Published in final edited form as:

Synlett. 2019 February ; 30(3): 343–347. doi:10.1055/s-0037-1612011.

Stereoselective Synthesis of Maresin-like Lipid Mediators

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Abstract

Maresin-L1 (14*S*,22-dihydroxy-docosa-4*Z*,7*Z*,10*Z*,12*E*,16*Z*,19*Z*-hexaenoic acid) and maresin-L2 (14*R*,22-dihydroxy-docosa-4*Z*,7*Z*,10*Z*,12*E*,16*Z*,19*Z*-hexaenoic acid) were chemically synthesized. They were identical to activated macrophage produced counterparts and their total synthesis was highly Stereoselective, as revealed by chiral LC-UV-MS/MS analysis. The synthesis involved the following steps: (1) kinetic resolution of a racemic allylic alcohol by the asymmetric epoxidation; (2) transformation of the epoxy alcohol to γ -hydroxyenal derivative; and (3) the Wittig reaction to furnish the *Z*-olefin.

Graphical Abstract

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Supporting Information
YES

Primary Data
NO

Keywords

maresin-like; diHDHA; chiral LC-UV-MS/MS; lipid mediators; stereoselective organic synthesis

Introduction

Macrophages were recently found to produce two new lipid mediators, maresin-L1 (14*S*,22-dihydroxy-docosa-4*Z*,7*Z*,10*Z*,12*E*,16*Z*,19*Z*-hexaenoic acid (14*S*,22-diHDHA), **1**) and its enantiomer maresin-L2 (14*R*,22-diHDHA) (**2**) (Figure 1).¹ Like maresins,^{2–6} maresin-Ls are inflammation-resolving and reparative as well as produced by macrophages from omega-3 essential fatty acid, docosahexaenoic acid (DHA), through enzymatic 14-hydroxylation. The formation of maresin-L1 involves 12- or 15-lipoxygenase catalyzed 14*S*-hydroxylation, similar to maresins; whereas, for maresin-L2, it involves cytochrome P450 catalyzed 14*R*-hydroxylation. Additionally, the unique biosynthetic pathways of maresin-Ls involve a hydroxylation through the P450 enzyme(s) at the 22-carbon of the polyunsaturated linear chain containing six double-bonds and twenty-two carbons. Macrophages produced about ten times more maresin-L1 than maresin-L2.¹ Maresin-Ls act as autocrine/paracrine factors to reduce the diabetes-impairment on macrophage production of hepatocyte growth factor and promotion of healing-required migration of epithelial cells and fibroblasts and stem cell transmigration. Maresin-Ls mitigated inflammatory activation of macrophages from diabetic mice. Thus, maresin-Ls may contribute to the roles of macrophages in wound healing and might be able to be used to restore impaired reparative function of macrophages for the healing of diabetic wounds.

So far, the potential physio-pathological functions and therapeutic value of a maresin-Ls were assessed *in vitro* using a minute amount of maresin-Ls prepared via biosynthesis with enzymes or macrophages and isolated by high pressure chiral liquid chromatography.¹ The knowledge gained is limited due to the lack of sufficient quantity of pure maresin-L1 or maresin-L2 for the studies *in vivo* and *in vitro*. However, there currently is no total organic synthesis method for the preparation of maresin-Ls. Therefore, we have established the stereoselective synthesis to fill this important scientific gap. Furthermore, we verified the stereochemistry of these synthetic enantiomers by matching them to the macrophage-produced counterparts using aqueous reversed-phase chiral lipid chromatography coupled with ultraviolet spectrometry and tandem mass spectrometry (acLC-UV-MS/MS).

Synthesis of maresin-Ls in (*S*)- and (*R*)-forms.

Maresin-L1 (**1**) and -L2 (**2**) are dihydroxy derivatives of DHA with a *Z,E*-dienyl alcohol sub-structure (Figure 1).¹ Among the possible methods for the construction of such a sub-structure, we selected a method comprising the following three steps: (1) the highly efficient kinetic resolution of a racemic allylic alcohol possessing the trimethylsilyl (TMS) group by asymmetric epoxidation;^{7,8} (2) transformation of the resulting epoxy alcohol to a γ -hydroxyenal derivative via the reaction with Et₂AlCN;⁹ and (3) the Wittig reaction to give the *Z*-olefin.¹⁰ The retro-synthesis of **1** along this line is outlined in Scheme 1. Thus, allylic alcohol *rac*-**7** in racemic form was envisioned as an early intermediate, which should be

transformed to enantiomerically enriched aldehyde (*S*)-**4** via epoxide (*S*)-**6**. Finally, the Wittig reaction of (*S*)-**4** with phosphonium salt **3** afforded the target compound **1**.

The sequence of reactions yielding epoxides (*S*)- and (*R*)-**6** is shown in Scheme 2. Racemic aldehyde **8** prepared by a literature method¹¹ was subjected to Wittig reaction with phosphonium salt **9**¹² using NaN(TMS)₂, a standard base, in THF at an initial temperature of –78 °C to afford olefin **10** stereoselectively. Subsequent desilylation gave diol **11** in 69% yield from **8**. The primary hydroxy group of **11** was regioselectively silylated with TBSCl and imidazole in CH₂Cl₂ to produce racemic alcohol *rac*-**7** in 69% yield. The kinetic resolution of *rac*-**7** by the Sharpless asymmetric epoxidation using L-(+)-DIPT/Ti(O-*i*-Pr)₄ afforded epoxy alcohol (*S*)-**12** and (*R*)-**7** with >99% ee and 91% ee, respectively, as determined by ¹H NMR analysis of the derived MTPA ester for the former and by chiral HPLC for the latter. After chromatographic separation on silica gel, the former was converted to (*S*)-**6** with TBSOTf, and the latter was transformed to (*R*)-**6** by epoxidation using the D-(–)-DIPT/Ti(O-*i*-Pr)₄, followed by silylation. During the epoxidation, the enantiomeric purity was increased to >99% ee as a result of the kinetic resolution between (*R*)-**7** and the minor (*S*)-isomer in the ratio of 95.5:4.5.

Further transformation toward maresin-L1 (**1**) was initiated with the reaction of epoxide (*S*)-**6** with Et₂AlCN to afford nitrile (*S*)-**5** in a high yield (Scheme 3). Subsequently, the nitrile was reduced to aldehyde (*S*)-**4** in a good yield. On the other hand, phosphonium salt **3** was synthesized by a method disclosed in Scheme 4. In brief, an alkylation of *t*-Bu acetate **15** with **16** afforded **17** along with unidentified by-product(s). Without separation, the mixture was subjected to the Castro-Stephens coupling¹³ with alkynyl alcohol **18**. Under these reaction conditions, **17** was selectively transformed to the more polar diynyl alcohol **19**, which was purified by silica gel chromatography in 39% yield from acetate **15**. The triple bonds in **19** were semi-hydrogenated with P-2 nickel¹⁴ under hydrogen to give alcohol **20**, which was then converted to phosphonium salt **3** in two steps.

The Wittig reaction of aldehyde (*S*)-**4** with **3** was carried out under the standard conditions using a slight excess of **3**. Subsequently, the product (*S*)-**13** was treated with TBAF to produce diol (*S*)-**14** in 84% yield from (*S*)-**4**. Finally, hydrolysis of the *t*-Bu ester was performed using excess LiOH in aqueous MeOH to give maresin-L1 (**1**) in 59% yield. The structure of the product was confirmed by ¹H, ¹³C, and APT (attached proton test) NMR spectroscopy. Similarly, epoxide (*R*)-**6** was converted to maresin-L2 (**2**).¹⁵

Verification of the stereochemistry and purity of synthesized maresin-L1 (**1**) and maresin-L2 (**2**) using chiral acLC-UV-MS/MS with macrophage-produced maresin-Ls (Figure 2).

The acLC-UV-MS/MS study was performed as we described previously.¹ The macrophage-produced maresin-Ls were prepared following the established procedures.^{1,6} The methods are described in the attached Supporting Information. The acLC-MS/MS and UV spectra of chemically synthesized maresin-Ls (**1** and **2**) were in agreement with their structures (Figures 2F to 2K); the purity of maresin-L1 (**1**) or maresin-L2 (**2**) was 96.6% or 98.0%, respectively, based on its relative peak area (%) in the acLC-MS chromatogram at deprotonated molecular ion *m/z* 359. Furthermore, the chemical synthesized maresin-L1 (**1**)

or maresin-L2 (**2**) matched its counterpart that was produced by activated macrophages as shown by their acLC-MS/MS chromatograms and spectra (Figure 2).

In conclusion, maresin-L1 (**1**) and -L2 (**2**) were chemically synthesized using a method shown in Scheme 1. The stereochemistry was accurately constructed. Our chiral LC-UV-MS/MS analysis indicated that each maresin-L chemically synthesized was highly enantiomerically pure (96%) and identical to its macrophages-produced counterpart. Biomedical studies will be facilitated by the organic synthesis. Furthermore, other stereospecific analogs of maresin-Ls could be synthesized in the future for the determination of the structure–bioactivity relationship and structure optimization of therapeutic candidates derived from maresin-L molecular templates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Information

This work was supported by USA NIH grants 2R01DK087800–06A1 and 1R21AG060430–01 (S. H.) and by an unrestricted departmental grant from Research to Prevent Blindness, Inc., New York, NY (S. H. & N. G. B.). This work was also supported by KAKENHI Grant Number JP15H05904, JP15H05898, JP15H05897, JP15H04648 and the Kobayashi International Scholarship, as well as by USA NIH grant 1R01NS104117–01A1 (N. G. B.).

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- (15). To an ice-cold solution of phosphonium salt 3 (967 mg, 1.58 mmol) in THF (20 mL) was added a solution of $\text{NaN}(\text{TMS})_2$ (1.0 M in THF, 1.54 mL, 1.54 mmol). After 5 min at 0 °C, a solution of aldehyde (S)-4 (250 mg, 0.570 mmol) in THF (5 mL) was added to the mixture at -78 °C. The cooling bath was removed and the mixture was stirred at rt for 10 min before addition of saturated NH_4Cl solution. The mixture was extracted with EtOAc and the product was purified by chromatography on silica gel to afford (S)-13, which was used for the next reaction without further purification. The ^1H NMR spectrum is attached in the ESI. To a solution of the above product in THF (20 mL) was added TBAF (1.0 M in THF, 4.80 mL, 4.80 mmol) dropwise. After 30 min at rt, the reaction was stopped by adding saturated NH_4Cl solution. The resulting mixture was extracted with EtOAc and the product was purified by chromatography on silica gel to afford diol ester (S)-14 (198 mg, 84% over two steps): $[\alpha]_{\text{D}}^{20} -21$ (c 0.95, CHCl_3). The ^1H and ^{13}C -APT NMR spectra are attached in the ESI. A mixture of ester (S)-14 (100 mg, 0.240 mmol) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (300 mg, 7.16 mmol) in MeOH (2.5 mL) and H_2O (2.5 mL) was stirred at rt of 22 h and diluted with MacIlvaine's phosphate buffer (pH 5.0, 80 mL). The mixture was extracted with Et₂O and the product was purified by chromatography on silica gel to afford acid 1 (51 mg, 59%), which was further purified by using recycle HPLC (LC-Forte/R equipped with YMC-Pack SIL-60, EtOAc/hexane = 2:1, 20 mL/min): $[\alpha]_{\text{D}}^{20} +4$ (c 0.52, MeOH). The ^1H and ^{13}C -APT NMR spectra and HRMS analysis are attached in the ESI.

Figure 1.
Maresin-L1 and maresin-L2

Figure 2.

Characterization by acLC-MS/MS. Selected reaction monitoring chromatograms include (A) and (B) for chemically-synthesized maresin-L1 (1) and -L2 (2), respectively; (C) for macrophage(Mφ)-produced 1 and 2; (D) for the co-injection of Mφ-produced 1 and 2 plus synthesized (1); and (E) for the co-injection of Mφ-produced 1 and 2 plus synthesized 2. (F), (G), and (H), respectively, are acLC-MS/MS spectra of synthesized 1 and 2, and Mφ-produced 1 and 2. (I) is the interpretation of MS/MS fragmentation. (J) and (K) are UV spectra for 1 and 2, respectively. Deprotonated molecular ion: m/z 359. Signature ion of maresin-Ls: m/z 329 (see panel I).

Scheme 1. Retrosynthesis of maresin-L1 (**1**)^a AE/KR: Asymmetric Epoxidation/Kinetic Resolution.

Scheme 2. Synthesis of enantioenriched hydroxy intermediates **6** of (*R*)- and (*S*)-forms

Scheme 3.
Synthesis of maresins-L1 and -L2

Scheme 4.
 Synthesis of phosphonium salt **3**