JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 245–252.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1039

Research Article

Convenient syntheses of [20,20,20-$^2H_3$]-arachidonic acid and [20,20,-$^2H_2$]-20-hydroxyeicosatetraenoic acid

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Summary

Deuterated arachidonic acid and 20-HETE were prepared in good overall yields and high stereoselectivities. Key transformations include a *trans*-specific vinyl dibromide reduction and Suzuki cross-couplings to a lithium borate or a 9-BBN borane. These standards are three and two mass units higher, respectively, than their naturally occurring counterparts and are useful in mass spectrometry analysis. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: ^{2}H-labeled standards; *Z*-vinyl bromide; Suzuki cross-coupling; stereoselective

Introduction

Upon release from phospholipid stores, arachidonic acid (AA) is rapidly oxidized to a plethora of structurally diverse autacoids, *inter alia*, prostanoids (PGs), leukotrienes (LTs), epoxyeicosatrienoic acids (EETs), and 20-hydroxyeicosatetraenoic acid (20-HETE, also known as 20-OH-AA).[1] The latter, a metabolite of the cytochrome P450 branch of the eicosanoid cascade,[2] is a potent vasoconstrictor, angiogenic factor, and proinflammatory agent.[3–5]

Mass spectrometry (MS) has been used extensively to quantify AA[6] and 20-HETE[7–9] and to correlate their levels with normo-[10] and pathophysiology[11–13] as well as their further metabolic conversions.[14–16] The deuterium labeled standards typically used in such studies are prepared by semi-hydrogenation of acetylenic intermediates[17,18] or by Wittig olefinations.[19,20] The complications

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Received 21 November 2005
Revised 26 November 2005
Accepted 28 November 2005

associated with these procedures, e.g. label scrambling, over or incomplete reduction, positional isomerization, and moderate stereoselectivity, generally necessitate tedious HPLC purification of the products.[21] Herein, we describe an efficient, highly stereoselective route to AA-$^2H_3$ (**1**) and 20-HETE-$^2H_2$ (**2**) (Figure 1) utilizing inexpensive, commercially available deuterated precursors. In both cases, the modest level and location of the isotope substitution minimizes chromatographic differentiation with respect to natural material.[22]

Results and discussion

Our strategy, summarized in Scheme 1, commenced with the readily available methyl 14,15-dihydroxyeicosatrienoate[23] (**3**) which was cleaved with lead tetraacetate at low temperature and the resultant aldehyde was immediately converted via Corey-Fuchs olefination[24] to vinyl dibromide **4** in good overall yield (Scheme 1). Selective *trans*-reduction using *n*-$Bu_3SnH/Pd(Ph_3P)_4$ led exclusively to *Z*-vinyl bromide **5**, the key intermediate. The stereochemistry of the $\Delta^{14,15}$-olefin in **5** was confirmed by the 7 Hz coupling at 6.17 ppm in the ^1H NMR. Subsequent Suzuki cross-coupling of **5** with lithium borate **10** and borane **13** furnished all-*cis* **6** and **7**, respectively. Free alcohol **8** was smoothly generated from the **7** via *n*-Bu_4NF desilylation in good yield. Finally, saponification of **6** and **8** afforded free acids **1** and **2**, respectively.

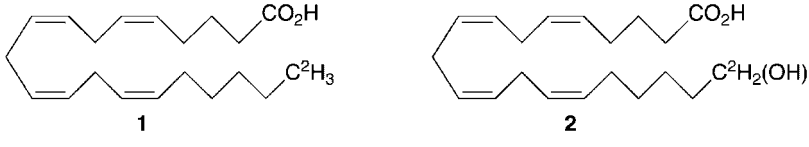
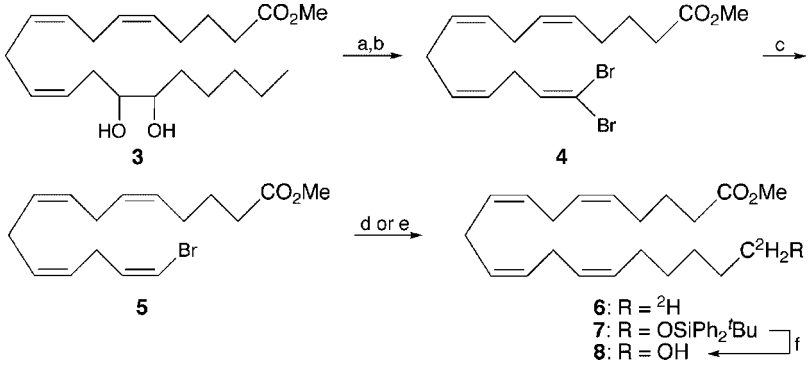
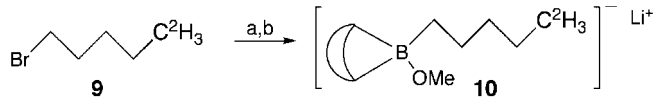


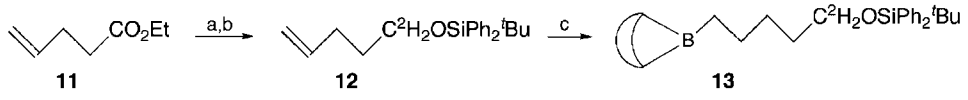
Figure 1. Chemical structures of deuterated arachidonic acid and 20-HETE



Scheme 1. Reagents and conditions: (a) $Pb(OAc)_4$, CH_2Cl_2; (b) CBr_4/PPh_3; (c) *n*-$Bu_3SnH/Pd(PPh_3)_4$, C_6H_6; (d) 10, $Pd(dppf)Cl_2$, K_3PO_4, DMF; (e) 13, $Pd(dppf)Cl_2$, $AsPh_3$, $CsCO_3$, DMF; (f) *n*-Bu_4NF, THF



Scheme 2. Reagents and conditions: (a) NaI, H_3CCOCH_3; (b) *t*-BuLi, 9-MeO-9-BBN



Scheme 3. Reagents and conditions: (a) $LiAl^2H_4$; (b) *t*-$BuPh_2SiCl$; (c) 9-BBN

To access lithium borate **10** (Scheme 2), commercial [5,5,5-$^2H_3$]-1-bromopentane (**9**) was first converted to the corresponding iodide, metallated with *t*-BuLi, and then added to 9-MeO-9-BBN.

Borane **13** was prepared by silylation of the known alcohol[25] obtained via $LiAl^2H_4$ reduction of ethyl 4-pentenoate (**11**) (Scheme 3). Silyl ether **12** was then subjected to hydroboration with 9-BBN.

Conclusions

Efficient and readily scalable syntheses of deuterated arachidonic acid and 20-HETE were achieved in high isotopic and positional purity. These analogs are three and two mass units higher, respectively, than their naturally occurring counterparts and are useful standards for mass spectrometry analysis.

Experimental

Methyl 15,15-dibromopentadeca-5(Z),8(Z),11(Z),14(Z)-tetraenoate (**4**)

Lead tetraacetate (1.385 g, 3.125 mmol) was added portionwise over 5 min to a −20°C solution of methyl 14,15-dihydroxyeicoa-5(*Z*),8(*Z*),11(*Z*)-trienoate[23] (**3**) (1.00 g, 2.841 mmol) in anhydrous dichloromethane (40 ml) under an argon atmosphere. After 30 min, the reaction mixture was filtered through a small pad of silica gel (70–230 mesh) and the pad was washed with another portion of dichloromethane (40 ml). The combined organic filtrate was evaporated *in vacuo* and the residue was purified by SiO_2 flash column chromatography using 40% ethyl acetate in hexanes as eluent to give methyl 14-oxotetradeca-5(*Z*),8(*Z*),11(*Z*)-trienoate (0.672 g, 94%) as a labile, colorless oil that was used immediately in the next reaction without further purification. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.60–1.78 (m, 2H), 2.00–2.15 (m, 2H) 2.30 (t, $J = 7.4$ Hz,

2H), 2.70–2.85 (m, 4H), 3.18–3.28 (m, 2H), 3.65 (s, 3H), 5.28–5.75 (m, 6H), 9.65 (t, $J = 1.8$ Hz, 1H).

Triphenylphosphine (2.82 g, 10.75 mmol) and carbon tetrabromide (1.963 g, 5.913 mmol) were stirred together at 0°C under an argon atmosphere in anhydrous dichloromethane (50 ml) for 15 min.[24] Into this was cannulated a solution of the above aldehyde (0.672 g, 2.688 mmol) in dichloromethane (10 ml). After another 2 h, all volatiles were evaporated and the residue was purified by SiO_2 flash column chromatography using 3% ether in hexanes to give dibromide **4** (0.870 g, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.36 (t, $J = 7.3$ Hz, 1H), 5.54–5.30 (m, 6H), 3.67 (s, 3H), 2.92–2.78 (m, 6H), 2.32 (t, $J = 7.3$ Hz, 2H), 2.18–2.06 (m, 2H), 1.71 (quintet, $J = 7.3$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.89, 25.78, 25.92, 26.71, 31.54, 33.55, 51.62, 89.50, 124.31, 127.63, 128.76, 128.82, 129.19, 130.45, 136.68, 174.11.

Methyl 15-bromopentadeca-5(Z),8(Z),11(Z),14(Z)-tetraenoate (**5**)

The reduction was initiated by addition of the first quarter-portion of tetrakis(triphenylphosphine)palladium(0) (0.494 g, 0.428 mmol) to a solution of dibromide **4** (0.870 g, 2.143 mmol) and tributyltin hydride (738 µl, 2.786 mmol) in anhydrous benzene (30 ml) under an argon atmosphere. Thereafter, another quarter-portion was added every 4 h. After a total of 16 h, water (10 ml) was added and the mixture was extracted with ether (3×30 ml). The combined ethereal extracts were washed with water (2×20 ml), brine (15 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by SiO_2 flash column chromatography using 0.5–1.0% ether in hexanes to furnish *Z*-bromide **5** (0.538 g, 77%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.17 (dt, $J = 1.5$, 7.0 Hz, 1H), 6.07 (apparent q, $J = 7.0$ Hz, 1H), 5.50–5.32 (m, 6H), 3.66 (s, 3H), 2.98 (t, $J = 6.4$ Hz, 2H), 2.86 (t, $J = 6.1$ Hz, 2H), 2.81 (t, $J = 5.4$ Hz, 2H), 2.32 (t, $J = 7.3$ Hz, 2H), 2.18–2.06 (m, 2H), 1.70 (quintet, $J = 7.3$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): 24.83, 25.70, 25.84, 26.63, 28.38, 33.46, 51.49, 108.05, 125.54, 127.83, 128.48, 128.85, 129.09, 129.75, 133.02, 173.96.

Methyl [20,20,20-$^2H_3$]-arachidonate (**6**)

A mixture of [5,5,5-$^2H_3$]-1-bromopentane (**9**) (250 mg, 1.62 mmol; methyl-D_3 98%, Cambridge Isotopes Laboratories) and NaI (500 mg, 3.33 mmol) were refluxed for 4 h in dry acetone (10 ml), then cooled to 0°C, filtered, and the filter cake was rinsed with pentane (10 ml). The combined filtrates were carefully evaporated under reduced pressure and the crude [5,5,5-$^2H_3$]-1-iodopentane was used directly in the next step (270 mg, 83%). ^{1}H NMR

($CDCl_3$, 300 MHz): δ 1.30–1.40 (m, 4H), 1.82 (q, $J = 7.0$ Hz, 2H), 3.18 (t, J = 7.0 Hz, 2H).

t-BuLi (0.82 ml, 1.7 M solution in pentane, 1.07 mmol) was added dropwise to a −78°C solution of the above [5,5,5-$^2H_3$]-1-iodopentane (100 mg, 0.49 mmol) in ether (5 ml). After 3 min, 9-MeO-9-BBN (1.58 ml, 1 M solution in hexanes, 1.24 mmol) was added followed by THF (5 ml).[26] The reaction mixture was stirred for 10 min at −78°C, brought to room temperature, and stirred for 1 h. To this reaction mixture was added aqueous 3 M K_3PO_4 (2 ml). After 5 min, the crude mixture of lithium borate **10** was used immediately in the next step.

The solution of lithium borate **10** from above was cannulated into a stirring mixture of vinyl bromide **5** (55 mg, 0.17 mmol), [1,1′-bis(diphenylphosphino)-ferrocene]palladium(II) chloride [Pd(dppf)Cl_2] (13 mg, 0.015 mmol), and $AsPh_3$ (10 mg, 0.032 mmol) in DMF (5 ml). The resultant dark red, heterogeneous mixture was degassed by sparging with argon for 10 min and then stirred overnight at RT. The reaction mixture was diluted with ether (100 ml), washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by SiO_2 flash chromatography using 2% EtOAc/hexanes provided **6** (40 mg, 74%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24–1.37 (m, 6H), 1.64–1.76 (m, 2H), 2.01–2.14 (m, 4H), 2.31 (t, $J = 7.6$ Hz, 2H), 2.78–2.85 (m, 6H), 3.56 (s, 3H), 5.25–5.42 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 22.44, 24.90, 25.75, 26.68, 27.35, 29.47, 31.56, 33.57, 51.61, 127.66, 127.77, 128.29, 128.33, 128.70, 129.00, 129.05, 130.63, 174.21.

[20,20,20-$^2H_3$]-Arachidonic acid (**1**)

LiOH (0.4 ml, 1 M solution in water, 0.37 mmol) was added to a 0°C solution of methyl ester **6** (40 mg, 0.12 mmol) in THF/H_2O (5 ml, 4:1) under an argon atmosphere. After 12 h at RT, the reaction mixture was cooled to 0°C, acidified to pH 4.5 using 1 M aqueous oxalic acid, and extracted with EtOAc (2×25 ml). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by SiO_2 flash chromatography using 50% EtOAc/hexanes provided **1** (33 mg, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27–1.37 (m, 6H), 1.64–1.76 (m, 2H), 2.03–2.11 (m, 4H), 2.31 (t, $J = 7.3$ Hz, 2H), 2.78–2.84 (m, 6H), 5.30–5.41 (m, 8H); HRMS (CI, CH_4) calculated for $C_{20}H_{30}D_3O_2$ (M + 1) 308.2669, found 308.2667.

Methyl [20,20-$^2H_2$]-20-(tert-butyldiphenylsilyloxy)eicosa-5(Z),8(Z),11(Z),14(Z)-tetraenoate (**7**)

Ethyl 4-pentenoate (**11**) (500 mg, 3.9 mmol) in ether (5 ml) was added to a stirring, 0°C suspension of $LiAl^2H_4$ (81 mg, 1.9 mmol; 98 atom % D, Aldrich

Chem. Co.) in ether (10 ml) under an argon atmosphere. After 45 min, the reaction mixture was quenched with a saturated aqueous Na_2SO_4 solution (5 ml) and then stirred for 30 min at room temperature. The ether layer was decanted and the aqueous layer was extracted with ether (2×25 ml). The combined ethereal extracts were washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude [1,1-$^2H_2$]-4-penten-1-ol[25] (302 mg, 88%) was used in the next step without further purification.

t-$BuPh_2SiCl$ (1.4 g, 5.1 mmol) in CH_2Cl_2 (5 ml) was added to a 0°C mixture of the above 4-penten-1-ol (300 mg, 3.4 mmol) and imidazole (694 mg, 10.21 mmol) in CH_2Cl_2 (15 ml) under an argon atmosphere. After 4 h at RT, the reaction mixture was filtered and the filter cake was rinsed with CH_2Cl_2 (10 ml). The combined filtrates were concentrated *in vacuo* and the residue was purified by SiO_2 flash chromatography using 5% EtOAc/hexanes to afford silyl ether **12** (1.04 g, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.04 (s, 9H), 1.64 (t, $J = 7.6$ Hz, 2H), 2.15 (q, $J = 7.0$ Hz, 2H), 4.91–5.02 (m, 2H), 5.73–5.86 (m, 1H), 7.34–7.44 (m, 6H), 7.65–7.68 (m, 4H).

9-BBN (4.5 ml, 0.5 M solution in THF, 2.23 mmol) was added to a stirring, room temperature solution of 1-(*tert*-butyldiphenylsilyloxy)pent-4-ene (**12**) (594 mg, 1.81 mmol) in THF (5 ml) under an argon atmosphere. After 2 h, the excess 9-BBN was quenched with water (2 ml). Without purification, the crude solution of borane **13** was cannulated into a mixture of vinyl bromide **5** (490 mg, 1.51 mmol), [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) chloride (123 mg, 0.15 mmol), $AsPh_3$ (92 mg, 0.30 mmol), and $CsCO_3$ (788 mg, 2.42 mmol) in DMF (5 ml). The reaction mixture was degassed by sparging with argon for 10 min and then allowed to stir overnight at room temperature. Following dilution with ether (100 ml), the reaction mixture was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by SiO_2 flash chromatography using 5% EtOAc/hexanes to give **7** (603 mg, 69%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.04 (s, 9H), 1.33–1.36 (m, 4H), 1.52–1.55 (m, 2H), 1.67–1.72 (m, 2H), 2.03–2.11 (m, 4H), 2.31 (t, $J = 7.6$ Hz, 2H), 2.78–2.84 (m, 6H), 3.65 (s, 3H), 5.32–5.42 (m, 8H), 7.36–7.40 (m, 6H), 7.64–7.67 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.25, 24.82, 25.49, 25.69, 25.71, 25.73, 26.60, 26.94, 27.29, 29.45, 32.33, 33.39, 51.39, 61.51, 127.65, 127.74, 127.94, 128.21, 128.56, 128.93, 128.95, 129.56, 130.29, 134.15, 135.59, 174.23.

Methyl [20,20-$^2H_2$]-20-hydroxyeicosa-5(Z),8(Z),11(Z),14(Z)-tetraenoate (**8**)

n-Bu_4NF (1 ml, 1 M solution in THF, 1.03 mmol) was added to a 0°C solution of methyl ester **7** (540 mg, 0.94 mmol) in THF (10 ml) under an argon atmosphere. After 3 h at RT, the reaction mixture was diluted with saturated aqueous NH_4Cl (10 ml) and extracted with ether (3×50 ml). The combined ethereal extracts were washed with water, brine, dried over Na_2SO_4, and

concentrated *in vacuo*. Purification of the residue by passage through a pad of silica gel using 30% EtOAc/hexanes provided **8** (261 mg, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27–1.37 (m, 6H), 1.64–1.76 (m, 2H), 2.03–2.11 (m, 4H), 2.31 (t, $J = 7.6$ Hz, 2H), 2.78–2.84 (m, 6H), 3.66 (s, 3H), 5.30–5.41 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.84, 25.47, 25.69, 25.72, 26.62, 27.29, 29.53, 32.54, 33.51, 51.59, 62.21, 127.89, 128.01, 128.23, 128.25, 128.55, 128.96, 129.00, 130.21, 174.21.

[20,20-$^2H_2$]-20-Hydroxyeicosa-5(Z),8(Z),11(Z),14(Z)-tetraenoic acid (**2**)

LiOH (0.5 ml, 1 M solution in water, 0.45 mmol) was added to a 0°C solution of **8** (53 mg, 0.15 mmol) in THF/H_2O (5 ml, 4:1) under an argon atmosphere. After 12 h at RT, the reaction mixture was cooled to at 0°C, acidified to pH 4.5 using 1 M aqueous oxalic acid, and extracted with EtOAc (2×25 ml). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was passed through a short pad of silica gel using 50% EtOAc/hexanes to give **2** (41 mg, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36–1.42 (m, 4H), 1.55 (t, $J = 6.7$ Hz, 2H), 1.64–1.76 (m, 2H), 2.05–2.16 (m, 2H), 2.34 (t, $J = 7.3$ Hz, 2H), 5.32–5.45 (m, 8H); HRMS (CI, CH_4) calculated for $C_{20}H_{31}D_2O_3$ (M + 1) 323.2555, found 323.2556.

Acknowledgements

Financial support provide by the Robert A. Welch Foundation and NIH (GM31278, DK38226).

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