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Total Synthesis of the Anti-inflammatory and Pro-resolving Lipid Mediator MaR1$_{n\text{-}3\ DPA}$ Utilizing an sp^3-sp^3 Negishi Cross-coupling Reaction**

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Abstract

The first total synthesis of the lipid mediator MaR1$_{n\text{-}3\ DPA}$ (**5**) has been achieved in 12% overall yield over 11 steps. The stereoselective preparation of **5** was based on a Pd-catalyzed sp^3-sp^3 Negishi cross-coupling reaction and a stereo controlled Evans-Nagao acetate aldol reaction. LC-MS/MS results with synthetic material matched the biologically product **5**. This novel lipid

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mediator displayed potent pro-resolving properties stimulating macrophage efferocytosis of apoptotic neutrophils.

Keywords

sp^3-sp^3 cross-coupling; total synthesis; palladium; natural products; inflammation

The critical role of inflammatory processes in health and diseases has long been recognized.[1] The detailed molecular mechanisms and biological events that regulate the progression and resolution of inflammation have recently emerged.[2,3] These studies have established that the resolution of inflammation is a biosynthetically active process, where stereospecific di- and trihydroxy-containing polyunsaturated fatty acids derived lipid mediators, named specialized pro-resolving mediators (SPMs), resolve inflammation, protect organs, stimulate resolution and induce tissue regeneration.[3] The resolvins, [4] protectins [4–10] and maresins [10,11] are examples of different families of SPMs. Among the many SPMs identified,[2] those derived from the dietary n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA), have attracted significant interest from biomedical researchers.[3] Examples of this class of SPMs includes resolvin D1 (**1**), resolvin D2 (**2**), protectin D1 (**3**) and maresin 1 (MaR1, **4**) (Figure 1).

These stereospecific tri- or di-hydroxylated polyunsaturated natural products exhibit very potent agonist activities, most often in the low nanomolar range, in a wide range of *in vivo* animal inflammation models. The precise molecular understanding and stereoselective mode of action of resolution of inflammation has been explained with these local mediators as pharmacological agents.[3] Several SPMs have entered clinical development programs.[2,3]

In 2013 Dalli et al. reported that n-3 docosapentaenoic acid (n-3 DPA) was converted biosynthetically in both human and murine leukocytes to several new SPMs.[10] One of the products identified was denoted $MaR1_{n\text{-}3\ DPA}$ (**5**), given its relation to maresin 1 (**4**) (Figure 1). This SPM was produced in pico- to nanogram amounts *in vivo*. The initial structural assignment of $MaR1_{n\text{-}3\ DPA}$ (**5**) was based on biosynthetic results and LC-MS/MS fragmentation data.[10] The exact stereochemical determination of the double bond geometry in the conjugated triene system and the absolute configurations of the C-7 and C-14 groups remained to be established. Hence, total synthesis became necessary for exact structural determination of **5**, but also for providing sufficient material for confirmation and further biological studies.

The sensitive *E*, *E*, *Z*-triene connected to either one or two secondary allylic alcohols is a common feature found in several of the SPMs.[2] In our retrosynthetic analysis of **5** a palladium catalyzed sp^3-sp^3 coupling reaction[12] is a key step leading back to alkyne **6**, alcohol **7** and 4-ethoxy-4-oxobutylzinc bromide (**8**) (Figure 2).

The reliability of this type of coupling reaction under transition metal catalysis has recently been considerably improved due to the work of Organ and co-workers.[13] As of today, no examples of palladium catalyzed Negishi sp^3-sp^3 coupling reactions, using alkylzinc reagents, have been applied to the total synthesis of natural products.[14] So far, most

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protocols have been based on magnesium-[15] or copper-reagents[16] in the presence of nickel-complexes.[17] We were tempted by this disconnection since intermediate **7** can easily be prepared employing a stereoselective Evans-Nagao acetate aldol reaction[18,19] between aldehyde **11**[20] and the chiral auxiliary **12**.

Our synthesis of **5** started with the preparation of alkyne **6** using an efficient protocol. First, (*S*)-(−)-α-hydroxy-γ-butyrolactone (**10**) was TBS-protected to **13**, which was reduced to the corresponding lactol that was converted to the alkyne **14** in a Colvin rearrangement. A Swern oxidation of **14** produced multi-grams of TBS-protected 3-hydroxy-4-pentynal (**15**) in overall 47% yield (Scheme 1). The Wittig-salt **9** was prepared from *cis*-3-hexen-1-ol (**16**) in 90% yield using a literature procedure,[21] see Scheme 1. Then a *Z*-selective Wittig reaction between **9** and aldehyde **15** afforded alkyne **6** in 83% yield after purification by chromatography. The stereochemical purity of **6** was determined by GC and ^{1}H NMR analyses. Overall, TBS-protected alkyne **6** was efficiently synthesized in 39% yield from **10**.

Next, commercially available pyridinium-1-sulfonate (**17**) was converted to (2*E*, 4*E*)-5-bromopenta-2,4-dienal (**11**) employing a two-step protocol.[22] Then **11** was reacted with **12** in an Evans-Nagao acetate aldol reaction under conditions developed by Olivo and co-workers.[23] This yielded a 15.3:1 ratio(HPLC analysis) of the desired diasteromer**19**.[23b] Protection (TBSOTf, 2,6-lutidine) and purification by chromatography afforded stereochemically pure **20** (HPLC and NMR analyses). Removal of the auxiliary in **20** was achieved with $LiBH_4$ yielding primary alcohol **7** in 88% yield. Alcohol **7** and alkyne **9** were then coupled in a Sonogashira reaction at ambient temperature in the presence of catalytic amounts of $Pd(PPh_3)_4$ and CuI affording **21** in 68% yield. All attempts to improve this reaction were unsuccessful.[24] An Apple reaction afforded the primary bromide **22** in near quantitative yield from **21**. This high yielding protocol allowed the preparation of **22** in 20% yield over seven steps (Scheme 2).

Several sp^3-sp^3 cross-coupling reactions between the primary bromide **22** with methyl 3-(9-borabicyclo[3.3.1]nonan-9-yl) propanoate were explored.[25] Unfortunately, all efforts to accomplish this coupling were unsuccessful as only trace amounts of **23** was formed.[25d] To our delight, when the palladium-based PEPPSI™-IPr catalyst **24** was employed in the presence of bromide **22** and 4-ethoxy-4-oxobutylzinc bromide (**8**), the desired ethyl ester**23** was formed consistently in 68–72% yield after chromatography. To the best of our knowledge, this is the first reported application of the sp^3-sp^3 palladium-catalysed cross-coupling protocol developed by Organ and co-workers in a synthesis of a natural product. Removal of the TBS-protection groups with tetra-*n*-butyl ammonium fluoride yielded **25** that wa as reduced employing a modified Lindlar procedure.[23b] This yielded ester **26** in 75% yield over the two steps (Scheme 3.) Basic hydrolysis of **26** afforded synthetic $MaR1_{n\text{-}3\ DPA}$ (**5**) in 86% yield with UV-, MS- and NMR-data in accord with the structure. The chemical purity of synthetic **5** was determined to be >98% by HPLC analyses.

To determine if synthetic **5** matched authentic $MaR1_{n\text{-}3\ DPA}$ (**5**), biologically formed **5** from human macrophages was employed.[10] Human macrophage induced formation of $MaR1_{n\text{-}3\ DPA}$ (**5**) showed retention time (T_R) of 14.1 min (Figure 3A). The retention time of synthetic **5** was identical (T_R = 14.1 min) with biologically produced **5**, see Figure 3B.

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Figure 3C depicts the co-injection of synthetic and biologically obtained material added at equal amounts. These experiments demonstrated that synthetic **5** co-elutes with natural occurring **5**. In addition, the MS/MS spectra for both natural and synthetic **5** were essentially identical, see Supporting information, and in accord with literature.[10]

Next incubation of synthetic material **5** with human macrophages was performed; a very potent stimulation of macrophage efferocytosis of apoptotic human neutrophils was observed (Figure 4). This is a key step in the resolution of inflammation;[3] an action that was shared with the DHA derived MaR1 (**4**).[11,26] Of note, the ethyl ester **26** also stimulated macrophage efferocytosis of apoptotic human neutrophils albeit to a lower extent (Figure 4).

In summary, multi mg of the potent anti-inflammatory and pro-resolving lipid mediator $MaR1_{n\text{-}3\ DPA}$ (**5**) was stereoselectively prepared in 11 steps and 12% overall yield from cheap, commercially available salt **17**. Our synthesis compares very favorable to those syntheses published of the maresin SPM class of natural products.[27] The palladium catalyzed sp^3-sp^3 cross-coupling reaction using the Pd-PEPPSI™-IPr catalyst **24**was applied for the first time to the synthesis of a natural product. The synthetic material displayed identical chromatographic properties with biologically produced **5**. These efforts confirmed the structure of the bioactive natural $MaR1_{n\text{-}3\ DPA}$ (**5**) to be (7*S*, 8*E*, 10*E*, 12*Z*, 14*S*, 16*Z*, 19*Z*)-7,14-dihydroxydocosa-8, 10, 12, 16, 19-pentaenoic acid and its potent biological actions in human macrophage efferocytosis. The results presented for (**5**) will be useful for future developments towards new pro-resolving and anti-inflammatory agents.[28] Further biological studies with synthetic material are ongoing and will be reported.

Experimental Section

Experimental procedures and ^1H-,^{13}C-spectra data, HRMS and UV/VIS spectra, HPLC chromatograms of **1** and all intermediates as well as LC/MS-MS of authentic $MaR1_{n\text{-}3DPA}$ (**1**) are available in the Supporting Information. Blood was obtained from healthy human volunteers giving informed consent (protocol #199-P-001297 approved by the Partners Human Research Committee).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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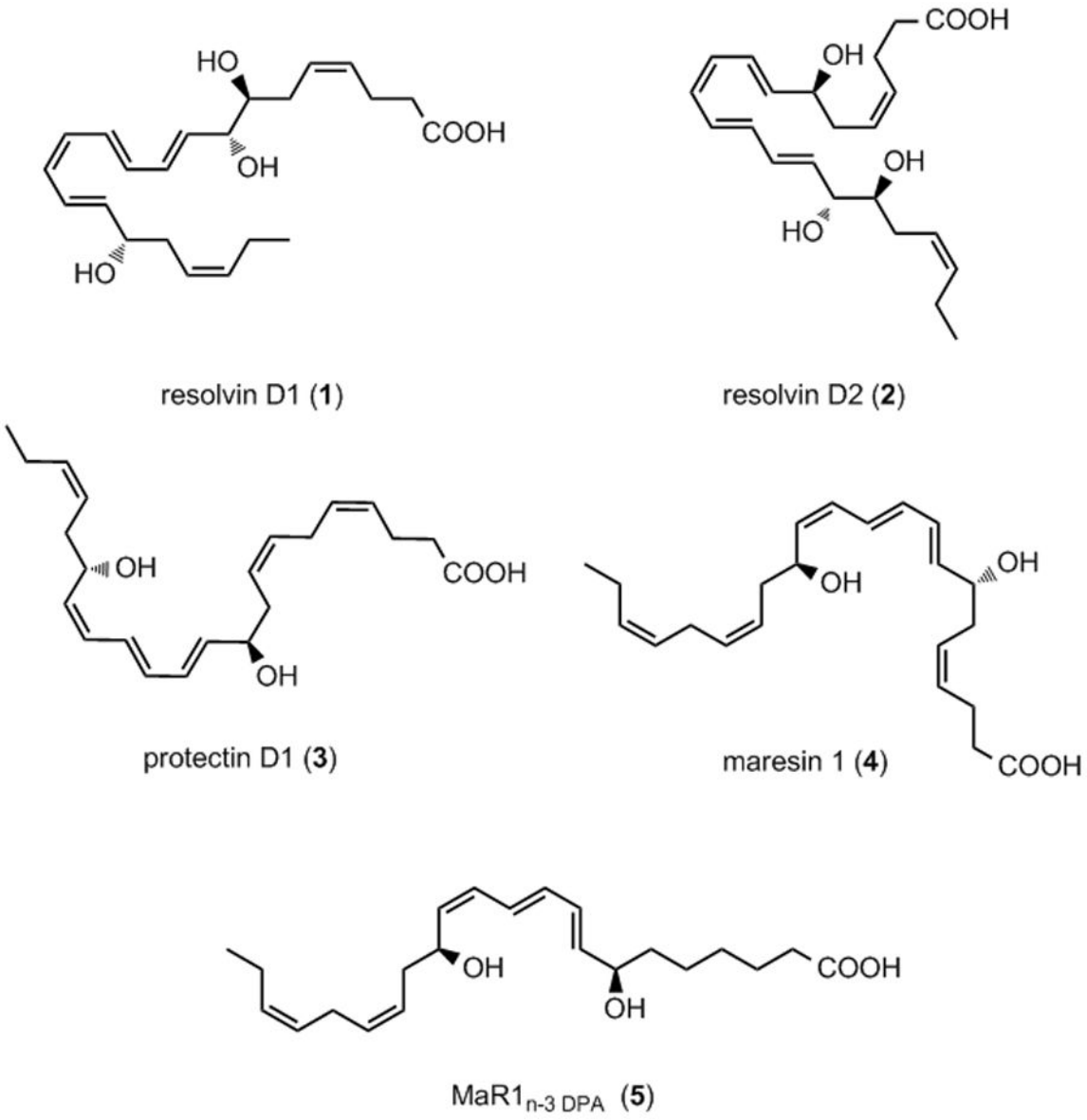

Figure 1.
SPMs exhibiting potent anti-inflammatory and pro-resolving actions.

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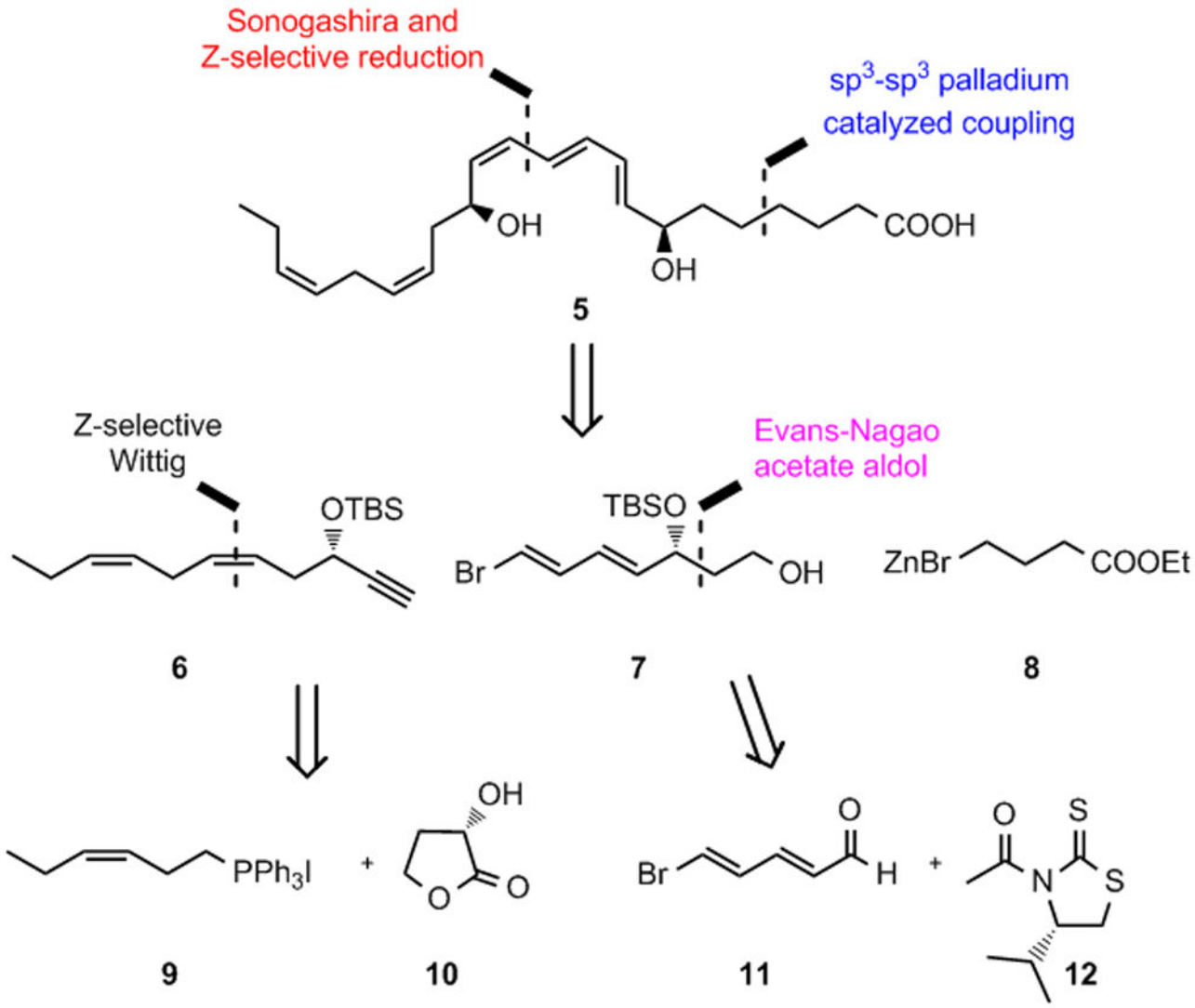

Figure 2.
Retrosynthetic analysis of $MaR1_{n\text{-}3\ DPA}$ (**5**).

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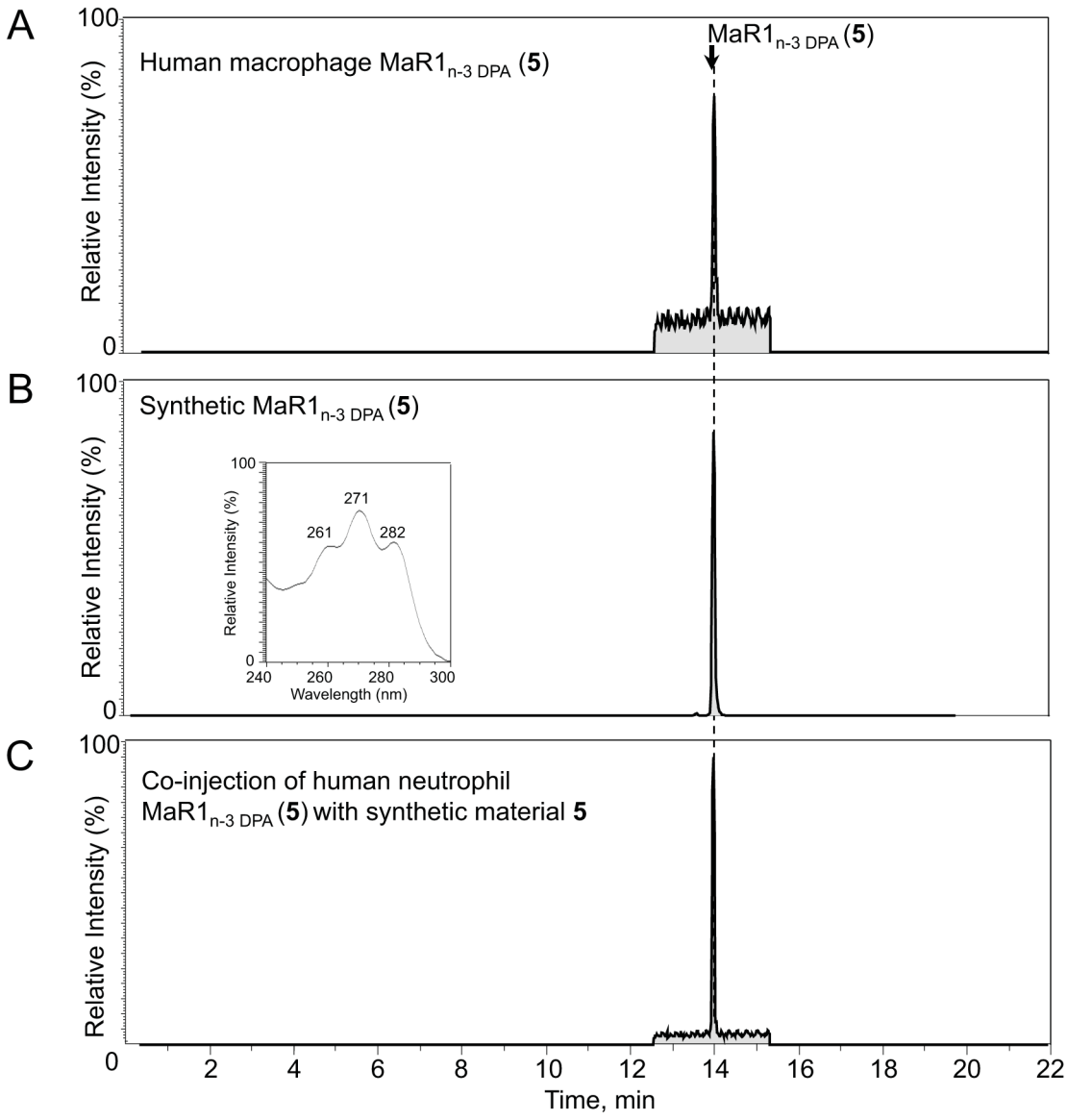

Figure 3.

Matching of synthetic **5** and authentic MaR1$_{n\text{-}3\ DPA}$ (**5**). See Supporting information for details.

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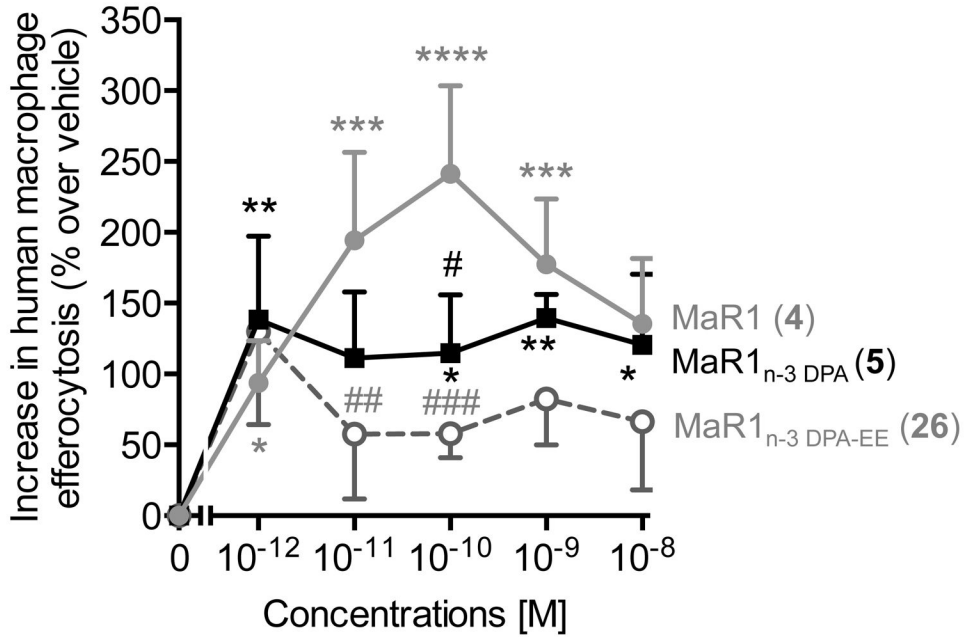
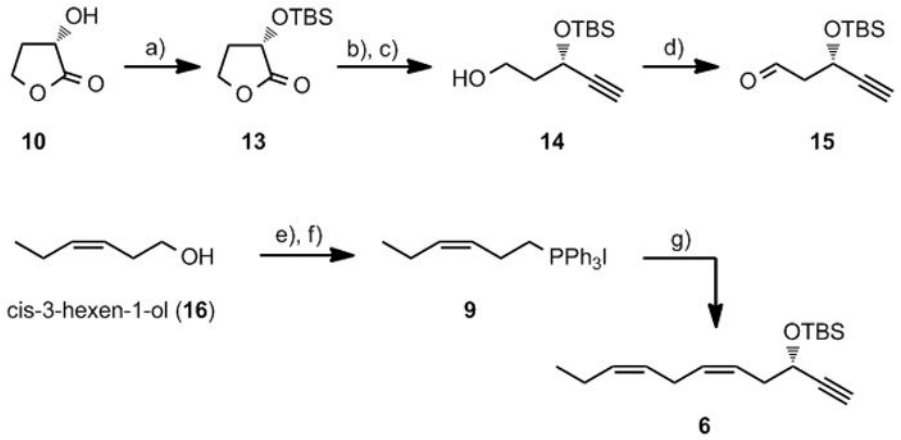


Figure 4.

$MaR1_{n\text{-}3\ DPA}$ (5) enhances human macrophage efferocytosis. Results are percent increase over vehicle and expressed as mean ± s.e.m. n = 4 with 3 – 4 determinations for each. **P* <0.05, ***P* <0.01, ****P* <0.001 and *****P* <0.0001 vs. vehicle; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$ <0.001 vs. maresin 1 (MaR1, **4**). See Supporting information for details



Scheme 1.

Reagents and conditions: a) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 97%; b) DIBAL-H, CH_2Cl_2, −78 °C; c) LDA, $TMSCHN_2$, THF, −78 °C, 57%; d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 85%; e) I_2, PPh_3, imidazole, CH_2Cl_2; f) PPh_3, MeCN, Δ, 90%; g) NaHMDS, HMPA, THF, **15**, −78 °C, 83%.

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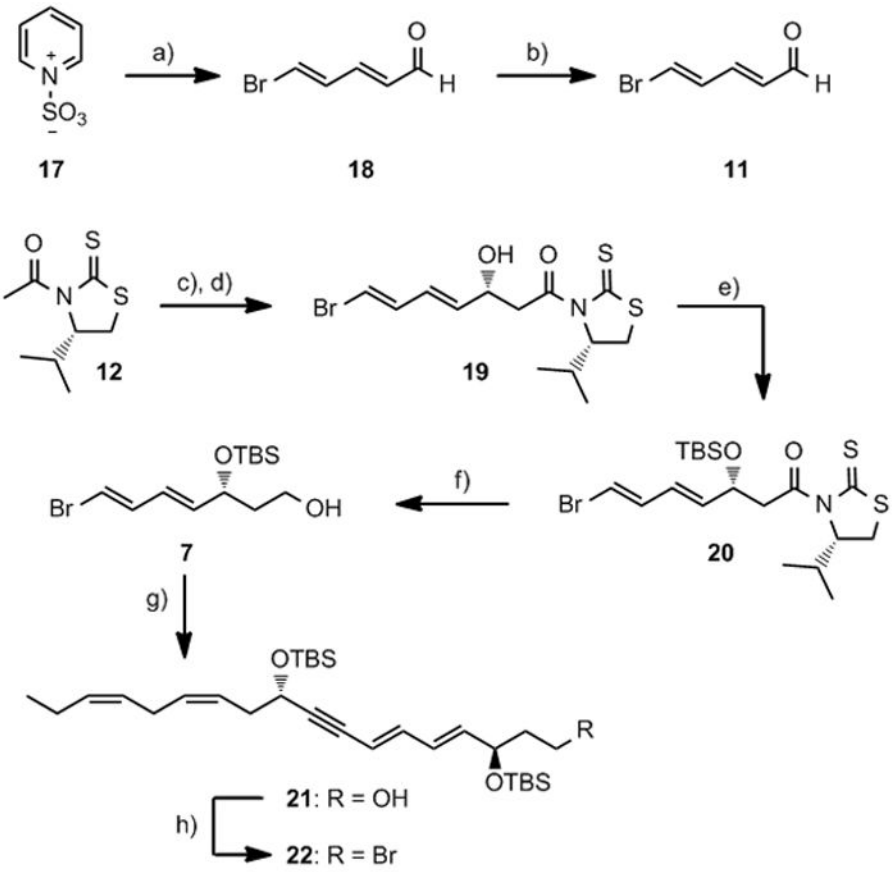

Scheme 2.

Reagents and conditions: a) KOH (aq.), −20 °C tort.; b) Br_2, PPh_3, CH_2Cl_2, 0 °C, 41%; c) (*i*-Pr)$_2$NEt, $TiCl_4$, CH_2Cl_2, −78 °C; d) aldehyde **11**, 86%; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 97%; f) $LiBH_4$, Et_2O, MeOH, 88%; g) $Pd(PPh_3)_4$, CuI, **6**, Et_2NH, 68%; h) CBr_4, PPh_3, 2,6-lutidine, CH_2Cl_2, 97%.

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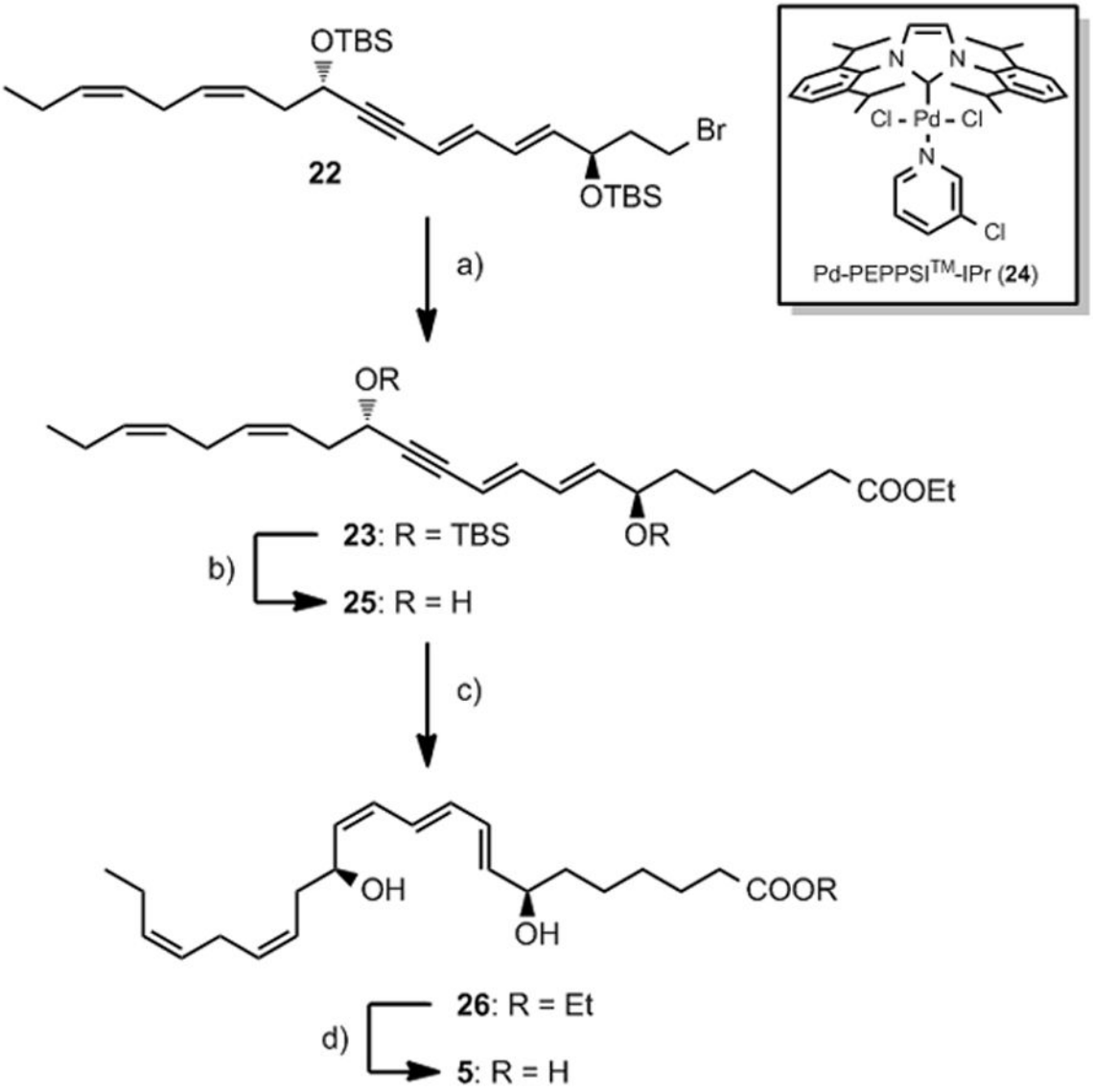

Scheme 3.

Reagents and conditions: a) Pd-PEPPSI™-IPr, LiCl, **8**, THF, NMP, 68%; b) TBAF, THF, 0 °C, 97%; c) Lindlar, H_2, EtOAc, pyridine, 1-octene, 77%, d) LiOH, THF, MeOH, 0 °C, 86%.