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Chemical synthesis and biological evaluation of ω-hydroxy polyunsaturated fatty acids

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ABSTRACT

ω-Hydroxy polyunsaturated fatty acids (PUFAs), natural metabolites from arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were prepared via convergent synthesis approach using two key steps: Cu-mediated C—C bond formation to construct methylene skipped poly-ynes and a partial alkyne hydrogenation where the presence of excess 2-methyl-2-butene as an additive that is proven to be critical for the success of partial reduction of the poly-ynes to the corresponding *cis*-alkenes without over-hydrogenation. The potential biological function of ω-hydroxy PUFAs in pain was evaluated in naive rats. Following intraplantar injection, 20-hydroxyeicosatetraenoic acid (20-HETE, ω-hydroxy ARA) generated an acute decrease in paw withdrawal thresholds in a mechanical nociceptive assay indicating pain, but no change was observed from rats which received either 20-hydroxyeicosapentaenoic acid (20-HEPE, ω-hydroxy EPA) or 22-hydroxydocosahexaenoic acid (22-HDoHE, ω-hydroxy DHA). We also found that both 20-HEPE and 22-HDoHE are more potent than 20-HETE to activate murine transient receptor potential vanilloid receptor1 (*m*TRPV1).

Polyunsaturated fatty acids (PUFAs) are mainly converted to oxylipin metabolites by cytochrome P450 (CYP) enzymes that catalyze hydroxylation or epoxidation.[1] Arachidonic acid (ARA), an ω-6 PUFA, is metabolized by the CYP enzymes to hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs). While EET regioisomers can be found in roughly similar amounts, 20-HETE is a product of ω-hydroxylation that is a major regioisomer of HETEs derived from ARA. All of these metabolites are important lipid mediators that play critical roles in various diseases.[1,2] Interestingly, EETs and HETEs generally have opposing biological functions, e.g., EETs are known to be anti-inflammatory and anti-hypertensive, but 20-HETE shows the opposite effect.[2] Among CYP enzymes, isoforms of the CYP2 family, such as CYP2C and CYP2J, are frequently implicated in production of EETs, but isoforms of the CYP4 family, such as CYP4A and CYP4F, produce 20-HETE. The same CYP isoforms also metabolize both ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to the corresponding epoxy- and hydroxy-PUFAs.[3] Recently, beneficial effects from dietary supplements of fish oil including ω-3 fish oil prescriptions have triggered interests in the biological functions of their metabolites.[4] For example, epoxides from DHA can reduce pain perception,[5] blood pressure,[6] and angiogenesis.[7] Herein, we are exploring biological roles of ω-hydroxy PUFAs (20-HEPE and 22-HDoHE) derived from EPA and DHA, respectively, compared to the relatively well-studied 20-HETE derived from ARA.

20-HETE has been shown to have detrimental effects in several diseases such as hypertension,[8] cancer,[9] and cardiovascular and kidney diseases,[10] despite its low *in vivo* concentrations due to reincorporation into membrane phospholipid pools, and plasma protein binding similar to other fatty acids.[1] In addition, significantly increased 20-HETE resulting from chronic administration of rofecoxib to mice suggests that it may contribute to the cardiovascular risks associated with coxibs and nonselective nonsteroidal anti-inflammatory drugs (NSAIDs).[11] There is also growing evidence that 20-HETE is a potent agonist of a transient receptor potential vanilloid receptor 1 (TRPV1)[12] whose activation by endogenous lipid mediators is closely associated with pain.[13]

Abbreviations: ARA, arachidonic acid; COX, cyclooxygenase; CYP 450, cytochrome P450; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FLIPR, fluorescent imaging plate reader; 22-HDoHE, 22-hydroxydocosahexaenoic acid; 20-HEPE, 20-hydroxyeicosapentaenoic acid; 20-HETE, 20-hydroxyeicosatetranoic acid; PUFA, polyunsaturated fatty acids; TRPV1, transient receptor potential vanilloid 1.

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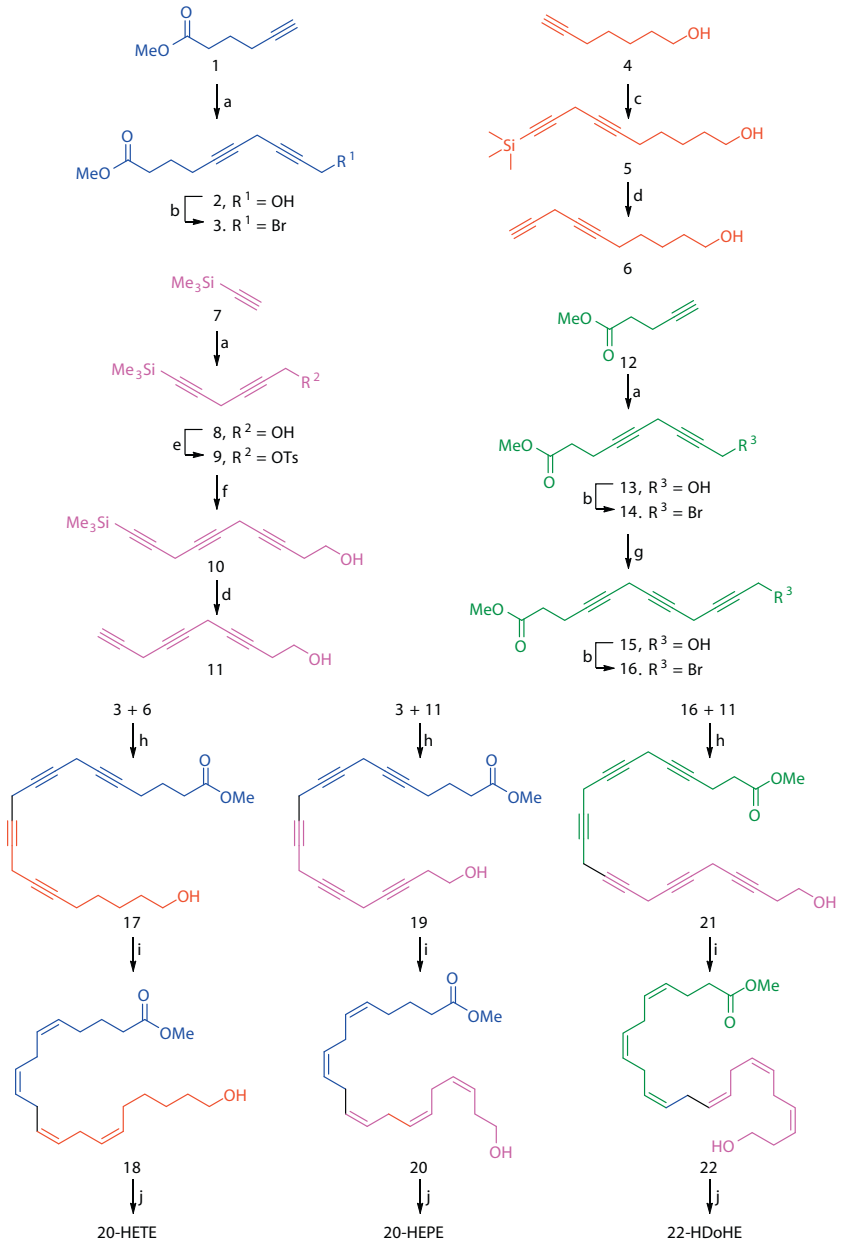
However, little is known about the biological roles of ω-hydroxy metabolites derived from ω-3 PUFAs due in part to their difficult synthesis and therefore limited availability. These are 20-hydroxyeicosapentaenoic acid (20-HEPE) and 22-hydroxydocosahexaenoic acid (22-HDoHE) derived from EPA and DHA, respectively. While several methods for the chemical and biosynthesis of 20-HETE have been reported,[14] there is only one example for 20-HEPE chemical synthesis.[15] To our knowledge, even though bioconversion of 22-HDoHE using fungi or enzymes has been recently reported,[14f–h] its chemical synthesis, which renders the ease of scale-up compared to its bioconversion, has not yet been reported. These compounds can be also used as standards for expanding metabolite analysis of oxylipins in the ARA cascade to the ω-3 PUFAs such as EPA and DHA. In particular, the chemical synthetic approach to form these molecules will provide a facile route to heavy atom standards.[16] Thus, we report here the practical chemical syntheses of all three ω-hydroxy PUFAs and their initial biological evaluation *in vitro* and *in vivo*.

Our convergent synthetic approach of the ω-hydroxy PUFAs, 20-HETE, 20-HEPE, and 22-HDoHE, is summarized in Scheme 1. During their total syntheses, serial Cu-mediated C—C bond formation to construct required methylene skipped poly-ynes and their partial hydrogenation to obtain the desired *cis*-double bonds of each ω-hydroxy PUFA have been used as two key reaction steps. All of the compounds were prepared by partially sharing common fragments **3**, **6**, **11**, and **16**, which were also obtained via serial Cu-mediated C—C bond formation reactions from the corresponding terminal alkynes **1**, **4**, **7**, and **12**, respectively. While the preparation of the fragments **3**, **6**, and **16** was straightforward, the initial attempt of bromination of **8** to prepare **11** in a similar



Scheme 1. Synthetic routes of compounds 20-HETE, 20-HEPE, and 22-HDoHE. Reagents and conditions: (a) CuI, NaI, Cs_2CO_3, 4-chloro-2-butyn-1-ol, DMF, rt, 12 h; (b) PPh_3, CBr_4, DCM, 0 °C, 2 h; (c) CuI, NaI, Cs_2CO_3, 3-bromo-1-(trimethylsilyl)-1-propyne, DMF, rt, 12 h; (d) 1 M TBAF in THF, AcOH, THF, rt, 12 h; (e) Et_3N, DMAP, *p*-TsCl, DCM, 0 °C to rt, 12 h; (f) CuI, NaI, Cs_2CO_3, 3-butyn-1-OH, DMF, rt, 12 h; (g) CuI, NaI, Cs_2CO_3, propargyl alcohol, DMF, rt, 12 h; (h) CuI, NaI, Cs_2CO_3, DMF, rt, 12 h; (i) Lindlar catalyst, 2-methyl-2-butene:MeOH:pyridine (4:4:1), H_2, rt, 18 h–2 d (note: 1:1 MeOH/EtOAc was used instead of MeOH for both **20** and **22**); (j) 1 N NaOH, MeOH, rt, 5 h.

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