ORGANIC LETTERS

2004
Vol. 6, No. 3
349−352

Synthesis of Site-Specifically Labeled Arachidonic Acids as Mechanistic Probes for Prostaglandin H Synthase

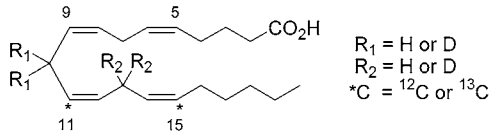
Sheng Peng,[†] Chris M. McGinley,[†] and Wilfred A. van der Donk*

Roger Adams Laboratory, Department of Chemistry, University of Illinois, 600 S. Mathews Avenue, Urbana, Illinois 61801

vddonk@scs.uiuc.edu

Received November 5, 2003

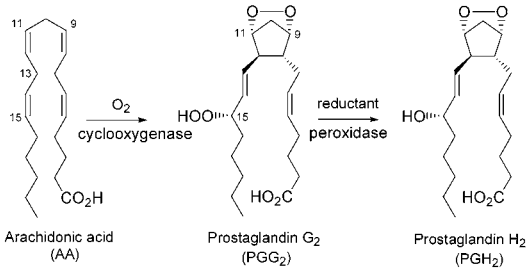
ABSTRACT



Prostaglandin H synthase catalyzes the first committed step in the biosynthesis of prostaglandins and thromboxane. Herein we report the synthesis of four site-specifically labeled arachidonic acids for investigation of the radical intermediate formed during this enzymatic reaction. Two compounds were prepared using a common C9–C11 fragment, while another target was synthesized using a previously reported advanced intermediate. An alkyne coupling followed by hydrogenation and Wittig reaction was used to prepare the final labeled substrate.

Prostaglandin H synthase, or cyclooxygenase (COX), catalyzes the conversion of arachidonic acid into prostaglandin H_2 in the first committed step in the biosynthesis of prostaglandins and thromboxane (Scheme 1).[1] These compounds have been implicated in numerous processes affecting human health, including inflammation and cardiovascular disease. However, at present, the reaction mechanism for the oxidation of arachidonic acid (AA) has not been fully elucidated. The first step of the enzymatic reaction has been previously shown to involve abstraction of a hydrogen from the C-13 position of AA by a tyrosyl radical.[2,3] This results in formation of either a pentadienyl or allyl radical depending on the conformation of the substrate when bound to the enzyme.

Scheme 1



In previous work we have demonstrated the use of site-specifically deuterium-labeled substrates to investigate the structure of the original radical intermediate by using EPR spectroscopy. These studies showed that in COX-2, the isozyme linked to inflammation, the initial hydrogen abstraction leads to formation of a pentadienyl radical intermediate spanning C11−C15.[4] The EPR spectrum shows coupling of the unpaired electron to six different hydrogens. Using deuterium labeling, four of these were assigned to protons

[†] These authors contributed equally to this work.

(1) (a) Rouzer, C. A.; Marnett, L. J. *Chem. Rev.* **2003**, *103*, 2239−2304. (b) Kulmacz, R. J.; van der Donk, W. A.; Tsai, A. L. *Prog. Lipid Res.* **2003**, *42*, 377−404 (c) Smith, W. L.; DeWitt, D. L.; Garavito, R. M. *Annu. Rev. Biochem.* **2000**, *69*, 145−182.

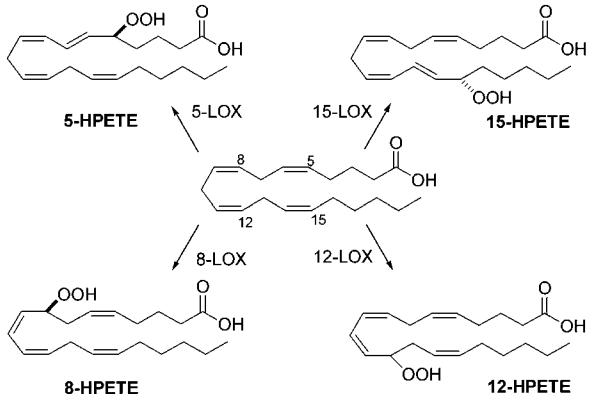
(2) (a) Karthein, R.; Dietz, R.; Nastainczyk, W.; Ruf, H. H. *Eur. J. Biochem.* **1988**, *171*, 313−320. (b) Tsai, A.-L.; Kulmacz, R. J. *Prostaglandins Other Lipid Mediators* **2000**, *62*, 231−254.

(3) (a) Tsai, A.-L.; Kulmacz, R. J.; Palmer, G. *J. Biol. Chem.* **1995**, *270*, 10503−10508. (b) Tsai, A.-L.; Palmer, G.; Xiao, G. S.; Swinney, D. C.; Kulmacz, R. J. *J. Biol. Chem.* **1998**, *273*, 3888−3894.

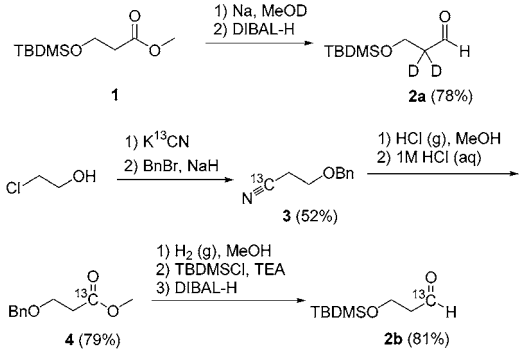
(4) (a) Peng, S.; Okeley, N. M.; Tsai, A.-L.; Wu, G.; Kulmacz, R. J.; van der Donk, W. A. *J. Am. Chem. Soc.* **2001**, *123*, 3609−3610. (b) Peng, S.; Okeley, N. M.; Tsai, A.-L.; Wu, G.; Kulmacz, R. J.; van der Donk, W. A. *J. Am. Chem. Soc.* **2002**, *124*, 10785−10786.

10.1021/ol0361711 CCC: $27.50
Published on Web 01/14/2004

Scheme 2



Scheme 3



at C11, C13, C15, and one of the protons at C16. The remaining two strongly coupled hydrogens were proposed to be located at C10. Similar studies using the COX-1 isozyme produced a signal of unknown structure under certain conditions,[5] which possibly is associated with an allyl radical. Herein we report the synthesis of two arachidonic acids site-specifically labeled with ^{13}C at positions 11 and 15 that were designed to test the allyl radical hypothesis. Furthermore, we prepared [10,10-$^{2}H_2$]-arachidonic acid to confirm the location of the two remaining strongly coupled protons in the signal observed in COX-2.

The labeled arachidonic acids described herein are also useful mechanistic probes for the human lipoxygenases (LOXs). A number of lipoxygenases in vertebrates are known that differ in the regioselectivity of oxidation (Scheme 2).[6] Their nomenclature is based on the position of hydroperoxidation of arachidonic acid.[7] 5-LOX abstracts an hydrogen atom from C7 to generate 5-hydroperoxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid (5-HPETE), the precursor to the leukotrienes that play key roles in inflammation and bronchoconstriction. The 8- and 12-LOX enzymes abstract a hydrogen atom from position 10 to produce hydroperoxides at positions 8 and 12, respectively. At present, two 15-LOX isozymes have been identified in humans, 15-LOX-1[8] and 15-LOX-2.[9] The former protein has been implicated to play a role in atherogenesis.[10,11] Both enzymes abstract a hydrogen atom from C13 and produce 15*S*-HPETE.

Soybean lipoxygenase, a 15-LOX, has received much attention for the unusually large kinetic isotope effects (KIE, 30−80) that are observed with either linoleic acid[12] (LA) or arachidonic acid[13] as substrate. Holman and co-workers showed that the human 15-LOX-1 also displays similarly large isotope effects with linoleic acid.[14] To date, however, no studies have disclosed kinetic isotope effects of the human lipoxygenases with their natural substrate, arachidonic acid. The preparation of arachidonic acids deuterium-labeled at C10 and C13 reported herein will allow such studies on 8-, 12-, and 15-LOX. We elected to prepare dideuterated compounds instead of our previous syntheses of stereospecifically mono-deuterium-labeled arachidonic acids[4] since it has been shown with soybean lipoxygenase that the large primary kinetic isotope effect on abstraction of the *pro-S* hydrogen atom leads to a decrease in stereospecificity of the enzyme.[15] That is, with [11*S*-^{2}H]-LA as substrate, the enzyme abstracts to some extent the hydrogen atom rather than the deuterium atom from C11. Obviously, this provides difficulties for assessing the KIE, and hence substrates labeled at both enantiotopic positions were prepared that will alleviate these complications.

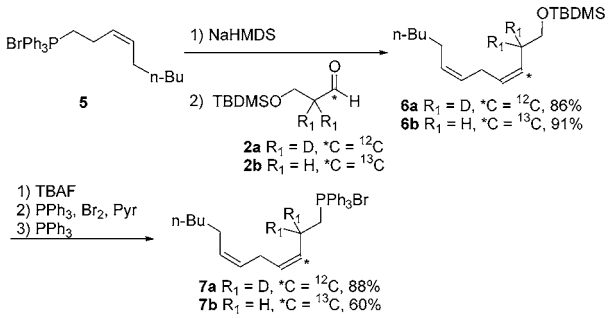
The preparation of [10,10-$^{2}H_2$]-arachidonic acid and [11-^{13}C]-arachidonic acid proceeded via the same C9−C11 fragment **2**. For the former target, ring opening of β-propiolactone by methanol, followed by protection of the ensuing alcohol with *tert*-butyldimethylsilyl chloride, yielded methyl ester **1** (Scheme 3). Quantitative deuterium incorporation at the α-position was achieved by stirring with Na metal in MeOD. Selective reduction to the aldehyde **2a** using DIBAL-H afforded the C9−C11 fragment without detectable loss in deuterium incorporation.

The synthesis of the C9−C11 fragment for [11-^{13}C]-arachidonic acid involved direct displacement of 2-chloroethanol with [^{13}C]-potassium cyanide followed by benzylation to afford nitrile **3** in good yield (Scheme 3). [^{13}C]-Potassium cyanide was chosen as the labeled starting material

(5) Tsai, A.-L.; Palmer, G.; Wu, G.; Peng, S.; Okeley, N. M.; van der Donk, W. A.; Kulmacz, R. J. *J. Biol. Chem.* **2002**, *277*, 38311−38321.
(6) Brash, A. R. *J. Biol. Chem.* **1999**, *274*, 23679−23682;
(7) Kuhn, H.; Schewe, T.; Rapoport, S. M. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1986**, *58*, 273−311
(8) Sigal, E.; Grunberger, D.; Craik, C. S.; Caughey, G. H.; Nadel, J. A. *J. Biol. Chem.* **1988**, *263*, 5328−5332.
(9) Brash, A. R.; Boeglin, W. E.; Chang, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6148−6152.
(10) Young, R. N. *Eur. J. Med. Chem.* **1999**, *34*, 671−685.
(11) Cyrus, T.; Witztum, J. L.; Rader, D. J.; Tangirala, R.; Fazio, S.; Linton, M. F.; Funk, C. D. *J. Clin. Invest.* **1999**, *103*, 1597−1604; Cyrus, T.; Pratico, D.; Zhao, L.; Witztum, J. L.; Rader, D. J.; Rokach, J.; FitzGerald, G. A.; Funk, C. D. *Circulation* **2001**, *103*, 2277−2282. Funk, C. D.; Cyrus, T. *Trends Cardiovasc. Med.* **2001**, *11*, 116−124. Cathcart, M. K.; Folcik, V. A. *Free Radical Biol. Med.* **2000**, *28*, 1726−1734. Steinberg, D. *J. Clin. Invest.* **1999**, *103*, 1487−1488. Belkner, J.; Stender, H.; Kuhn, H. *J. Biol. Chem.* **1998**, *273*, 23225−23232.

(12) (a) Glickman, M. H.; Wiseman, J. S.;. Klinman, J. P *J. Am. Chem. Soc.* **1994**, *116*, 793−794. (b) Hwang, C. C.; Grissom, C. B. *J. Am. Chem. Soc.* **1994**, *116*, 795−796. (c) Glickman, M. H.; Klinman, J. P. *Biochemistry* **1995**, *34*, 14077−14092. (d) Glickman, M. H.; Klinman, J. P. *Biochemistry* **1996**, *35*, 12882−12892.
(13) Peng, S.; van der Donk, W. A. *J. Am. Chem. Soc.* **2003**, *125*, 8988−8989.
(14) Lewis, E. R.; Johansen, E.; Holman, T. R. *J. Am. Chem. Soc.* **1999**, *121*, 1395−1396.
(15) Rickert, K. W.; Klinman, J. P. *Biochemistry* **1999**, *38*, 12218−12228.

Scheme 4

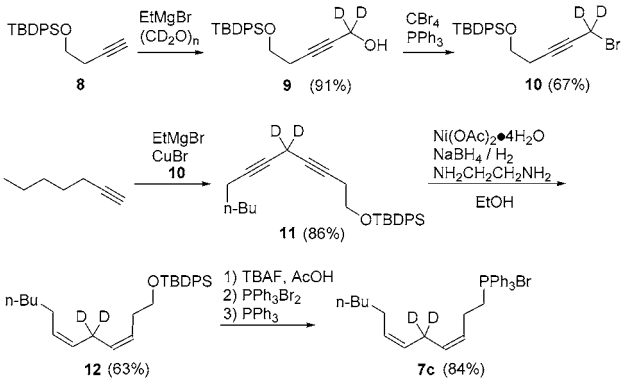


because of its commercial availability and relatively low cost. Treatment of nitrile **3** with a saturated HCl/MeOH solution, followed by hydrolysis yielded methyl ester **4** in good yield. After deprotection and silylation, the methyl ester was reduced to the aldehyde **2b** using DIBAL-H.

Wittig olefination of aldehydes **2a** and **2b** with phosphonium salt **5**[16] employing salt-free conditions[17] resulted in dienes **6** with 95:5 *Z*-selectivity (Scheme 4). The isomers were not separated at this stage but carried on through the synthesis; the minor isomers were removed by HPLC after generation of the final arachidonic acid products. Deprotection of **6a** and **6b** using TBAF, followed by bromination and refluxing with triphenylphosphine, afforded phosphonium salts **7a** and **7b**.

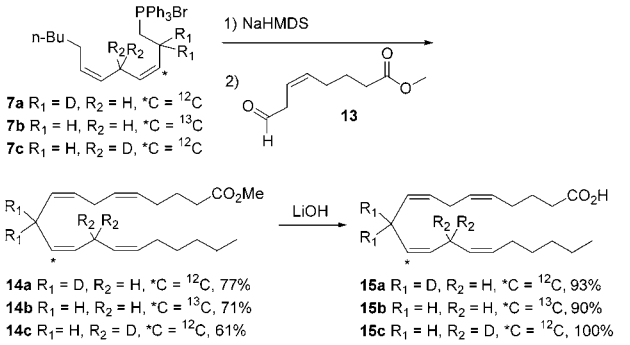
Phosphonium salt **7c** was required for the synthesis of [13,13-$^2H_2$]-arachidonic acid. Its preparation started with the protected alkyne **8** (Scheme 5). Deprotonation with ethylmagnesium bromide followed by addition to deuterium-labeled paraformaldehyde produced the protected pent-2-yn-1,5-diol **9** in good yield. This alcohol was reacted with carbon tetrabromide and triphenylphosphine to yield propargyl bromide **10**. A copper acetylide, formed by treating 1-heptyne with ethylmagnesium bromide and copper(I) bromide, was reacted with bromide **10**, yielding the skipped diyne **11** in 86% yield. Hydrogenation in the presence of nickel(II) acetate yielded skipped diene **12**, which was deprotected with TBAF in acidic solution. The alcohol was subsequently brominated and converted to phosphonium salt **7c**.

Scheme 5



magnesium bromide followed by addition to deuterium-labeled paraformaldehyde produced the protected pent-2-yn-1,5-diol **9** in good yield. This alcohol was reacted with carbon tetrabromide and triphenylphosphine to yield propargyl bromide **10**. A copper acetylide, formed by treating 1-heptyne with ethylmagnesium bromide and copper(I) bromide, was reacted with bromide **10**, yielding the skipped diyne **11** in 86% yield. Hydrogenation in the presence of nickel(II) acetate yielded skipped diene **12**, which was deprotected with TBAF in acidic solution. The alcohol was subsequently brominated and converted to phosphonium salt **7c**.

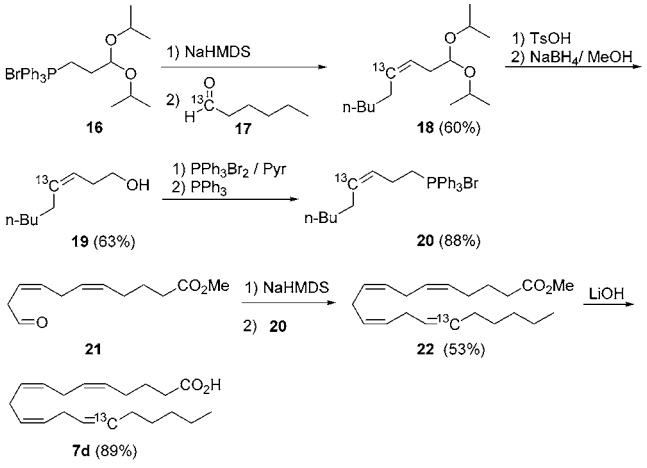
Scheme 6



The three isotopically labeled phosphonium salts **7a**−**c** were olefinated with aldehyde **13**[4] to afford the labeled methyl arachidonates **14** (Scheme 6). Hydrolysis with lithium hydroxide followed by HPLC purification yielded the final products, [10,10-$^2H_2$]- and [13,13-$^2H_2$]-arachidonic acid, and [11-^{13}C]-arachidonic acid.

The synthesis of [15-^{13}C]-arachidonic acid was achieved from commercially available [1-^{13}C]-hexanoic acid. Following esterification and lithium aluminum hydride reduction, [1-^{13}C]-hexanal was obtained by PCC oxidation. A Wittig reaction between the labeled aldehyde **17** and triphenylphosphonium salt **16** yielded protected aldehyde **18** in modest yield (Scheme 7). Deprotection with *p*-toluenesulfonic acid produced the β,γ-unsaturated aldehyde, which was reduced to alcohol **19**. Reaction with dibromotriphenylphosphorane yielded labeled 1-bromo-non-3-ene, which was converted in good yield to the phosphonium salt **20**. We previously

Scheme 7



(16) Pommier, A.; Pons, J.-M.; Kocienski, P. J. *J. Org. Chem.*, **1995**, *60*, 7334−7339.

(17) (a) Greenwald, R.; Chaykovsky, M.; Corey, E. J. *J. Am. Chem. Soc.* **1963**, *28*, 1128−1129. (b) Bestmann, H. J.; Stransky, W.; Vostrowsky, O. *Chem. Ber.* **1976**, *109*, 1694−1700.

reported a facile and high yielding synthesis of skipped aldehyde **21**.[4] Reaction of this aldehyde with phosphonium salt **20** yielded [15-^{13}C]-methyl arachidonate **7d**. Final hydrolysis with lithium hydroxide yielded the target compound, [15-^{13}C]-arachidonic acid **7d**, in excellent yield.

Herein, we described the synthesis of four site-specifically labeled arachidonic acids. The synthesis of both dideuterated and ^{13}C-labeled compounds was carried out in high efficiency and isotopic purity. These compounds can be used as mechanistic probes for cyclooxygenase and lipoxygenases. Specifically, the dideuterated compounds can be employed for kinetic isotope effect studies with 12- and 8-LOX, which both abstract hydrogen atoms from C10, and for 15-LOX and COX-1 and COX-2, which abstract a hydrogen atom from C13. Mechanistic studies of the labeled substrates with these enzymes are underway and will be published in due course.

Acknowledgment. This work was supported in part by GM44911 from the National Institutes of Health. NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence, funded in part by the W. M. Keck Foundation, NIH (PHS 1 S10 RR10444), and NSF (CHE 96-10502). Mass spectra were recorded on a Voyager mass spectrometer funded in part by the National Institutes of Health (RR 11966).

Supporting Information Available: Experimental procedures and spectral characterization of all synthetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0361711