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Stereocontrolled Total Synthesis of the Potent Anti-inflammatory and Pro-resolving Lipid Mediator Resolvin D3 and its Aspirin-Triggered 17*R*-Epimer

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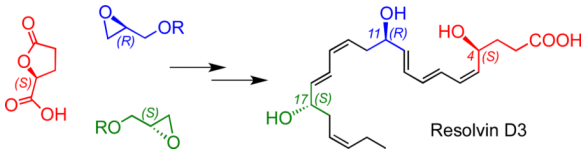
Abstract



The first total synthesis of stereochemically pure resolvin D3 and aspirin-triggered resolvin D3 is reported. These enzymatic metabolites of docosahexaenoic acid (DHA) have potent anti-inflammatory and pro-resolving actions. The convergent synthetic strategy is based on enantiomerically pure starting materials and it is highly stereocontrolled.

Recent investigations on the resolution of inflammation have revealed the key regulatory roles of some new types of lipid metabolites produced locally via enzymatic pathways from polyunsaturated fatty acids.[1] These studies identified a number of anti-inflammatory and pro-resolving lipid mediators,[2] including the lipoxins[3] formed by arachidonic acid, and the E-series resolvins obtained from eicosapentaenoic acid (EPA).[1,4] Several related molecules derived from docosahexaenoic acid (DHA) have also been identified, including the D-series resolvins,[1,5] the protectins,[1,6] and the maresins.[7] Notably, the discovery and study of these potent metabolites of EPA and DHA, which are important omega-3 fatty acids, provided key molecular and biological insights for their well-known benefits against inflammatory diseases.[8]

An important aspect of these lipid mediators is that they are formed biosynthetically via enzymatic processes, and are often extremely potent (nM to pM range), acting via specific receptors and signaling pathways. Despite some common features, each type of these molecules has a unique structure and distinct biological role. They usually contain multiple C=C bonds with defined *Z/E* geometry, as well as several hydroxy substituents with specific *R/S* configurations. Since they are produced in very small quantities *in vitro* or *in vivo*, their complete stereochemical characterization requires direct matching with stereochemically

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pure materials produced via total synthesis. These synthetic efforts are also essential for elucidating the biological functions of these molecules.

Thus, we have recently developed the first total synthesis that led to the first stereochemical and biological characterization for several of these new lipid mediators.[1] Some of our efforts[1] with resolvin E1 (RvE1),[9] resolvin D1 (RvD1),[10] resolvin D2 (RvD2),[11] neuroprotectin D1/protectin D1 (NPD1/PD1),[12] and maresin 1 (MaR1)[13] have been disclosed, while additional studies will be reported in due course.

Herein, we detail our synthetic work towards resolvin D3 (RvD3), an important member of the D-series resolvins[1,5] with potent pro-resolving properties, such as reduction of human neutrophil transendothelial migration.[5] Notably, the endogenous production of RvD3 was shown to be elevated during ischemic injury of the kidney,[14] and in a colitis model with *fat-1* transgenic mice[15] that produce increased levels of DHA.

The postulated biosynthesis[1,5] of RvD3 from DHA is summarized in Scheme 1. The first step involves the conversion of DHA to (17*S*)-hydroperoxy DHA (17*S*-HpDHA) catalyzed by a lipoxygenase enzyme (LOX), such as 15-LOX. A second lipoxygenation of this intermediate or its reduced hydroxy product (17*S*-HDHA) leads to a new hydroperoxide at the C-4 position, which is converted enzymatically to the 4*S*,5*S* epoxide that undergoes enzymatic hydrolysis to form RvD3 **1**.

A related biosynthetic pathway[1,5] involves the initial oxygenation of DHA with COX-2 in the presence of aspirin to form the (17*R*)-hydroperoxy DHA (17*R*-HpDHA) and its reduced hydroxy product 17*R*-HDHA. Similar lipoxygenation and further transformation of these 17R metabolites leads to the (17*R*)-epimer of RvD3, named aspirin-triggered RvD3 or AT-RvD3 **2**.[1,5]

Our convergent and stereocontrolled synthetic strategy for RvD3 **1** is outlined in Scheme 2. While the structures of RvD3 and AT-RvD3 were initially deduced by using mass spectrometry,[5] their detailed *Z/E* geometry and *R/S* configuration remained to be established. Towards this goal, and based on the above biosynthetic hypothesis, we first targeted the total synthesis of the RvD3 isomer shown in Scheme 1, namely (4*S*,11*R*,17*S*)-trihydroxy-5*Z*,7*E*,9*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid.

In order to prevent Z/E isomerization, our aim was to follow a synthetic route allowing for the sensitive triene and diene moieties to be formed in the final steps of the synthesis. By taking advantage of a mild Zn/Cu/Ag alkyne hydrogenation process[16] at the final stage of the synthesis, we envisioned a selective reduction of the bis-acetylenic precursor **3** to create both the 5*Z*,7*E*,9*E*-triene and 13*Z*,15*E*-diene units of **1**. For the synthesis of **3** we relied on the highly efficient Pd-catalyzed Sonogashira coupling reaction[17] of alkyne **4** and vinyl halide **5**, which is available from alcohol **6** via oxidation and olefination. Compound **6** can be produced in stereochemically pure form starting with the addition of alkynyl lithium **7** to enantiomerically pure (*S*)-glycidol derivative **8**. The synthesis of the key intermediate **4** involves another Sonogashira reaction[17] between alkyne **9** and *E,E*-dienyl iodide **10**, which can be prepared from alcohol **11** that is available via the addition of alkynyl lithium **12** to (*R*)-glycidol derivative **13**. Finally, the synthesis of the alkyne intermediate **9** relies on precursor **14**, which is accessible from the commercially available chiral starting material *(S)*-γ-carboxy butyrolactone **15**.

Scheme 3 shows the detailed synthesis of the three key intermediates, alkenyl iodides **5** and **10**, and alkyne **9**. The synthesis of **5** begun with the addition of lithiated 1-butyne to the *(S)*-epoxide **8** in the presence of boron trifluoride to afford alcohol **16**. Silylation with TBDPS

and clean deprotection of the primary alcohol with camphorsulfonic acid (CSA) gave **17**, which was selectively hydrogenated under Lindlar conditions to afford intermediate **18**. Swern oxidation[18] of this alcohol to the corresponding aldehyde, followed by Takai olefination[19] led to the vinyl iodide **19**. Although this TBDPS-protected compound can be utilized in the final assembly of RvD3, we have found that the final TBDPS removal from the bis-alkynyl product with TBAF leads to product decomposition. Therefore, we converted **19** to the corresponding TBS-protected intermediate **5**.[20]

The synthesis of the dienyl iodide **10** relied on the addition of lithiated TMS-acetylene to the *(R)*-protected glycidol **13**, followed by TBS protection to give **20**, Selective removal of the primary TBS group converted **20** to alcohol **11**. Oxidation under Swern conditions,[18] followed by Wittig olefination with triphenylphosphoranylidene acetaldehyde afforded the α,β-unsaturated aldehyde **22**. Takai olefination[19] of **22** afforded **10** as a labile 9:1 mixture of *E/Z* alkenyl iodides in 84% yield.

Alkyne **9** was prepared from (*S*)-γ-carboxy butyrolactone **15**,[21] starting with ring opening under acid-catalyzed methanol treatment to give the diester **23** in high yield. Selective reduction of the ester adjacent to the hydroxyl group was achieved with borane dimethyl sulfide and a catalytic amount of sodium borohydride to give diol **24**.[22] TBS di-protection, followed by selective removal of the primary TBS group gave alcohol **14**. Swern oxidation,[18] afforded aldehyde **25** in very high yield. The direct transformation of **25** to alkyne **9** was achieved via the Corey-Fuchs homologation,[23] by using LDA as the base to preserve the integrity of the ester moiety.[24] An alternative approach for this transformation employing the Bestmann-Ohira homologation,[25] afforded the product in lower yields and with the added disadvantage of multistep preparation of the reagent.

The final stages of the synthesis of RvD3 from intermediates **5**, **9** and **10** are shown in Scheme 4. Sonogashira coupling[17] of alkyne **9** with dienyl iodide **10** afforded isomerically pure product **26** in excellent yield. Removal of the TMS group gave the terminal alkyne **4**. The second Sonogashira reaction[17] between **4** and vinyl iodide **5** to form **27** also proceeded in high yield. However, the removal of the silyl protective groups was problematic, forming the expected ester derivative **28** in a 3:1 ratio with lactone **29**, as well as the free acid **3** which was esterified with diazomethane back to **28**. Although this type of desilylation has been more efficient in other related lipid derivatives,[1] presumably in **27** the closer proximity of the C-4 hydroxyl group with the ester moiety favors such side reactions. Treatment of **28** with freshly prepared Zn/Cu/Ag amalgam[16] led to the selective hydrogenation of both alkyne groups to the Z-alkenes, but still formed a mixture of ester and lactone products. Basic hydrolysis followed by HPLC purification led to a single stereoisomer of RvD3 **1**, albeit in low yield. Direct hydrogenation of compound **3** gave similar results.

The same type of strategy was employed for the total synthesis of AT-RvD3 **2**, summarized in Scheme 5. The (*R*)-glycidol derivative **13** was converted to alcohol **30** which was transformed to the vinyl iodide **31**. Sonogashira coupling of **31** with alkyne **4** gave the bis-acetylenic compound **32**, which was deprotected and hydrogenated, as above, to afford AT-RvD3 **2**.

The structure and complete stereochemistry of RvD3 **1** and AT-RvD3 **2** were confirmed by LC/MS and NMR, including 600MHz 2-D COSY. The spectroscopic and biological properties of these synthetic compounds, matched those of biogenically-derived molecules from DHA, and helped to unambiguously establish their *Z/E* and *R/S* configurations for the first time.[26]

Using synthetic materials, the biological profile of this type of lipid mediator has been investigated further. Preliminary data revealed that RvD3 **1** and AT-RvD3 **2** are potent immunoresolvents, regulating neutrophil actions, and promoting macrophage-mediated activities.[26]

In summary, the first total synthesis of RvD3 **1** and AT-RvD3 **2** has been achieved, helping to fully assign their stereochemistry. The reported strategy is convergent and highly stereocontrolled, forming these molecules in stereochemically pure form by using enantiomerically pure starting materials. The synthetic availability of these lipid mediators will help elucidate their role during inflammation and confirm their novel anti-inflammatory and pro-resolving properties. Overall, these data offer new insights for the biological roles of aspirin and DHA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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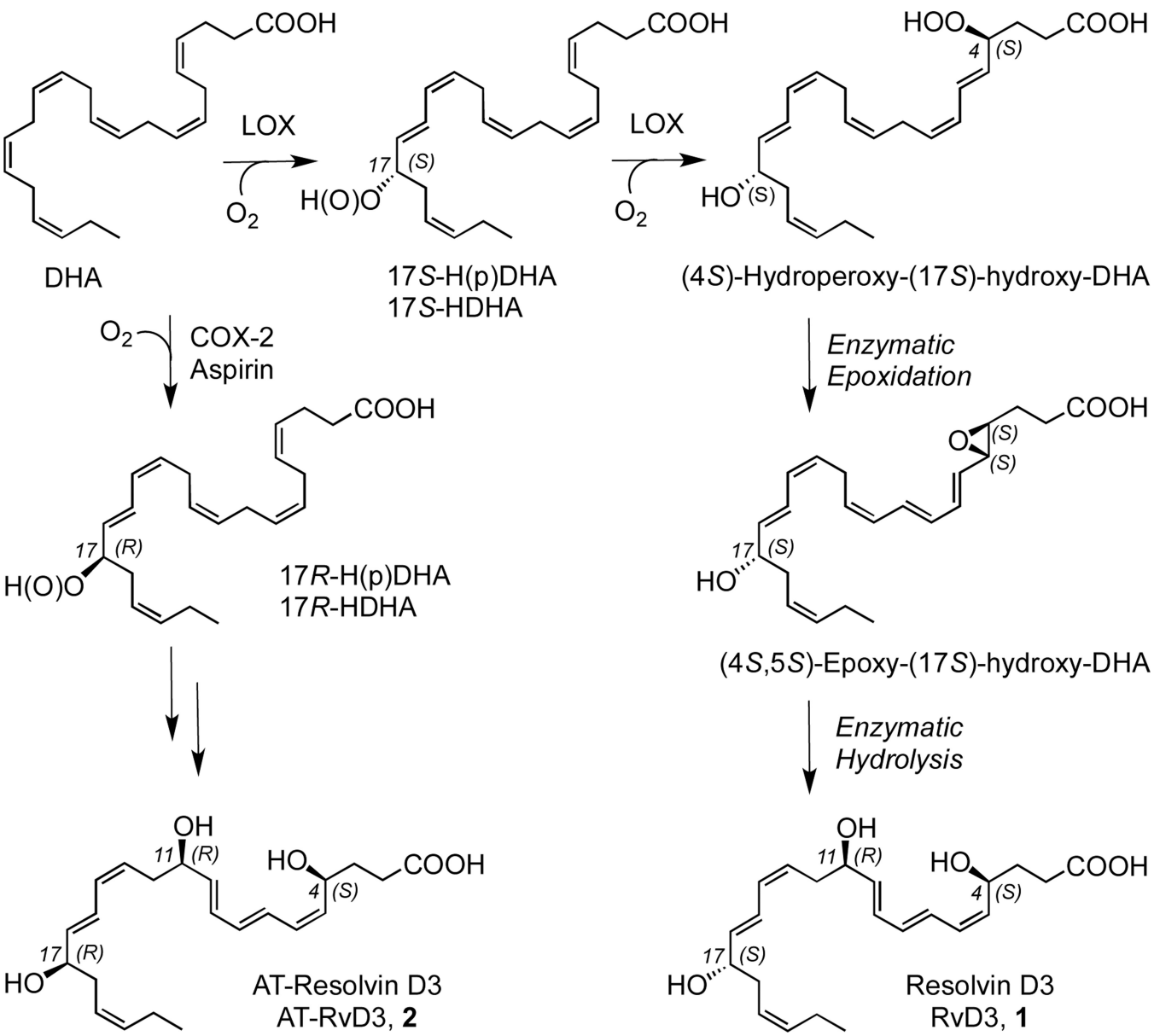
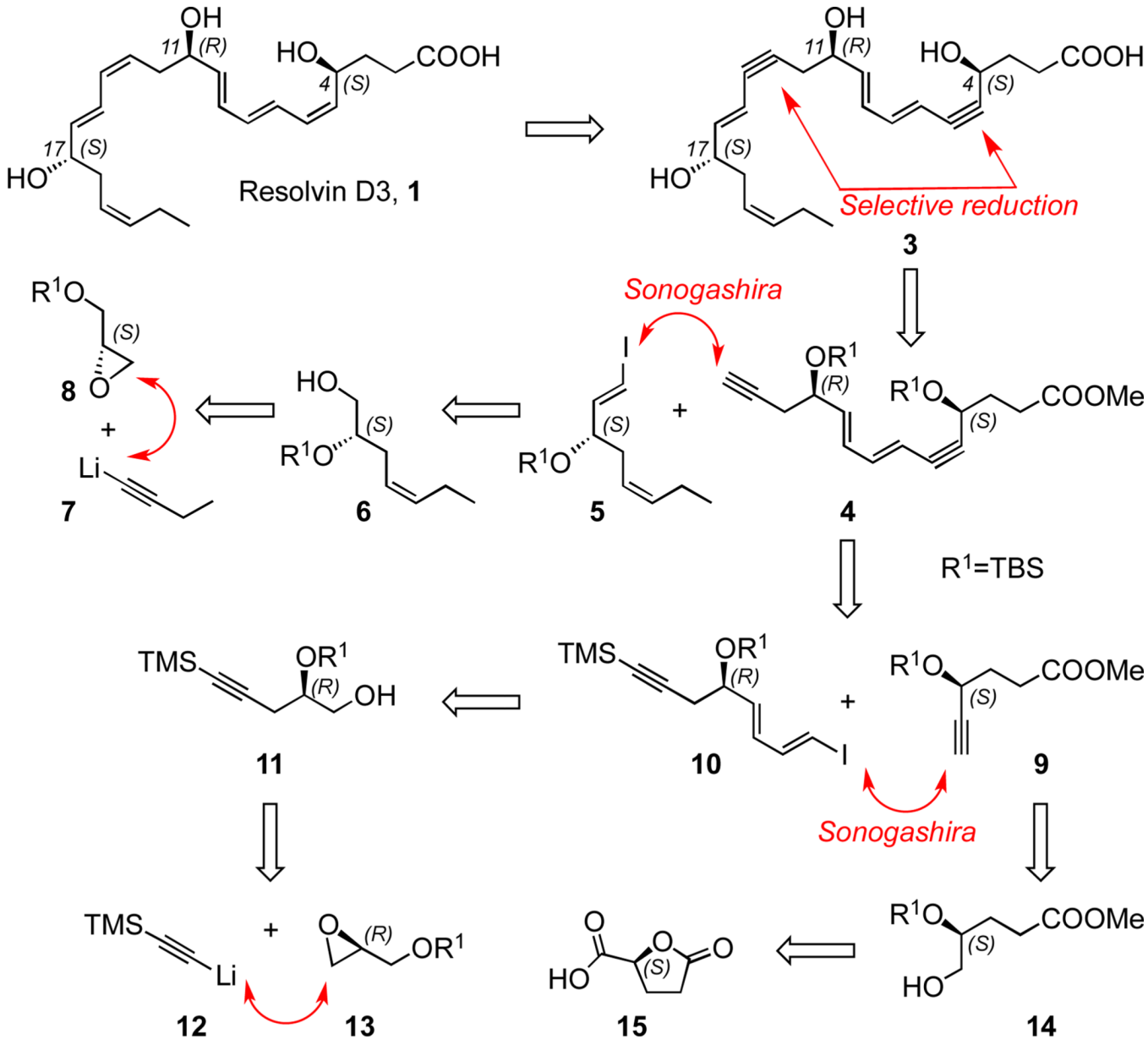
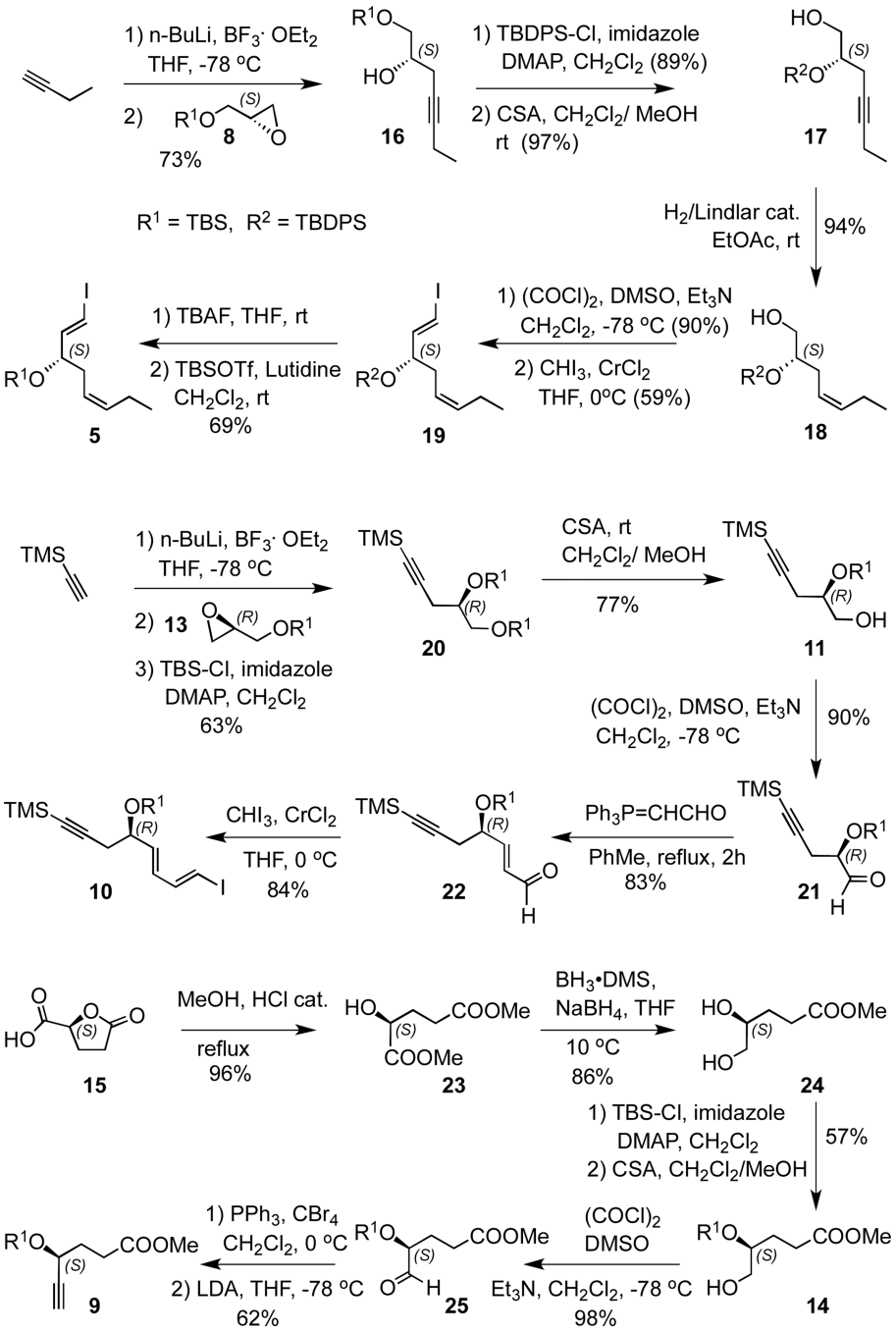


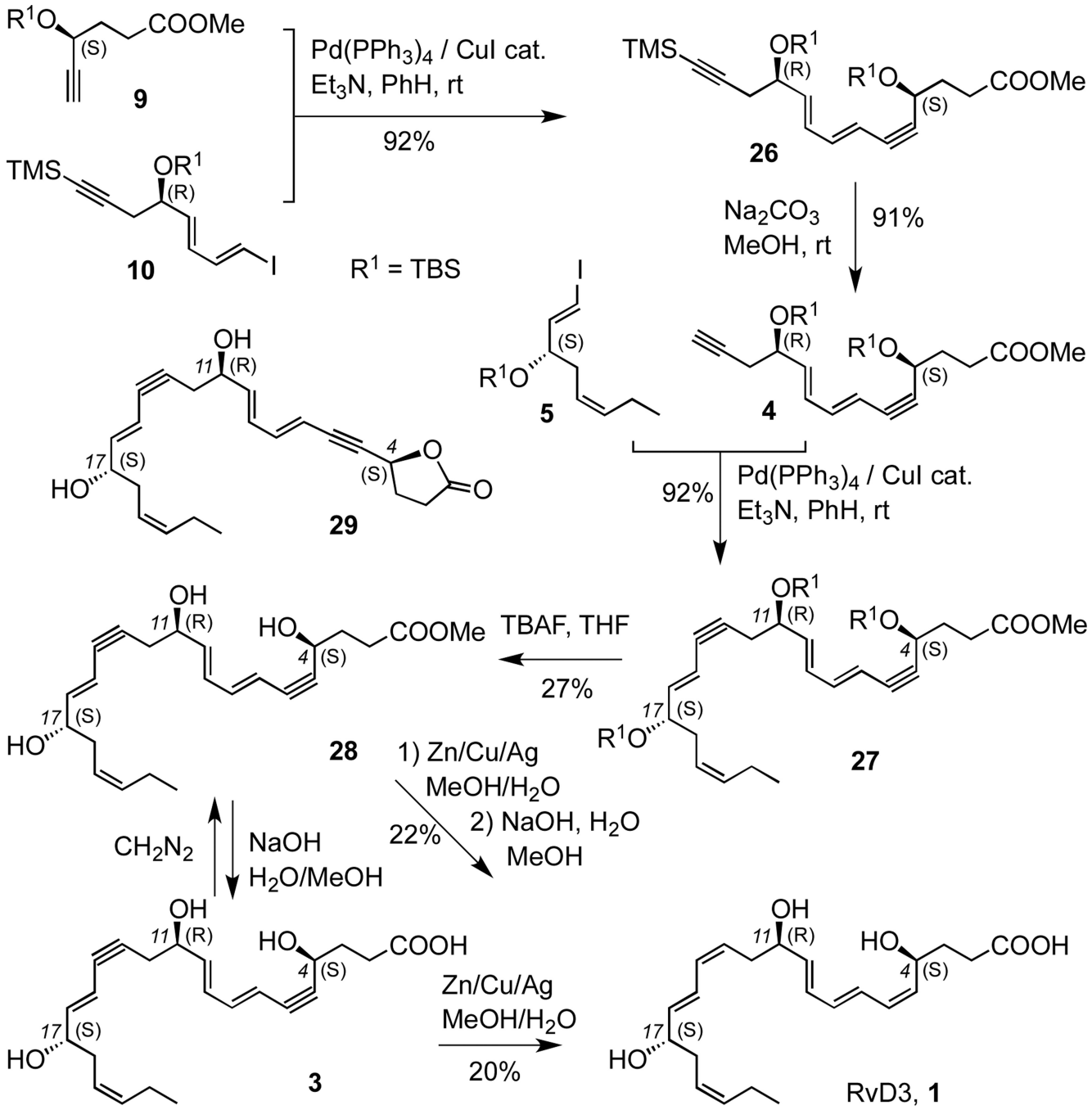
Scheme 1.
Biosynthesis of resolvin D3 and AT-resolvin D3



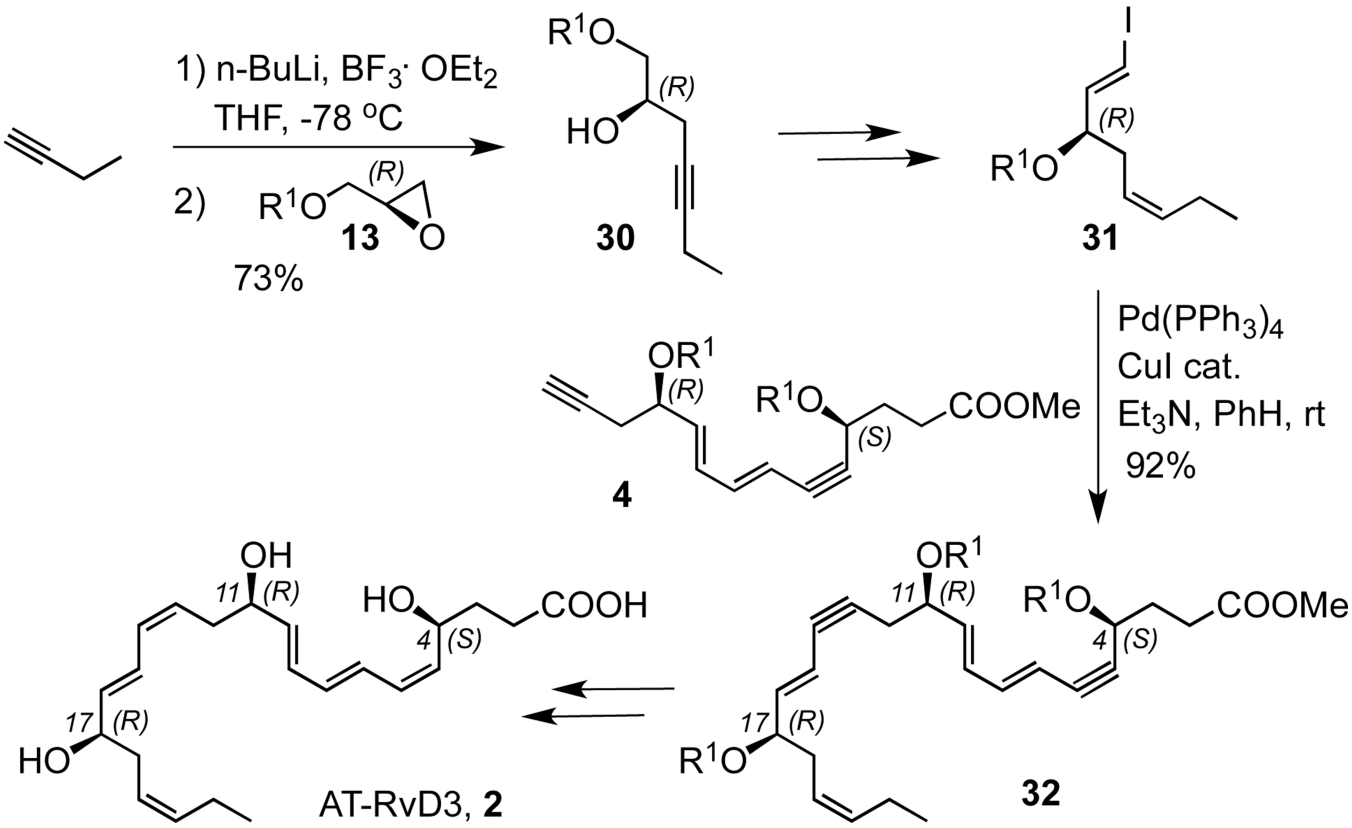
Scheme 2.
Retrosynthetic analysis of resolvin D3



Scheme 3.
Synthesis of key intermediates **5**, **9** and **10**



Scheme 4.
Synthesis of RvD3 from intermediates **5**, **9** and **10**



Scheme 5.
Total synthesis of AT-resolvin D3 (**2**)