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SYNTHESIS AND FUNCTIONAL PHARMACOLOGICAL EFFECTS ON HUMAN BRONCHI OF 20-HYDROXYEICOSATETRAENOIC ACID

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We have synthesized 20-hydroxyeicosatetraenoic acid (20-HETE) following a new route and delineated its functional effects in human bronchi. Isometric tension measurements were performed, and they demonstrated that synthetic 20-HETE induced a concentration-dependent relaxant effect in ASM on resting tone and on bronchi pre-contracted with methacholine.

Keywords: 20-hydroxyeicosatetraenoic acid, synthesis, human bronchi, Cu-mediated C-C bond formation, histamine, methacholine.

20-HETE (Scheme 1) is a bioactive eicosanoid synthesized in several mammalian tissues from free arachidonic acid (AA) by cytochrome P450 (CYP) ω-hydroxylase. 20-HETE is an important modulator of vascular, kidney, gastrointestinal, and bronchial cell reactivity [1, 2]. The CYP-450 enzymes are predominantly detected in the liver, heart, vasculature, gastrointestinal tract, kidney, and lung [2, 3]. Moreover, its production may be affected *in vivo* under certain pathological conditions [4]. In guinea pig, 20-HETE induces an increase in airway smooth muscle (ASM) basal tone [5], an effect largely due to a direct activation of various surface membrane ionic conductance such as type 6 canonical transient receptor potential (TRPC6) [5, 6]. In contrast, 20-HETE relaxes human bronchi preconstricted with histamine and methacholine; these effects are correlated with a direct activation of BK_{Ca} channels in ASM [7]. One of the challenging issues in this field is that there are presently no specifically identified or cloned 20-HETE receptors. Therefore, we wanted to synthesize 20-HETE following a new method to assess its pharmacological and physiological effects on human bronchi derived from selective lobectomy.

We synthesized 20-HETE (Scheme 1) in a convergent way from 4 synthons **1**, **4**, **8**, and **11** [8]. The chemistry relied on repetitive Cu-mediated C-C bond formation between propargylic halides and alkynes to install four alkyne groups along the C20 linear chain [9]. The resulting tetraynic system would then be partially hydrogenated to the corresponding tetraenic molecule in which all the alkenes have the desired Z geometry.

We also synthesized the tetrayne **14** following an alternative pathway with the same four building blocks **3**, **5**, **8**, and **13**.

We then proceeded to collect the isomeric tension measurements. The mechanical effects induced by specific agonists and eicosanoids were measured as previously reported [7]. Bronchial rings were mounted in isolated organ baths, containing 6 mL of Krebs solution at 37°C, continually gassed with 95% O_2: 5% CO_2 mixture and to which an initial load of 0.8 g was applied. Tissues were allowed to equilibrate for 1 h in Krebs solution and washed out every 15 min. Passive and active tensions were assessed using transducer systems (Radnoti Glass Tech., Monrovia, CA) coupled to Polyview software (Grass-Astro-Med Inc, West Warwick, RI) for facilitating data acquisition and analysis. Results are expressed as means ± S. E. M. with *n* indicating the number of experiments. Statistical analyses were performed using the Student t test. Differences were considered significant when $p < 0.05$. Data curve fittings were performed using Sigma Plot 10.0 (SPSS-Science, Chicago, IL) to determine IC_{50} values [7].

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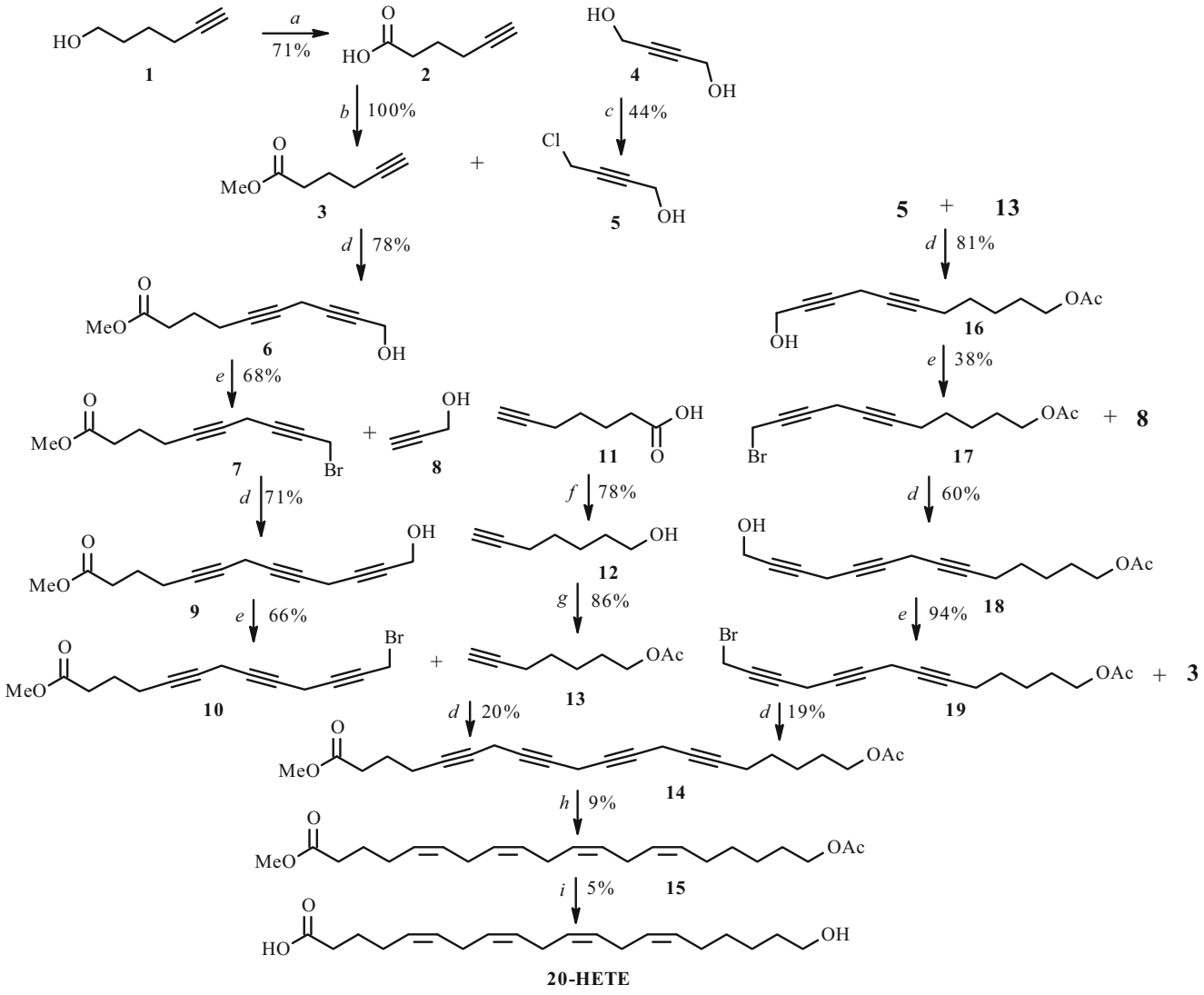


Scheme 1. Synthesis of 20-HETE

a. Jones reagent, 0°C to room temperature; *b*. MeOH, *p*-TSA, CH_2Cl_2, reflux; *c*. $SOCl_2$, Py, benz, room temperature; *d*. K_2CO_3, CuI, NaI, DMF, room temperature, *e*. PBr_3, Et_2O, room temperature; *f*. $LiAlH_4$, THF, 0°C; *g*. Ac_2O, DMAP, CH_2Cl_2, room temperature; *h*. H_2, Pd (5%), $CaCO_3$, EtOAc, quinoline, room temperature; *i*. 0.5 N aq. LiOH, room temperature.

We then found that 20-HETE relaxes human bronchial tone from the following investigation. Tension measurements were performed on distal human bronchial rings to test the effect of 20-HETE on resting tone. Tissues were subjected to 0.8 g basal tone, and cumulative concentrations of 20-HETE (0.3–10 μM) resulted in concentration-dependent relaxing effects (Fig. 1*a*); 3 μM 20-HETE yielded a mean relaxation of 2.3 ± 0.2 g on human bronchi. Vehicle, ethanol, had no significant effect on the resting tone (0.1–0.3%). Cumulative concentrations of 20-HETE (0.01–10 μM) resulted in concentration-dependent relaxing effects with an IC_{50} value of 0.49 μM (Fig. 1*b*).

Then, the effects of 20-HETE were assessed on bronchi precontracted with 1 μM MCh. Once the plateau phase of precontracted tissues was reached, addition of cumulative concentrations of 20-HETE resulted in concentration-dependent relaxing effects on the active tone (Fig. 2*a*). Figure 2*b* quantifies the mean concentration-dependent relaxing effects induced by 20-HETE on 1 μM MCh precontracted bronchi, with an IC_{50} value of 0.72 μM. Thus the effect of 20-HETE pretreatment was assessed on histamine and metacholine precontracted bronchi. Figure 2c depicts two sequential recordings in which 3 μM 20-HETE pretreatment resulted in a decrease of 48% of the amplitude of contraction induced by 1 μM histamine, when compared to the control response (Fig. 2*c*). Quantitative analysis demonstrated that the 20-HETE pretreatment significantly reduced the contractile responses to 1 μM histamine or 1 μM MCh on human bronchi (Fig. 2*d*).

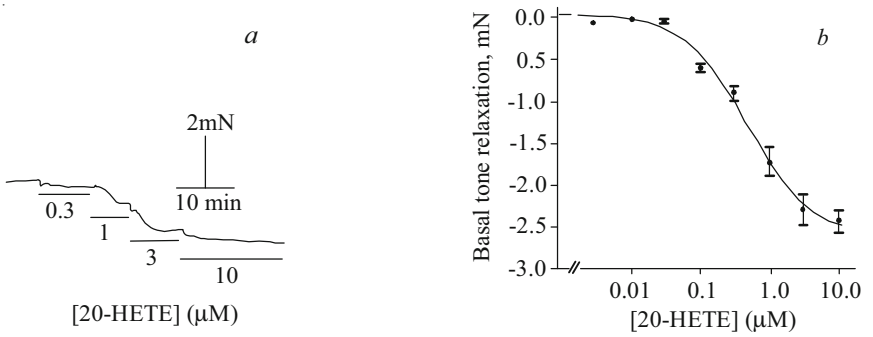


Fig. 1. 20-HETE-induced concentration dependent relaxations of human bronchial tone: Relaxing effect induced by cumulative addition of 20-HETE on the resting tension from a distal human bronchi (*a*); Cumulative concentration response curve (CCRC) to 20-HETE on resting tone (*b*). Each point represents the mean ±S.E.M. with n = 12.

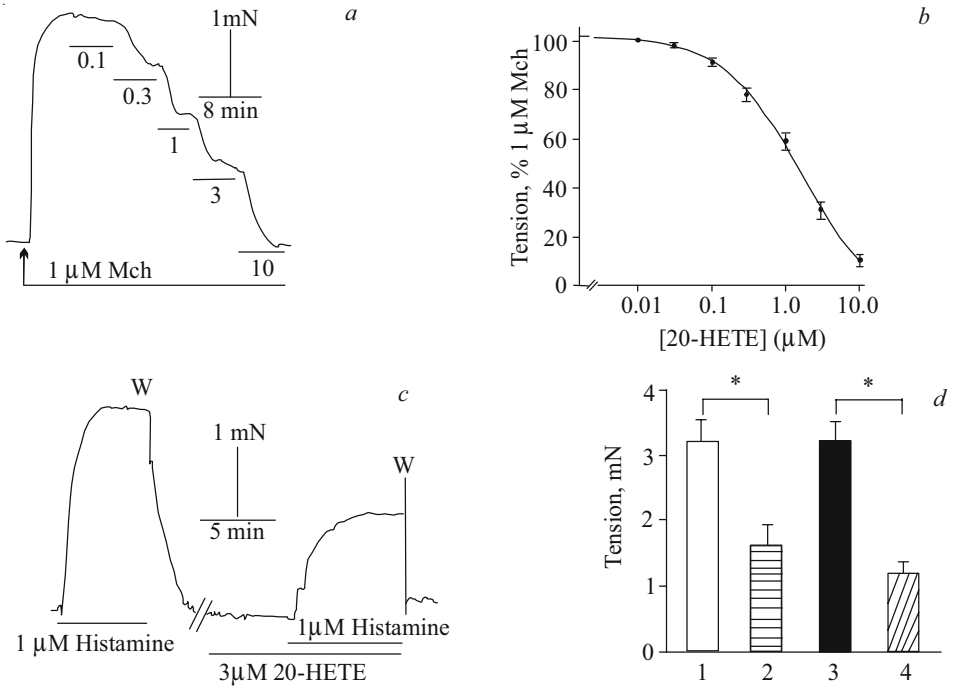
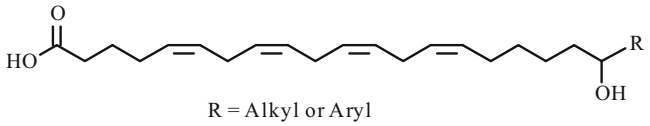


Fig. 2. Effect of 20-HETE on human bronchi precontracted with pharmacological agonists: Typical recording showing the relaxing effect induced by 20-HETE on bronchi precontracted with 1 μM methacholine (MCh) (*a*); CCRS to 20-HETE on precontracted bronchi, n = 15 (*b*); Sequential recordings to 1 μM histamine before and after 3 μM 20-HETE pretreatment on bronchial rings (*c*); Bar histogram quantifying the mean inhibition effect induced by 3 μM 20-HETE: 1 – Histamine, 2 – 20-HETE + Histamine, 3 – MCh, 4 – MCh + 20-HETE (*d*). (CCRC *p < 0.05; n = 12).

In conclusion, the present study provides additional evidence that 20-HETE is easily amenable to *de novo* synthesis [8, 9]. This hydroxyl-eicosanoid is able to modulate the mechanical properties of ASM in human bronchi. Synthetic 20-HETE induces concentration-dependent relaxations on resting tone and precontracted human bronchi with properties (IC_{50} value = 0.49 μM) similar to those previously described for this hydroxyl-arachidonic acid derivative [7]. Hence, 20-HETE pretreatment was shown to modulate the histaminic as well as the muscarinic responses. Despite the fact that stability and solubility are still an issue, these alternative strategies open the path for the synthesis of pharmacological analogs with potential therapeutic value

for specific clinical applications. We are now in a position to synthesize several 20-HETE derivatives following our synthetic route to study their biological properties toward bronchial tissues.



R = Alkyl or Aryl

EXPERIMENTAL

Hex-5-ynoic Acid (2) [10]. Hex-5-yn-1-ol (**1**) (9.8 g, 100 mmol) was dissolved in acetone (1000 mL) and cooled to 0°C. Fresh Jones reagent was added dropwise to the solution under vigorous stirring until the reacting mixture remained orange. The mixture was allowed to reach room temperature, and more Jones reagent was added to maintain the orange color. One hour after the end of the addition, isopropyl alcohol (100 mL) was added to neutralize the remaining reagent. The mixture was filtered through Celite, and the solvent was evaporated under reduced pressure. The crude blue oil was dissolved in Et_2O (500 mL), washed with water (2 × 250 mL), and dried ($MgSO_4$). The ether was evaporated, and the crude oil obtained was distilled under reduced pressure (bp. 120–125°C, 20 mm Hg) to yield a colorless oil (8.0 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.52 (2H, t, J = 7.0), 2.29 (2H, td, J = 7.0 and 2.5), 1.98 (1H, t, J = 2.5), 1.85 (2H, quin, J = 7.0).

Methyl Hex-5-ynoate (3). Hex-5-ynoic acid (**2**) (8.0 g, 71 mmol) was dissolved in MeOH (13 mL) and CH_2Cl_2 (DCM) (40 mL). *p*-TSA (116 mg, 0.6 mmol) was added, and the solution was stirred under reflux for 36 h. A saturated solution of $NaHCO_3$ (50 mL) was added to the mixture, and the organic layer was separated. The remaining aqueous layer was extracted again with DCM (2 × 20 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under vacuum. The crude product was purified by flash chromatography on silica gel using Et_2O and hexane (1:4) as eluting solvent to yield the methyl ester (**3**, 9.2 g, 100%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.68 (3H, s), 2.46 (2H, t, J = 7.0), 2.26 (2H, td, J = 2.5 and 7.0), 1.97 (1H, t, J = 2.5), 1.85 (2H, quin, J = 7.0).

4-Chloro-but-2-yn-1-ol (5) [9]. But-2-yn-1,4-diol (**4**) (30 g, 0.35 mol) was dissolved in benzene (35 mL) and pyridine (31 mL, 0.38 mol). The solution was stirred, and thionyl chloride (28 mL, 0.38 mol) was added slowly over a period of 8 h. The mixture was stirred overnight at room temperature. The solution was poured on ice (250 mL), and the organic phase was separated. The aqueous phase was extracted with Et_2O (4 × 100 mL). The combined organic phases were washed with saturated $NaHCO_3$ (200 mL) and water (200 mL). The resulting solution was dried ($MgSO_4$) and evaporated under vacuum. The residual oil was distilled under reduced pressure (bp. 80°C, 5 mm Hg) to yield a colorless oil (16.9 g, 44%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.33 (2H, t, J = 2.0), 4.18 (2H, t, J = 2.0).

Methyl 10-Hydroxydeca-5,8-diynoate (6) [9]. Before being used, K_2CO_3, CuI, and NaI were all dried in the oven at 80°C overnight. Methyl hex-5-ynoate (**3**) (7.0 g, 55 mmol) was added to a suspension of K_2CO_3 (15.4 g, 82.5 mmol), CuI (10.6 g, 55 mmol), NaI (16.6 g, 82.5 mmol), and 4-chloro-but-2-yn-1-ol (**5**) (5.8 g, 55 mmol) in DMF (100 mL). The solution was stirred under an argon atmosphere for 40 h at room temperature. The reaction mixture was diluted in EtOAc (300 mL) and filtered through Celite. The organic phase was then washed with saturated NH_4Cl (2 × 250 mL) and with brine (250 mL). The solution was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude product was purified by a really quick (10 min) pad chromatography on silica gel using EtOAc and hexane (1:1). The title product was obtained as a colorless oil (8.4 g, 78%). Longer flash purification of this compound promoted extensive degradation. This remark applies to all poly-yne compounds mentioned below. ^{1}H NMR (300 MHz, $CDCl_3$, ppm, J/Hz): 4.26 (2H, t, J = 2.0), 3.68 (3H, s), 3.18 (2H, quin, J = 2.0), 2.43 (2H, t, J = 7.0), 2.24 (2H, tt, J = 2.0 and 7.0), 1.82 (2H, quin, J = 7.0).

Methyl 10-Bromodeca-5,8-diynoate (7) [11]. The alcohol **6** (8.4 g, 43 mmol) was dissolved in anhydrous Et_2O (150 mL) under an argon atmosphere. The mixture was stirred at room temperature, and a solution of PBr_3 (5.8 mL, 61 mmol) in anhydrous Et_2O (20 mL) was added dropwise. The mixture was stirred overnight. The reaction was cooled down to 0°C, and water (300 mL) was added slowly. The aqueous phase was separated and extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with brine (200 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by a really quick (10 min) pad chromatography on silica gel using EtOAc and hexane (3:17). The bromide compound was obtained as a colorless oil (7.6 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.91 (2H, t, J = 2.0), 3.68 (3H, s), 3.20 (2H, quin, J = 2.0), 2.43 (2H, t, J = 7.0), 2.24 (2H, tt, J = 2.0 and 7.0), 1.82 (2H, quin, J = 7.0).

Methyl 13-Hydroxytrideca-5,8,11-triynoate (9). The bromide **7** (7.6 g, 30 mmol) was added to a suspension of K_2CO_3 (6.1 g, 44 mmol), CuI (5.6 g, 30 mmol), and NaI (6.7 g, 44 mmol) in DMF (75 mL). Propargyl alcohol (**8**) (1.7 g, 31 mmol) was then added to the mixture, and the solution was stirred under an argon atmosphere for 40 h at room temperature. The reaction mixture was diluted in EtOAc (300 mL) and filtered through Celite. The organic phase was then washed with saturated NH_4Cl (2 × 250 mL) and with brine (250 mL). The solution was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude product was purified by a really quick (10 min) pad chromatography on silica gel using EtOAc and hexane (2:3). The title product was obtained as a colorless oil (4.9 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.25 (2H, t, J = 2.5), 3.67 (3H, s), 3.20 (2H, quin, J = 2.5), 3.12 (2H, quin, J = 2.5), 2.43 (2H, t, J = 7.5), 2.23 (2H, tt, J = 2.5 and 7.5), 1.81 (2H, quin, J = 7.5).

Methyl 13-Bromotrideca-5,8,11-triynoate (10) [12]. The alcohol **9** (4.94 g, 21.3 mmol) was dissolved in anhydrous Et_2O (100 mL) under an argon atmosphere. The mixture was stirred at room temperature, and a solution of PBr_3 (2.83 mL, 29.8 mmol) in anhydrous Et_2O (15 mL) was added dropwise. The mixture was stirred overnight. The reaction was cooled down to 0°C, and water (400 mL) was added slowly. The aqueous phase was separated and extracted with Et_2O (3 × 150 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by a really quick (10 min) pad chromatography on silica gel using EtOAc and hexane (1:9). The title compound was obtained as a colorless oil (4.14 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.91 (2H, t, J = 2.5), 3.68 (3H, s), 3.23 (2H, quin, J = 2.5), 3.12 (2H, quin, J = 2.5), 2.43 (2H, t, J = 7.5), 2.27–2.20 (2H, m), 1.81 (2H, quin, J = 7.5).

Hept-6-yn-1-ol (12) [13]. $LiAlH_4$ (2.4 g, 63 mmol) was placed in suspension in THF (150 mL) in a dry flask under an argon atmosphere at 0°C. Then a solution of hept-6-ynoic acid (**11**, 4.0 g, 32 mmol) in THF (20 mL) was added dropwise with stirring. The mixture was allowed to warm to room temperature and was stirred overnight. HCl (110 mL, 1N) was added very slowly at 0°C. The aqueous layer was separated and extracted with Et_2O (5 × 120 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using EtOAc and hexane (1:1). A colorless oil was obtained (2.8 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.64 (2H, t, J = 6.5), 2.20 (2H, td, J = 6.5 and 2.5), 1.94 (1H, t, J = 2.5), 1.63–1.41 (6H, m).

Hept-6-ynyl Acetate (13). 6-Heptyn-1-ol (**12**, 2.77 g, 24.6 mmol) was dissolved in DCM (100 mL) and triethylamine (5.00 g, 49.2 mmol). Ac_2O (3.30 g, 32.1 mmol) was added dropwise, and then DMAP (150 mg, 1.23 mmol) was added afterward. The solution was stirred at room temperature for 5 h. The reaction mixture was washed with saturated NH_4Cl (3 × 50 mL) and brine (50 mL). The organic phase was dried ($MgSO_4$) and evaporated under vacuum. The crude product was purified by flash chromatography on silica gel using EtOAc and hexane (1:9) to obtain the protected alcohol as a colorless light oil (3.10 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.06 (2H, t, J = 6.5), 2.20 (2H, td, J = 6.5 and 2.5), 2.05 (3H, s), 1.94 (1H, t, J = 2.5), 1.67–1.46 (6H, m).

Methyl 20-Acetoxyeicosa-5,8,11,14-tetraynoate (14). The bromide **10** (4.1 g, 14 mmol) and the acetate **13** (2.2 g, 15 mmol) were dissolved in DMF (20 mL), then added to a suspension of K_2CO_3 (2.9 g, 21 mmol), CuI (2.6 g, 14 mmol), and NaI (3.9 g, 21 mmol) in DMF (100 mL). The solution was stirred under an argon atmosphere for 24 h at room temperature. The reaction mixture was diluted in EtOAc (350 mL) and filtered through Celite. The organic phase was then washed with saturated NH_4Cl (2 × 300 mL) and with brine (300 mL). The solution was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude product was purified by a quick (20 min) flash chromatography on silica gel using EtOAc and hexane (3:17). The title product was obtained as a colorless oil (0.98 g, 20%). A longer flask purification is necessary to remove all of the impurities in the mixture and to proceed with the next step. This resulted in a lower yield due to the degradation of the compound. The title compound degrades in the presence of air and light at room temperature (giving orange impurities). Consequently, **14** was stored as a frozen solution in benzene. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.06 (2H, t, J = 6.5), 3.68 (3H, s), 3.15–3.11 (6H, m), 2.43 (2H, t, J = 7.5), 2.23–2.17 (4H, m), 2.05 (3H, s), 1.81 (2H, quin, J = 7.5), 1.65–1.45 (6H, m).

Methyl (5*Z*,8*Z*,11*Z*,14*Z*)-20-Acetoxyeicosa-5,8,11,14-tetraenoate (15). Lindlar′s catalyst (Pd 5% on $CaCO_3$) (187 mg, 0.088 mmol) was weighed in a dry round bottom flask under an argon atmosphere. EtOAc (4 mL) was added to form a suspension, and a stream of H_2 was bubbled through the solvent for 10 min. The tetrayne **14** (125 mg, 0.35 mmol) and quinoline (0.004 mL, 0.035 mmol) were dissolved together in EtOAc (4 mL), and the solution was added in the flask. A stream of H_2 was again bubbled in the reaction for 5 min, and the mixture was stirred at room temperature for 1 h. The resulting mixture was diluted in EtOAc (25 mL) and filtered through Celite. The solution was washed with HCl (25 mL, 1N) and with brine (25 mL). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using EtOAc and hexane (1:19). The tetraene product was obtained as a colorless oil

(17 mg, 9%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.42–5.33 (8H, m), 4.05 (2H, t, J = 7.0), 3.66 (3H, s), 2.86–2.74 (6H, m), 2.32 (2H, t, J = 7.5), 2.14–1.98 (4H, m), 2.04 (3H, s), 1.77–1.62 (4H, m), 1.56–1.27 (4H, m). GC-MS (*m/e*, *rel intensity*): 376 (M^+, 4), 316 (4), 119 (36), 105 (43), 74 (100).

(5*Z*,8*Z*,11*Z*,14*Z*)-20-Hydroxyeicosa-5,8,11,14-tetraenoic Acid (20-HETE). The tetraene **15** (15.1 mg, 0.041 mmol) was dissolved in THF (5 mL), and lithium hydroxide (0.25 mL, 0.5 N, 0.10 mmol) was added under an argon atmosphere. The solution was stirred for 16 h at room temperature. The reaction mixture was diluted in water (20 mL), and 1N HCl was added until the pH reached 2. The solution was then extracted with DCM (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. The crude product was purified by semipreparative HPLC on a C18 column using a gradient of acetonitrile in water. The desired product came out of the column at around 60% of acetonitrile in water. 20-HETE was obtained as a colorless oil (0.7 mg, 5%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.42–5.36 (8H, m), 3.68 (2H, t, J = 6.5), 2.86–2.76 (6H, m), 2.36 (2H, t, J = 7.0), 2.15–2.01 (4H, m), 2.04 (3H, s), 1.76–1.55 (4H, m), 1.54–1.31 (4H, m).

11-Hydroxyundeca-6,9-diynyl Acetate (16). Hept-6-ynyl acetate (**13**) (1.25 g, 8.10 mmol) and 4-chloro-but-2-yn-1-ol (**5**, 1.18 g, 11.34 mmol) were dissolved in dry DMF (15 mL). Sodium iodide (2.42 g, 16.21 mmol), copper iodide (3.09 g, 16.21 mmol), and potassium carbonate (1.68 g, 12.16 mmol) were added to the solution, which was stirred at 30°C for 12 h under argon. The solution was diluted in EtOAc (60 mL) and filtered through Celite. The organic phase was then washed with saturated NH_4Cl (2 × 30 mL) and brine (30 mL), dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography with EtOAc–hexane mixture (1:1) to afford a yellow oil (1.46 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.25 (1H, m), 4.07 (2H, t, J = 7.0), 3.18 (2H, t, J = 2.0), 2.18 (2H, m), 2.05 (3H, s), 1.72–1.40 (8H, m).

11-Bromoundeca-6,9-diynyl Acetate (17). 11-Hydroxyundeca-6,9-diynyl acetate (**16**, 1.46 g, 6.57 mmol) was dissolved in anhydrous ether (50 mL), and phosphorus tribromide (1.24 g, 4.60 mmol) (10% solution in anhydrous ether) was added dropwise. The reaction was stirred at room temperature for 12 h under argon, cooled down to 0°C, and water (100 mL) was added slowly. The aqueous phase was separated and extracted with Et_2O (3 × 30 mL). The combined organic phases were dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography with EtOAc–hexane mixture (1:9) to afford an oil (710 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.07 (2H, t, J = 7.0), 3.91 (2H, t, J = 2.0), 3.21 (2H, t, J = 2.0), 2.18 (2H, m), 2.04 (3H, s), 1.72–1.40 (8H, m).

14-Hydroxytetradeca-6,9,12-triynyl Acetate (18). 11-Bromoundeca-6,9-diynyl acetate (**17**, 660 mg, 2.31 mmol) and propargyl alcohol **8** (182 mg, 3.24 mmol) were dissolved in dry DMF (10 mL). Sodium iodide (692 mg, 4.62 mmol), copper iodide (879 mg, 4.62 mmol), and potassium carbonate (479 mg, 3.47 mmol) were added to the solution, which was stirred at 30°C for 12 h under argon. The solution was diluted in EtOAc (40 mL) and filtered through Celite. The organic phase was then washed with saturated NH_4Cl (2 × 20 mL) and brine (20 mL), dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by flask chromatography with EtOAc–hexane mixture (4:6) to afford a yellow oil (360 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.25 (2H, m), 4.07 (2H, t, J = 7.0), 3.20 (2H, br), 3.13 (2H, br), 2.18 (2H, m), 2.05 (3H, s), 1.72–1.40 (8H, m).

14-Bromotetradeca-6,9,12-triynyl Acetate (19). 14-Hydroxytetradeca-6,9,12-triynyl acetate (**18**, 360 mg, 1.38 mmol) was dissolved in anhydrous ether (15 mL); phosphorus tribromide (261 mg, 0.97 mmol) (10% solution in anhydrous ether) was added dropwise, and the reaction was stirred at room temperature for 12 h under argon. The reaction was cooled down to 0°C, and water (20 mL) was added slowly. The aqueous phase was separated and extracted with Et_2O (3 × 15 mL). The combined organic phases were dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to afford a crude oil (420 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.10 (2H, m), 3.91 (2H, m), 3.22 (2H, br), 3.13 (2H, br), 2.18 (2H, m), 2.05 (3H, s), 1.70–1.40 (8H, m).

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