates of cyclization by double reductive amination. [a]Reagents and conditions: (**a**) $(MeO)_3CH$ (80 eq), PPTS (1.2 eq), MeOH, 40–50 °C, 4 days; (**b**) Ac_2O, (5.0 eq), MeCN, 40 °C 16 h; (**c**) OsO_4 (0.15 eq), NMO (2.0 eq), aq. acetone, rt, 24 h, 30% in three steps; (**d**) $Pb(OAc)_4$ (2.1 eq), PhH, Na_2CO_3 (8.0 eq), rt, 10 min.; (**e**) $BnNH_2$ (1.1 eq), $NaBH_3CN$ (3.9 eq), AcOH (15 eq), EtOH, 0 °C to rt, 33 h, 10% in two steps; (**f**) MeOH:H_2O (9:1), 55 °C, 24 h, 79–90%; (**g**) CHF_2CO_2H (20 eq), MeCN-H_2O, rt, 24 h, 84–94%; (**h**) $MeNH_2\cdot$ HCl (1.1 eq), $NaBH_3CN$ (3.9 eq), AcOH (15 eq), EtOH, 0 °C to rt, 16 h, 11% in two steps. Compound number with asterisk: practically synthesized; compound number without asterisk: designed molecules (not synthesized) for DFT calculations

chemistry. These results encouraged us to analyze cyclization by double reductive amination with computational chemistry. Syntheses of many kinds of azalides with a medium-sized azalactone from 13- to 17-membered, such as 8a-, 9a-, and 11a-azalide have been reported, [29-36] but synthesis of 10a-azalide such compounds **4–7**, **10**, and **11** has not been reported yet. In 1982, Ōmura et al. [37] reported that the framework of the 16-membered macrolide, JM/LM-A_3, except an aldehyde group at the C-18 position, was quite stable under hydride reduction conditions for reductive amination.

Research purposes of this computational study are to clarify the following three questions.

(i) Compound **2** possesses two aldehyde groups at the C-10 and C-13 positions (Scheme 1). Which aldehyde is attacked by an amine in the first reductive amination step, the C-10 position or C-13?

Table 1 Relative energy[a] and distance[a] of compounds **8a** and **8b** by MMFF94s and B3LYP/6–31G(d)

MMFF94s in vacuo			B3LYP/6–31G(d) with PCM (ethanol)			
	8a	8b	8a		8b	
No.[b]	Energy[c]	Energy[c]	Energy[d]	CHO-HN	Energy[d]	CHO-HN
1	0.00	0.00	3.81	10.36	**0.33**	**2.31**
2	0.23	0.43	3.81	10.36	5.24	10.43
3	0.68	0.49	4.74	10.34	5.46	10.37
4	0.83	0.49	3.68	10.27	5.45	10.38
5	1.03	0.61	3.68	10.27	**1.13**	**2.31**
6	1.20	0.72	4.87	10.61	**1.44**	**2.30**
7	1.49	0.73	4.57	10.33	4.54	2.30
8	1.61	0.81	5.14	10.35	5.78	9.87
9	1.77	0.89	4.09	10.38	**0.00**	**2.26**
10	1.83	1.04	6.43	9.31	6.44	10.38

[a]Energy difference: kcal/mol; CHO-HN: Å

[b]The number in order of stable conformational isomers by MMFF94s.

[c]Relative energy when No. 1 is set to 0.00 kcal/mol as a reference.

[d]Relative energy when No. 9 of compound **8b** (− 2,648.247662 a.u.) is set to 0.00 kcal/mol as a reference

Bold values conformations are supposed to be energy stable and possible hydrogen bonding

(ii) In the second reductive amination step, the compound with an introduced amino group at C-10 or C-13 needs to adopt specific conformations for cyclization in which the remaining aldehyde group and the introduced amino group are located near each other. Are these specific conformations observed in the low-energy conformers?

(iii) Is the cyclization reaction exothermic or endothermic?

Accumulating answers to these three questions would be useful to reveal scope and limitation of medium-sized ring formation using double reductive amination and lead to a prediction of medium-sized ring formation by computational study rather than synthetic experiments in near future.

As the chemical structures of reactant **2** and product **4** in the cyclization reaction were too large to perform density functional theory (DFT) calculations, their chemical structures had to be somewhat simplified. First, we used a simpler reactant **3** without a neutral sugar moiety, instead of **2**. As the neutral sugar moiety of **2** seems to be far from the cyclization site, this simplification would give little influence on calculations. When we replaced benzylamine with methylamine hydrochloride, compound **2** gave azalactone **10** (Scheme 1) in 11% yield (*via* two steps) [24] by double reductive amination. This yield was almost the same as compound **4**. Therefore, we used azalactone **12** (a simplified compound of **10**) as a product in our calculations. Compound **12** differs from **10** only in that the former has no neutral sugar moiety such as **3**.

To examine questions (i) and (ii), we first constructed the three-dimensional structures of model compounds **8a** and **8b** (Scheme 1) corresponding to compounds containing an introduced methylamino group at the C10 and C13, respectively, of model reactant **3**. We next performed conformational search of the model compounds **8a** and **8b** in vacuo by CONFLEX algorithm [38, 39] using CONFLEX 7 program (supple #1) with MMFF94s force field [40–46]. We obtained a total of more than 40,000 different conformers for each compound. Finally, we re-optimized the top 10 most stable isomers optimized by MMFF94s of **8a** and **8b** by DFT method (B3LYP) using the 6–31G(d) basis set in vacuo (Table 1S (supple)) and in ethanol environment (polarizable continuum model (PCM) [47]) using Gaussian 09 program (supple #2). We aimed to compare the energies and structures of **8a** and **8b** more accurately in the experimental conditions based on DFT calculations (supple #3).

The results are shown in Table 1. From the results of DFT calculations, we found that compound **8b** reacted at the C-13 gave the most stable structure (No. 9), suggesting that **8b** would be a product in the first reductive amination step. In addition, all of four lower energy conformations (No. 1, 5, 6, and 9) (bold values) of **8b** showed a short distance of ~ 2.3 Å between a hydrogen atom in the methylamino group and an oxygen atom in the aldehyde group as shown in Table 1, indicating the hydrogen bonding formation between these two groups. This hydrogen bonding formation could keep these two functional groups in proximity and facilitate the cyclization reaction. On the other hand, no stable conformations with a hydrogen bonding between methylamino and aldehyde groups could be found in the compound **8a** reacted at the C-10. Thus, we could clarify questions (i) and (ii) as follows. Reaction of **3** and methylamine with hydride reagent in ethanol gives precursor **8b**, that is, an aldehyde at the C-13 is attacked by an amine in the first reductive amination step. This finding is also reasonable from steric hindrance viewpoints, as the C-13 of **3** is less crowded. In addition, the precursor **8b** could adopt the energetically stable conformations where the methylamino and aldehyde groups were in proximity to each other for cyclization. The most stable three-dimensional conformation of compound **8b** (No. 9) is shown in Fig. 2.

Next, we try to clarify question (iii). The lowest energy structure of cyclized product **12** (Scheme 1) was determined by the same procedure applied for compounds **8a** and **8b**. In addition, we also examined cyclized forms of the precursor **8b**, i.e., **9**α and **9**β (Scheme 1). We expected that the cyclization step of **8b** would be a rate-limiting step in the second reductive amination step and considered **9**α or **9**β as virtual intermediates to roughly estimate activation energy.

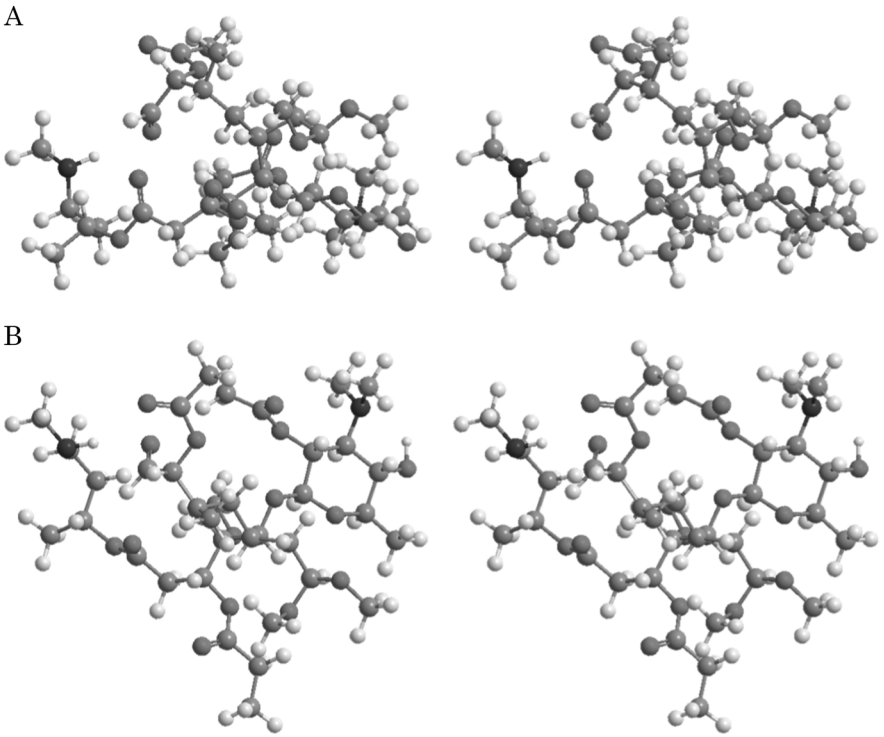


Fig. 2 Stereo view of the most stable three-dimensional conformation of compound **8b** (No. 9). **A** Relative position between an *N*-Me group and an aldehyde group is visually easy to understand. **B** The view of B is obtained by 90° rotation of A around the horizontal axis. An overall framework of **8b** is easy to understand

Table 2 Energy* profile of sequence cyclization, compound **8b** to **12** *via* virtual intermediate **9α**

	H_2	H_2O	**8b**	**9α**	**12**	ΔEcycle	ΔEtotal**
In vacuo	− 1.175482	− 76.408953	− 2648.225569	− 2,648.223537	− 2,573.004145	1.28	− 7.56
Ethanol	− 1.175565	− 76.415980	− 2,648.247662	− 2,648.246052	− 2,573.026376	1.01	− 12.00

*Total energy of each molecule (a.u.)

**{(− 2,573.026376–76.415980)–(− 2,648.247662–1.175565)} × 627.51 = − 12.00 (in ethanol)

The results of DFT calculations for **9α**, **9β** and **12** are provided (Table 2S (supple)). These results indicated that the energy of the most stable conformation of **9α** (No. 3) was lower than that of **9β** (No. 1), suggesting that **9α** was suitable as an intermediate. In consideration of energies of H_2 and H_2O molecules, we obtained an energy profile for cyclization of **8b** to **12** through virtual intermediate state **9α** in ethanol environment as shown in Table 2 and Fig. 3. The activation energy from the initial state consisting of **8b** and H_2 to **9α** was a relatively small value of +1.01 kcal/mol. The most stable energy level in ethanol of the final state including cyclized product **12** and H_2O was remarkably lower (−12.00 kcal/mol) than the initial state consisting of **8b** and H_2. This finding strongly suggested that this reductive amination (the second step) proceeded toward energy stable direction, which means, the reaction was exothermic. The relatively small value of activation energy and the exothermicity might explain that the reaction could occur at around room temperature.

As described above, we examined the model cyclization of **8b** to **12** through intermediate state **9α** using DFT calculations. These calculations were found to be very time-consuming, although we used somewhat simplified structures. If we had used more simplified structures, we could have had lots of merit such as time saving. Thus, we finally examined whether we could get appropriate results or not in application of more simplified structures. Mono reductive aminated molecules **13a** and **13b** were first designed instead of **8a** and **8b**, respectively. Compounds **13a** and **13b** had only an ester bond and a tetrahydropyran ring (Scheme 1). Relative energy and focused distance of **13a** and **13b** were obtained (Table 3S (supple)) using a similar computational method used for **8a** and **8b**. These results were notably similar to those of compounds **8a** and **8b**. Compound **13b** corresponding to **8b** gave the most stable structure and possessed lower energy conformations where the methylamino and aldehyde groups were in proximity to each other for cyclization (Fig. 2S (supple)). Accordingly, we could obtain the same conclusion that methylamine was supposed to attack the C-13 aldehyde preferably compared with the C-10 aldehyde as in the case of **8a** and **8b**. Next, we examined the second reductive amination step using more

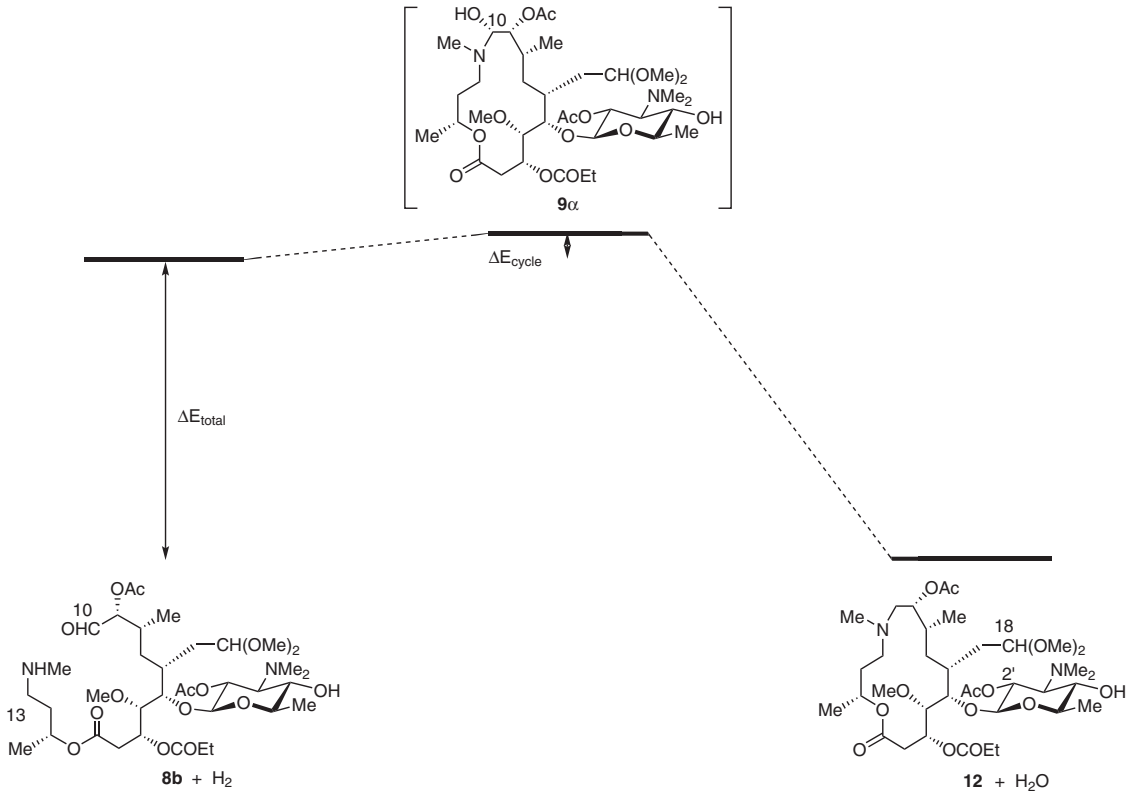


Fig. 3 An energy profile of cyclization reaction with a precursor **8b**

simplified model compounds **14**α, **14**β, and **15** corresponding to **9**α, **9**β, and **12** (Table 4S (supple)). The resulting energy profile for cyclization of **13b** to **15** was also very similar to that for cyclization of **8b** to **12**, that is, the activation energy was estimated to be + 1.87 kcal/mol and the most stable energy level in ethanol of the final state including cyclized product **15** and H_2O was remarkably lower (−12.23 kcal/mol) than the initial state consisting of **13b** and H_2 (Table 5S and Fig. 3S (supple)). These results led us to the same conclusion that this reductive amination (the second step) proceeded toward energy stable direction as in the case from **8b** to **12**. Thus, it may be useful to use dramatically simplified forms like **13a** and **13b** instead of compounds **8a** and **8b**, in order to reduce calculation time. On the other hand, fully simplified compound **16** provided us with only limited information (supple #4), because a yield of cyclization reaction was known to be greatly affected by a substituent [48].

In conclusion, our computational study indicated the following facts. (i) The first step of practical reductive amination reaction from **2** to **10** was determined as an attack of methylamine at the C-13 aldehyde. (ii) In the second step of reductive amination for cyclization, the methylamino group at the C-13 and the aldehyde group at the C-10 are located very closely due to hydrogen bonding. (iii) The sequential cyclization was categorized as exothermic. (iv) Using adequate simplified forms would reduce the calculation time. From now on, applying the above computational study to novel molecules may make it possible to predict to some extent the easiness or difficulty of constructing a medium-sized ring by double reductive amination without information by synthetic experiments. On the other hand, we have to accumulate further information on the possibility of double reductive amination for cyclization reaction of 8- to 14-membered rings and 16- or more membered rings using both theoretical calculations and synthetic experiments. This would allow us to discuss correlations between calculations and experiments in near future.

As formation of 15- or 16-membered azalactone by double reductive amination is low yield but practical for medicinal chemists (not for process chemists), application of these reactions in novel medicinal chemistry of biologically important macromolecules is expected to be effective also in future.

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