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Synthesis of deuterium-labeled analogs of the lipid hydroperoxide-derived bifunctional electrophile 4-oxo-2(*E*)-nonenal

Jasbir S. Arora^a, Tomoyuki Oe^b, and Ian A. Blair^{a,*}

^aCenter for Cancer Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA

^bDepartment of Bio-analytical Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Aobaku, Sendai 980-8578, Japan

Abstract

Lipid hydroperoxides undergo homolytic decomposition into the bifunctional 4-hydroxy-2(*E*)-nonenal and 4-oxo-2(*E*)-nonenal (ONE). These bifunctional electrophiles are highly reactive and can readily modify intracellular molecules including glutathione (GSH), deoxyribonucleic acid (DNA) and proteins. Lipid hydroperoxide-derived bifunctional electrophiles are thought to contribute to the pathogenesis of a number of diseases. ONE is an α,β -unsaturated aldehyde that can react in multiple ways and with glutathione, proteins and DNA. Heavy isotope-labeled analogs of ONE are not readily available for conducting mechanistic studies or for use as internal standards in mass spectrometry (MS)-based assays. An efficient onestep cost-effective method has been developed for the preparation of C-9 deuterium-labeled ONE. In addition, a method for specific deuterium labeling of ONE at C-2, C-3 or both C-2 and C-3 has been developed. This latter method involved the selective reduction of an intermediate alkyne either by lithium aluminum hydride or lithium aluminum deuteride and quenching with water or deuterium oxide. The availability of these heavy isotope analogs will be useful as internal standards for quantitative studies employing MS and for conducting mechanistic studies of complex interactions between ONE and DNA bases as well as between ONE and proximal amino acid residues in peptides and proteins.

Keywords

4-oxo-2-nonenal; oxidative stress; lipid peroxidation; deuterium labeling; DNA-adducts; protein adducts; pentyl furan

Introduction

Reactive oxygen species (ROS) and oxidative stress have received increasing attention because of their potential role in re-perfusion injury,¹ cancer² and age-related diseases.³

Oxidative stress results in ROS-mediated conversion of polyunsaturated lipids into hydroperoxides. Subsequent homolytic decomposition of the lipid hydroperoxides leads to the intermediate formation of 4-hydroperoxy-2(*E*)-nonenal which decomposes to the α,β -unsaturated aldehydes, 4-oxo-2(*E*)-nonenal (ONE) and 4-hydroxy-2(*E*)-nonenal (HNE).⁴⁻⁶ ONE is more neurotoxic and more reactive toward proteins than HNE.⁷ It is also a particularly potent cytotoxin and genotoxin,^{8,9} which reacts with deoxyribonucleic acid (DNA) bases such as 2'-deoxyguanosine (dGuo) to form heptanone-etheno-dGuo adducts.¹⁰ ONE also reacts with histone proteins leading to the formation of novel cyclic peptide adducts.¹¹ The ONE adducts with hemoglobin,¹² *N*-acetyl-cysteine^{13,14} and GSH¹⁵ could be useful as biomarkers various oxidative stress-related diseases.

The 4-oxo group of ONE inductively enhances the electrophilicity of C-3 making it more susceptible to nucleophilic attack than HNE. This enhanced electrophilicity provides an explanation for the greater cytotoxicity and DNA reactivity of ONE when compared with HNE. In order to comprehensively study the role of ONE in its interactions with both small molecules and macromolecule, it is necessary to have reliable and efficient methods available for the synthesis of specifically heavy isotope-labeled analogs. We have previously reported a multi-step synthesis of ONE and HNE starting from ethyl (triphenylphosphoranylidene) acetate.¹⁶ Herein, we report the synthesis of the deuterium-labeled version of ONE with selective labeling at C-9, C-2, C-3 and C-2/C-3 positions.

Experimental

Materials and instruments

NBS, sodium thiosulfate, citric acid, magnesium, bromoethane, propionaldehyde diethylacetal, hexanal, LAH, MnO₂ and citric acid were purchased from Aldrich. 2-pentyl furan was purchased from Paulter and Baumann. NMR spectra were determined at 25°C using a Varian UNITY 500 instrument with Tetramethylsilane (TMS) as a reference internal standard. HRMS was performed using a Micromass Autospec in the Chemistry department of University of Pennsylvania.

Synthesis section

ONE from 2-pentylfuran (4a)—To a solution of 2-pentyl furan **3a** (2.5 g, 18.1 mmol) and pyridine (2.8 mL, 35.1 mmol) in THF–acetone–H₂O (5:4:1, 60 mL) was added NBS (3.86 g, 21.7 mmol) dissolved in THF–acetone–H₂O (5:4:1, 20 mL) at –20°C. The solution was stirred for 1 h at –20°C and 6 h at room temperature and then poured into a mixture of ethyl acetate and aqueous Na₂S₂O₃. The organic layer was separated and washed with aqueous citric acid solution (pH 3). The organic layer was dried and evaporated under reduced pressure. The residue was purified on a silica gel column using 10% ethyl acetate in hexane to afford the title compound (1.23 g, 44%). ¹H-NMR (500 MHz, CDCl₃) δ : 0.9 (*t*, *J* = 6.9 Hz, 3H, C(9)-CH₃), 1.34–1.38 (*m*, 4H, C(7,8)-CH₂), 1.66 (*p*, *J* = 7.5 Hz, 2H, C(6)-CH₂), 2.68 (*t*, *J* = 7.4 Hz, 2H, C(5)CH₂), 6.76 (*dd*, *J* = 6.9, 16.2 Hz, 1H, C(8)-CH), 6.87 (*d*, *J* = 16.2 Hz, 1H, C(7)-CH), 9.78 (*d*, *J* = 6.9 Hz, 1H, CH). ¹³C-NMR (CDCl₃) δ : 13.7, 22.3, 23.2, 31.1, 41.0, 137.1, 144.9, 193.4, 200.0.

9-[²H₃]-ONE (4b)—*n*-Butyl lithium (19.5 mL, 2.5 M in hexane, 48.8 mmol) was added to furan **1** (5.90 mL, 81.21 mmol) in anhydrous THF (75 mL) under argon at 0°C. The resulting brown solution was brought to room temperature and stirred for additional 3 h. Bromopentane **2** (2 g, 13.1 mmol) was added to the reaction mixture at 0°C. The reaction mixture was stirred for additional 4 h at room temperature and quenched by adding saturated NH₄Cl solution. The mixture was extracted with ethyl acetate, dried (sodium sulfate) and evaporated under reduced pressure. Without further purification of deuterated 2-pentyl furan **3b** (2.5 g, 17.7 mmol), added pyridine (2.8 mL, 35.1 mmol) and a mixture of THF-acetone-H₂O (5:4:1, 60 mL). To the above solution was slowly added NBS (3.78 g, 21.2 mmol) dissolved in THF-acetone-H₂O (5:4:1, 20 mL) at –20°C. The solution was stirred for 1 h at –20°C and 6 h at room temperature and then poured into a mixture of ethyl acetate and aqueous Na₂S₂O₃. The organic layer was separated and washed with aqueous citric acid solution (pH 3). The organic layer was dried and evaporated under reduced pressure. The residue was purified on a silica gel column using 10% ethyl acetate in hexane to afford the title compound **4b** as yellow oil (1.32 g, 48%). ¹H-NMR (500 MHz, CDCl₃)δ: 1.30–1.32 (*m*, 4H, C(7,8)-CH₂), 1.66 (*p*, *J* = 7.5 Hz, 2H, C(6)-CH₂), 2.68 (*t*, *J* = 7.5 Hz, 2H, C(5)-CH₂), 6.78 (*dd*, *J* = 7.5, 16 Hz, 1H, C(2)-H), 6.87 (*d*, *J* = 16 Hz, 1H, C(3)-H), 9.78 (*d*, *J* = 7.5 Hz, 1H, CH). ¹³C-NMR (CDCl₃)δ: 14.0, 22.5, 23.5, 31.4, 41.3, 137.4, 144.7 (*t*), 193.5, 200.3. HRMS (*m/z*): [M+H]⁺ calcd for C₉[²H₃]H₁₁O₂, 158.1260; found, 158.1260.

4-Hydroxy-non-2-ynal-dimethylacetal (6)—In a three-necked flask containing magnesium (2.43 g, 0.1 mol) and THF (50 mL) was slowly added a solution of bromoethane (10.83 g, 0.1 mol) in THF (50 mL) under an atmosphere of argon. The solution of Grignard was cooled to –10°C while stirring, resulting in the precipitation of ethyl magnesium bromide. The suspension was stirred intensely at –10°C and to it was added a solution of propionaldehyde diethylacetal **5** (12.81 g, 0.1 mol) in THF (50 mL). The solution was warmed to 0°C and the stirring was continued for further 2 h. A solution of hexanal (10.01 g, 0.1 mol) in THF (50 mL) was added to the above suspension at –15°C slowly. The suspension was stirred for further 4 h at –10°C. After the completion of reaction, saturated ammonium chloride was added while cooling. The organic phase was extracted with ether (3 × 200 mL). Dried the organic layer with sodium carbonate and evaporated under reduced pressure. Purification of the residue by column chromatography using 10% ethyl acetate in hexanes as an eluent resulted in pure **6** (18.24 g, 80%). ¹H-NMR (500 MHz, CDCl₃)δ: 0.89 (*t*, *J* = 7 Hz, 3H, CH₃), 1.24–1.72 (*m*, 14H, 4xCH₂+2xCH₃), 3.59 (*t*, *J* = 7 Hz, 2H, CH₂), 3.72 (*t*, *J* = 7 Hz, 2H, CH₂), 4.43 (*s*, 1H, CH), 5.30 (*s*, 1H, CH).

4-Hydroxy-non-2-enal-dimethylacetal (7)—To a solution of **6** (10 g, 43.8 mmol) in anhydrous ether (200 mL) at –25°C was added lithium aluminum anhydride (3.32 g, 87.5 mmol) slowly, under an atmosphere of argon. The suspension was stirred for 6 h at –25°C and then carefully hydrolyzed by adding 10 mL of saturated ammonium chloride solution. The temperature of the reaction mixture was kept below –5°C during the addition. The precipitates were filtered. Washed the mother liquor with water, dried the ether layer with sodium carbonate and evaporated the solvent under reduced pressure. The purification of the residue by column chromatography using 15% ethyl acetate in hexanes resulted in pure **7** as yellow oil (6.55 g, 65%). ¹H-NMR (500 MHz, CDCl₃)δ: 0.88–0.89 (*m*, 3H, CH₃), 1.22 (*t*, *J*

= 7 Hz, 6H, 2xCH₃), 1.30–1.32 (*m*, 6H, 3xCH₂), 1.58 (*m*, 2H, CH₂), 3.49–3.52 (*m*, 2H, CH₂), 3.63–3.66 (*m*, 2H, CH₂), 3.97 (brs, 1H, OH), 4.15 (*s*, 1H, CH), 4.89–4.90 (*m*, 1H, CH), 5.67–5.76 (*m*, 1H, CH), 5.85–5.89 (*m*, 1H, CH). Using similar reaction conditions as for **7** and following a combination of lithium aluminum hydride or lithium aluminum deuteride with water or deuterium oxide compounds **9**, **11** and **13** were synthesized.

2-[²H]-4-hydroxy-non-2-enal-dimethylacetal (9)—Reduction of **6** was conducted using lithium aluminum deuteride and the quenching was performed by a saturated solution of deuterated ammonium chloride in deuterium oxide. Yield 65%, yellow oil. ¹H-NMR (500 MHz, CDCl₃)δ: 0.89 (*t*, *J* = 7 Hz, 3H, CH₃), 1.22 (*t*, *J* = 7 Hz, 6H, 2xCH₃), 1.28–1.32 (*m*, 6H, 3xCH₂), 1.53 (*p*, *J* = 7 Hz, 2H, CH₂), 3.47–3.53 (*m*, 2H, CH₂), 3.62–3.67 (*m*, 2H, CH₂), 3.96 (brs, 1H, OH), 4.14 (*d*, *J* = 5 Hz, 1H, CH), 4.89 (*s*, 1H, CH), 5.84 (*s*, 1H, CH).

3-[²H]-4-hydroxy-non-2-enal-dimethylacetal (11)—Reduction of **6** was conducted using lithium aluminum deuteride and the quenching was conducted using a saturated solution of ammonium chloride in water. Yield 65%, yellow oil. ¹H-NMR (500 MHz, CDCl₃)δ: 0.88 (*t*, *J* = 7 Hz, 3H, CH₃), 1.22 (*t*, *J* = 7 Hz, 6H, 2xCH₃), 1.30–1.37 (*m*, 6H, 3xCH₂), 1.58–1.54 (*m*, 2H, CH₂), 3.47–3.53 (*m*, 2H, CH₂), 3.61–3.67 (*m*, 2H, CH₂), 4.14 (*s*, 1H, CH), 4.89 (*d*, *J* = 5 Hz, 1H, CH), 5.68 (*s*, 1H, CH).

2,3-[²H₂]-4-hydroxy-non-2-enal-dimethylacetal (13)—Reduction of **6** was performed using lithium aluminum deuteride and the quenching was conducted using a saturated solution of deuterated ammonium chloride in deuterium oxide. Yield 65%, yellow oil ¹H-NMR (500 MHz, CDCl₃)δ: 0.88 (*t*, *J* = 7 Hz, 3H, CH₃), 1.22 (*t*, *J* = 7 Hz, 6H, 2xCH₃), 1.28–1.32 (*m*, 6H, 3xCH₂), 1.52–1.53 (*m*, 2H, CH₂), 3.47–3.53 (*m*, 2H, CH₂), 3.62–3.67 (*m*, 2H, CH₂), 3.94 (brs, 1H, OH), 4.14–4.15 (*m*, 1H, CH), 4.93 (*s*, 1H, CH).

4-Oxo-non-2-ena-dimethylacetal (8)—To a solution of **7** (6 g, 0.026 mol) in hexanes was added MnO₂ (34.02 g, 0.39 mol). The suspension was allowed to stir under an atmosphere of argon for 24 h. The solid was filtered and the mother liquor was evaporated under reduced pressure. Purification of the residue by column chromatography using 5% ethyl acetate in hexanes resulted in pure **8** (3.568 g, 60%). ¹H-NMR (500 MHz, CDCl₃)δ: 0.89 (*t*, *J* = 7 Hz, 3H, CH₃), 1.21–1.24 (*t*, *J* = 7 Hz, 6H, 2xCH₃), 1.30–1.33 (*m*, 4H, 2xCH₂), 1.62 (pentet, *J* = 7.5 Hz, 2H, CH₂), 2.56 (*t*, *J* = 7 Hz, 2H, CH₂), 3.50–3.56 (*m*, 2H, CH₂), 3.62–3.68 (*m*, 2H, CH₂), 5.04–5.05 (*m*, 1H, CH), 6.33 (*d*, *J* = 16.5 Hz, 1H, CH), 6.62 (*dd*, *J* = 4.5, 16.25 Hz, 1H, CH). Using the same procedure, compounds **10**, **12** and **14** were synthesized from **9**, **11** and **13**, respectively. Yields and NMR spectra are provided below.

2-[²H]-4-oxo-non-2-enal-dimethylacetal (10)—Yield, 60%, yellow oil. ¹H-NMR (500 MHz, CDCl₃)δ: 0.89 (*t*, *J* = 7 Hz, 3H, CH₃), 1.23 (*t*, *J* = 7.5 Hz, 6H, 2xCH₃), 1.28–1.33 (*m*, 4H, CH₂), 1.62 (*p*, *J* = 7 Hz, 2H, CH₂), 2.57 (*t*, *J* = 7 Hz, 2H, CH₂), 3.50–3.56 (*m*, 2H, CH₂), 3.62–3.68 (*m*, 2H, CH₂), 5.04 (*s*, 1H, CH), 6.33 (*s*, 1H, CH).

3-[²H]-4-oxo-non-2-enal-dimethylacetal (12)—Yield 60%, yellow oil. ¹H-NMR (500 MHz, CDCl₃)δ: 0.89 (*t*, *J* = 7 Hz, 3H, CH₃), 1.23 (*t*, *J* = 7 Hz, 6H, 2xCH₃), 1.33–1.34 (*m*,

4H, 2xCH₂), 1.60 (pentet, $J = 7.5$ Hz, 2H, CH₂), 2.56 (t , $J = 7.5$ Hz, 2H, CH₂), 3.5–3.56 (m , 2H, CH₂), 3.6–3.68 (m , 2H, CH₂), 5.04 (d , $J = 4.5$ Hz, 1H, CH), 6.62 (s , 1H, CH).

2,3-[²H₂]-4-oxo-non-2-enal-dimethylacetal (14)—Yield 60%, yellow oil. ¹H-NMR (500 MHz, CDCl₃) δ : 0.89 (t , $J = 7$ Hz, 3H, CH₃), 1.23 (t , $J = 7$ Hz, 6H, 2xCH₃), 1.28–1.33 (m , 4H, 2xCH₂), 1.60–1.65 (m , 2H, CH₂), 2.57 (t , $J = 7$ Hz, 2H, CH₂), 3.50–3.56 (m , 2H, CH₂), 3.62–3.68 (m , 2H, CH₂), 5.04 (s , 1H, CH).

Synthesis of ONE (4a) from 8—Made a suspension of **8** (3 g, 13 mmol) in threefold volume (9 mL) of 1% aqueous citric acid and allowed the reaction to run for another 24 h. After the completion of the reaction, the solution was extracted with dichloromethane (3 $\times$ 25 mL). Dried the organic layer (Na₂SO₄) and evaporated under reduced pressure. Purification of the residue using 5% ethyl acetate in hexanes resulted in **4a** as yellow oil (1.2 g, 60%). The analytical data of this compound is similar to the ONE synthesized from 2-pentyl furan. Using the same procedure, labeled ONE analogs **15**, **16** and **17** were synthesized from **10**, **12** and **14**, respectively. Yields, NMR spectra and HRMS data are provided below.

2-[²H]-ONE (15)—Yield 60%, yellow oil. ¹H-NMR (500 MHz, CDCl₃) δ : 0.90 (t , $J = 7$ Hz, 3H, CH₃), 1.32–1.33 (m , 4H, 2xCH₂), 1.66 (p , $J = 7$ Hz, 2H, CH₂), 2.69–2.70 (t , $J = 7$ Hz, 2H, CH₂), 6.86 (s , 1H, CH), 9.78 (s , 1H, CH). ¹³C-NMR (CDCl₃) δ : 13.9, 22.5, 23.4, 31.3, 41.3, 137.0 (t), 144.9, 193.5, 200.3. HRMS (m/z): [M+H]⁺ calcd for C₉H₁₃[²H]O₂, 156.1135; found, 156.1132.

3-[²H]-ONE (16)—Yield 60%, yellow oil. ¹H-NMR (500 MHz, CDCl₃) δ : 0.89 (t , $J = 7$ Hz, 3H, CH₃), 1.29–1.33 (m , 4H, 2xCH₂), 1.66 (p , $J = 7.5$ Hz, 2H, CH₂), 2.69 (t , $J = 7$ Hz, 2H, CH₂), 6.76–6.77 (m , 1H, CH), 9.79 (d , $J = 7.5$ Hz, 1H, CH). ¹³C-NMR (CDCl₃) δ : 14.0, 22.5, 23.5, 31.4, 41.3, 137.4, 144.7 (t), 193.5, 200.3. HRMS (m/z): [M+H]⁺ calculated for C₉H₁₃²HO₂, 156.1135; found, 156.1143.

2,3-[²H₂]-ONE (17)—Yield 60%, yellow oil. ¹H-NMR (500 MHz, CDCl₃) δ : 0.90 (t , $J = 7$ Hz, 3H, CH₃), 1.32–1.33 (m , 4H, 2xCH₂), 1.67 (p , $J = 7.5$ Hz, 2H, CH₂), 2.68 (t , $J = 7.5$ Hz, 2H, CH₂), 9.78 (s , 1H, CH). ¹³C-NMR (CDCl₃) δ : 14, 22.5, 23.5, 31.4, 41.3, 137.1 (t), 144.6 (t), 193.6, 200.3. HRMS (m/z): [M+H]⁺ calculated for C₉H₁₂[²H₂]O₂, 157.1198; found, 157.1202.

Results and discussion

2-Pentyl furan **3a** was used to synthesize the unlabeled ONE **4a** by ring opening of the 2-substituted furan using *N*-bromosuccinimide (NBS) in a solvent mixture consisting of acetone, THF and water containing pyridine as a base, the conditions reported by Kobayashi *et al.*¹⁷ for the ring opening of 2-substituted furans. A similar ring opening, for the synthesis of ONE, was reported recently (24% yield);¹⁸ however, in our hands the yields of the ring opening of the 2-substituted furans with modified conditions (Figure 1) was higher (44–48%). The analytical data of **4a** was same as has been reported earlier.¹⁶ The synthesis of C-9 labeled ONE started with reaction of the anion of furan **1**, generated with BuLi, with

C-5 deuterium-labeled bromopentane to provide the labeled 2-pentyl furan analog **3b** (Figure 1). Intermediate **3b** was subjected to ring opening using NBS and the product was purified using column chromatography. The ^1H -NMR spectrum of **4b** did not show any C-9 methyl protons at 0.9 ppm that were observed for the unlabeled ONE **4a** (Figure 2). The ^{13}C -NMR spectrum revealed a very weak triplet at 13 ppm instead of a singlet in **4a** at 13.7 ppm (Figure 3) confirming the incorporation of three deuterium atoms at C-9 position of ONE. High-resolution (HR) mass spectrometry (MS) of $[\text{M}+\text{H}]^+$ further supported the formation of **3b** with complete labeling at C-9.

In a second synthetic strategy (Figure 4), the acetylenic hydrogen of commercially available propionaldehyde diethylacetal **5** was abstracted with ethyl magnesium bromide to convert **5** to propionaldehyde diethylacetal magnesium bromide *in situ*. The addition of hexanal to this intermediate resulted in the formation of 4-hydroxy-non-2-ynal dimethylacetal **6** in 80% yield. Dimethylacetal **6** was exploited to synthesize ONE and its deuterium-labeled versions. Addition of lithium aluminum hydride to dimethylacetal **6** followed by aqueous work-up gave 4-hydroxy-non-2-enal-dimethylacetal **7**, which was oxidized with manganese dioxide to provide **8** in 60% yield. Deprotection of **8** under acidic conditions gave ONE **4a**. In a slightly modified procedure, the reduction in dimethylacetal **6** with lithium aluminum hydride and work-up with deuterium oxide, saturated with deuterated ammonium chloride, gave intermediate **9**, which had deuterium at C-2 of 4-hydroxy-non-2-enal-dimethylacetal. The ^1H -NMR spectrum of **9** showed the absence of the multiplet at 5.67 ppm as compared with the ^1H -NMR spectrum of **7**, confirming the incorporation of deuterium at C-2. Oxidation of **9** and subsequent deprotection of the deuterium-labeled intermediate **10** gave labeled ONE **15** with deuterium at C-2. The ^1H -NMR spectrum of **15** showed a singlet at 9.78 ppm for the aldehyde hydrogen instead of the doublet observed in **4a** (Figure 2). The signal at 6.76 ppm observed in **4a** was also absent confirming the presence of deuterium at C-2 in **15**. The ^{13}C -NMR spectrum of **15** showed a weak triplet at 137 ppm, whereas the unlabeled ONE **4a** had a strong singlet at 137 ppm (Figure 3). HRMS confirmed the incorporation of one deuterium into **15**.

For the synthesis of ONE **16** with a deuterium at C-3, the dimethylacetal **6** was reduced with lithium aluminum deuteride and the reaction was quenched by the addition of a saturated solution of ammonium chloride in water to give intermediate **11** with deuterium incorporated at C-3. Subsequent oxidation with MnO_2 to **12** followed by deprotection resulted in the formation **16**. The ^1H -NMR spectrum of **16** shows the absence of signals from the C-3 proton at 6.87 ppm (Figure 2) and the presence of weak triplet in the ^{13}C -NMR spectrum at 144.7 ppm (Figure 3). ONE **17** having deuterium atoms at both C-2 and C-3 was synthesized by reduction of dimethylacetal **6** with lithium aluminum deuteride with a work-up using deuterium oxide saturated with deuterium-labeled ammonium chloride. This yielded intermediate **13** having deuterium labeling at both C-2 and C-3 positions. Subsequent oxidation with MnO_2 and acidic deprotection resulted in the formation of ONE **17**. The ^1H -NMR spectrum of **17** did not show any signals in the region 6–8 ppm. In addition, the aldehyde proton appeared as singlet (Figure 2). The ^{13}C -NMR spectrum of **17** showed two weak triplets at 137.1 and 144.6 due to carbon deuterium coupling instead of the corresponding singlets seen in unlabeled ONE **4a** at the same positions (Figure 3).

HRMS of the labeled compound further confirmed the incorporation of two deuterium atoms into ONE **17**.

In summary, we have demonstrated that deuterium-labeled analogs of ONE can be synthesized in good yields starting from the commercially available propionaldehyde diethylacetal. The availability of these heavy isotope analogs will be useful as internal standards for quantitative studies employing MS and for conducting mechanistic studies of complex interactions between ONE and DNA bases⁹ as well as between ONE and proximal amino acid residues in peptides¹⁹ and proteins.¹¹

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Figure 1.
Synthesis of deuterium-labeled (C-9) and -unlabeled ONE from furan derivatives.

Figure 2.
Comparison of ^1H -NMR spectra between deuterium-labeled and -unlabeled ONE.

Figure 3.
Comparison of ^{13}C -NMR spectra between deuterium-labeled and -unlabeled ONE.

Figure 4.

Synthesis of C-2, C-3 and C-2/C3-deuterium-labeled ONE and -unlabeled ONE.