Journal of Labelled Compounds and Radiopharmaceuticals

Received 12 July 2008, Revised 23 July 2008, Accepted 12 August 2008 Published online 9 October 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1540

Synthesis of 2-(5Z,8Z,11Z,14Z)-Icosa-5,8,11,14-tetraenamidoethyl-d_4 dihydrogen phosphate, tetra-deuterated pAEA†

Kejun Cheng,[a] Bijali Saha,[b] A. Mahadevan,[b] Raj K. Razdan,[b] George Kunos,[c] Arthur E. Jacobson,[a] and Kenner C. Rice[a]*

A labile intermediate phospho-anandamide (2-(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenamidoethyl dihydrogen phosphate, pAEA) has been identified in mouse brain and macrophages, but its precise quantitation was difficult because of its low concentration and chemical instability. We report the synthesis of tetra-deuterated pAEA from 2-aminoethyl dihydrogen phosphate-1,1,2,2-d^4 and (5Z,8Z,11Z,14Z)-2,5-dioxopyrrolidin-1-yl icosa-5,8,11,14-tetraenoate. The compound will be used to quantitate the pAEA necessary for a novel biosynthetic pathway.

Keywords: deuterium labeling; deuterated phospho-anandamide; pAEA; endocannabinoid

Introduction

Endocannabinoids are endogenous lipid ligands that interact with the same receptors that recognize the Δ^9-tetrahydrocannabinol in marijuana to produce similar biological effects. The first endocannabinoid was isolated from porcine brain and identified as arachidonoyl ethanolamide (anandamide, AEA) in 1992.[1] Shortly thereafter a two step biosynthetic process was proposed to account for the *in vivo* generation of AEA, whereby a transacylase transfers the arachidonic acid from the *sn*-1 position of phosphatidylcholine to the nitrogen in phosphatidylethanolamine (PE) to generate *N*-arachidonoyl PE (NAPE), which is then hydrolyzed by a NAPE-specific phospholipase D (NAPE-PLD) to yield AEA.[2] Unexpectedly, tissue AEA levels were found unaffected by genetic deletion[3] or knockdown of NAPE-PLD,[4] suggesting the existence of alternative biosynthetic pathways involved in the conversion of NAPE to anandamide. One such recently identified pathway involves the hydrolysis of NAPE by a phospholipase C (PLC) to yield the labile intermediate phospho-anandamide (pAEA), which is then dephosphorylated by a phosphatase.[4,5] pAEA was definitively identified in mouse brain and macrophages using HPLC/ESI-MS/MS, but its precise quantitation was difficult because of its low concentration and chemical instability.[4,5] In order to facilitate the quantitation of pAEA that is necessary for the further analysis of this novel biosynthetic pathway, we have introduced a method for the synthesis of tetra-deuterated pAEA.

Results and discussion

Based on the procedure for the preparation of the analogous C^{14}-labelled compound,[6] 2-aminoethanol-1,1,2,2-d^4 (**1**, Scheme 1) was added to a solution of dilute phosphoric acid and water was removed to obtain the intermediate 2-aminoethyl dihydrogen phosphate-1,1,2,2-d^4 (**2**). Compound **2** was obtained as a crystalline solid from a water–ethanol mixture. (5Z,8Z,11Z,14Z)-2,5-dioxopyrrolidin-1-yl icosa-5,8,11,14-tetraenoate (**3**) was added to an alkaline solution of **2** to give, after workup, 2-(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenamidoethyl-d_4 dihydrogen phosphate (tetra-deuterated pAEA, **4**) as a waxy solid. The availability of the tetra-deuterated compound **4** in reasonable overall yield via a simple two-step reaction will enable the detailed examination of interesting biosynthetic pathways.

Experimental

General

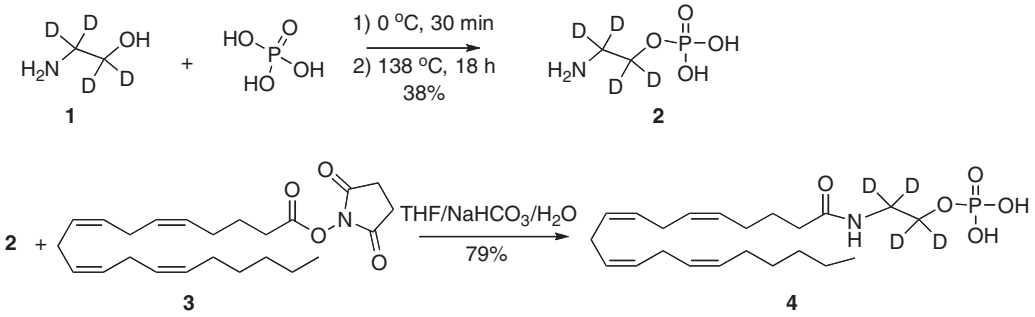
The starting material 2-aminoethanol-1,1,2,2-d^4 was obtained from CDN Isotopes (88 Leacock St., Pointe-Claire; Quebec, Canada H9R 1H1; www.CDNISOTOPES.com). Chemical ionization

[a]*Drug Design and Synthesis Section, Chemical Biology Research Branch, Department of Health and Human Services, 5625 Fishers Lane, Room 4N03, National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, MSC 9415, Bethesda, MD 20852, USA*

[b]*Organix Inc., 240 Salem Street, Woburn, MA 01801, USA*

[c]*Neuroendocrinology Section, Laboratory of Physiologic Studies, Department of Health and Human Services, 5625 Fishers Lane, Room 2S24, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, MSC 9413, Rockville, MD 20852, USA*

**Correspondence to: Kenner C. Rice, Drug Design and Synthesis Section, Chemical Biology Research Branch, Department of Health and Human Services, 5625 Fishers Lane, Room 4N03, National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, MSC 9415, Bethesda, MD 20852, USA.*
E-mail: kr21f@nih.gov.



Scheme 1.

mass spectra (CIMS) were obtained using a Finnigan 4600 mass spectrometer unless otherwise noted. ^{2}H nuclear magnetic resonance (^{2}H NMR, 500 MHz) was recorded on a Bruker Avance 500 instrument in deuterium depleted water (2–3 ppm deuterium, Cambridge Isotope Laboratories, Inc.) with the values given in ppm (D_2O as internal standard at 4.80 ppm). ^{31}P nuclear magnetic resonance (^{31}P NMR) spectra were recorded on a Varian Gemini-300 instrument in D_2O with the values given in ppm (H_2O as internal standard at 4.80 ppm). The melting point was determined on a Büchi B-545 melting point apparatus and is uncorrected. Combustion analyses were determined at Atlantic Microlabs, Atlanta, GA.

*2-Aminoethyl dihydrogen phosphate-1,1,2,2-d^4 (**2**)*

To an ice-cold solution of 2-aminoethanol-1,1,2,2-d^4 (**1**, 1.0 g, 15.4 mmol) in water (20 mL), was added dropwise, over 10 min, a solution of phosphoric acid (3.0 g, 30.8 mmol) in water (20 mL). After the mixture was stirred at 0°C for 30 min, most of the water was removed in *vacuo*. A desiccant flask holding phosphorus pentoxide was attached, and the flask assembly was evacuated to a pressure of 3–5 mm. The reaction flask was heated to 138°C (oil bath) and kept at this temperature for 18 h. After cooling to room temperature, the residue was dissolved in water (7 mL) and ethanol (3 mL) was added. The white crystalline solid that formed was filtered, washed with 95% ethanol, and dried in *vacuo* to give **2** (445 mg). The filtrate was concentrated and dissolved in a water–ethanol mixture to give a second crop of crystals of **2** (400 mg, 38.0% overall). Mp 238–239°C; ^{2}H NMR (H_2O, deuterium depleted): δ 3.99 (s, 2D), 3.17 (s, 2D); ^{31}P NMR (D_2O): δ 1.47; CIMS (*m/z*) calcd. for $C_2H_3D_4NO_4P$ $(M\text{-}H)^-$: 144.0364; Found: 144.0367; Anal. calcd. for $C_2H_4D_4NO_4P$: C, 16.56; H+D, 5.56; H, 9.65; Found: C, 16.60; H+D, 5.51; H, 9.62.

*2-(5Z,8Z,11Z,14Z)-Icosa-5,8,11,14-tetraenamidoethyl-d$_4$ dihydrogen phosphate (**4**)*

To a homogeneous stirred solution of 2-aminoethyl dihydrogen phosphate-1,1,2,2-d^4 (**2**, 85 mg, 0.58 mmol), sodium bicarbonate (126 mg, 1.5 mmol) in water (2 mL), a solution of (5Z,8Z,11Z,14Z)-2,5-dioxopyrrolidin-1-yl icosa-5,8,11,14-tetraenoate[7] (**3**, 178.5 mg, 0.45 mmol) in THF (3.5 mL) was added dropwise under nitrogen and the stirring was continued overnight at room temperature. The reaction mixture was acidified to pH 2 with 0.1N HCl and extracted with CH_2Cl_2 (3 × 30 mL) and little $CHCl_3$ (3 mL). The organic layer was washed with water (2 × 20 mL), dried (Na_2SO_4) and concentrated *in vacuo* to afford **4** as a waxy light yellow solid (184 mg, 79%). ^{1}HNMR (Jeol Eclipse, 300 MHz, $CDCl_3$) δ 5.26–5.36 (m, 8H), 2.76–2.80 (m, 6H), 2.25 (brt, 2H), 2.0–2.06 (m, 4H), 1.66–1.68 (m, 2H), 1.24–1.33 (m, 6H), 0.87 (t, J = 6.7 Hz, 3H); MS (Agilent 1100 Series HPLC-MS, CI) *m/z* 430 (M−1) and 432 (M+1). Anal. calcd. for $C_{22}H_{34}D_4NO_5P\bullet 0.7CHCl_3$: C, 52.93; H and D as H, 7.58; N, 2.72. Found: C, 52.90; H and D as H, 7.71; N, 2.65. The presence of chloroform was confirmed by the NMR of **4** in DMSO.

Acknowledgement

We would like to thank Dr Klaus Gawrisch and Dr Walter Teague of the Laboratory of Membrane Biochemistry and Biophysics (LMBB) in NIAAA for ^{2}H NMR spectral data. The authors also express their appreciation to Noel Whittaker and Wesley White of the Laboratory of Analytical Chemistry for mass spectral data and ^{31}P NMR spectral data. The work of the Drug Design and Synthesis Section, CBRB, NIDA, & NIAAA, was supported by the NIH Intramural Research Programs of the National Institute on Drug Abuse (NIDA) and the National Institute of Alcohol Abuse and Alcoholism, and the work of the Neuroendocrinology Section, Laboratory of Physiologic Studies, was supported by the NIH Intramural Research Programs of the National Institute of Alcohol Abuse and Alcoholism. The authors (B.S., A.M., R.K.R.) thank NIDA Grant DA-05488 for financial support.

References

[1] W. A. Devane, L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, *Science* **1992**, *258*, 1946–1949.
[2] V. Di Marzo, A. Fontana, H. Cadas, S. Schinelli, G. Cimino, J.-C. Schwartz, D. Piomelli, *Nature* **1994**, *372*, 686–691.
[3] D. Leung, A. Saghatelian, G. M. Simon, B. F. Cravatt, *Biochemistry* **2006**, *45*, 4720–4726.
[4] J. Liu, L. Wang, J. Harvey-White, D. Osei-Hyiaman, R. K. Razdan, Z. Zhou, A. Chen, B. Huang, H. Y. Kim, G. Kunos, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 13345–13350.
[5] J. Liu, L. Wang, J. Harvey-White, B. X. Huang, H.-Y. Kim, S. Luquet, R. D. Palmiter, G. Krystal, R. Rai, A. Mahadevan, R. K. Razdan, G. Kunos, *Neuropharmacology* **2008**, *54*, 1–7.
[6] J. H. Weissburger, W. C. Schneider, *J. Org. Chem.* **1961**, *26*, 1658–1660.
[7] T. Sheskin, L. Hanus, J. Slager, Z. Vogel, R. Mechoulam, *J. Med. Chem.* **1997**, *40*, 659–667.