Synthesis of both enantiomers of the docosahexaenoic ester of 13-hydroxyoctadecadienoic acid (13-DHAHLA)

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ABSTRACT

Recently, several different classes of endogenous lipids have been reported that display antidiabetic and anti-inflammatory effects. Due to their minute presence in human samples, access to synthetic material of each enantiomer becomes necessary for exact structural elucidation and extensive biological evaluation. Herein we report the multi-milligram synthesis of both enantiomers of the docosahexaenoic ester of 13-hydroxyoctadecadienoic acid (13-DHAHLA) from commercially available starting materials.

Keywords: 13-DHAHLA; 13-HODE; FAHFAs; Schwarz's reagent; Boland reduction.

1. Introduction

Fatty acid esters of hydroxy fatty acids (FAHFAs) are a new class of endogenous lipids that were first uncovered in adipose tissue,¹ but has since been found in many other tissues, blood and breast milk.² Elevated levels of FAHFAs have been associated with antidiabetic and anti-inflammatory effects.¹ Very recently, the biological significance and strategies for the chemical syntheses of FAHFAs were reviewed.³ The FAHFAs can be categorized into subfamilies based on the composition of the fatty acid and hydroxy fatty acid, and regioisomers that differ in the position and configuration of the ester linkage connecting the two fatty acids. Most of the FAHFAs contain saturated lipid chains. Notably, in 2016 Kuda and co-workers identified omega-3 polyunsaturated fatty acid (PUFA) derived FAHFAs.⁴ One of these compounds contained docosahexaenoic acid (DHA) esterified to (9Z,11E)-13-hydroxyoctadeca-9,11-dienoic acid (13-HODE). This novel compound, which was termed 13-DHAHLA,^{4,5} was uncovered from serum and white adipose tissue of both mice and diabetic patients supplemented with n-3 PUFAs. Interestingly, 13-DHAHLA displayed anti-inflammatory and pro-resolving properties.⁴ Moreover, in low micromolar concentration this novel FAHFA inhibited lipopolysaccharide-triggered activation of macrophages and human peripheral blood mononuclear cells, and enhanced phagocytic activity toward zymosan A particles. These experiments were performed with a racemic mixture of 13-DHAHLA. The total synthesis of each enantiomer is needed for further biological and pharmacological investigations.

Figure 1. Structure of 13-DHAHLA.

2. Synthesis

The synthetic strategy for the synthesis of 13(*S*)-DHAHLA is depicted in Figure 2. The trimethylsilyletanoic ester in **1** was installed to allow for a selective hydrolysis of this group in the presence of an ester group on C-13 after a Steglich esterification of DHA with **1**. Other key steps were a Sonogashira-cross coupling reaction between alkyne **2** and vinyl iodide **3**, followed by a *Z*-selective alkyne reduction. Epoxide **4** was used in a reaction with Grignard reagent **5** for the synthesis of alkyne **6**. Switching **4** for its enantiomer would give access to 13(*R*)-DHAHLA.

Figure 2. Retrosynthetic analysis of 13(*S*)-DHAHLA.

The synthesis of fragment **6** was achieved in 31% yield over six steps as outlined in Scheme 1. Commercially available *n*-butylmagnesium chloride (**5**) and a catalytic amount of CuI were added to *tert*-butyldimethylsilyl (*S*)-glycidyl ether (**4**) to afford the secondary alcohol **7** in 78% yield. Protection and selective removal of the primary silyl group using HF-pyridine gave the primary alcohol **8** in 59% yield. Next, oxidation with Dess-Martin periodinane and a subsequent Corey-Fuchs reaction afforded alkyne **6** via vinyl dibromide **9** in 68% yield over three steps. The alkyne ester **2** was prepared in two steps and 58% yield from commercially available methyl 9-hydroxynonanoate (**10**), using a Dess-Martin oxidation and treating the resulting aldehyde with the Ohira-Bestmann reagent in methanol in the presence of K₂CO₃.

Scheme 1. Synthesis of fragments **6** and **2**. Reagents and conditions: (a) CuI (0.1 equiv.), THF, -50 °C-rt., 78%; (b) TBSCl, imidazole, DMF; (c) HF·pyridine, THF, pyridine, 16 h, overall yield for two steps 59%; (d) Dess-Martin periodinane, NaHCO₃, CH₂Cl₂; (e) CBr₄, PPh₃, Zn, CH₂Cl₂, overall yield for two steps 70%; (f) MeLi, THF, -78 °C, 97%; (g) Dimethyl(1-diazo-2-oxo propyl)-phosphonate, K₂CO₃, MeOH, overall yield for two steps 58%.

We next applied a recently reported method for an *in situ* formation of the Schwartz's reagent⁶ for the conversion of alkyne **6** into the vinyl iodide **3**. The labile vinyl iodide **3** was immediately reacted in a Sonogashira cross-coupling reaction with alkyne **2**. Purification of the resulting product was not successful, but removal of its silyl group with TBAF provided pure alcohol **11** in 34% yield over the four steps. Next, the conjugated alkyne in **11** was reduced stereoselectively using Boland's method⁷ in 88% yield. Hydrolysis of the ester group gave 13(*S*)-HODE, which due to its biological properties has been the focus for numerous synthetic and chemo-enzymatic syntheses.⁸ The fluoride sensitive trimethylsilyl ethyl ester protecting group was installed on 13(*S*)-HODE to give alcohol **1**. A Steglich esterification with DHA provided 13(*S*)-DHAHLA after removal of the trimethylsilyl ethyl ester with fluoride. The enantiomer, 13(*R*)-DHAHLA, was prepared from *tert*-butyldimethylsilyl (*R*)-glycidyl ether (*ent*-**4**) using the same protocol as described for 13(*S*)-DHAHLA.⁹

Scheme 2. Synthesis of 13(*S*)-DHAHLA. Reagents and conditions: (a) Cp₂ZrCl₂ (1.5 equiv.), LiAlH(O-*t*Bu)₃ (1.5 equiv.), THF; (b) I₂ (1.5 equiv.) (c) **2**, Pd(PPh₃)₄ (0.04 equiv.), CuI (0.05 equiv.), benzene, Et₃NH; (d) TBAF, THF, 4 °C, 16 h, 34% overall yield from **6**; (e) Zn(Cu/Ag), MeOH, H₂O, 18 h, 88%; (f) LiOH, MeOH, THF, H₂O, 80%; (g) TMSCH₂CH₂OH, *N,N'*-dicyclohexylcarbodiimide (1.2 equiv.), DMAP (0.5 equiv.), CH₂Cl₂, 68%; (h) DHA (2.0 equiv.), *N,N'*-dicyclohexylcarbodiimide (2.5 equiv.), DMAP (0.75 equiv.), CH₂Cl₂, 87%; (i) TBAF, THF, rt., 2 h, 50%.

3. Conclusions

A stereoselective synthesis of 13(*S*)-DHAHLA has been achieved in 2% overall yield over 15 steps. Its enantiomer, 13(*R*)-DHAHLA, was synthesized from *tert*-butyldimethylsilyl (*R*)-glycidyl ether using the same sequence and in a similar yield. A recent and convenient protocol for making vinyl iodides was used.⁶ Matching of the synthetic compounds with endogenous material, and investigations of the biological effects of each enantiomer, are currently ongoing and will be reported elsewhere.

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Supplementary material.

Supplementary data to this article can be found online at <https://>

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9. Spectroscopic data obtained for 13(*S*)-DHAHLA: $[\alpha]_D^{25} = +1.4$ ($c = 0.5$, hexane). UV (hexane) λ_{max} 235. ^1H NMR (600 MHz, CDCl_3) δ 6.50 (dd, $J = 15.3$ and 11.1 Hz, 1H), 5.83 (t, $J = 11.1$ Hz, 1H), dd, $J = 15.1$ and 7.5 Hz, 1H), 5.51-5.27 (m, 14H), 2.90-2.77 (m, 10H), 2.43-2.32 (m, 6H), 2.22-2.12 (m, 2H), 2.12-2.02 (2H), 1.70-1.54 (m, 2H), 1.69-1.54 (m, 4H), 1.37-1.25 (m, 14H), 0.97 (t, $J = 7.5$ Hz, 3H), 0.88 (m, 3H). ^{13}C NMR (150 MHz, CDCl_3) δ 178.5, 172.7, 133.8, 132.2, 131.1, 129.3, 128.7, 128.4, 128.4, 128.3, 128.3 (2xC), 128.2, 128.2, 128.2, 128.0, 127.7, 127.2, 75.1, 34.7, 34.7, 33.9, 31.7, 29.9, 29.5, 29.1, 29.1, 29.0, 27.9, 25.8 (2xC), 25.8, 25.7, 25.0, 24.8, 23.0, 22.7, 20.7, 14.4 and 14.2. HRESITOFMS: m/z 629.4540 $[M+\text{Na}]^+$ (calcd for $\text{C}_{40}\text{H}_{62}\text{NaO}_4$, 629.4540). Spectroscopic data obtained for 13(*R*)-DHAHLA: $[\alpha]_D^{25} = -1.2$ ($c = 0.5$, hexane). All other spectroscopic data of 13(*R*)-DHAHLA were identical to that of its enantiomer, 13(*S*)-DHAHLA.