

## Research Article

# Synthesis of $^{15}\text{N}$ -, $^{13}\text{C}$ -, and $^2\text{H}$ -labeled methanandamide analogs

Fen-Mei Yao<sup>1</sup>, Sonya L. Palmer<sup>1</sup>, Atmaram D. Khanolkar<sup>1</sup>,  
Xiaoyu Tian<sup>1</sup>, Jianxin Guo<sup>1</sup> and Alexandros Makriyannis<sup>1,2,3,\*</sup>

<sup>1</sup> *Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA*

<sup>2</sup> *Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA*

<sup>3</sup> *Center for Drug Discovery, University of Connecticut, Storrs, CT 06269, USA*

## Summary

Four isotopically labeled, metabolically stable analogs of arachidonyl-ethanolamide (anandamide), an endogenous cannabinoid ligand, were synthesized via a five-step reaction sequence starting from arachidonic acid. These stable methanandamide derivatives will serve as probes for studying the conformational properties of anandamide in model membrane systems using solid-state NMR spectroscopy. The synthetic methods described can be applied to the preparations of other anandamide analogs with isotopic labeling in different positions of the molecule, which could be utilized in biochemical and pharmacological experiments. Copyright © 2002 John Wiley & Sons, Ltd.

**Key Words:** anandamide; isotopically labeled; conformation

\*Correspondence to: A. Makriyannis, Department of Pharmaceutical Sciences, Center for Drug Discovery, University of Connecticut, Storrs, CT 06269, USA.  
E-mail: makriyan@uconnvm.uconn.edu

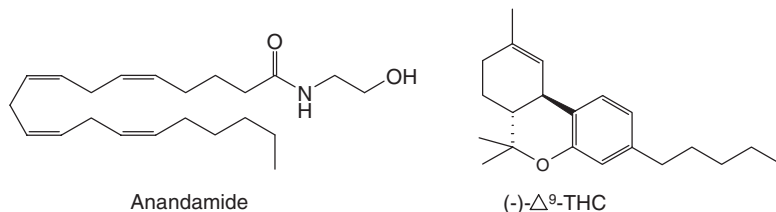
Contract/grant sponsor: National Institute on Drug Abuse; contract/grant number: DA03801, DA07215, and DA9158

## Introduction

Anandamide (arachidonylethanolamide, AEA, Table 1) is an endogenous cannabinoid receptor ligand initially isolated from porcine brain in 1992. Like other cannabimimetic agents, anandamide was shown to modulate cAMP levels and inhibit the electrically evoked twitch response of the mouse vas deferens.<sup>1</sup> Anandamide was also shown to produce the four characteristic *in vivo* effects of cannabimimetics, which include analgesia, hypothermia, hypoactivity, and catalepsy; however, it was found to be somewhat less potent than (-)- $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC, Table 1), the most widely known prototypical cannabinoid.<sup>2, 3, 4</sup>

The structure of anandamide differs greatly from the tricyclic structure of the plant-derived classical cannabinoids, such as  $\Delta^9$ -THC. This has stimulated vigorous research in order to explain their pharmacological similarity and provide a reasonable structural basis for comparison of these dissimilar cannabimimetic ligands. The currently known cannabinoid receptors, CB1 and CB2, have not yet been structurally characterized and only limited information is available regarding their active sites. Also, no crystallographic data is available outlining the receptor–bond ligand. For this reason, the information on the conformational properties of this highly flexible molecule and the conformation it adopts when bound to the receptor site can greatly help in understanding the molecular features involved in anandamide–cannabinoid receptor interaction.<sup>5</sup> Recently, molecular dynamics calculations were used to characterize the conformational mobility of a variety of anandamide analogs. The studies involving anandamide and its analogs identified an energetically favored loop-like conformation, which can be structurally correlated to the classical cannabinoids.<sup>6</sup> Also, Welsh *et al.* used constrained molecular dynamics simulations on

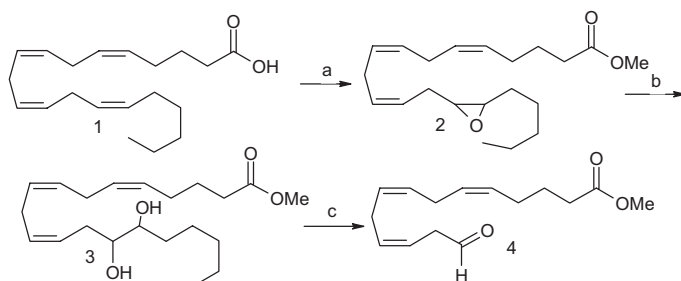
**Table 1** Natural and Endogenous Cannabinoids



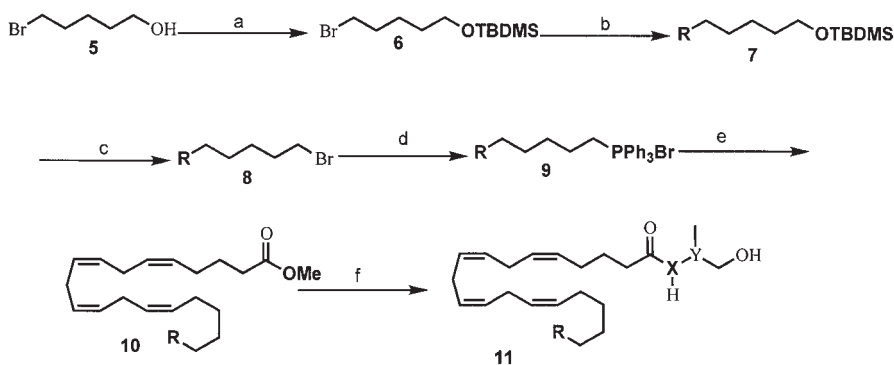
anandamide, in combination with a systematic distance comparison search, to reveal a specific low-energy conformer whose spatial disposition of the pharmacophoric elements closely matches that of hexahydrocannabinol, a synthetic  $\Delta^9$ -THC analog.<sup>7</sup> Finally, Reggio *et al.* have used conformational memories to explore the conformational space of anandamide in both polar and nonpolar environments and found that anandamide in water favors hybrid extended/U-shaped conformations, whereas, in chloroform, the extended (angle-iron) conformation is predominant.<sup>5</sup> Thus, the computational work on the conformational properties of anandamide revealed substantially different results depending on the method being used. These discrepancies argued in favor of an experimental approach for studying the conformational properties of anandamide in solution, in the membrane bilayer and at the receptor active site. In our laboratory, we have designed solid-state NMR experiments to study the conformational properties of anandamide in a membrane environment. Such work necessitated the availability of anandamide and related analogs specifically labeled with <sup>13</sup>C, <sup>15</sup>N, and <sup>2</sup>H stable isotopes. Complementary to our earlier work focusing on labeled anandamide analogs,<sup>8</sup> this paper discusses the synthesis of metabolically stable methanandamide derivatives (**11a–d**) for the use in pharmacological and NMR experiments. The methods reported here are also easily applicable to the synthesis of other isotopically labeled anandamides and related analogs of potential usefulness in pharmacological or biochemical experiments.

## Results and discussion

Preparation of the title compound was carried out as outlined in Schemes 1 and 2. The entry point for the synthesis of the <sup>13</sup>C-20 labeled analogs was the known intramolecular self-epoxidation of arachidonic acid developed by Corey *et al.*<sup>9</sup> to form the 14,15-epoxide regioselectively. This was followed by reconstruction of the double bond via the Wittig reaction using a <sup>13</sup>C-labeled phosphonium salt. Thus, arachidonic acid was treated with carbonyldiimidazole and the resulting arachidonyl imidazolide was reacted with a large excess of ethereal hydrogen peroxide at 0°C to afford the corresponding epoxy acid, which was esterified with carbonyldiimidazole/methanol to give the epoxy ester **2**. The epoxide was opened under acidic conditions to the diol and then oxidized to the unstable aldehyde ester **4**. The  $\omega$ -labeled



**Scheme 1.** (a): (i)  $\text{CO}(\text{imidazole})_2$ , 1.05eq; (ii) 5.4  $\text{MH}_2\text{O}_2$ -ether; (iii)  $\text{CO}(\text{imidazole})_2$ , MeOH,  $\text{CH}_2\text{Cl}_2$ , (b) 10%  $\text{HClO}_4$ -ether, and (c)  $\text{Pb}(\text{OAc})_4$ - $\text{CH}_2\text{Cl}_2$ ,  $-30^\circ\text{C}$



**Scheme 2.** (a) TBDMSCl/imidazole in THF,  $0^\circ\text{C}$ -RT, (b) RLi, CuCN, ether,  $-20^\circ\text{C}$ -RT, (c)  $\text{PPh}_3\text{Br}_2$ - $\text{CH}_2\text{Cl}_2$ , RT, (d)  $\text{PPh}_3$ , in a sealed vial,  $110^\circ\text{C}$ , 12 h, (e): (i)  $n\text{-BuLi}$ -THF,  $-78^\circ\text{C}$ ; (ii) 4 in THF,  $-78^\circ\text{C}$ -RT, (f) 2-amino-propanol, NaCN (Cat.), MeOH, in a sealed vial,  $50^\circ\text{C}$ , overnight. 11a R =  $^{-13}\text{CH}_3$ , X =  $^{15}\text{N}$ , Y = CH; 11b R =  $^{-13}\text{CH}_3$ , X = N, Y = CH; 11c R =  $^{-13}\text{C}_2\text{H}_5$ , X = N, Y = CH; 11d R =  $^{-13}\text{CH}_3$ , X = N, Y =  $^{13}\text{CH}$

phosphonium salt **9** was prepared in a straightforward method outlined in Scheme 2 starting with 5-bromopentanol. Its hydroxyl was protected with a *t*-butyldimethylsilyl group before it was subjected to a cross-coupling reaction with isotopically labeled methyl lithium ( $\text{LiCD}_3/\text{Li}^{13}\text{CH}_3$ ) to introduce the isotope-labeled C-20 methyl.

The Wittig reaction between the ylides derived from **9** and **4** led to the desired *cis*-isomer exclusively.<sup>10,11</sup> The  $\omega$ -labeled arachidonate methyl ester was subjected to differently labeled 2-amino-propanol isotopomers

in coupling reactions catalyzed by sodium cyanide in methanol to give the desired labeled compounds. The head group  $^{15}\text{N}$ -,  $^{13}\text{C}$ -labeled analogs were synthesized by direct amidation of arachidonate methyl ester with DL- $^{15}\text{N}$ -2-aminopropanol (**12**) obtained from the reduction of the corresponding amino acid with  $\text{LiAlH}_4$ .<sup>12</sup>

## Conclusion

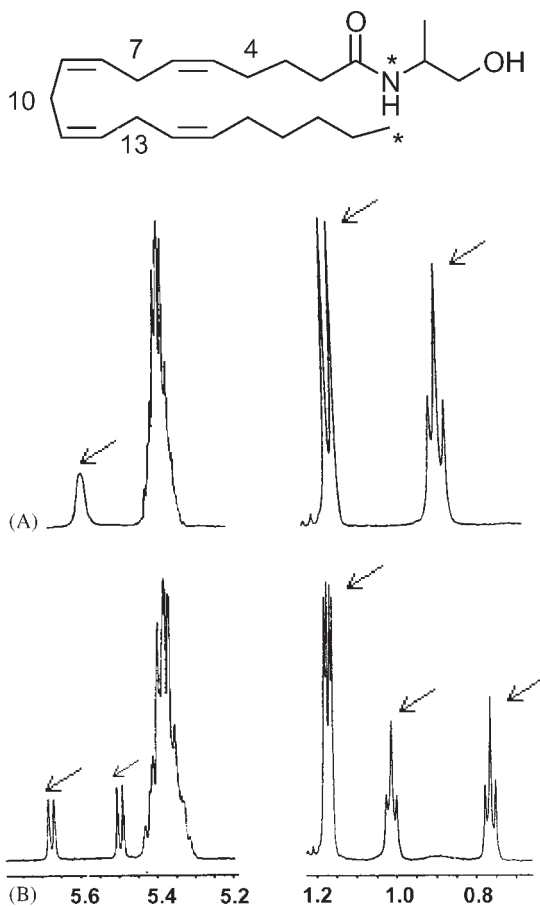
Four isotopically labeled anandamide analogs, **11a–d**, were synthesized. Their structures were confirmed by HRMS and NMR spectroscopy. Evidence for the  $^{15}\text{N}$ ,  $^{13}\text{C}$ , and  $^2\text{H}$  isotopic labeling was provided by the respective high-field  $^1\text{H}$ -NMR spectra (Figures 1–4). These compounds are currently being used to study the conformation of anandamide in the membrane bilayer using REDOR NMR experiments.<sup>13</sup> Preliminary experimental results to be reported elsewhere show that anandamide in the membrane bilayer adopts an extended conformation in which the individual allylic fragments are orthogonal to each other.

## Experimental

$^1\text{H}$ -NMR spectra were recorded on a Bruker AMX-500 MHz spectrometer using TMS as the internal standard in  $\text{CDCl}_3$ . All chemical shifts are reported in ppm. Mass spectra were obtained on a ZAB-SE mass spectrometer via FAB. Arachidonic acid was purchased from Nu-Chek-Prep, Inc. The 2- $^{13}\text{C}$ -aminopropanol was obtained from Cambridge Isotope Laboratories, Inc.

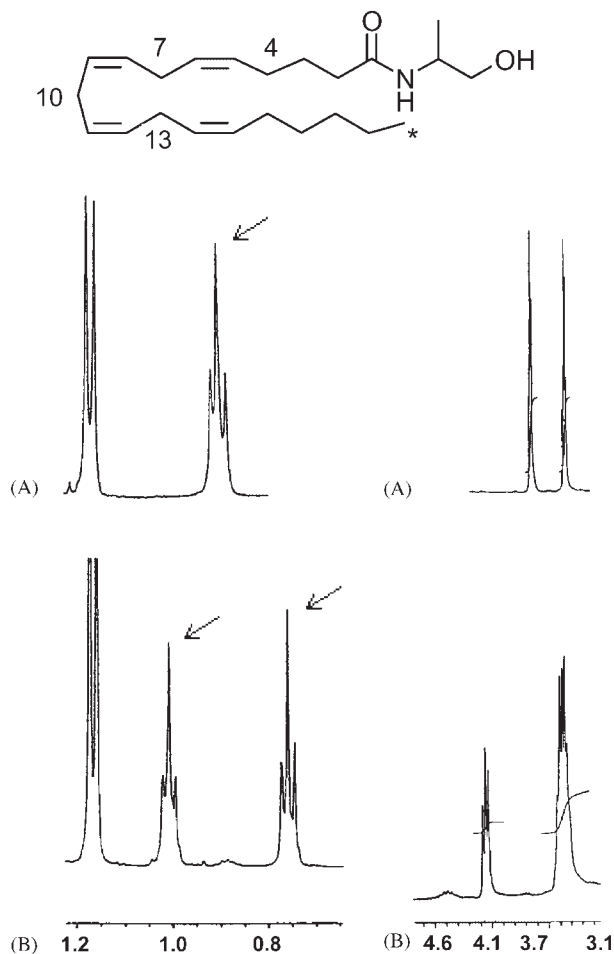
### *Methyl 14,15-epoxyeicosa-cis-5,8,11-trienoate (2)*

1,1'-Carbonyl-diimidazole (1.96 g, 12.09 mmol) in 15 ml dry methylene chloride was treated with arachidonic acid (3.5 g, 11.51 mmol) under argon with stirring at an ambient temperature. After 40 min of stirring, the resulting arachidonyl imidazolide solution was added dropwise over a 5 min period to ethereal hydroperoxide (4.56 M, 80 ml) at  $0^\circ\text{C}$ .  $\text{KHSO}_4$  (22.73 g, 166.94 mmol) was added to the reaction when half the imidazolide had been added. The mixture was stirred for an additional 5 min, filtered, washed with water and brine, and dried over  $\text{Na}_2\text{SO}_4$ . Evaporation under reduced pressure afforded 3.64 g of a mixture of arachidonic acid and the peroxy acid.



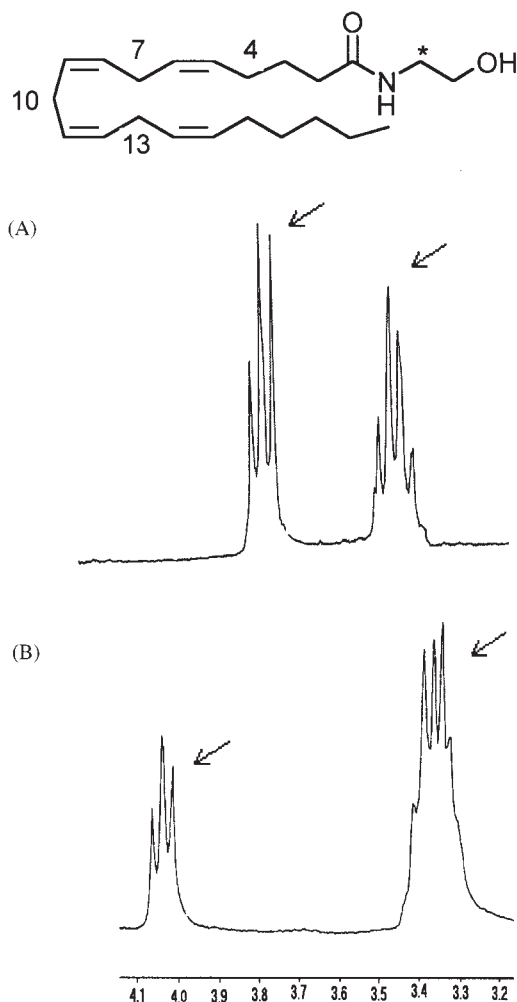
**Figure 1.**  $^1\text{H-NMR}$  spectrum of 2'-methylanandamide for the C-20, C2'-methyl and N-protons. The  $^{15}\text{N}$ -labeled compounds exhibit a  $J^{1\text{H}-^{15}\text{N}} = 89.1$  Hz: (A) molecule without isotopic labeling and (B) molecule with  $^{13}\text{C}$ -20 and  $^{15}\text{N}$ -double labeling

The above epoxide acid (3.64 g) was added to 1,1'-carbonyldiimidazole (3.73 g, 23.02 mmol) in 25 ml of dry methylene chloride with stirring under argon at ambient temperature. After stirring for 30 min, methanol (4.2 ml, 103.62 mmol) was added, and the reaction was stirred for 4 h at ambient temperature (monitored by TLC, 30% ethyl acetate in petroleum ether). The volatiles were evaporated on a rotary evaporator and the residue partitioned between ether and water. The combined ether layers were washed with brine and dried ( $\text{Na}_2\text{SO}_4$ ). The residue upon evaporation in a vacuum (4.5 g) was purified by chromatography on silica gel (10% ethyl acetate–petroleum ether) to



**Figure 2.**  $^1\text{H}$ -NMR spectra of 2'-methylanandamide for the C-20 and C2'-methyl protons. The  $^1\text{H}$ -spectrum for  $^{13}\text{C}$ -20 labeled compounds exhibit a  $J^{1\text{H}}\text{-}^{13}\text{C} = 124.5$  Hz: (A) molecule without isotopic labeling and (B) molecule with  $^{13}\text{C}$ -20 labeling

afford 1.2 g of methyl arachidonate and 2.2 g of the title epoxy ester (57%, based on arachidonic acid): Oil,  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ):  $\delta$ 5.47 (m, 2H, 11, 12-vinyl H), 5.36 (m, 4H, 5, 6, 8, 9-vinyl H), 3.66 (s, 3H,  $\text{OCH}_3$ ), 2.94 (m, 2H, 13- $\text{CH}_2$ ); 2.84 (t, 2H,  $J = 5.9$  Hz, 10- $\text{CH}_2$ ), 2.80 (t, 2H,  $J = 5.5$  Hz, 7- $\text{CH}_2$ ), 2.40 (m, 1H, 14-CHO), 2.32 (2H, t,  $J = 7.5$  Hz, 2- $\text{CH}_2$ ), 2.24 (m, 1H, 15-CHO), 2.10 (m, 2H, 4- $\text{CH}_2$ ), 1.70 (m, 2H,  $J = 7.5$  Hz 3- $\text{CH}_2$ ), 1.51 (br.m, 2H, 16- $\text{CH}_2$ ), 1.33 (br. m, 6H, 1719- $\text{CH}_2$ ), 0.90 (t, 3H,  $J = 7.1$  Hz, 20- $\text{CH}_3$ ).

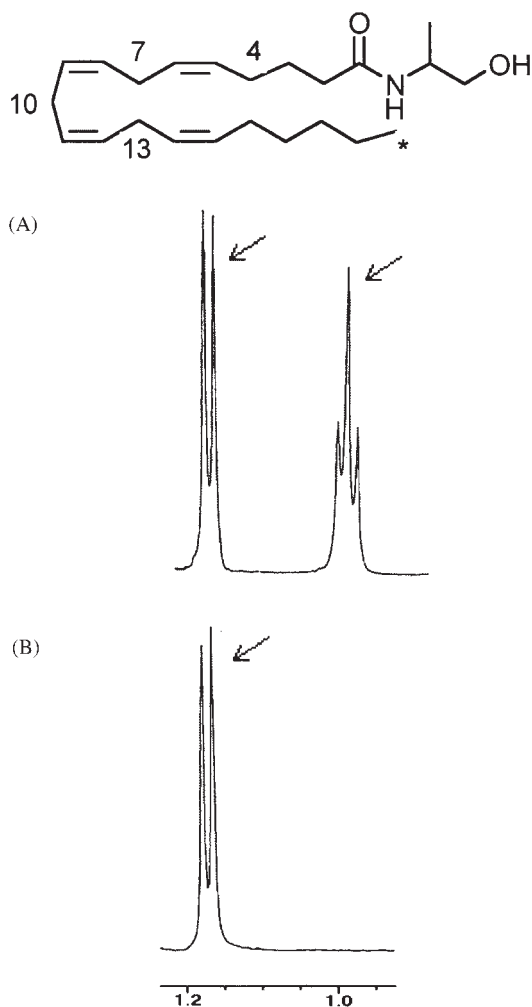


**Figure 3.**  $^1\text{H}$ -NMR spectra of anandamide for the C1', C2'-methylene protons. In the  $^1\text{H}$  spectrum of the  $^{13}\text{C}$ -labeled anandamide, the frequency due to the H- $^{13}\text{C}$  protons is a doublet of triplets  $J^{\text{H}-^{13}\text{C}} = 125.1$  Hz: (A) molecule without isotopic labeling and (B) molecule with  $^{13}\text{C}$ -2' labeling

### *Methyl 14,15-dihydroxyeicosa-cis-5,8,11-trienoate (3)*

To a stirred solution of the above epoxy ester (2.2 g, 6.59 mmol) in 50 ml of ether at  $0^\circ\text{C}$  was added 20 ml of cold 10% aqueous  $\text{HClO}_4$  dropwise. The ice bath was removed, and the reaction mixture was stirred at ambient temperature until hydrolysis appeared to be complete ( $\sim 6$  h) by TLC (10% ethyl acetate in petroleum ether). The reaction





**Figure 4.**  $^1\text{H}$ -NMR spectra of 2'-methylanandamide for the C2'-methyl and 20-methyl protons.  $^2\text{H}$ -labeling at the C-20 carbon results in the disappearance of the terminal methyl  $^1\text{H}$  frequencies: (A) molecule without isotopic labeling and (B) molecule with  $^2\text{H}_3$ -20 double labeling

mixture was diluted with ether and the ether layer was washed with aqueous  $\text{NaHCO}_3$  and brine. After drying ( $\text{Na}_2\text{SO}_4$ ), ether was removed and the residue was chromatographed on silica gel (30% ethyl acetate–petroleum ether) to afford 1.5 g of the diol (65%) as an oil,  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ):  $\delta$  5.5 (m, 2H, 11, 12-vinyl H), 5.36 (m, 4H, 5, 6, 8, 9-vinyl H), 3.67 (s, 3H,  $\text{OCH}_3$ ), 3.46 (m, 2H, 14, 15-CHO), 2.82 (m, 4H, 7, 10- $\text{CH}_2$ ), 2.32 (t, overlap, 4H,  $J = 7.4$  Hz, 2, 13- $\text{CH}_2$ ), 2.20 (m, 2H, OH), 2.09 (m,

2H, 4-CH<sub>2</sub>), 1.69 (q, 2H,  $J = 7.4$  Hz, 3-CH<sub>2</sub>), 1.49 (m, 2H, 16-CH<sub>2</sub>), 1.31 (br. M, 6H, 17-19-CH<sub>2</sub>), 0.89 (m, 3H, 20-CH<sub>3</sub>).

#### *Methyl 14-oxotetradeca-cis-5, 8,11-trienoate (4)*

To a stirred solution of the diol (500 mg, 1.42 mmol) in 5 ml of anhydrous CH<sub>2</sub>Cl<sub>2</sub> at  $-30^{\circ}\text{C}$  was added dropwise to a solution of lead tetraacetate (692 mg, 1.56 mmol) in 5 ml of CH<sub>2</sub>Cl<sub>2</sub> and the resulting mixture was stirred for 30 min then quenched with ethylene glycol. Solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>) to afford 230 mg (64%) the title aldehyde.

### **Bromohexane-1-<sup>13</sup>C and Bromohexane-1-C<sup>2</sup>H<sub>3</sub>**

#### *5-t-Butyl dimethylsilyloxy-1-bromo-pentane (6)*

To a stirred solution of 5-bromo-pentanol (**9**) (2.2 g, 13.7 mmol) and imidazole (3.75 g, 53.7 mmol) in 5 ml of anhydrous DMF at  $0^{\circ}\text{C}$  was added a solution of *t*-butyldimethylchlorosilane (4.57 g, 30.3 mmol) in 5 ml of anhydrous DMF under argon. TLC showed the reaction to be complete in 1 h (5% ethyl acetate–petroleum ether). The reaction mixture was poured into ice water, and extracted with ether. The combined organic phase was washed with brine and dried (Na<sub>2</sub>SO<sub>4</sub>). After evaporation on a rotary evaporator, the residue was purified by chromatography (3% ethyl acetate–petroleum ether) to afford 3.29 g (85%) of the title compound: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) $\delta$ 3.61 (2H, t,  $J = 5.8$  Hz, CH<sub>2</sub>O); 3.41 (2H, t,  $J = 6.7$  Hz, CH<sub>2</sub>Br), 1.87 (3H, q,  $J = 7.0$  Hz, 1.49 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 0.89 (9H, s, *t*Bu), 0.05 (6H, s, Me<sub>2</sub>).

### **1-*t*-Butyl dimethylsilyl-6-<sup>13</sup>C (or C<sup>2</sup>H<sub>3</sub>)-hexanol (7)**

#### <sup>13</sup>CH<sub>3</sub>Li

To a stirred suspension of lithium (366.8 mg, 52.8 mmol) in 15 ml anhydrous ether was added <sup>13</sup>CH<sub>3</sub>I (5.0 g, 35.2 mmol) dropwise while keeping the reaction mixture gently refluxing. After the addition was complete, the reaction mixture was refluxed for 2 h.

A flask charged with CuCN (1.578 g, 17.615 mmol) was gently heated with a heat gun under vacuum to remove residual moisture, and then flushed with argon. After cooling to room temperature under argon, the solid was suspended in 5 ml of anhydrous THF and was allowed to cool to  $-78^{\circ}\text{C}$ . Freshly prepared  $^{13}\text{C}_3\text{Li}$  (or commercially available  $\text{CD}_3\text{Li}$ ) was added via cannula using a positive pressure of argon. After the addition was complete, the mixture was warmed to  $0^{\circ}\text{C}$  and stirred for 5 min at this temperature. The dark green opaque mixture was then cooled to  $-78^{\circ}\text{C}$  and a solution of **6** (3.12 g, 10.7 mmol) in 25 ml of anhydrous THF was added via cannula. The resulting mixture was warmed to  $-20^{\circ}\text{C}$ , stirred for 4 h, then allowed to warm to room temperature and stirred overnight. The reaction was quenched with  $\text{NH}_3\cdot\text{H}_2\text{O}\text{-NH}_4\text{Cl}$  aqueous solution (1:2), extracted with ether, washed with brine, and dried ( $\text{Na}_2\text{SO}_4$ ). After purification by chromatography on silica gel (5% ether:petroleum ether) affording 1.43 g (60%) of the title product:  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) ( $^{13}\text{C}$ -labeled)  $\delta$ 3.59 (2H, t,  $J = 6.5$  Hz, 1- $\text{CH}_2$ ), 1.49 (2H, m, 2- $\text{CH}_2$ ), 1.281.07 (6H, m, 3- and 5- $\text{CH}_2$ ), 0.88 (3H, dt,  $J = 124.5$  and  $6.5$  Hz,  $^{-13}\text{C}_3$ ) 0.81 (9H, s, tBuSi), 0.04 (6H, s,  $\text{Me}_2\text{Si}$ ). ( $^2\text{H}_3$ -labeled)  $\delta$ 3.59 (2H, t,  $J = 6.5$  Hz, 1- $\text{CH}_2$ ), 1.49 (2H, p,  $J = 6.5$  Hz, 2- $\text{CH}_2$ ) 1.30 (6H, m, 3- and 5- $\text{CH}_2$ ), 0.81 (9H, s, tBuSi), 0.04 (6H, s,  $\text{Me}_2\text{Si}$ ).

*Bromohexane-6- $^{13}\text{C}$  (or  $\text{C}^2\text{H}_3$ ) (8)*

To a stirred suspension of triphenyldibromo-phosphorane (2.18 g, 4.95 mmol) in anhydrous methylene chloride at room temperature was added a solution of **4** (827 mg, 3.81 mmol) in anhydrous methylene chloride under argon. The mixture was stirred at room temperature for 30 min and then diluted with ether and filtered through a pad of silica gel topped with celite. The solvent was removed by distillation under atmospheric pressure to give the expected product as an oil (900 mg, 69%):  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) ( $^{13}\text{C}$ -labeled):  $\delta$ 3.31 (2H, t,  $J = 6.8$  Hz, 1- $\text{CH}_2$ ), 1.75 (2H, p,  $J = 7.3$  Hz, 2- $\text{CH}_2$ ), 1.33 (2H, m, 3- $\text{CH}_2$ ), 1.20 (4H, m, 4, 5- $\text{CH}_2$ ), 0.88 (3H, dt,  $J = 124.5$  and  $6.8$  Hz,  $^{13}\text{C}_3$ ). ( $\text{D}_3$ -labeled) ( $\text{CDCl}_3$ )  $\delta$ 3.31 (2H, t,  $J = 6.8$  Hz, 1- $\text{CH}_2$ ), 1.75 (2H, p,  $J = 7.3$  Hz, 2- $\text{CH}_2$ ), 1.33 (2H, m, 3- $\text{CH}_2$ ), 1.20 (4H, m, 4, 5- $\text{CH}_2$ ).

*1-Triphenylphosphoniumyl-hexane bromide-6-<sup>13</sup>C (or 6-C<sup>2</sup>H<sub>3</sub>) (9)*

A solution of **8** (800 mg, 4.81 mmol), and triphenylphosphine (1.299 g, 4.95 mmol) in 5 ml of acetonitrile was heated in a sealed Reacti-vial with stirring at 110°C for 48 h. After evaporation of acetonitrile under reduced pressure and the excess of triphenylphosphine was removed by triturating with ether leaving 1.8 g of the title phosphonium salt **9**. <sup>1</sup>H-NMR (<sup>13</sup>C -labeled in methanol-d<sub>4</sub>): δ7.907.61 (5H, m, tri-Ph), 3.46 (2H, m, 1-CH<sub>2</sub>), 1.71 (2H, m), 1.60 (2H, q, *J* = 7.0 Hz), 1.35(4H, m), 0.93 (3H, dt, *J* = 126 and 6.8 Hz, -<sup>13</sup>CH<sub>3</sub>). (**D<sub>3</sub>**-labeled in CDCl<sub>3</sub>): 7.90–7.61 (15H, m, 3Ph H), 3.75 (2H, m, 1-CH<sub>2</sub>), 1.62 (4H, m, 2, 3-CH<sub>2</sub>), 1.21 (4H, m, 4, 5-CH<sub>2</sub>).

*Methyl-20-<sup>13</sup>C-eicosa-cis-5,8,11, 14-tetraenoate (or 20-C<sup>2</sup>H<sub>3</sub>) (10)*

A solution of the phosphonium salt (**9**) (832 mg, 1.95 mmol) in anhydrous THF under argon was cooled to –76°C and treated with *n*-BuLi (1.06 ml, 2 M in hexane, 2.11 mmol) with stirring. The resulting deep red solution was stirred at –76°C for 30 min, and the freshly prepared aldehyde (**4**, 1.62 mmol) in anhydrous THF (3 ml) was added dropwise. The mixture was stirred for 1 h at –76°C and then allowed to warm to room temperature gradually over 3 h. Adding a phosphate buffer (pH 7) quenched the reaction, and the volatiles were evaporated under reduced pressure. The residue was dissolved in ethyl ether, and the solution was washed with water, brine and dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of the ether and chromatography of the residual oil on silica gel (5% diethyl ether–petroleum ether) afforded 100 mg of the title compound (30%, based on the diol ester). <sup>1</sup>H-NMR (CDCl<sub>3</sub>), (<sup>13</sup>C-labeled) δ5.38 (8H, m, 5, 6, 8, 9, 11, 12, 14, 15-vinyl H), 3.67 (3H, s, OCH<sub>3</sub>), 2.82 (6H, m, 7, 10, 13-CH<sub>2</sub>), 2.32 (2H, t, *J* = 7.5 Hz, 2-CH<sub>2</sub>), 2.132.03 (4H, m, 4, 16-CH<sub>2</sub>), 1.71 (2H, p, *J* = 7.5 Hz, 3-CH<sub>2</sub>), 1.371.29 (6H, 1719-CH<sub>2</sub>), 0.89 (3H, dt, *J* = 124.5 and 6.8 Hz, 20-<sup>13</sup>CH<sub>3</sub>). (<sup>2</sup>H<sub>3</sub>-labeled): 5.38 (8H, m, 5, 6, 8, 9, 11, 12, 14, 15-vinyl H), 3.67 (3H, s, -OCH<sub>3</sub>), 2.82 (6H, m, 7, 10, 13-CH<sub>2</sub>), 2.32 (2H, t, *J* = 7.4 Hz, 2-CH<sub>2</sub>), 2.13–2.03 (4H, 4, 16-CH<sub>2</sub>), 1.71 (2H, p, *J* = 7.4 Hz, 3-CH<sub>2</sub>), 1.28 (6H, m, 1719-CH<sub>2</sub>).

*DL-<sup>15</sup>N-2-amino-propanol (12)*

A solution of DL-<sup>15</sup>N-alanine (200 mg, 2.245 mmol) in 10 ml of anhydrous THF was cooled to 0°C and 4.5 ml of a 1.0 M solution of

$\text{LiAlH}_4$  in THF added dropwise. The reaction mixture was stirred at  $0^\circ\text{C}$  to room temperature for 5 h. TLC showed it was complete. The reaction was quenched with aqueous  $\text{NaHCO}_3$ , extracted with ethyl acetate, dried over  $\text{Na}_2\text{SO}_4$  to afford 150 mg of the titled aminoalcohol after evaporation, which was used in the next step without purification.

*DL-20- $^{13}\text{C}$ - $^{15}\text{N}$ -(eicosa-cis-5,8,11,14-tetraenoyl)-1'-hydroxy-2'-propyl-amine (11a)*

A solution of **10** (40 mg, 0.125 mmol), sodium cyanide (0.90 mg, 0.0184 mmol), and DL- $^{15}\text{N}$ -amino -propanol (150 mg, crude) in methanol in a sealed Reacti-vial was heated to  $50^\circ\text{C}$  with oil bath and stirred overnight. The solvent was evaporated on a rotary evaporator and the residue was purified by chromatography (5% methanol in  $\text{CH}_2\text{Cl}_2$ ) affording 20 mg (43.9%) of the title compound:  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ 5.58 (1H, dd,  $J = 89.1$  and  $7.2$  Hz,  $^{15}\text{N-H}$ ), 5.42–5.32 (8H, m, 5, 6, 8, 9, 11, 12, 14, 15-vinyl H), 4.07 (1H, m,  $^{15}\text{N-CH-}$ ), 3.66 (1H, m, 2'- $\text{CH-OH}$ ), 3.53 (1H, m, 2'- $\text{CH-OH}$ ), 2.84–2.74 (6H, m, 7, 10, 13- $\text{CH}_2$ ), 2.19 (2H, t,  $J = 7.5$  Hz, 2- $\text{CH}_2$ ), 2.13 (2H, m, 4/16- $\text{CH}_2$ ), 2.05 (2H, m, 16/4- $\text{CH}_2$ ), 1.72 (2H, p,  $J = 7.5$  Hz, 3- $\text{CH}_2$ ), 1.34 (6H, m, 17,19- $\text{CH}_2$ ), 1.17 (3H, dd,  $J = 6.8$  and  $2.8$  Hz,  $^{15}\text{NHCCH}_3$ ), 0.89 (3H, dt,  $J = 124.5$  and  $6.8$  Hz, 20- $^{13}\text{CH}_3$ ). FAB-MS:  $\text{MH}^+$  calc. 364.3216; found 364.3213.

*DL-20- $^{13}\text{C}$ -(eicosa-cis-5,8,11,14-tetraenoyl)-1'-hydroxy-2'-propylamine (11b)*

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 5.65 (1H, br.m., N-H), 5.42–5.32 (8H, m, 5,6,8,9,11,12,14,15-vinyl-H), 4.06 (1H, m, NCH-), 3.65 (1H, m, -CHO), 3.52 (1H, m, - $\text{CH}'\text{O}$ ), 2.97 (1H, br.m, -OH), 2.84–2.74 (6h, m, 7, 10, 13- $\text{CH}_2$ ), 2.19 (2H, t,  $J = 7.5$  Hz, 2- $\text{CH}_2$ ), 2.13 (2H, m, 4/16- $\text{CH}_2$ ), 2.05 (2H, m, 16/4- $\text{CH}_2$ ), 1.72 (2H, p,  $J = 7.5$  Hz, 3- $\text{CH}_2$ ), 1.38–1.27 (6H, m, 17-19- $\text{CH}_2$ ), 1.17 (3H, d,  $J = 6.9$  Hz,  $\text{NCCH}_3$ ), 0.89 (3H, dt,  $J = 124.5$  and  $6.8$  Hz, 20- $^{13}\text{CH}_3$ ). FAB-HRMS:  $\text{MH}^+$ , 363.3090; calc.  $\text{MH}^+$ : 363.3092.

*20- $\text{C}^2\text{H}_3$ -(eicosa-cis-5,8,11,14-tetraenoyl)-1'-hydroxy-2'-propylamine (11c)*

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 5.56 (1H, br.m, N-H), 5.42–5.32 (8H, m, 5, 6, 8, 9, 11, 12, 14, 15-vinyl H), 4.06 (1H, m, N-CH), 3.65 (1H, dd,  $J = 10.8$  and

3.2 Hz, 2'-CHO), 3.52 (1H, dd,  $J = 10.8$  and 6.2 Hz, 2'-CH'O), 2.85–2.75 (6H, m, 7, 10, 13-CH<sub>2</sub>), 2.19 (2H, t,  $J = 7.5$  Hz, 2-CH<sub>2</sub>), 2.13 (2H, m, 4/16-CH<sub>2</sub>), 2.05 (2H, m, 16/4-CH<sub>2</sub>), 1.72 (2H, p,  $J = 7.5$  Hz, 3-CH<sub>2</sub>), 1.38–1.27 (6H, m, 17–19-CH<sub>2</sub>), 1.17 (3H, d,  $J = 6.9$  Hz, N-CCH<sub>3</sub>). FAB-MS: MH<sup>+</sup> calc. 365.3789; found 365.3786.

*2'-<sup>13</sup>C-(eicosa-cis-5,8,11,14-tetraenoyl) ethanolamine (11d)*

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): 6.18 (1H, m, CON-H), 5.45–5.28 (8H, m, 5, 6, 8, 9, 11, 12, 14, 15-vinyl H), 3.70 (2H, dt,  $J = 125.1$  and 5.1 Hz, CON-<sup>13</sup>CH<sub>2</sub>), 3.35 (2H, m, 2'-CH<sub>2</sub>O), 2.85–2.77 (6H, m, 7, 10, 13-CH<sub>2</sub>), 2.20 (2H, t,  $J = 7.5$  Hz, 2-CH<sub>2</sub>), 2.11–1.99 (4H, m, 4, 16-CH<sub>2</sub>), 1.70 (2H, p,  $J = 7.5$  Hz, 3-CH<sub>2</sub>), 1.28 (6H, m, 17/19-CH<sub>2</sub>), 0.87 (3H, m, 20-CH<sub>3</sub>). FAB-HRMS: MH<sup>+</sup> calc. 363.3092; found 363.3089.

### Acknowledgements

This research was supported by grants DA03801, DA07215 and DA9158 from the National Institute on Drug Abuse. The authors would also like to thank Drs. Spiro Pavlopoulos, Gerard Leclerc, and Chen Li for their valuable contributions to this paper.

### References

1. Devane WA, Dysarz FA, Johnson RM, Melvin LS, Howlett AC. *Science* 1992; **258**: 1946–1949.
2. Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR. *J Pharmacol Exp Ther* 1994; **270**: 219–227.
3. Varga K, Lake K, Martin BR, Kunos G. *Eur J Pharmacol* 1995; **278**: 279–283.
4. Welch SP, Dunlow LD, Patrick GS, Razdan RK. *J Pharmacol Exp Ther* 1995; **273**: 1235–1244.
5. Barnett-Norris J, Guarnier F, Hurst DP, Reggio PH. *J Med Chem* 1998; **41**: 4861–4872.
6. Thomas BF, Adams IB, Mascarella SW, Martin BR, Razdan RK. *J Med Chem* 1996; **39**: 472–482.
7. Tong W, Collantes ER, Welsh WJ. *J Med Chem* 1998; **41**: 4207–4215.
8. Xie X-Q, Yang H, Choi G, Lin S, Makriyannis A. *J Label Compd Radiopharm* 2002; **45**: 775–784.

9. Corey EJ, Niwa H, Falch JR. *J Am Chem Soc* 1979; **101**: 1586–1587.
10. Ryan WJ, Banner WK, Wiley JL, Martin BR, Razdan RK. *J Med Chem* 1997; **40**: 3617–3625.
11. Labelle M, Falgueyret JP, Riendeau D, Rokach J. *Tetrahedron* 1990; **46**: 6301–6310.
12. Kulkarni SU, Patil VK. *Heterocycles* 1992; **18**: 163–167.
13. Garbow JR, Breslav M, Antohi O, Naider F. *Biochemistry* 1994; **33**: 10094–10099.