Synthesis of [9-^{3}H]-*trans*-4-Hydroxy-2-nonenal

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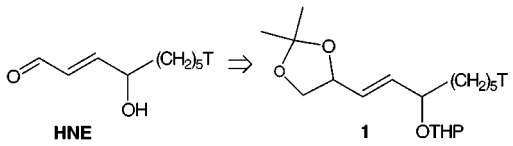
Because polyunsaturated lipids are prone to oxidative damage, aerobic organisms wage a continual and, ultimately, losing battle against oxidative injury.[1] The failure of a redoubtable array of enzymatic and chemical antioxidant defenses has pathological consequences that contribute to aging and a host of degenerative disease processes. Oxidation not only damages lipids but also generates reactive electrophilic products that chemically modify biological nucleophiles.[2,3] An especially cytotoxic aldehyde, *trans*-4-hydroxy-2-nonenal (HNE), is created by free-radical oxidative cleavage of arachidonate and linoleate esters.[3] HNE forms hemiacetal-stabilized Michael adducts of protein-based thiol,[3] imidazole, and amino groups,[4−7] as well as pyrroles that incorporate protein amino groups.[8] Such covalent modifications may result in enzyme inhibition,[4,5,7,9−12] neurodegeneration,[13−15] or receptor-mediated uptake by macrophages, e. g., of low-density lipoprotein (LDL) that occurs during the initial stages of atherosclerosis.[16−22]

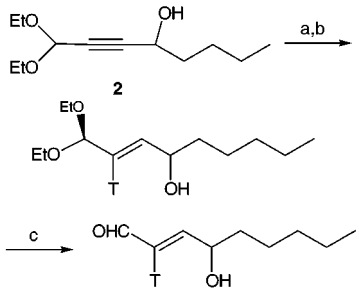
To facilitate studies on the reactions of HNE with biological nucleophiles and on the chemistry of the resulting covalent adducts, we developed a synthesis of tritium-labeled HNE. To rule out the possibility of radiolabel loss by exchange, tritium was appended to the terminal methyl of an *n*-pentyl chain. Because HNE is prone to decomposition, the synthesis was designed to generate this reactive aldehyde from a stable precursor **1**, which is suitable for long-term storage. Furthermore, to minimize the necessity for manipulation of radioactive intermediates, the introduction of tritium was delayed until the last step of the synthesis of **1**.



Results and Discussion

Previous Syntheses of Tritium Labeled HNE. [2-^{3}H]-*trans*-4-Hydroxy-2-nonenal was prepared (Scheme 1) by hydroalumination of alkyne **2** followed by hydrodealumination by treatment with T_2O and, finally, hydrolysis of the acetal.[23] Isotope exchange during reactions of [2-^{3}H]-*trans*-4-hydroxy-2-nonenal is anticipated since several tautomerization reactions are possible. Indeed, some loss (7%) of label was observed during Michael addition of *N*-acetylcysteine to [2-^{2}H]-*trans*-4-hydroxy-2-nonenal in dilute aqueous NaOH.

Scheme 1[a]



[a] Key: (a) $LiAlH_4$; (b) T_2O; (c) H^+, H_2O.

[4-^{3}H]-*trans*-4-Hydroxy-2-nonenal is readily available (Scheme 2) by reduction of ketone **3** with $NaBT_4$.[24,25] However, complete loss of tritium is anticipated for the reaction of [4-^{3}H]-*trans*-4-hydroxy-2-nonenal with primary amines that affords 2-pentylpyrroles, a reaction that occurs when proteins react with HNE.[8] This "pyrrolyzation" of proteins is especially interesting because we found that such protein modifications are present in

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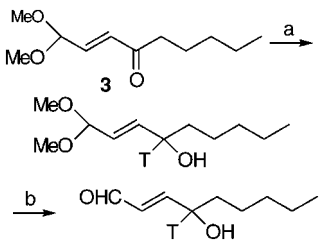
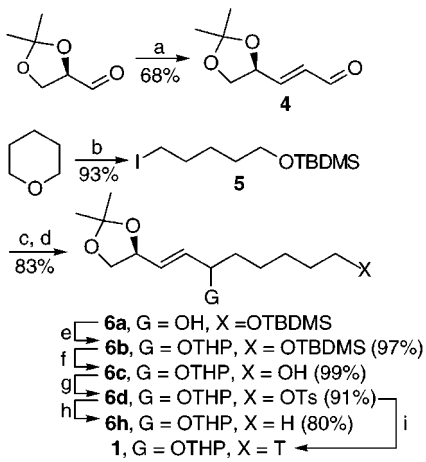
Scheme 2[a]



[a] Key: (a) $NaBT_4$; (b) H^+, H_2O.

Scheme 3[a]



[a] Key: (a) $Ph_3P{=}CHCHO$; (b) NaI, TBDMSCl, $CaCO_3$, THF; (c) **5**, Mg, Et_2O; (d) **4**; (e) DHP, PPTS, CH_2Cl_2; (f) Bu_4NF, THF; (g) Ts_2O, CH_2Cl_2; (h) $NaBH_4$, DMSO; (i) $NaBT_4$, DMSO.

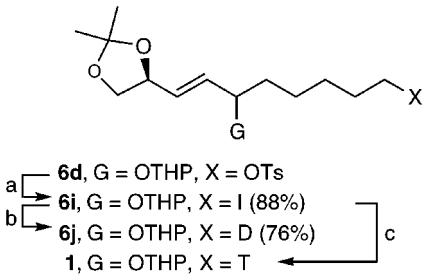
the neurofibrillary tangles that are a hallmark of Alzheimer's disease.[13]

A Stable Tritiated Precursor. Carbons 1−4 of the target molecule were provided by a synthetic equivalent **4** of fumaric dialdehyde in which one of the formyl groups is present in latent form as a 3,3-dimethyl-2,4-dioxolanyl moiety (Scheme 3). Wittig olefination of 2,3-*O*-isopropylidene-D-glyceraldehyde[26] with (formylmethylene)triphenylphosphorane delivered 3-(3,3-dimethyl-2,4-dioxolanyl)-prop-2-enal (**4**). Carbons 5−9 of the target molecule were provided by the TBDMS ether **5** of 5-iodopentanol that is readily available from TBDMS chloride, NaI, and tetrahydropyran.[27] Addition to aldehyde **4** of a Grignard reagent prepared from iodide **5** provided alcohol **6a**. Protection of the secondary alcohol as a THP derivative **6b** and desilylation afforded the primary alcohol **6c**. Tritium was introduced by reduction of the corresponding tosylate **6d** with $NaBT_4$ in DMSO.

An alternative route for converting tosylate **6d** into isotopically labeled precursors of HNE was also explored (Scheme 4). Thus, reduction of the derived iodide **6i** with zinc−copper couple in the presence of D_2O afforded the precursor **6j** for [9-^{2}H]-*trans*-4-hydroxy-2-nonenal. This approach allows the tritium in the key intermediate **1** to be derived from T_2O, which is less expensive than $NaBT_4$ and is available with high specific activity.

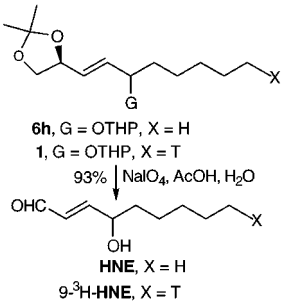
Generation of HNE by Oxidative Cleavage of 3-Decene-1,2,4-triol. Completion of our synthesis of

Scheme 4[a]

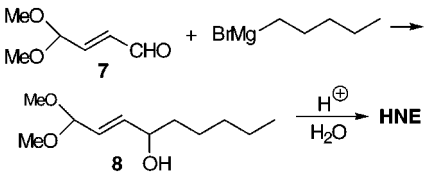


[a] Key: NaI, acetone; (b) Zn(Cu), D_2O; (c) An(Cu), T_2O.

Scheme 5



Scheme 6



HNE and its 9-^{3}H derivative was accomplished by an efficient one-pot procedure that we have exploited previously to generate the chemically sensitive δ-hydroxy-β,γ-unsaturated aldehyde array present in levuglandins. Thus, exposure of **6h** or **1** to a solution of $NaIO_4$ in aqueous acetic acid generated the corresponding γ-hydroxy-α,β-unsaturated aldehyde in excellent yield (Scheme 5).

Previously, HNE was prepared by a related synthetic strategy involving generation of the acetal intermediate **8** by addition of a pentyl Grignard reagent to the monoacetal **7** of fumaric dialdehyde followed by deprotection of the resulting hydroxy acetal **8** (Scheme 6).[24] However, we abandoned this approach for preparing a chemically stable tritiated precursor of HNE because intermediates containing the dimethyl acetal masking group were too susceptible to adventitious hydrolysis. The 3,3-dimethyl-2,4-dioxolanyl moiety that serves as a latent formyl group in our intermediates is much more robust than the dimethyl acetal.

Experimental Section

General Methods. All proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini spectrometer operating at 300 MHz. Proton chemical shifts are reported in parts per million (ppm) on the δ scale relative to tetramethylsilane (δ 0.00) or $CHCl_3$ (δ 7.24). ^{1}H NMR spectral data are tabulated in terms of multiplicity of proton absorption (s, singlet; d, doublet; dd, doublet of doublet; t triplet; m multiplet; br broad), coupling constants (Hz), and number of protons. Carbon magnetic resonance (^{13}C NMR) spectra were recorded on a Varian Gemini spectrometer operating at 75 MHz. These spectra are reported

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in ppm on the δ scale relative to $CDCl_3$ (δ 77.0). Proton and carbon NMR samples were analyzed as solutions in $CDCl_3$. All high-resolution mass spectra were recorded on a Kratos AEI MS25 RFA high-resolution mass spectrometer at 20 eV. All solvents were distilled under a nitrogen atmosphere prior to use. Tetrahydrofuran was distilled over potassium and benzophenone. Methylene chloride, diethyl ether, and *N*,*N*-dimethyl formamide (DMF) were distilled over calcium hydride. Chloroform and carbon tetrachloride were distilled over P_2O_5.

Chromatography was performed with ACS-grade solvents (ethyl acetate, hexane). High-performance liquid chomatography (HPLC) was performed with HPLC-grade solvents using a Waters Associates system consisting of a Waters M6000A solvent delivery system and a Waters U6K injector. The eluants were monitored using a Waters R401 differential refractometer. Thin-layer chromatography (TLC) was performed on glass plates precoated with silica gel (Kieselgel 60 F_{254}, E. Merck, Darmstadt, West Germany); R_f values are quoted for plates of thickness 0.25 mm. The plates were visualized by viewing the developed plates under short-wavelength UV light or with iodine or by heating the plates after spraying with vanillin−sulfuric acid. Flash column chromatography was performed on 230−400 mesh silica gel supplied by E. Merck. All reactions performed in an inert atmosphere were in argon unless otherwise specified. (Formylmethylene)triphenylphosphorane was prepared as described previously[28] or obtained from Lancaster Synthesis. Isopropylidene-D-glycerladehyde[26] and 1-[(*tert*-butyldimethylsilyl)oxy]-5-iodopentane (**5**)[27] were prepared as described previously.

(*E*)-3-(3,3-Dimethyl-2,4-dioxolanyl)prop-2-enal (4). To a stirred suspension of (formylmethylene)triphenylphosphorane (1.98 g, 6.5 mmol) in toluene (30 mL) at 0 °C[29] was added 2,3-*O*-isopropylidene-D-glyceraldehyde (845.0 mg, 6.5 mmol)[26] dropwise. The resulting mixture was stirred for 39 h at 0−3 °C. Then the solvent was removed on a rotary evaporator. The residue was extracted with ethyl ether. Evaporation of the extracts gave a crude product that was purified by flash chromatography on silica gel (ethyl acetate−hexanes 15:85) to yield compound **4** (690 mg, 68%): R_f = 0.19 (ethyl acetate−hexanes 15:85); ^{1}H NMR ($CDCl_3$) δ 9.57 (d, J = 7.8 Hz, 1H), 6.74 (dd, J = 15.7, 5.4 Hz, 1H), 6.33 (ddd, J = 15.7, 7.8, 1.2 Hz, 1H), 4.77 (m, 1H), 4.23 (dd, J = 15.6, 6.7 Hz, 1H), 3.71 (dd, J = 6.7, 8.2 Hz, 1H), 1.44 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, APT) δ 193.00 (CHO), 153.06 (CH=CH), 132.38 (CH=CH), 110.55 (−C−), 74.83 (CH), 68.70 (CH_2), 26.42 (CH_3), 25.62 (CH_3); HRMS (EI) *m*/*z* calcd for $C_7H_9O_3$ (M^+ − Me) 141.0552, found 141.0552.

(1*E*)-1-(3,3-Dimethyl-(2,4-dioxolanyl)-8-(1,1,2,2-tetramethyl-1-silapropoxy)oct-1-en-3-ol (6a). Magnesium turnings (296.5 mg, 12.2 mmol) and 2.0 mL of dry ethyl ether were placed in a 125 mL, flame-dried, three-necked flask with mechanical stirrer and reflux condenser under an argon atmosphere at room temperature. A few drops of a solution of iodide **5** (2.0 g, 6.1 mmol) in dry diethyl ether (1 mL) were added. After formation of the Grignard reagent began, another 30 mL of dry ethyl ether were added and the remaining iodide solution was added dropwise. After complete addition, the resulting mixture was stirred for another 1 h and then chilled to 0 °C, followed by slow addition of sufficient 3-(3,3-dimethyl-2,4-dioxolanyl)prop-2-enal (**4**) (350 mg, 2.24 mmol) to consume all of the Grignard reagent as determined with Michler's ketone. After the mixture was stirred for another 30 min, saturated aqueous NH_4Cl was added, the product was then extracted into Et_2O and dried with anhydrous $MgSO_4$, and solvent was removed by rotary evaporation. The crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 2:8), affording **6a** (667 mg, 83% yield based on **4**): R_f = 0.23 (ethyl acetate−hexanes 2:8); ^{1}HNMR (**6a***R* + **6a***S*, $CDCl_3$) δ 5.81 (dd, J = 15.2, 6.0 Hz, 1H), 5.64 (ddd, J = 1 5.3, 7.1, 2.6 Hz, 1H), 4.49 (dd, J = 14.0, 7.4 Hz, 1H), 4.05−4.13 (m, 2H), 3.57 and 3.55 (2t, J = 3.5 Hz, J = 4.1 Hz, 1H, from two diasteromers), 1.25−1.60 (8H), 1.40 (s, 3H), 1.37 (s, 3H), 0.87 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (**6a***R* + **6a***S*, $CDCl_3$, APT) δ 137.43, 137.36 (CH, two diasteromers) 127.88, 127.79 (CH), 109.40 (−C−), 76.63 (CH), 72.04 (CH), 69.48 (CH_2), 63.17 (CH_2), 37.16, 37.04 (CH_2), 32.78 (CH_2), 26.71 (CH_3), 26.01 (CH_2), 25.93, 25.77 (CH_2), 25.23 (CH_3), 25.17 (CH_3), 18.41 (−C−), −5.22 (CH_3); HRMS *m*/*z* calcd for $C_{18}H_{35}O_4Si$ (M^+ − CH_3) 343.2305, found 343.2380.

(7*E*)-1-[[8-(3,3-Dimethyl-2,4-dioxolanyl)-6-(2-oxanyloxy)-oct-7-enyl]oxy)-1,1,2,2-tetramethyl-1-silapropane (6b). A small amount of pyridinium *p*-toluenesulfonate (PPTS) was added to a solution of compound **6a** (50 mg, 0.14 mmol) and dihydropyran (18.0 mg, 0.21 mmol) in dry methylene chloride (2 mL).[30] The resulting solution was stirred overnight at room temperature, water (2 mL) was added, and the resulting mixture was extracted with ethyl ether. The organic solution was washed with half-saturated brine. The solvent was removed by rotary evaporation. The crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 8:92), affording **6b** (60.0 mg, 97%): R_f = 0. 20 (ethyl acetate−hexanes 1:9); ^{1}H NMR ($CDCl_3$) δ 5.55−5.85 (m, 2H), 4.55−4.80 (m, 1H), 4.42−4.54 (m, 1H), 3.98−4.12 (m, 2H), 3.76−3.88 (m, 1H), 3.35−3.60 (m, 2H), 3.56 (t, J = 6.4 Hz, 1H), 1.20−1.80 (14H), 1.40 (s, 3H), 1.35 (s, 3H), 0.89 (s, 9H), 0.02 (s, 6H); HRMS *m*/*z* calcd for $C_{23}H_{43}O_5Si$ (M^+ − CH_3) 427.2880, found 427.2862.

(7*E*)-8-(3,3-Dimethyl-2,4-dioxolanyl)-6-(2-oxanyloxy)oct-7-en-1-ol (6c). *n*-Bu_4NF (135 μL, 1 M in THF, 0.135 mmol) was added dropwise to a stirred solution of the silyl ether **6b** (20.0 mg, 0.045 mmol) and THF (0.5 mL).[31] The resulting mixture was stirred at room temperature overnight. Water (1 mL) was then added. The resulting mixture was extracted with ethyl ether, washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on silica gel (ethyl acetate−hexanes 3:7) to afford **6c** (14.5 mg, 99%): R_f = 0.19 (ethyl acetate−hexanes 3:7); ^{1}H NMR ($CDCl_3$) δ 5.55−5.86 (m, 2H), 4.44−4.68 (m, 2H), 4.02−4.16 (m, 2H), 3.76−3.88 (m, 1H), 3.52−3.68 (m, 2H), 3.36−3.54 (m, 1H), 1.20−1.80 (14H), 1.39 (s, 3H), 1.36 (s, 3H); HRMS calcd for $C_{17}H_{29}O_5$ (M^+ − CH_3) 313.2015, found 313.2019.

[(7*E*)-8-3,3-Dimethyl-2,4-dioxolanyl)-6-(2-oxanyloxy)oct-7-enyl]oxy Tosylate (6d). A solution of the alcohol **6c** (70.0 mg, 0.21 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (51.0 mg, 0.42 mmol) in dry CH_2Cl_2 (3 mL) was stirred 10 min at 0 °C. Then, *p*-toluenesulfonic anhydride (138.0 mg, 0.42 mmol) was added.[32] The resulting mixture was stirred for 18 h at 0−5 °C. The solvent was removed on a rotary evaporator. The residue was triturated with ethyl ether (3 × 10 mL). After rotary evaporation of the solvent, the residue was purified by flash chromatography on silica gel (ethyl acetate−hexanes 3:7) to give **6d** (91.8 mg, 91%): R_f = 0.36 (ethyl acetate−hexanes 4:6); ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 5.52−5.84 (m, 2H), 4.44−4.10 (m, 2H), 4.00−4.10 (m, 2H), 3.98 (t, J = 6.3 Hz, 2H), 3.80 (m, 1H), 3.55 (m, 1H), 2.43 (s, 3H), 1.20−1.90 (14H), 1.40 (s, 3H), 1.36 (s, 3H); HRMS calcd for $C_{24}H_{35}O_7S$ (M^+ − Me) 467.2103, found 467.2121.

(2*E*)-2-[[3-(3,3-Dimethyl-2,4-dioxolanyl)-1-pentylprop-2-enyl]oxy]oxane (6h). Method A. Sodium borohydride (1.8 mg, 0.048 mmol) in dimethyl sulfoxide (0.5 mL) was added to **6d** (8.0 mg, 0.016 mmol).[33] The reaction mixture was heated at 85 °C for 2 h and diluted with water (2 mL). The resulting solution was extracted with diethyl ether (3 × 5 mL). The combined ether extracts were dried with anhydrous magnesium sulfate, and solvent was removed by rotary evaporation. The crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 8:9) to yield compound **6h** (4.0 mg, 80%): R_f = 0.4 (ethyl acetate−hexanes 4:6); ^{1}H NMR ($CDCl_3$) δ 5.54−5.88 (m, 2H), 4.56−4.70 (m, 1H), 4.50 (m, 1H), 4.05 (m, 2H), 3.84 (m, 1H), 3.42 (m, 1H), 1.40−1.90 (14H), 1.39 (s, 3H), 1.36 (s, 3H), 0.85 (t, J = 6.4 Hz, 3H); HRMS calcd for $C_{18}H_{32}O_4$ (M^+) 312.2300, found 312.2290, calcd for $C_{17}H_{29}O_4$ 297.2067 (M^+ − Me), found 297.2057.

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Method B. Magnesium turnings (1.0 g, 41.4 mmol) and dry ethyl ether (2.0 mL) were placed in a flame-dried three-necked 125 mL flask with a mechanical stirrer and reflux condenser under an argon atmosphere at room temperature. A few drops of a solution of 1-bromopentane (2.5 g, 16.5 mmol, freshly distilled) in dry diethyl ether (1 mL) were added. After the formation of Grignard reagent began, another 30 mL of dry ether were added. The remaining bromide solution was then added dropwise. After completion of the addition, the reaction mixture was stirred overnight and then chilled to 0 °C, followed by dropwise addition of aldehyde **4** (300.0 mg, 1.92 mmol) until all of the organometallic was consumed as determined with Michler's ketone. After the mixture was stirred for 30 min, saturated aqueous NH_4Cl was added, and the resulting mixture was extracted with diethyl ether. The extract was dried with anhydrous $MgSO_4$. The solvent was removed on a rotary evaporator. The crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 35:65) to give **(1*E*)-1-(3,3-dimethyl-2,4-dioxolanyl)oct-1-en-3-ol** (400.0 mg, 91.3% yield based on **4**): R_f = 0.28 (ethyl acetate−hexanes 35:65); ^{1}H NMR ($CDCl_3$) δ 5.78 (ddd, J = 16.0, 6.3, 1.8 Hz, 1H), 5.64(ddd, J = 16.6, 6.5, 2.8 Hz, 1H), 4.48 (dd, J = 13.8, 7.3 Hz, 1H), 4.05 (m, 2H), 3.56 (td, J = 8.0, 1.8 Hz, 1H), 1.70 (m, 2H), 1.20−1.60 (14H), 1.39 (s, 3H), 1.36 (s, 3H), 0.85 (t, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, APT, two diasteromers) δ 137.48, 137.42 (CH), 127.81, 127.74 (CH), 90.39 (−C−), 76.65, 76.58 (CH), 72.10, 71.98 (CH), 69.49, 69.47 (CH_2), 31.12, 31.02 (CH_2), 31.73 (CH_2), 26.70 (CH_3), 26.01 (CH_3), 25.08, 25.04 (CH_2), 22.60 (CH_2), 14.05 (CH_3); HRMS calcd for $C_{13}H_{24}O_3$ (M^+) 228.1725, found 228.1729.

A small amount of pyridinium *p*-toluenesulfonate (PPTS) was added to a solution of the above alcohol (52.0 mg, 0.23 mmol) and dihydropyran (29.0 mg, 0.35 mmol) in dry methylene chloride (1 mL). The resulting solution was stirred overnight at room temperature, diluted and extracted with ethyl ether, and washed with half-saturated brine. The solvent was removed on a rotary evaporator. The crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 1:90) to afford **6h** (60 mg, 97%): R_f = 0.20 (ethyl acetate−hexanes 12:88).

[9^{3}H]-(2*E*)-2-[[3-(3,3-Dimethyl-2,4-dioxolanyl)-1-pentyl-prop-2-enyl]oxy]oxane (1). Tosylate **6d** (8.0 mg, 1.67 μmol) was dissolved in dimethyl sulfoxide (200 μL) and added to sodium borotritide (0.63 mg, 1.67 μmol, 25 mCi) in a vial under an argon atmosphere.[34] The reaction mixture was stirred magnetically and heated at 85 °C for 2 h and then cooled to room temperature, diluted by adding water (1 mL), and extracted with diethyl ether (2 × 3 mL). The ether extracts were dried with anhydrous magnesium sulfate, and solvent was evaporated by rotary evaporation. The crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 1:9) to yield tritiated precursor **1** (322.7 μCi): R_f = 0.20 (ethyl acetate−hexanes 12:88). TLC showed an R_f identical to that of the unlabeled analogue **6h**.

[9-^{3}H]-(2*E*)-4-Hydroxynon-2-enal. The hot tritiated precursor **1** was mixed with **6h** (cold analogue), and then the mixture was purified again by flash chromatography on silica gel (ethyl acetate−hexanes 1:9) to give a mixture (**1** + **6h**) (32.0 mg) that exhibited a specific activity of 3.35 mCi/mmol. Sodium periodate (3.0 mg, 0.014 mmol) was dissovled in acetic acid/water (2:1, v/v, 300 μL). The solution was added to the mixture of **1** + **6h** (3 mg, 0.0096 mmol). The reaction mixture was heated at 40 °C for 4 h and then neutralized to pH = 7 by the portionwise addition of saturated aqueous sodium bicarbonate, extracted with diethyl ether, and dried with anhydrous magnesium sulfate. The solvent was removed on a rotary evaporater, and the crude product was purified by flash chromatography on silica gel (ethyl acetate−hexanes 3:7) to afford [9-^{3}H]-HNE (1.4 mg, 93.3%, 3.35 mCi/mmol): R_f = 0.23 (ethyl acetate−hexanes 3:7). The structure was confirmed by its ^{1}H NMR spectrum, which was indistinguishable from that of unlabeled HNE.

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Supporting Information Available: Details of the syntheses of **6i** from **6d**, **6j** from **6i**, and **HNE** from **6h**, as well as NMR spectra of all new compounds (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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