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Biogenesis-Guided Synthesis and Structural Revision of Sarocladione Enabled by Ruthenium-Catalyzed Endoperoxide Fragmentation

*Yuhan Ning, Hailong Tian, and Jinghan Gui**

Abstract: *Sarocladione is the first 5,10:8,9-diseco-steroid with a 14-membered macrocyclic diketone framework to have been isolated from a natural source. Herein we report a biomimetic synthesis of sarocladione in only two or seven steps from inexpensive, commercially available ergosterol. The key feature of this synthesis was a novel ruthenium-catalyzed endoperoxide fragmentation, which transformed various saturated endoperoxides into olefinic diketones by cleavage of two C–C bonds. This synthesis allowed us to unambiguously determine the structure of sarocladione and provided experimental support for its revised biosynthetic origin. This work also vividly demonstrates that consideration of the biogenesis is a powerful tool for elucidating the structures of natural products.*

Chemical synthesis is a critical tool for determining the structures of natural products and revising incorrectly assigned structures.[1] Furthermore, detailed analysis of natural product biosynthesis has proved to be a highly rewarding tactic in that such analysis not only can inspire the design of concise laboratory syntheses[2] but also can provide valuable hints of potential structural misassignments prior to chemical synthesis,[3] thereby avoiding unnecessary, tedious syntheses of incorrectly assigned structures.[4]

In this study, we targeted sarocladione (**1**, Figure 1 A), which is the first 5,10:8,9-diseco-steroid to have been isolated from a natural source. In 2012, Gao and co-workers reported the isolation of this unique compound from the endolichenic fungal stain *Sporormiella irregularis*.[5] They assigned the absolute stereochemical configurations for 4 of the 7 stereogenic centers in **1**, although the relative or absolute configurations of the remaining stereocenters (all in the macrocyclic ring) remain undetermined. More recently, it was isolated by Yang and co-workers from the deep-sea-derived fungus *Sarocladium kiliense* and named sarocladione.[6] These investigators provided a tentative assignment of the relative and absolute stereochemical configuration of **1** based on 2D NMR data and computational predictions, in further view of biosynthetic considerations. Although other compounds isolated from *S. kiliense* are known to exhibit significant cytotoxicity against HeLa-S3 cancer cells, the biological activity of **1** has not been elucidated, possibly because of the limited amounts of the compound that are available from the natural sources. Therefore, an efficient and practical synthetic route to sarocladione and its analogues would be highly desirable.

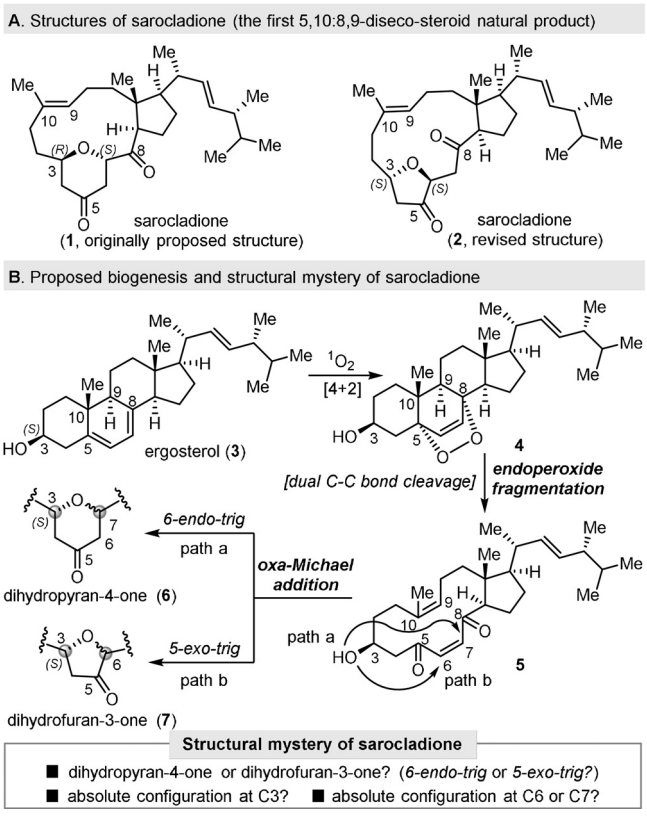

Figure 1. A) Sarocladione structures and B) proposed biosynthetic pathway and structural ambiguities.

Gao and co-workers proposed that sarocladione is biosynthesized from ergosterol (**3**) as follows (Figure 1 B).[5] First, a [4+2] cycloaddition reaction between **3** and singlet oxygen generates ergosterol peroxide **4** (also a natural product),[7] which undergoes an endoperoxide fragmentation reaction that cleaves the C5-C10 and C8-C9 bonds, generating macrocyclic diketone **5**. A subsequent intramolecular oxa-Michael addition reaction of **5** affords sarocladione (**1**). Upon close inspection of this proposed pathway, three facts attracted our attention because they suggested some ambiguities about the structure assigned to sarocladione: (1) the absolute configuration at C3 of **3** is opposite to that of **1**, which suggests either that the configuration at C3 of **1** was misassigned or that the biosynthetic pathway involves a ster-

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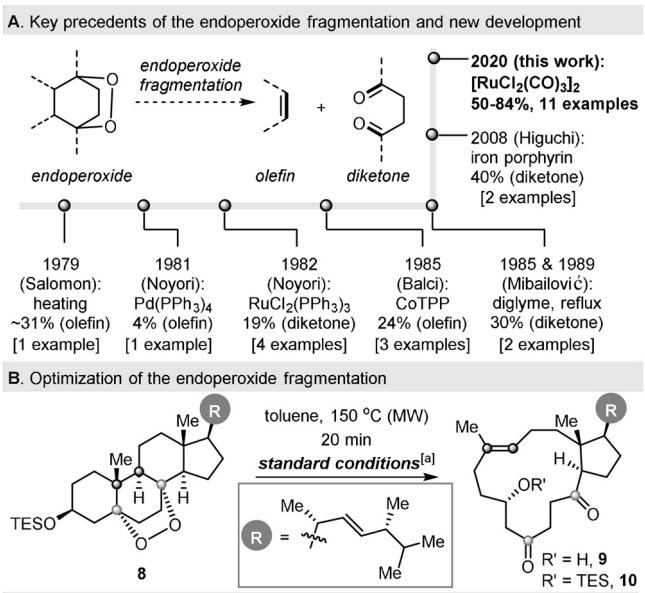
eoinversion at C3; (2) mechanistically, the oxa-Michael addition reaction of **5** could proceed not only in a 6-*endo*-trig manner (via attack at C7) to give dihydropyran-4-one **6** but also in a 5-*exo*-trig manner (via attack at C6) to give dihydrofuran-3-one **7**; and (3) in either case, the absolute configuration at C7 of **6** or at C6 of **7** remains to be determined. Intrigued by these ambiguities, we set about to synthesize this unique natural product with the goal of unveiling its true structure and facilitating a comprehensive investigation of its biological activity.

Herein, we report that we have achieved a biomimetic synthesis of sarocladione in only two or seven steps from ergosterol, an inexpensive, commercially available sterol. The synthesis was enabled by the development of a novel ruthenium-catalyzed endoperoxide fragmentation reaction,[8] which transformed the A/B/C tricyclic ring system of the classical steroids into 14-membered macrocyclic diketones by cleavage of two C–C bonds. Moreover, the synthesis allowed us to unambiguously determine that the structure of sarocladione is in fact not **1** but **2** (Figure 1 A), as indicated by X-ray crystallographic analysis.

The key reaction in the biosynthesis of sarocladione is the endoperoxide fragmentation, which is not without precedent in synthetic chemistry. However, most of the reported methods suffer from a narrow substrate scope and low yields and are therefore of limited utility (Figure 2 A). In 1979, Coughlin and Salomon reported the thermal decomposition of 2,3-dioxabicyclo[2.2.2]octane to give succinaldehyde and ethylene.[9] Shortly thereafter, the groups of Noyori and Balci reported catalytic decomposition reactions of saturated bicyclic endoperoxides with catalysis by Pd-$(PPh_3)_4$,[10] $RuCl_2(PPh_3)_3$,[11] or CoTPP (tetraphenylporphyrine).[12] In the 1980s, Mihailović reported the thermal fragmentation of steroidal 5α,8α-peroxides in refluxing diglyme to afford diketone products in low yields.[13] More recently, Higuchi and colleagues studied fragmentation of 2,3-dioxabicyclo[2.2.1]heptane with catalysis by iron porphyrin to generate malondialdehyde and ethylene as products.[14] Despite this progress, a general method for endoperoxide fragmentation remains elusive, and we decided to explore this transformation in the context of the synthesis of sarocladione.

Inspired by the work of Noyori and Balci, we planned to search for a suitable catalyst for this fragmentation. After extensive experimentation, we found that heating a solution of endoperoxide **8** and 5 mol% $[RuCl_2(CO)_3)]_2$ in toluene produced desired macrocyclic diketone **9** in 71% yield (Figure 2 B, entry 1), after in situ removal of the triethylsilyl (TES) ether protecting group for ease of purification. Notably, subjecting the C3-OH derivative of **8** to the standard conditions furnished **9** directly, albeit in a lower yield (40%). Reaction of **8** at 120 °C in the microwave also gave **9**, albeit in a slightly lower yield over a longer time (4 h, entry 2). When conventional heating was used instead of microwave heating, the yield dropped to 61% (entries 3 and 4). Control experiments showed that most of the starting material was recovered in the absence of $[RuCl_2(CO)_3)]_2$ (entry 5) and that adding 1.0 equiv of TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxyl) to the reaction mixture dramatically reduced the conversion (entry 6), indicating that the reaction probably



entry	variations from the "standard conditions"	catalyst (5 mol%)	**8** (%)[b]	**10** (%)[b]
1	none	$[RuCl_2(CO)_3]_2$	0	71[c]
2	120 °C (MW), 4 h	$[RuCl_2(CO)_3]_2$	0	66[c]
3	150 °C (sealed tube)[d]	$[RuCl_2(CO)_3]_2$	0	61[c]
4	120 °C (reflux)[d], 9 h	$[RuCl_2(CO)_3]_2$	0	61[c]
5	4 h	-	92	4
6	TEMPO (1.0 equiv)	$[RuCl_2(CO)_3]_2$	76	14
7	-	*meso*-CoTPP	no reaction	
8	-	$RuCl_2(PPh_3)_3$	86	4
9	4 h	$RuCl_2(PPh_3)_3$	32	44
10	4 h	$RuCl_2(PPh_3)_4$	57	22
11	4 h	$RuCl_2(CO)_2(PPh_3)_2$	84	16
12	4 h	$[RuCl_2(mesitylene)]_2$	72	22
13	4 h	$[RuCl_2(p\text{-cymene})]_2$	68	24
14	4 h	$[RuCl_2(C_6H_6)]_2$	82	16
15	4 h	$[RuCl_2(norbornadiene)]_n$	88	10
16[e]	diglyme, reflux, 9 h	-	56	28
17[f]	DCM, 50 °C, 2 h	$RuCl_2(PPh_3)_3$	no reaction	

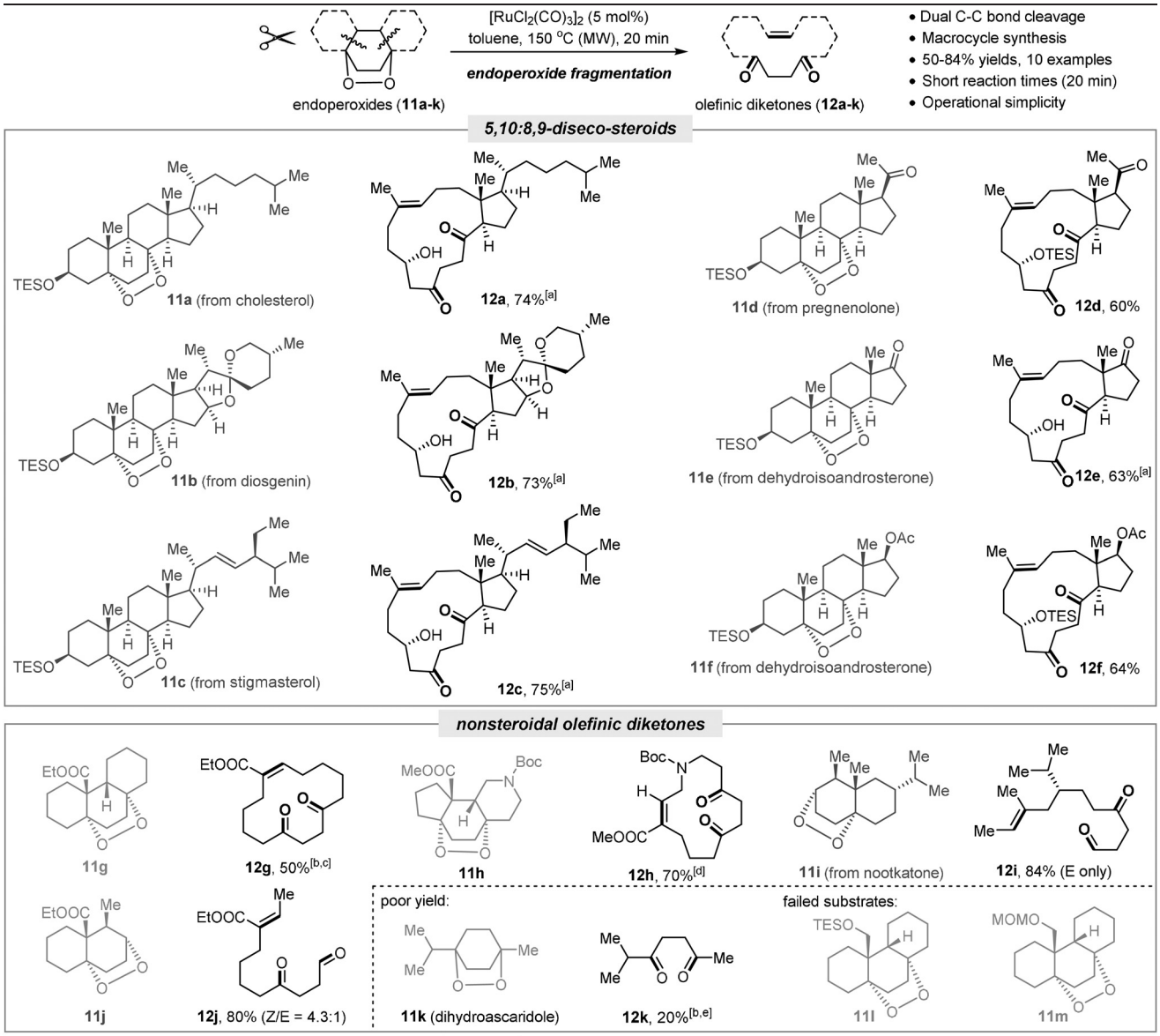
[a] Standard conditions: **8** (50 mg), $[RuCl_2(CO)_3]_2$ (5 mol%), toluene (0.025 M), 150 °C (MW), 20 min. [b] Isolated yields. [c] Isolated yields of **9** after treatment of the reaction mixture with HF·Py. [d] Conventional heating instead of microwave heating. [e] Mibailović's reported conditions (ref. 13a). [f] Noyori's reported conditions (ref. 11).

Figure 2. A) Precedents for endoperoxide fragmentation and new developments and B) optimization of reaction conditions.

proceeded via a radical mechanism (see Supporting Information for a detailed discussion of the plausible mechanism).[15] Furthermore, no reaction occurred when the catalyst was changed to CoTPP (entry 7), and the use of various other ruthenium catalysts also resulted in little conversion (entries 8–15). Lastly, we compared the efficiency of our optimal conditions with previously reported conditions for this fragmentation. Specifically, we found that refluxing **8** in diglyme for 9 h (Mihailović's conditions)[13a] afforded **10** in only 28% yield (entry 16); and no reaction occurred when **8** was treated with $RuCl_2(PPh_3)_3$ in DCM at 50 °C (Noyori's conditions, entry 17).[11]

With the standard conditions in hand, we briefly explored the substrate scope of the endoperoxide fragmentation (Table 1). Steroidal endoperoxides **11a–f** derived from cho-

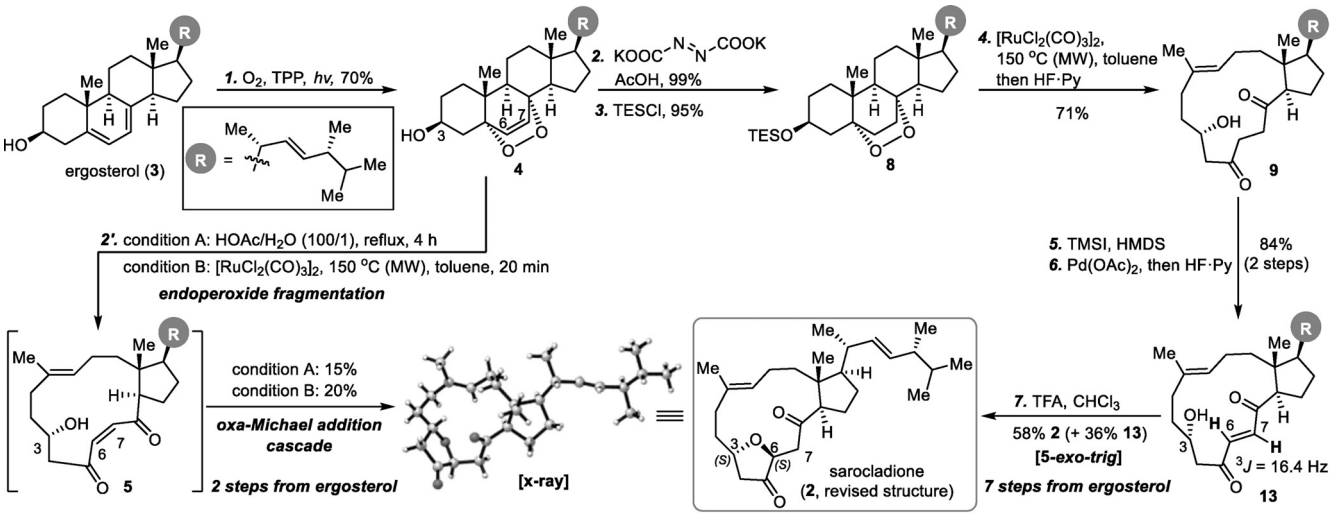
Table 1: Substrate scope of ruthenium-catalyzed endoperoxide fragmentation.



[a] Isolated yield of **12** after treatment of the reaction mixture with HF·Py. [b] ^{1}H NMR yield using 1,3,5-trimethoxybenzene as the internal standard. [c] 1,2-dichlorobenzene, 1 h. [d] 1 h. [e] C_6D_6, 120 °C (MW), 4 h.

lesterol, diosgenin, stigmasterol, pregnenolone, and dehydroisoandrosterone were amenable to the reaction, affording corresponding products **12a–f** in 60–75% yields. These results indicate that this reaction provides a convenient, general synthetic strategy for preparing 5,10:8,9-diseco-steroids from classical [6-6-6-5] steroids via cleavage of the C5-C10 and C8-C9 bonds. Furthermore, we tested the reaction on some nonsteroidal substrates. Pleasingly, tricyclic endoperoxides **11g** and **11h** smoothly gave corresponding 14- and 13-membered-ring macrocycles **12g** and **12h**, respectively, indicating the potential utility of this fragmentation for the synthesis of challenging macrocycles from easily accessible fused ring systems. Moreover, bicyclic endoperoxides **11i** and **11j** also proved to be viable substrates, furnishing long-chain olefinic diketones **12i** and **12j** in 84% and 80% yields, respectively. Notably, although a single stereoisomer of **12i** was formed, **12j** was produced as a 4.3:1 mixture of *Z* and *E* isomers. As a current limitation of the endoperoxide fragmentation, dihydroascaridole **11k** afforded the desired diketone **12k** in only 20% yield, and endoperoxides **11l** and **11m** with an ether substitute adjacent to the putative carbon radical (intermediate **S3**, Scheme S2, Supporting Information) did not give any desired products.

Having established a reliable method for accessing 5,10:8,9-diseco-steroids, we started the synthesis of saroladione with the preparation of known endoperoxide **4** (Scheme 1).[7] Selective diimide reduction[16] of the C6-C7 olefin of **4** followed by TES protection of the C3 hydroxyl group afforded saturated endoperoxide **8**. Subjection of **8** to the standard fragmentation conditions and subsequent in situ



Scheme 1. Biomimetic synthesis and structural revision of sarocladione.

removal of the TES group with HF-pyridine gave **9** in 71% yield. Saegusa oxidation[17] of **9** generated C6-C7 olefin **13** exclusively in the *E* configuration. To complete the synthesis, we attempted an intramolecular oxa-Michael reaction of **13**. Upon exploring various acidic and basic conditions (see Supporting Information for details), we found that stirring a solution of **13** and 1.0 equiv of trifluoroacetic acid in $CHCl_3$ afforded sarocladione in 58% yield, along with a 36% yield of recovered **13**. The spectroscopic data for our synthetic sarocladione were in good agreement with those reported by Gao[5] and Yang,[6] suggesting that our synthetic sample was identical to the natural product. Luckily, we were able to obtain an X-ray structure of the synthetic compound,[18] which clearly confirmed its structure to be **2**, which has an embedded dihydrofuran-3-one moiety rather than a dihydropyran-4-one moiety. Furthermore, the stereocenters at C3 and C6 of **2** were unambiguously established to be in the *S* configuration. Therefore, our synthesis provides strong experimental evidence that the oxa-Michael addition reaction in the sarocladione biosynthetic pathway proceeds in a 5-*exo*-trig manner rather than a 6-*endo*-trig manner as originally proposed.

Although we accomplished the synthesis of sarocladione in seven steps from ergosterol, this first-generation synthesis was far from ideal because four of the seven steps were concession steps:[19] reduction of the C6-C7 olefin, protection of the C3 hydroxyl group, and the two-step Saegusa oxidation to restore the C6-C7 olefin. Aiming for an ideal synthesis,[19] we then attempted to imitate the biosynthetic pathway depicted in Figure 1B by performing the key endoperoxide fragmentation in the presence of the C6-C7 olefin. Previous work by the groups of Noyori and Foote demonstrated that fragmentation of 2,3-unsaturated 1,4-endoperoxides with catalysis by $RuCl_2(PPh_3)_3$[11] or CoTPP[20] generally affords 1,2,3,4-diepoxides in high yields. Despite this documented undesired reactivity, we were able to identify two separate sets of reaction conditions for accessing sarocladione (**2**) directly from unsaturated endoperoxide **4** (Scheme 1). First, heating a solution of **4** in $HOAc/H_2O$ under reflux for 4 h gave **2** in 15% yield; and second, subjecting **4** to the standard fragmentation conditions produced **2** in 20% yield. On the basis of our first-generation synthesis, we surmised that the direct formation of **2** from **4** occurred via a cascade sequence involving endoperoxide fragmentation and oxa-Michael addition. Notably, this two-step, second-generation chemical synthesis successfully mimics the sarocladione biosynthesis pathway in the laboratory.

In conclusion, we have achieved the first biomimetic synthesis of the 5,10:8,9-diseco-steroid sarocladione, in either two or seven steps from ergosterol. Inspiration from consideration of the biogenesis of this natural product enabled us to develop a concise strategy for its chemical synthesis, which in turn allowed us to revise the previous, incorrectly assigned structure. Our work also revealed $[RuCl_2(CO)_3]_2$ to be an efficient catalyst for endoperoxide fragmentation, which provides easy access to 5,10:8,9-diseco-steroids and other olefinic diketones via cleavage of two C–C bonds.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biomimetic synthesis · endoperoxide fragmentation · natural products · ruthenium · structural revision

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