Branched Fatty Acid Esters of Hydroxy Fatty Acids: Endogenous Anti-Diabetic and Anti-inflammatory Lipids

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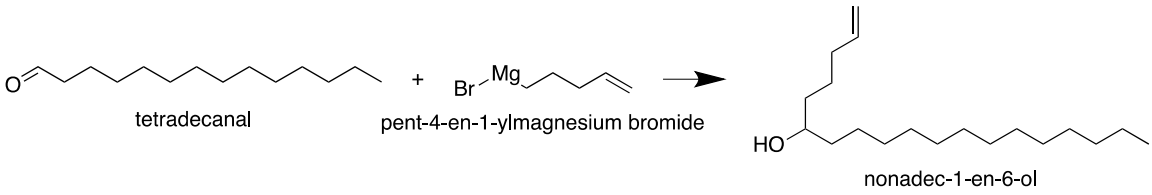
Extended experimental procedures

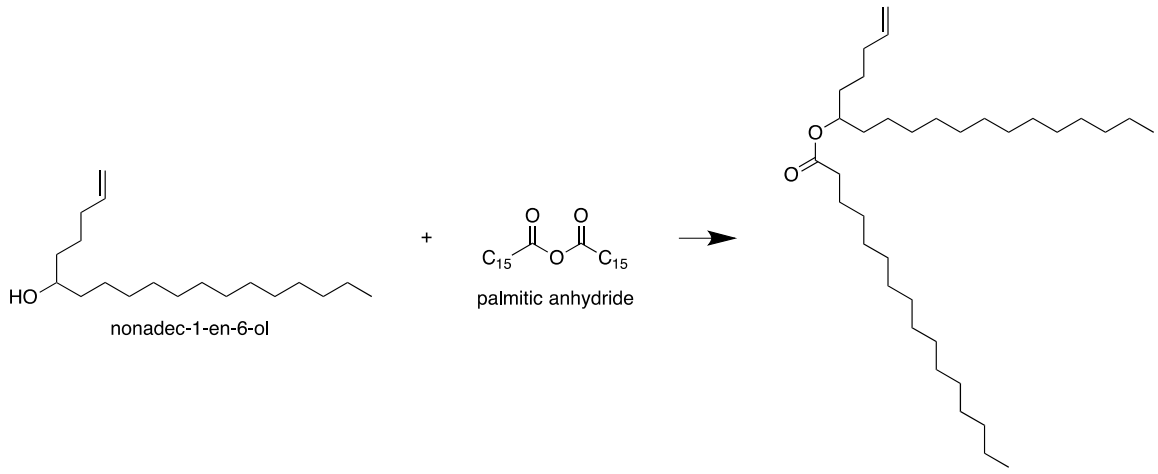
PAHSA Synthesis



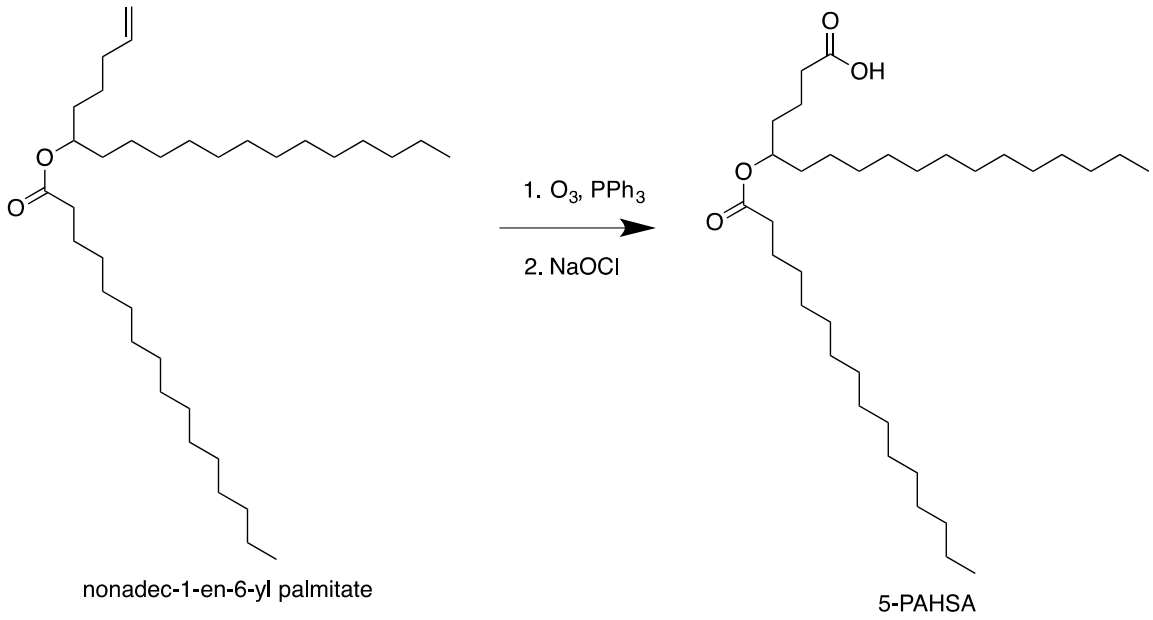
General materials.

Glassware was oven dried for at least 24 hours before use and the reactions were carried out under an atmosphere of nitrogen. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel F-254 plates and visualized by phosphomolybdic acid stain (PMA). Flash chromatography was performed using 230-400 mesh silica gel form EMD. ^{1}H NMR spectra were performed at 400 MHz.

Synthesis of nonadec-1-en-6-ol. To a stirred solution of tetradecanal (1.5 g, 7.1 mmoles, 1 eq) in THF (50 mL) on an ice bath was added pent-4-en-1-ylmagnesium bromide (21.2 mL, 10.6 mmoles, 1.5 eq, 0.5 M solution in THF) by syringe. The solution stirred overnight (16 hours) and over this time warmed to room temperature. The reaction was quenched by the addition of a saturated solution of ammonium chloride (1 mL), concentrated onto celite 545 (10 g), and purified by silica gel chromatography (15% EtOAc/hexanes). Pure fractions were combined and concentrated to afford a white solid (570 mg, 28%); Rf = 0.17, 10% EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$ (7.26 ppm)): 5.846-5.761 (m, 1H), 5.022-4.920 (m, 2H), 3.584 (s, 1H), 2.070-2.055 (d, 2H), 1.42-1.24 (m, 29H), 0.863 (t, 3H); m/z (ESI+) found [MNH_4^+] $C_{19}H_{42}NO^+$, 300.3259; calculated for $C_{19}H_{42}NO^+$: 300.3261, ΔPPM = 0.67.



Synthesis of nonadec-1-en-6-yl palmitate. To a stirred solution of nonadec-1-en-6-ol (570 mg, 2 mmoles, 1 eq) in CH_2CL_2 (20 mL) was added palmitic anhydride (1.2 g, 2.4 mmoles, 1.2 eq), 4-(dimethylamino)pyridine (122 mg, 1 mmole, 0.5 eq), and triethylamine (1.1 mL, 8 mmole, 4 eq). The solution stirred overnight (16 hours) at room temperature. The reaction was concentrated onto celite 545 (10 g), and purified by silica gel chromatography (10% EtOAc/hexanes). Pure fractions were combined and concentrated to afford a clear, colorless, oil (750 mg, 71%); Rf = 0.33, 15% EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$ (7.26 ppm)): 5.846-5.761 (m, 1H), 5.022-4.920 (m, 2H), 3.584 (s, 1H), 2.070-2.055 (d, 2H), 1.42-1.24 (m, 29H), 0.863 (t, 3H); m/z (ESI+) found [MH^+] $C_{35}H_{69}O_2^+$, 521.5302; calculated for $C_{35}H_{69}O_2^+$: 521.5292, ΔPPM = 1.92.



nonadec-1-en-6-yl palmitate

5-PAHSA

Synthesis of 5-PAHSA. Ozone was bubbled into a stirred solution of nonadec-1-en-6-yl palmitate (104 mg, 0.2 mmoles, 1 eq) in CH_2CL_2 (20 mL) at -78 °C until the solution turned blue. Nitrogen was then bubbled into the reaction until it was colorless and triphenyl phosphine (104 mg, 0.4 mmole, 2 eq) was added and the reaction warmed to room temperature. After 2 hours, the solution was concentrated. Sodium hypochlorite (112 mg of an 80% grade stock, 1 mmole, 5 eq.), sodium phosphate monobasic (138 mg, 1 mmole, 5 eq.), 2 methylbut-2-ene (1.6 mL,16 mmole, 80 eq.), water (3.6 mL) and tert-butanol (14 mL) were added and the reaction stirred overnight. The reaction was concentrated and taken up in methylene chloride and then washed with 10% HCl in a separatory funnel. The organic layer was dried with sodium sulfate, filtered and then concentrated using a rotovap. An waxy solid bordering on an oil remained in the flask. This residue was dissolved in a minimal amount of ethyl acetate and the purified by silica gel chromatography (20% EtOAc/hexanes). Pure fractions were combined and concentrated to afford a white solid (750 mg, 71%); Rf = 0.33, 15% EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$ (7.26 ppm)): 5.846-5.761 (m, 1H), 5.022-4.920 (m, 2H), 3.584 (s, 1H), 2.070-2.055 (d, 2H), 1.42-1.24 (m, 29H), 0.863 (t, 3H); m/z (ESI+) found [MH^-] $C_{34}H_{65}O_4^-$, 537.4905; calculated for $C_{34}H_{65}O_4^-$: 537.4888, ΔPPM = 3.16.

The synthesis of 9-PAHSA was carried out using the same route except that the first step uses dec-9-enal and nonylmagnesium bromide for the Grignard addition. 9-hydroxy

heptadecanoic acid (HHA) was made in two steps by addition of octylmagnesium bromide to dec-9-enal, followed by ozonolysis to afford the hydroxy fatty acid.