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Design and Synthesis of Cyclopropane Congeners of Resolvin E2, an Endogenous Proresolving Lipid Mediator, as Its Stable Equivalents

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Supporting Information

ABSTRACT: Lipid chemical mediator resolvins with highly potent anti-inflammatory activity can be leads to develop novel anti-inflammatory drugs; however, they are unstable in oxygen due to their characteristic polyunsaturated structures. To solve the problem, CP-RvE2 has been designed and synthesized in which the *cis*-olefin of RvE2 was replaced with a cyclopropane. CP-RvE2s were much more stable than RvE2 against autoxidation and equipotent or more potent than RvE2. CP-RvE2s were successfully identified as stable equivalents of RvE2.

Resolvins (Rvs), metabolites of typical ω-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), are anti-inflammatory and pro-resolving, i.e., inflammation-resolving, lipid mediators (Figure 1).[1] The anti-inflammatory effects of Rvs are even more potent

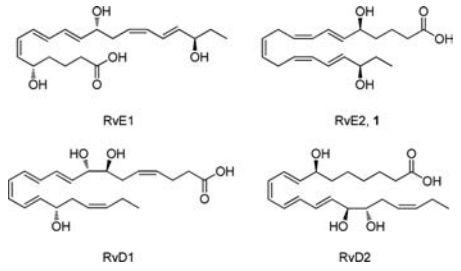

Figure 1. Structures of representative resolvins.

than clinically effective steroidal and nonsteroidal anti-inflammatory drugs and, therefore, are expected to be a prototype for the development of a new class of anti-inflammatory drugs.[2] A drawback of Rvs as a drug prototype, however, is that they are unstable to oxygen (autoxidation) due to their characteristic polyunsaturated structures. Thus, the development of stable equivalents of Rvs that can be used not only as drug leads but also as biological tools for investigating the physiological functions of Rvs and related signaling pathways is eagerly required. Here, we present the rational design, synthesis, and anti-inflammatory effects of cyclopropane analogues of resolvin E2 (RvE2) (**1**) α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**) as stable equivalents of RvE2.

Intensive studies of the mechanism of nonenzymatic autoxidation of poly-unsaturated fatty acids and lipid mediators clarified that the oxidative instability is often due to their 1,4-diene (skipped diene) structures.[3] As shown in Figure 2a, the central bisallylic position in the 1,4-diene structure is remarkably

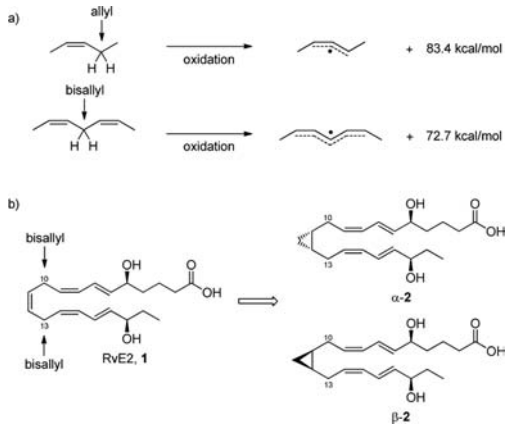

Figure 2. (a) Calculated energies of the radical at the allylic and bisallylic positions.[4] (b) Design of α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**) lacking the bisallylic structures as stable equivalents of RvE2.

more readily oxidized than the allylic position via radical hydrogen abstraction.[4] Based on these findings, we focused on the structure of RvE2, in which the two bisallylic C10 and C13 positions might be easily oxidized. Thus, we designed 11,12-methano analogues α-**2** and β-**2**, in which the C11−C12 *cis*-olefin of RvE2 was replaced with an α- or β-*cis*-cyclopropane (Figure 2b). Cyclopropane, the minimal saturated cyclic alkane, is an efficient bioisostere of olefin due to both its steric feature bearing olefin-like eclipsed adjacent substituents and its sp^2-like electronic feature of the ring-orbital.[5] Therefore, we expected that α-**2** and β-**2** lacking the unstable bisallylic structures could sterically and stereoelectronically mimic RvE2 as stable equivalents with the same biological activity as RvE2.

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We computationally investigated the susceptibility of RvE2 and the designed cyclopropane analogues to oxidation (Figure 3). The reactions of compounds **A** and **B**, simplified models of

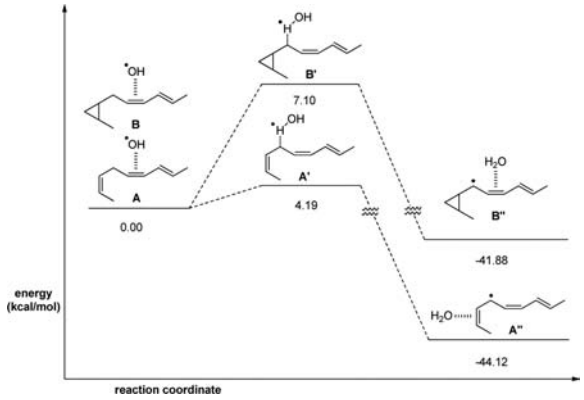
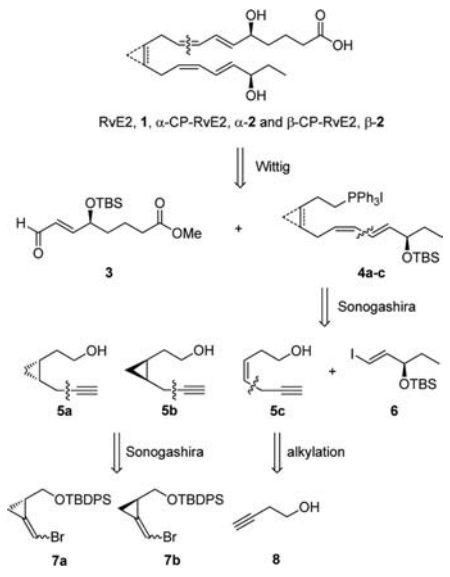


Figure 3. Energetics for the H abstractions of model compounds A and B calculated by UBHandHLYP/6-31+G(d,p). Energies were corrected by zero-point energy.

RvE2 and CP-RvE2, respectively, with a hydroxy radical, were theoretically calculated according to Eriksson's previously reported procedure.[6] The calculations revealed that both the transition state **A′**, derived from the RvE2 model compound **A**, and thereafter the formed bisallylic radical intermediate **A″** are significantly more stable than the corresponding transition state **B′** and radical intermediate **B″** generated from the CP-RvE2 model **B**, respectively. These calculations suggested that the cyclopropane analogues *α*-**2** and *β*-**2** could be more stable than RvE2 against oxidation, as expected.

Although total synthesis of RvE2 has already been reported by some groups,[7] we required a new synthetic route to effectively provide the cyclopropane analogues *α*-**2** and *β*-**2** as well as endogenous RvE2. Our retrosynthetic analysis common for *α*-**2**, *β*-**2**, and RvE2 is outlined in Scheme 1. Our analysis revealed that the targets **1**, *α*-**2**, and *β*-**2** would be provided using the Wittig reaction between an enal **3**[8] and phosphonium salts **4a**−**c**, which

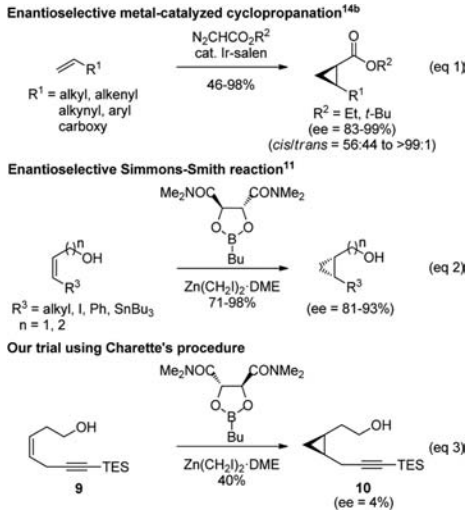
Scheme 1. Retrosynthetic Analysis of RvE2, 1 and the Cyclopropane Analogues *α*-2 and *β*-2



were likely to be obtained via Sonogashira coupling between alkynes **5a**−**c** and a vinyl iodide **6**.[9]

In this strategy, particularly to achieve the synthesis of *α*-**2** and *β*-**2**, preparation of the *cis*-substituted *α*- and *β*-cyclopropane units **5a** and **5b**, respectively, was the key issue because synthesis of chiral *cis*-cyclopropanes with the desired substituents is not readily achieved. Although enantio- and *cis*-selective synthesis of chiral 1,2-disubstituted cyclopropane has been studied extensively, only a few successful examples are known.[10−15] Katsuki reported a highly enantio- and *cis*-selective diazo cyclopropanation using an aryliridium−salen complex (Scheme 2,

Scheme 2. Katsuki's and Charette's Enantioselective Cyclopropanation and Our Trial



eq 1), although the substituents on the cyclopropane are rather limited[14] and preparation of the aryliridium−salen complexes might be difficult.[16] Charette reported an elegant enantioselective Simmons−Smith reaction with allyl or homoallyl alcohols as substrates providing the corresponding *cis*-cyclopropanes (Scheme 2, eq 2). This method is practically useful, and we successfully applied the reaction to prepare a series of proteasome inhibitors of chiral *cis*-cyclopropane structures.[17] Therefore, we investigated Charette asymmetric cyclopropanation to prepare the cyclopropane unit **5b** with homoallyl alcohol **9** as the substrate. This was unsuccessful, however, and the desired *cis*-cyclopropane **10** was not obtained in high enantiopurity (Scheme 2, eq 3).[18]

We recently developed an efficient synthetic procedure for chiral arylmethyl *cis*-cyclopropanes by Suzuki−Miyaura coupling of the chiral bromomethylenecyclopropane **7b**, followed by regio- and diastereoselective reduction of the olefin moiety (Scheme 3, eq 1).[19] Therefore, we expected that by applying the Sonogashira coupling reaction instead of the Suzuki−Miyaura coupling reaction we could obtain the desired cyclopropane units **5a** and **5b** from chiral bromomethylenecyclopropane **7a** and its enantiomer **7b**, respectively (Scheme 3, eq 2).

For the synthesis of *β*-CP-RvE2 (*β*-**2**), we examined Sonogashira coupling of bromomethylenecyclopropane **7b** (Scheme 4). When **7b** and TIPS-protected acetylene were treated with CuI, $PdCl_2(dppf)\cdot CH_2Cl_2$, and Et_3N in CH_3CN, the reaction effectively provided the corresponding coupling product in high yield. We found that the diastereoselective reduction of the olefin moiety of the product occurred with $Ni(OAc)_2\cdot 4H_2O$ to give the desired chiral *cis*-cyclopropane **11b** by preserving the

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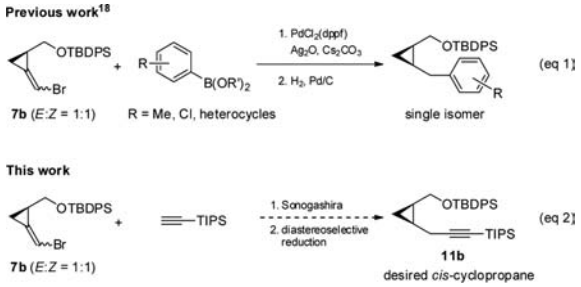
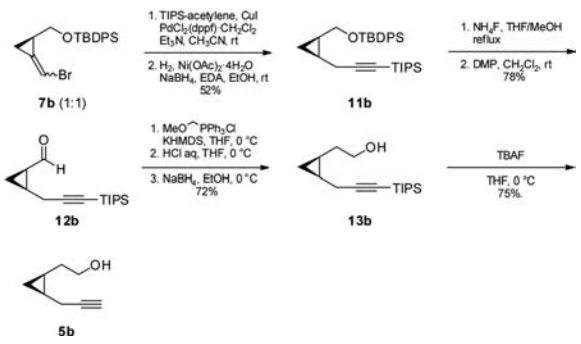
Scheme 3. Our Previous Work and This Work for the Synthesis of Chiral *Cis*-Substituted Cyclopropanes



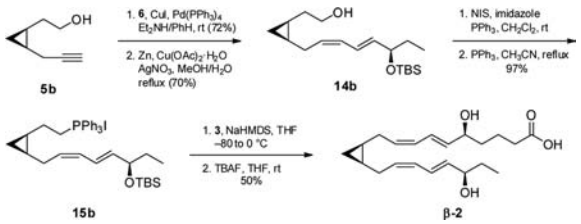
Scheme 4. Preparation of 5b



alkyne moiety as a single isomer.[20] After **11b** was converted into aldehyde **12b** via chemoselective desilylation and subsequent oxidation of the resulting primary alcohol, its Wittig reaction followed by acidic treatment gave the corresponding homologated aldehyde, whose reduction provided alcohol **13b**. Removal of the terminal silyl-protecting group of **13b** with TBAF afforded the desired β-cyclopropane unit **5b**. From the enantiomeric chiral bromomethylenecyclopropane **7a**, the corresponding α-cyclopropane unit **5a** was also prepared by the same procedure.

With *cis*-cyclopropane **5b** in hand, synthesis of β-**2** was investigated via couplings of **5b** with **6** and subsequently with **3** (Scheme 5). Thus, Sonogashira coupling of **5b** and vinyl iodide **6**,

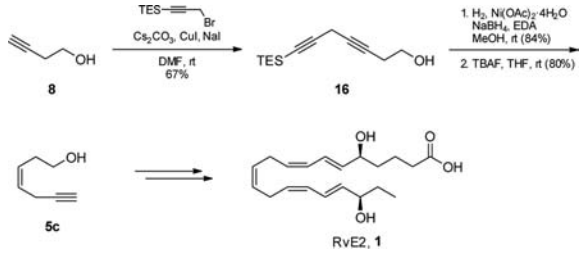
Scheme 5. Synthesis of β-CP-RvE2 (β-2)



followed by partial reduction of the alkyne moiety of the coupling product, gave diene **14b**.[21] After converting **14b** into the phosphonium salt **15b**, its Wittig reaction with enal **3** followed by treatment of the resulting coupling product with TBAF finally afforded β-**2**. Units **3**, **5a**, and **6** were assembled by the same procedure to give α-CP-RvE2 (α-**2**).

Similarly, RvE2 (**1**) was synthesized with the olefin unit **5c** (Scheme 6), and the spectral data were consistent with those of previous reports.[7a,b] Thus, we achieved the synthesis of α-CP-RvE2 (α-**2**), β-CP-RvE2 (β-**2**), and RvE2 (**1**) based on a common convergent synthetic strategy.

Scheme 6. Synthesis of RvE2 (1)



We evaluated the susceptibility of α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**) as well as synthetic RvE2 (**1**) to oxygen (Figure 4a).

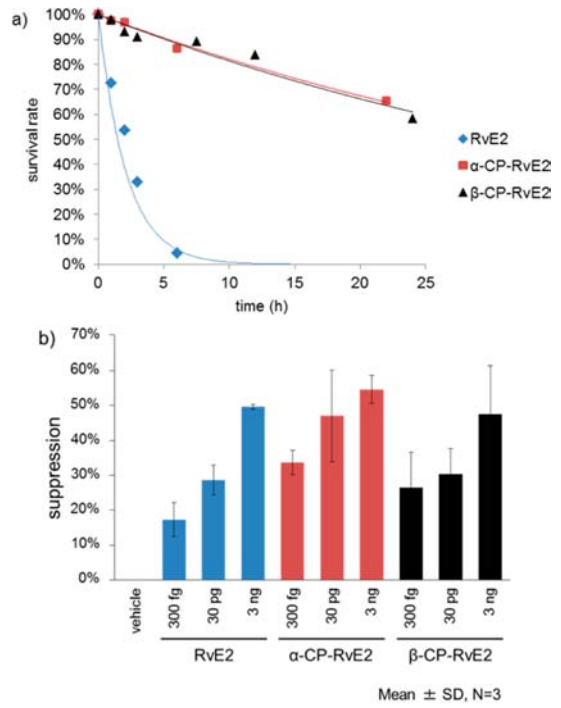


Figure 4. (a) Stability of RvE2 (**1**) α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**), against oxygen. (b) Anti-inflammatory activity of RvE2 (**1**) α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**).

The half-life of RvE2 in air atmosphere at room temperature was 1.5 h, while those of α-**2** and β-**2** were 35 h. These findings indicated that both α-**2** and β-**2** have greater stability than RvE2 against autoxidation, as expected.

Finally, we evaluated the anti-inflammatory activity of α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**) in comparison with that of RvE2 (**1**) using an in vivo mouse model of bacteria-induced peritonitis (Figure 4b).[22] Intraperitoneal injection of heat-killed *Propionibacterium acnes* (*P. acnes*), a Gram-positive bacterium, induced inflammation of the peritoneum, which resulted in a clear increase in the number of peritoneal exudate cells (PECs) after 24 h. RvE2 administered intraperitoneally at 12 h after the heat-killed *P. acnes* injection significantly reduced the number of PECs at 24 h. In this system, the anti-inflammatory activity was observed even at a low dose of 300 fg of RvE2 (~20% reduction in PEC number). The anti-inflammatory effect of RvE2 was dose-dependent, and β-**2** was almost equipotent to RvE2. To our delight, the anti-inflammatory effect of α-**2** was much higher than that of the proresolving lipid mediator RvE2.

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As described herein, we successfully identified α-CP-RvE2 (α-**2**) and β-CP-RvE2 (β-**2**) as stable equivalents of RvE2. To our knowledge, this is first report using a cyclopropane ring for bioisosteric stabilization of 1,4-diene- containing polyunsaturated fatty acids against autoxidation.

In conclusion, we designed α-CP-RvE2 (α-**2**) and β-CP-RvE2(β-**2**), which avoid the unstable bisallylic positions of RvE2, as stable equivalents of RvE2 on the basis of computational calculations. Efficient syntheses of α-**2** and β-**2** as well as RvE2 were achieved by adopting a convergent synthetic strategy in which the key cyclopropane units **5a** and **5b** were provided via Sonogashira coupling with bromomethylenecyclopropane **7a** and its enantiomer **7b**, inspired by our previous work on cyclopropane chemistry. The synthesized α-**2** and β-**2** were not only much more stable than RvE2 against autoxidation but also exhibited equal or much more anti-inflammatory activity than RvE2, resulting in the identification of α-**2** and β-**2** as stable equivalents of RvE2. This study presents a strategy using a cyclopropane for bioisosteric stabilization of 1,4-diene structures to overcome the instability of polyunsaturated compounds of pharmacological importance.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02612.

Detailed experimental procedures, calculation, oxidative stability, and biological assay (PDF)

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Notes

The authors declare no competing financial interest.

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