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Theoretical Study of the Rearrangement Reaction in Bisorbicillinoid Biosynthesis: Insights into the Molecular Mechanisms Involved†

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Bisorbibutenolide and bisorbicillinolide are polyketide compounds with complex skeletons that are formed by the dimerization of sorbicillin. These compounds have long been of interest, with several reports of their biosynthesis, biological activity, and total synthesis. In this study, we theoretically investigated the detailed biosynthetic mechanism of the rearrangement reaction to form bisorbicillinolide. We showed that the presence of water molecules facilitates the intramolecular aldol reaction, determined the rate-limiting steps, and revealed that a cyclopropane intermediate is formed during the rearrangement process. Although computational chemistry has been widely applied to the carbocation chemistry present in terpene biosynthesis, it has seldom been used to investigate the carbonyl chemistry responsible for polyketide biosynthesis. This study shows that computational chemistry is a useful tool for studying anionic skeletal rearrangement reactions.

Introduction

Natural products are an invaluable source of bioactive compounds with unique structural features and diverse biological activities for drug discovery and development.[1] One of the main characteristics of complex natural product biosynthesis is its use of key dimerization steps to form structurally diverse and biologically potent molecules.[2] These strategies can also be harnessed for total synthesis.[3–5] Dimerization processes can occur spontaneously or be facilitated by enzymes such as cytochrome P450s, flavin-containing monooxygenase(FMO), and laccases.[2] Regardless of its mechanism, dimerization often generates stereochemically complex molecules that are difficult to synthesize by conventional methods.

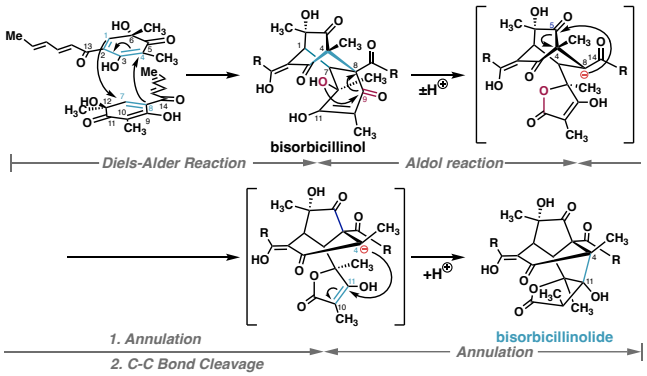

Fig. 1 Proposed biosynthetic pathway of bisorbicillinolide. R stands for side alkyl chain.

Bisorbicillinoids are complex polyketides derived from fungi such as *Trichoderma*[6,7] and *Penicillium*[8]. These natural products are known for their diverse biological activities, including antioxidant[9] and antiallergic[10] properties. Previous reports have proposed that the biosynthesis of bisorbicillinoids involves the Diels-Alder-type dimerization of two sorbicillinol derivatives (figure 1).[8,11–16] According to *in vitro* experiments, these dimerization reactions occur spontaneously.[17–20]

Despite growing interest in bisorbicillinoids and their biosynthetic pathways, the detailed reaction mechanism that occurs after dimerization remains elusive because of the high difficulty of determining detailed biosynthetic mechanisms solely by experimental methods. Computational chemistry has recently been established as a powerful tool for investigating complex biosynthetic reaction mechanisms, providing valuable insights into the structures and energies of transition states and intermediate species.[21] In particular, terpene-forming reactions are well-suited for investigation using computational chemistry, as evidenced by the numerous studies reported by Tantillo and coworkers.[22–26] We have also previously utilized computational chemistry in

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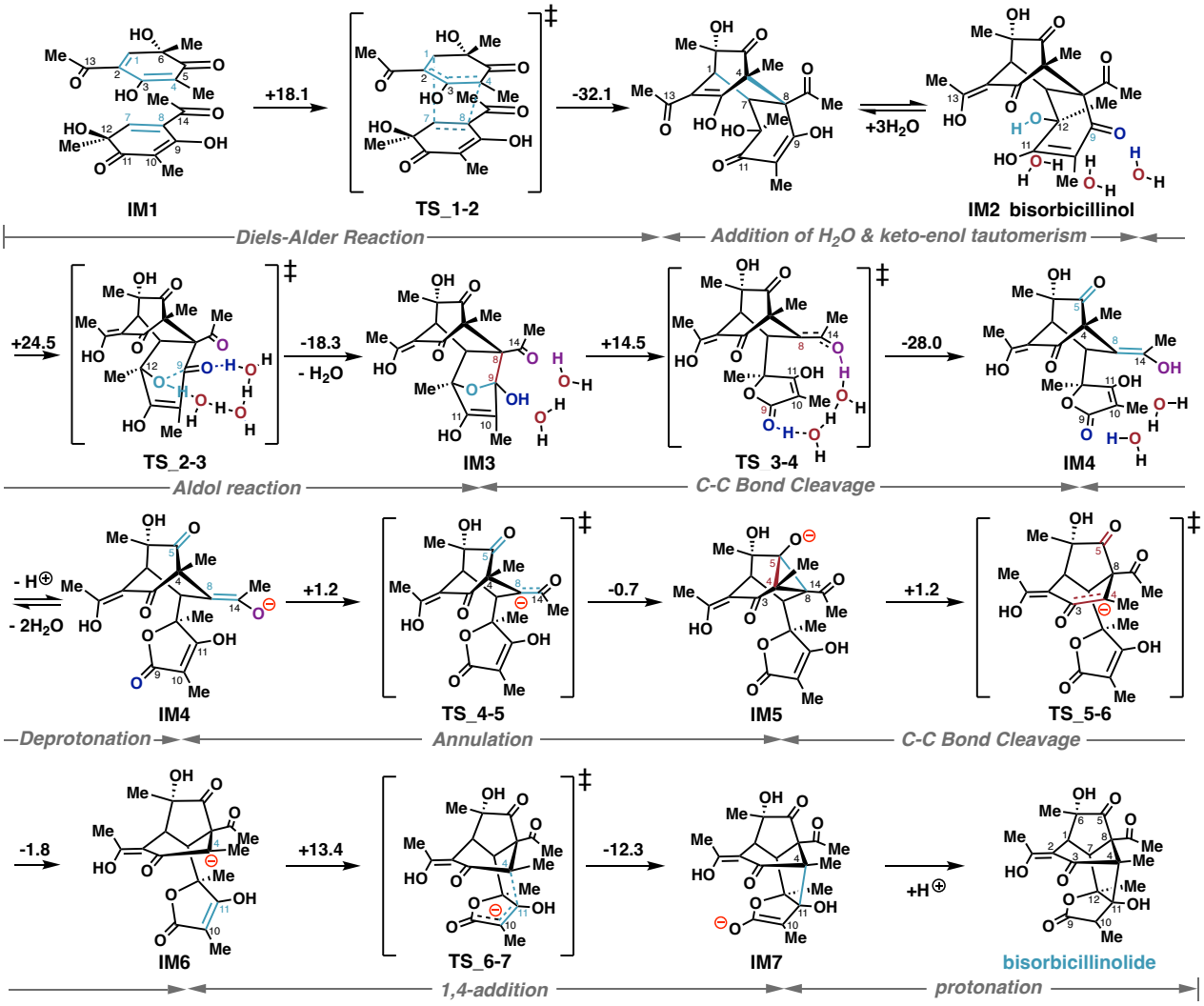

Fig. 2 DFT evaluation of the complete bisorbicillinolide biosynthetic pathway. Potential energy changes (kcal mol^{-1}, calculated at the ωB97XD/6-31G(d,p) level) are displayed on the arrows. A continuum solvation model (SMD) was employed to consider the effects of water.

the study of terpene-forming reactions, and have revealed detailed reaction mechanisms.[27,28] Although there are many computational chemistry studies in the field of terpene biosynthesis, such as *ab initio* properties (AIMD)[29], QM[27,28,30], QM/MM[31–37], and QM/MM MD[38] approaches, research on the biosynthesis of other natural products, such as polyketides[4,39], alkaloids[40,41], and those involving intramolecular cyclization reactions[4,42–44] remains scarce. In this study, we performed a detailed analysis of the bisorbicillinoid biosynthetic pathway using density functional theory (DFT), focusing specifically on the key rearrangement reaction step, to better understand the factors that govern this critical reaction step. This understanding could potentially inform the design of novel bisorbicillinoid analogs with enhanced biological properties, and may provide insight into tailored biosynthetic strategies for their production. Our study exemplifies how computational chemistry can contribute to the description of complex biosynthetic processes, and we hope it serves as a useful starting point for future investigations in natural product biosynthesis.

Experimental

All calculations were performed using the Gaussian 16 package.[45] Structure optimizations were done using the ωB97XD[46] functional with an ultrafine grid and the 6-31G(d,p) basis set without symmetry restrictions. All the biosynthetic calculations were performed in water solvent using the solvation model based on density (SMD)[47]. This level of theory has been reported to be suitable for studying polyketide biosynthesis, particularly in cases involving proton shuttling mediated by water molecules.[4] Vibrational frequency calculations at the same level of theory with optimization were performed to verify that each local minimum had no imaginary frequencies and that each TS had only one imaginary frequency. Intrinsic reaction coordinate (IRC) calculations[48–51] were performed for all TSs using GRRM17[52] based on Gaussian 16. Relative Gibbs free energies (ΔG_{rel}) are given for all discussions. To reduce computation cost, we truncated the alkyl side chains. Such model simplification has been performed in the previous literature.[53] After the formation of **IM4**, we performed

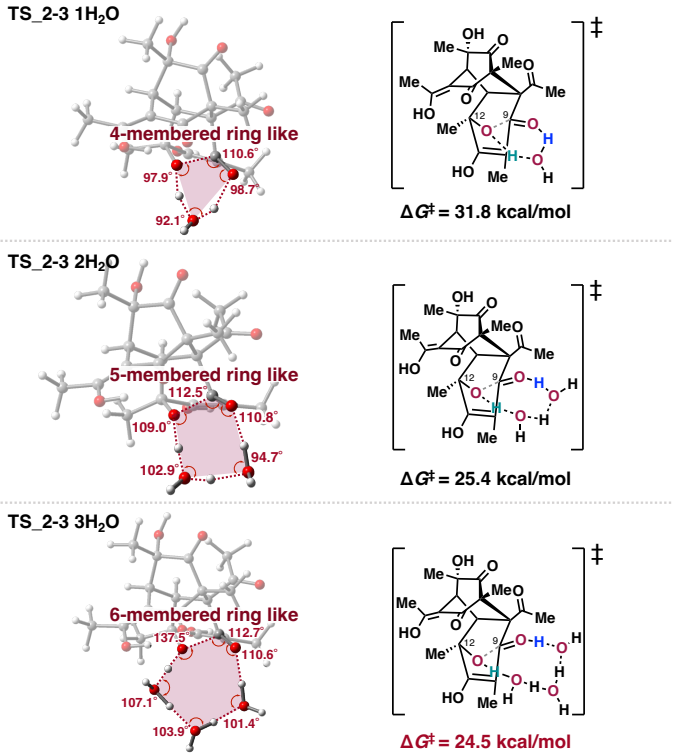

Fig. 3 Three dimensional structures of TS_2-3 obtained from the DFT calculations.

calculations for its anionic form, assuming that keto-enol tautomerism, especially at a position between two carbonyl groups, could easily occur in water. The conformational search and water sampling was conducted using Gromacs.[54] We selected those conformations that smoothly facilitate proton shuttling.

Results and Discussion

Figure 2 shows the results of our calculations. All activation energies were lower than 25 kcal mol^{-1}, suggesting that these reactions can occur under biomimetic conditions. First, Diels-Alder-type dimerization occurs between two sorbicillinol molecules(**IM1**), yielding a large stabilization energy of c.a. 14 kcal mol^{-1}. The corresponding activation energy of only 18.1 kcal mol^{-1} suggests that this is a spontaneous reaction, which is consistent with previous experimental results.[17–20] Subsequent keto-enol tautomerization of the C13 carbonyl group forms the intermediate **IM2**. Then, we introduced three water molecules into the system to facilitate the intramolecular Aldol reaction of **IM2** to **IM3** by enabling the attack of the C12 hydroxy group to the C9 carbonyl group. A series of proton transfers involving coordinated water molecules occurs during this step. The keto-enol tautomerization of the C10-C11 bond in **IM3**, with C8-C9 bond cleavage and formation of the C11 carbonyl group, leads to the formation of **IM4**. This step also involves protonation and deprotonation events mediated by water molecules. A complex ring rearrangement reaction then transforms **IM4** into **IM6** (*vide infra*). A final intramolecular 1,4-addition yields **IM7**, which is deprotonated to form bisorbicillinolide. The following discussion provides an in-depth analysis of the key steps in the bisorbicillinoid biosynthetic pathway.

The formation of bisorbicillinolide (**IM7**) involves a series of bond formations and cleavages, with the nucleophilic attack of anionic species generated during the process of carbonyl carbons. We examined the influence of the number of coordinated water molecules on the activation energy for each reaction step (Figure 3). The activation energy of the overall reaction with one water molecule was 31.8 kcal mol^{-1}, which was too high for the reaction to proceed under ambient conditions. The introduction of two and three water molecules lowered the activation energy to 25.4 and 24.5 kcal mol^{-1}, respectively, indicating a maximum stabilization of approximately 7.3 kcal mol^{-1}. This substantial difference in activation energy is attributed to the reduced distortion of the hydrogen bonding system, which includes the oxygen atoms connected to C12 and C9, with an increasing number of coordinated water molecules. An analysis of the transition state structures (TS) revealed that ring distortion is minimized when using three coordinated water molecules. Note that the addition of four water molecules led to a hydrogen-bonding network similar to that encountered with three water molecules, as the fourth water molecule did not participate in the hydrogen bonding network and bonded to other parts of the structure. However, we observed that the activation energy increased with the three water molecules in the conversion of **IM3** to **IM4**; the optimal number of water molecules for this step was two. The ring size and the angle between the carbonyl oxygen and the water molecules to be protonated play a crucial role in determining the activation energy of the reaction. Our results indicate that a smaller ring distortion favors the reaction, resulting in lower activation energy.

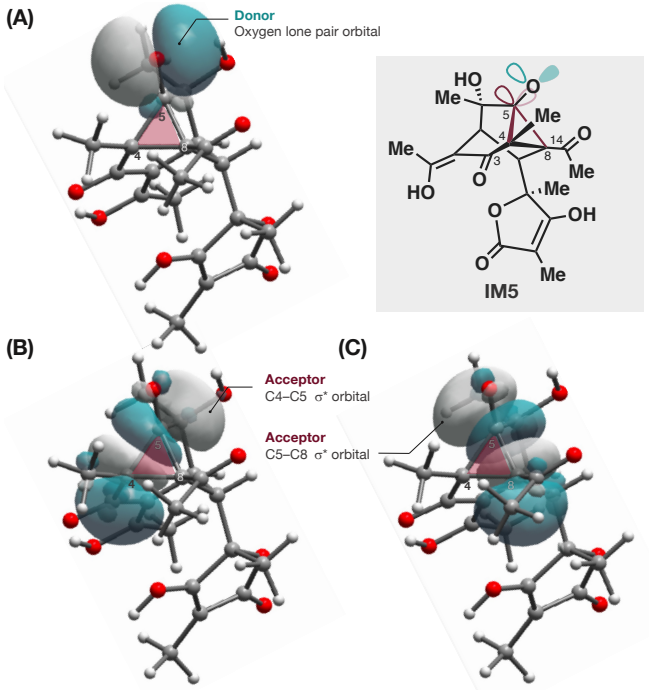

Fig. 4 Key representative orbitals computed by DFT calculations. (A) O5 oxygen lone pair orbital. (B) C4–C5 σ^* orbital. (C) C5–C8σ^* orbital.

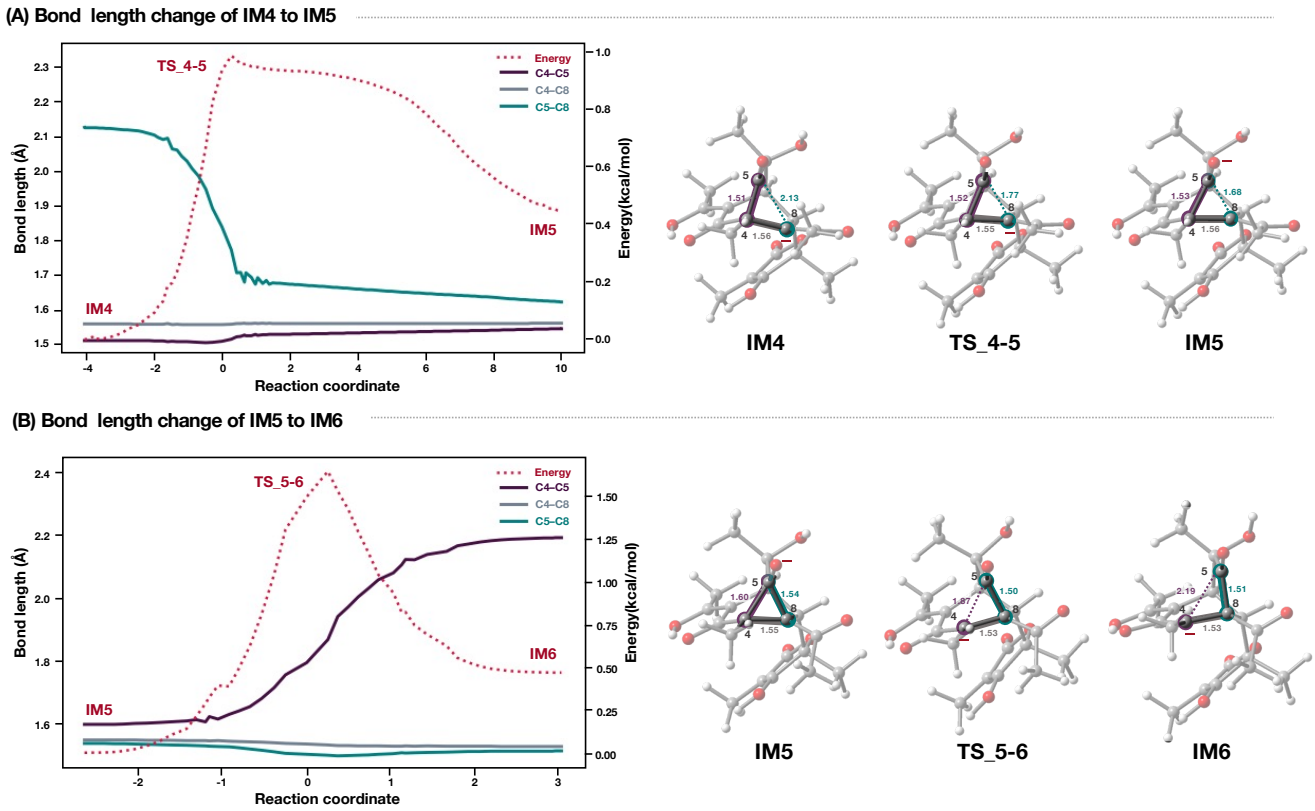

Fig. 5 Representative example of the evolution of key bond lengths in the conversion of (A) IM4 to IM5 and (B) IM5 to IM6. The three dimensional structures of IM4, TS_4-5, IM5, TS_5-6, and IM6 are also presented.

Next, we analyzed the rearrangement of **IM4** to **IM6**. Figure 5 shows the relevant change in bond distances (such as C4-C5, C4-C8, and C5-C8) and energy within this reaction step. Contrary to our expectations, the tetrahedral intermediate structure **IM5** was thermodynamically more stable than **IM4** or **IM6**. Furthermore, C4-C8 maintained a single-bond bond length throughout the reaction, without a significant double-bond character. The main factors that influenced the change in energy observed in this rearrangement reaction were the changes in the bond distances of C4-C5 and C5-C8. In our previous studies on terpene-forming reactions, non-classical cation species called cyclopropylcarbinyl cations [28,53,55,56] often appear via the formation of three-membered ring structures. However, those three-membered rings had bond lengths of approximately 1.7 Å because of hyperconjugation. In this rearrangement reaction, the three-membered ring has relatively short bond lengths and assumes a standard three-membered ring configuration, likely because of the positional relationship between the anion formed on the adjacent oxygen and the cyclopropane. We visualized the atomic orbitals using NBO calculations (Figure 4), revealing that the oxygen anion overlapped with the C4-C5 σ^* orbital and the C5-C8 σ^* orbital. Therefore, the cyclopropane ring likely acted as an electron-withdrawing group.

Conclusions

In this study, we performed a detailed analysis of the bisorbicillinoid biosynthetic pathway using computational methods. We determined that the optimal number of coordinated water molecules varies according to the reaction step. For the Aldol reaction of **IM2** to **IM3**, the presence of three water molecules minimizes ring distortion and lowers the activation energy, driving the reaction under biomimetic conditions. In contrast, the conversion of **IM3** to **IM4** is favored by the use of two coordinated water molecule. In addition, we characterized the detailed mechanism of the rearrangement reaction. Although this reaction was thought to be concerted, an intermediate cyclopropane ring structure, **IM5**, was observed. The presence of this intermediate can be attributed to the electron-withdrawing nature of the cyclopropane ring. Our study provides insights into the bisorbicillinoid biosynthetic pathway, in particular detailing the stepwise nature of the **IM4** - **IM6** rearrangement. Nevertheless, these findings are one piece of the complicated puzzle that is this field of study. We are fully aware that further studies considering aspects such as enzyme interactions on bisorbicillinoid biosynthesis is essential to gain a full understanding. The use of advanced computational techniques in conjunction with experimental validation remains crucial in this regard. We hope that our study contributes to the broader scientific endeavor of elucidating the biosynthetic pathways of natural products. While we believe that our work stands

on its own merits, we recognize that it is only one step in the ongoing journey to improve human health through the development of novel therapeutics derived from natural products.

Author Contributions

Conceptualization, H.S.; methodology, H.S.; validation, H.S.; formal analysis, M.N.; investigation, M.N.; data curation, M.N.; writing—original draft preparation, M.N.; writing—review and editing, H.S.; visualization, M.N.; supervision, H.S.; project administration, H.S.; funding acquisition, H.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

Notes and references

1 L. Katz and R. H. Baltz, *J. Ind. Microbiol. Biotechnol.*, 2016, **43**, 155–176.
2 J. Liu, A. Liu and Y. Hu, *Nat. Prod. Rep.*, 2021, **38**, 1469–1505.
3 J. Sun, H. Yang and W. Tang, *Chem. Soc. Rev.*, 2021, **50**, 2320–2336.
4 M. Nakajima, Y. Adachi and T. Nemoto, *Nat. Commun.*, 2022, **13**, 152.
5 M. Nakajima, T. Yamauchi, Y. Adachi and T. Nemoto, *Chem. Pharm. Bull.*, 2022, **70**, 735–739.
6 N. Abe, T. Murata and A. Hirota, *Biosci. Biotechnol. Biochem.*, 1998, **62**, 661–666.
7 N. Abe, T. Murata and A. Hirota, *Biosci. Biotechnol. Biochem.*, 1998, **62**, 2120–2126.
8 C. Derntl, F. Guzmán-Chávez, T. M. Mello-de Sousa, H.-J. Busse, A. J. M. Driessen, R. L. Mach and A. R. Mach-Aigner, *Front. Microbiol.*, 2017, **8**, 2037.
9 N. Abe and A. Hirota, *Chem. Commun.*, 2002, 662–663.
10 K. Sugaya, T. Terajima, A. Takahashi, J.-I. Onose and N. Abe, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 832–835.
11 L. Kahlert, E. F. Bassiony, R. J. Cox and E. J. Skellam, *Angew. Chem. Int. Ed Engl.*, 2020, **59**, 5816–5822.
12 N. Abe, K. Yamamoto, T. Arakawa and A. Hirota, *Chem. Commun.*, 2001, **23**, 2423.
13 N. Abe, O. Sugimoto, T. Arakawa, K. Tanji and A. Hirota, *Biosci. Biotechnol. Biochem.*, 2001, **65**, 2271–2279.
14 K. Sugaya, H. Koshino, Y. Hongo, K. Yasunaga, J.-I. Onose, K. Yoshikawa and N. Abe, *Tetrahedron Lett.*, 2008, **49**, 654–657.
15 A. M. Harned and K. A. Volp, *Nat. Prod. Rep.*, 2011, **28**, 1790–1810.
16 J. Meng, X. Wang, D. Xu, X. Fu, X. Zhang, D. Lai, L. Zhou and G. Zhang, *Molecules*, 2016, **21**, 715.
17 A. Sib and T. A. M. Gulder, *Angew. Chem. Int. Ed Engl.*, 2017, **56**, 12888–12891.
18 N. Abe, O. Sugimoto, K.-I. Tanji and A. Hirota, *J. Am. Chem. Soc.*, 2000, **122**, 12606–12607.
19 E. Gravel and E. Poupon, *European J. Org. Chem.*, 2008, **2008**, 27–42.
20 D. Barnes-Seeman and E. J. Corey, *Org. Lett.*, 1999, **1**, 1503–1504.
21 H. Sato, K. Saito and M. Yamazaki, *Front. Plant Sci.*, 2019, **10**, 802.
22 D. J. Tantillo, *Nat. Prod. Rep.*, 2011, **28**, 1035–1053.
23 D. J. Tantillo, *Nat. Prod. Rep.*, 2013, **30**, 1079–1086.
24 D. J. Tantillo, *Chem. Soc. Rev.*, 2010, **39**, 2847–2854.
25 D. J. Tantillo, *Angew. Chem. Int. Ed Engl.*, 2017, **56**, 10040–10045.
26 Y. J. Hong and D. J. Tantillo, *Chem. Soc. Rev.*, 2014, **43**, 5042–5050.
27 H. Sato, T. Hashishin, J. Kanazawa, K. Miyamoto and M. Uchiyama, *J. Am. Chem. Soc.*, 2020, **142**, 19830–19834.
28 H. Sato and M. Nakano, *Chem. Eur. J.*, 2022, **29**, e202203076.
29 S. R. Hare and D. J. Tantillo, *Beilstein J. Org. Chem.*, 2016, **12**, 377–390.
30 H. Sato, K. Narita, A. Minami, M. Yamazaki, C. Wang, H. Suemune, S. Nagano, T. Tomita, H. Oikawa and M. Uchiyama, *Sci. Rep.*, 2018, **8**, 2473.
31 M. Weitman and D. T. Major, *J. Am. Chem. Soc.*, 2010, **132**, 6349–6360.
32 D. T. Major and M. Weitman, *J. Am. Chem. Soc.*, 2012, **134**, 19454–19462.
33 K. Raz, S. Levi, P. K. Gupta and D. T. Major, *Curr. Opin. Biotech.*, 2020, **65**, 248–258.
34 S. Das, M. Shimshi, K. Raz, N. N. Eliaz, A. R. Mhashal, T. Ansbacher and D. T. Major, *J. Chem. Theory Comput.*, 2019, **15**, 5116–5134.
35 T. Ansbacher, Y. Freud and D. T. Major, *Biochemistry*, 2018, **57**, 3773–3779.
36 M. Dixit, M. Weitman, J. Gao and D. T. Major, *ACS Catal.*, 2018, **8**, 1371–1375.
37 M. Dixit, M. Weitman, J. Gao and D. T. Major, *ACS Catal.*, 2017, **7**, 812–818.
38 T. Lou, A. Li, H. Xu, J. Pan, B. Xing, R. Wu, J. S. Dickschat, D. Yang and M. Ma, *J. Am. Chem. Soc.*, 2023, **145**, 8474–8485.
39 F. Cen-Pacheco, A. J. Santiago-Benítez, K. Y. Tsui, D. J. Tantillo, J. J. Fernández and A. H. Daranas, *J. Org. Chem.*, 2021, **86**, 2437–2446.
40 M. W. Lodewyk and D. J. Tantillo, *Chirality*, 2020, **32**, 484–488.
41 H. Sato, M. Uchiyama, K. Saito and M. Yamazaki, *Metabolites*, 2018, **8**, 48–58.
42 S. Yamamoto, T. Matsuyama, T. Ozaki, J. Takino, H. Sato, M. Uchiyama, A. Minami and H. Oikawa, *J. Am. Chem. Soc.*, 2022, **144**, 20998–21004.
43 H. Zhang, A. J. E. Novak, C. S. Jamieson, X.-S. Xue, S. Chen, D. Trauner and K. N. Houk, *J. Am. Chem. Soc.*, 2021, **143**, 6601–6608.
44 D. J. Tantillo, *Org. Lett.*, 2016, **18**, 4482–4484.
45 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, *Gaussian 16 Revision C.01*, 2016, Gaussian Inc. Wallingford CT.
46 J.-D. Chai and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615–6620.
47 A. V. Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378–6396.
48 K. Fukui, *Acc. Chem. Res.*, 1981, **14**, 363–368.
49 M. Page, C. Doubleday and J. W. M. Jr., *J. Chem. Phys.*, 1990, **93**, 5634–5642.
50 K. Ishida, K. Morokuma and A. Komornicki, *J. Chem. Phys.*, 1977, **66**, 2153–2156.
51 C. Gonzalez and H. B. Schlegel, *J. Phys. Chem.*, 1990, **94**, 5523–5527.
52 S. Maeda, K. Ohno and K. Morokuma, *Phys. Chem. Chem. Phys.*, 2013, **15**, 3683–3701.
53 H. Sato, B. X. Li, T. Takagi, C. Wang, K. Miyamoto and others, *JACS Au*, 2021, **1**, 1231–1239.
54 M. Abraham, T. Murtola, R. Schulz, S. Páll, J. Smith, B. Hess and E. Lindahl, *SoftwareX*, 2015, **1-2**, 19–25.
55 H. Sato, K. Teramoto, Y. Masumoto, N. Tezuka, K. Sakai and others, *Sci. Rep.*, 2016, **5**, 18471.
56 Y. J. Hong and D. J. Tantillo, *Org. Biomol. Chem.*, 2015, **13**, 11140.