Accepted Manuscript

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PII: S0960-894X(14)00541-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.05.041
Reference: BMCL 21655

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 8 March 2014
Revised Date: 6 May 2014
Accepted Date: 8 May 2014

Please cite this article as: Suzuki, M., Takashima-Hirano, M., Ishii, H., Watanabe, C., Sumi, K., Koyama, H., Doi, H., Synthesis of ^{11}C-Labeled Retinoic Acid, [^{11}C]ATRA, via an Alkenylboron Precursor by Pd(0)-mediated Rapid *C*-[^{11}C]Methylation, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.05.041

Synthesis of ^{11}C-Labeled Retinoic Acid, [^{11}C]ATRA, via an Alkenylboron Precursor by Pd(0)-mediated Rapid *C*-[^{11}C]Methylation

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Abstract

Retinoids are a class of chemical compounds which include both natural dietary vitamin A (retinol) metabolites and active synthetic analogs. Both experimental and clinical studies have revealed that retinoids regulate a wide variety of essential biological processes. In this study, we synthesized ^{11}C-labeled all-trans-retinoic acid (ATRA), the most potent biologically active metabolite of retinol and used in the treatment of acute promyelocytic leukemia. The synthesis of ^{11}C-labeled ATRA was accomplished by a combination of rapid Pd(0)-mediated *C*-[^{11}C]methylation of the corresponding pinacol borate precursor prepared by 8 steps and hydrolysis. [^{11}C]ATRA will prove useful as a PET imaging agent, particularly for elucidating the improved therapeutic activity of ATRA (natural retinoid) for acute promyelocytic leukemia by comparing with the corresponding PET probe [^{11}C]Tamibarotene (artificial retinoid).

Retinoids are a class of chemical compounds which include both natural dietary vitamin A (retinol) metabolites and active synthetic analogs.[1] Both experimental and clinical studies have revealed that retinoids regulate a wide variety of essential biological processes such as vertebrate embryonic morphogenesis and organogenesis, cell growth arrest, differentiation, apoptosis, and homeostasis, as well as several associated disorders. Retinoids mediate their biological effects through binding to nuclear receptors known as retinoic acid receptors (RARs) and retinoid X receptors (RXRs), members of the nuclear hormone receptor superfamily. Retinoids (including all-*trans*-retinoic acid (ATRA) (**1**), 13-*cis*-retinoic acid (**2**), 9-*cis*-retinoic acid (**3**)) and their synthetic analogs (Etretinate (**4**), acyclic retinoid (**5**), and Tamibarotene (**6**)) are therapeutic agents used for applications in dermatology and oncology (Figure 1).[2] ATRA (**1**), the most potent biologically active metabolite of vitamin A which only binds to RARs, is used clinically in the treatment of acute promyelocytic leukemia (APL). The relationship of ATRA and its receptors to cancer, metabolic disease, and neurodegenerative diseases such as Alzheimer's and Parkinson's have been reported by many groups, but the mechanisms involved are still poorly understood.[3] Morin et al. previously reported the synthesis of ^{123}I-iodinated analogues of ATRA for SPECT imaging.[4] Thus, *in vivo* imaging of ATRA should prove very useful in helping to understand the relationship between retinoids, their receptors and the progression of a variety of diseases.

Herein, we describe the radiolabeling of ATRA for positron emission tomography (PET) imaging. PET is a powerful noninvasive molecular imaging technique that provides high sensitivity, good spatial resolution, and accurate quantification, and therefore, PET is widely used in diagnostic medicine and has the potential to significantly benefit drug discovery.[5] Labeled small molecules can provide clinical distribution data to facilitate compound selection at an early stage of the drug development or can offer valuable information for disease diagnosis by probing drug-receptor enzyme occupancies and disease mechanisms across multiple therapeutic areas (for example CNS, oncology, metabolic and immunological disorders). Radionuclides used in PET are comprised of short-lived radioisotopes including ^{11}C, ^{13}N, ^{15}O, and ^{18}F. Of these, carbon-11 (half-life = 20.4 min) is one of the most ideal isotopes for PET research, because carbon is found in all organic molecules. In this context, our group has been working on the development of Pd(0)-mediated rapid *C*-[^{11}C]methylation reactions using stannyl or boron compounds as precursors.[6] Based on our method which allows the rapid introduction of a ^{11}C-methyl group onto a vinyl carbon, we describe herein the first ^{11}C-labeling of ATRA using Pd(0)-mediated rapid *C*-[^{11}C]methylation of an alkenyl boron precursor.

ATRA has three vinyl methyl groups at C-5, C-9, and C-13. Of these, we decided to label a C-5 methyl of ATRA because the labeling at the C-9 and C-13 positions might yield geometric isomers when using a transition metal.

Synthesis of an alkenyl boron precursor for [^{11}C]ATRA involving two kinds of Horner-Wadsworth-Emmons (HWE) olefinations is shown in Scheme 1. Retinoids consisting of polyene structures are quite sensitive to light and heat and they tend to isomerize or decompose.[7] Therefore, the reactions were carried out under dim light beginning with the first HWE olefination. The derived geometric isomers were separated by column chromatography. The starting material 2-carboethoxy-3,3-dimethylcyclohexanone (**7**) was prepared according to Steiner's procedure.[8] Compound **7** was converted to an enol triflate **8** in 70% yield by treatment with NaH in the presence of triflic anhydride. Then, the ester of **8** was converted to the alcohol **9** in 72% yield with diisobutylaluminum hydride (DIBAL-H), and then quantitatively oxidized to aldehyde **10** by treatment with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine-*N*-oxide (NMO). The first HWE olefination was accomplished for **10** in 40% yield by using triethyl 3-methyl4-phosphonocrotonate (**16**) pretreated with ^{n}BuLi in the presence of DMPU. Then, the derived ester **11** was reduced to alcohol **12** in 85% yield by DIBAL-H in diethyl ether, which was very unstable at room temperature even in the dark. Therefore, **12** was quickly converted to the pinacol borate derivative **13** in 41% yield by Miyaura's method.[9] The resulting alcohol **13** was oxidized by TPAP-NMO in dichloromethane to quantitatively afford the aldehyde **14**. The second HWE olefination of **14** using the anion of **16** generated by NaH in THF afforded **15** in 36% yield from **13**. The pinacol borate precursor **15** was quickly stored at −78 to −30 °C in the dark to

limit decomposition. The 13-*cis*-pinacol borate precursor for [^{11}C]13-*cis*-RA was isolated by reverse-phase HPLC separation.

Radiosynthesis of [^{11}C]ATRA using rapid *C*-[^{11}C]methylation followed by rapid hydrolysis was also carried out under dim light.[10] [^{11}C]H_3I was prepared as previously described.[11] Palladium(0)-mediated rapid [^{11}C]methylation of the precursor **15** was conducted using [^{11}C]H_3I in the presence of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, K_2CO_3 (1:4:9) in DMF at 65 °C for 4 min followed by basic hydrolysis of the ethyl ester at 100 °C for 2 min to afford the desired [^{11}C]ATRA ([^{11}C]**1**) in 14% yield (HPLC analytical yield). To complete the hydrolysis within 2 minutes, heating of the reaction mixture was required. The radioactivity of the product was often significantly decreased and unknown compounds arising from decomposition were observed in the HPLC radiochromatogram of the reaction mixture. We considered that these phenomena were as a result of radiolysis. Therefore, sodium ascorbate, a radical scavenger used to prevent radiolysis, was added as a base instead of K_2CO_3 to prevent product decomposition by radiolysis during the reaction and work-up.[12] Consequently, rapid *C*-[^{11}C]methylation in the presence of sodium ascorbate as a base proceeded smoothly and rapid hydrolysis consistently afforded more than 1 GBq of the desired [^{11}C]**1** with >99% radiochemical purity in 36% yield (HPLC analytical yield) (Scheme 2). Total synthetic time including HPLC purification and formulation was 32 min. The decay-corrected radiochemical yield based on [^{11}C]H_3I was 25%. Additionally, the decomposition of the product was not

observed in the presence of sodium ascorbate and the radiochemical purity was maintained >99% at 90 min after the completion of synthesis. In this reaction, 13-*cis*-[^{11}C]retinoic acid ([^{11}C]**2**) and 9-*cis*-[^{11}C]retinoic acid ([^{11}C]**3**) were also formed (Figure 2). Other RI peaks were not identified however several peaks with retention times less than 10 min were also observed in the absence of hydrolysis and thus, are considered to be decomposition products.

In summary, the synthesis of ^{11}C-labeled ATRA was accomplished by a combination of rapid Pd(0)-mediated *C*-[^{11}C]methylation and hydrolysis. We hope that [^{11}C]ATRA will prove useful as a PET imaging agent, particularly for elucidating the improved therapeutic activity of Tamibarotene (**6**) for acute promyelocytic leukemia[13] by comparing with the corresponding PET probe [^{11}C]**6** previously reported by our group.[14, 15]

Acknowledgements

This work was supported in part by a consignment expense for the Molecular Imaging Program on "Research Base for Exploring New Drugs" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and in part by JSPS KAKENHI Grant Number (A) 13371294. We thank Professor Yuichi Hashimoto from the University of Tokyo, Japan, for his valuable advice on *in vivo* molecular imaging studies of retinoids and the use of related artificial compounds as drugs. We would also like to thank Mr. Masahiro Kurahashi (Sumitomo Heavy Industry Accelerator Service Ltd.) for operating the cyclotron.

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10. A mixture of **15** (6.0 mg, 13.6 μmol), $Pd_2(dba)_3$ (2.9 mg, 3.17 μmol), P(*o*-tolyl)$_3$ (3.8 mg, 12.7 μmol), and 0.2 M Na *L*-ascorbate aq. (50 μL, 10 μmol) in DMF (400 μL) was placed in the reaction vessel under dim light. [^{11}C]H_3I was transported by a stream of helium (30 mL/min) and trapped in the mixture at 30 °C for 2 min. It was heated at 65 °C for 4 min and then a solution of 6 M KOH in methanol-water (2:1, 600 μL) was added. It was heated at 100 °C for 2 min and then it was acidified (pH2) by HCOOH (500 μL) and diluted with acetonitrile (500 μL). The resulting mixture was injected onto a preparative HPLC (mobile phase, acetonitrile:0.2% HCOOH in water = 90:10; column, Cholester (COSMOSIL), 10 (i.d.) × 250 mm, 5 μm; flow rate, 5 mL/min; UV detection, 360 nm; retention time, 13 min). The desired fraction was collected into a flask containing 25% ascorbic acid (200 μL), and the organic solvent was removed under reduced pressure. The

desired radiotracer was dissolved in a mixture of polysorbate 80, propylene glycol, and saline (1:9:90 v/v/v, 4 mL). The total synthesis time including HPLC purification and radiopharmaceutical formulation for intravenous administration was 32 min. The isolated radioactivity was 1.5 GBq and the specific radioactivity was 44 GBq/μmol. The decay-corrected radiochemical yield based on [^{11}C]H_3I was 25%. The chemical identity of [^{11}C]**1** was confirmed by co-injection with an authentic sample of all-*trans*-retinoic acid using analytical HPLC (mobile phase, acetonitrile:0.2% HCOOH in water = 90:10; column, GeminiNX (Phenomenex), 4.6 (i.d.) × 150 mm, 5 μm; flow rate, 1 mL/min; UV detection, 360 nm; retention time, 6.1 min). The chemical purity analyzed at 360 nm was 92% and the radiochemical purity was greater than 98%. The identification of minor products, [^{11}C]**2** and [^{11}C]**3**, were also conducted by co-injection with the corresponding authentic samples, **2** and **3** obtained from the isomerization reaction of **1** under the similar conditions as used for rapid *C*-methylation.

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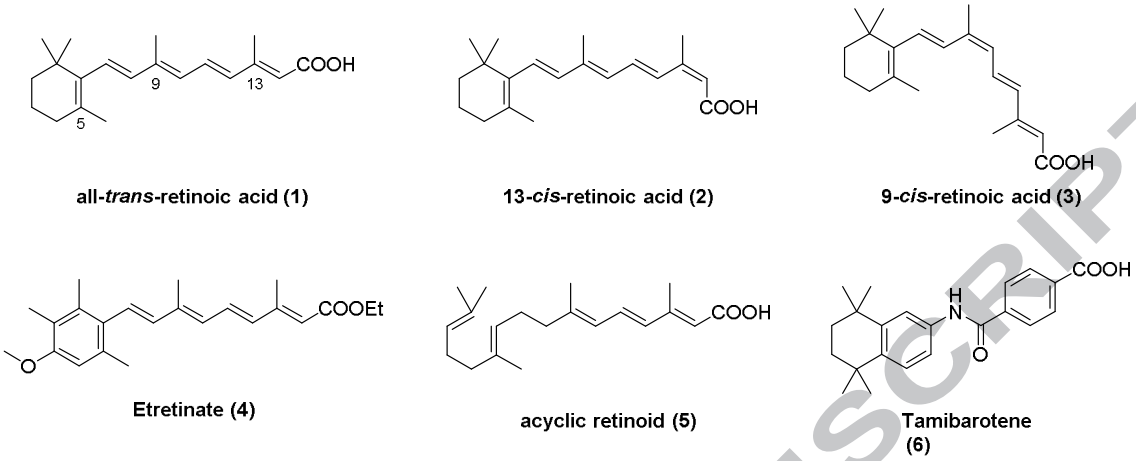
15. Molecular imaging studies will be reported in a separate paper.

1 Figure 1. Structures of retinoic acids and related compounds



all-*trans*-retinoic acid (1)

13-*cis*-retinoic acid (2)

9-*cis*-retinoic acid (3)

Etretinate (4)

acyclic retinoid (5)

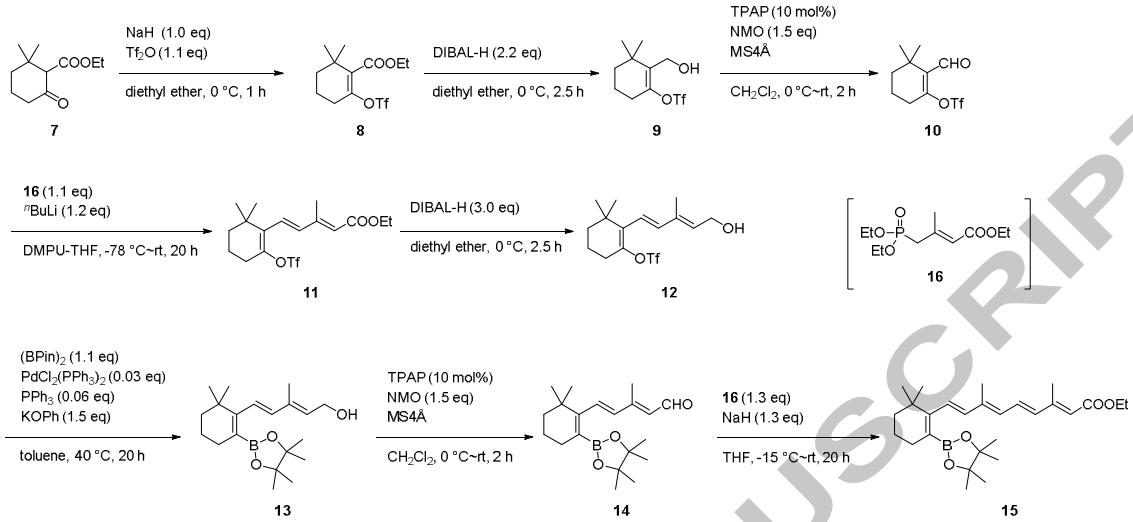
Tamibarotene (6)

2

3

4

1 Scheme 1. Synthesis of precursor



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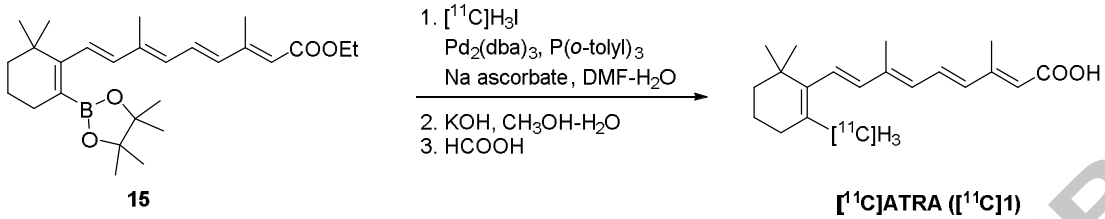
1 Scheme 2. Radiosynthesis of [^{11}C]ATRA (^{11}C-incorporated all-*trans*-retinoic acid)



15 **[^{11}C]ATRA ([^{11}C]1)**

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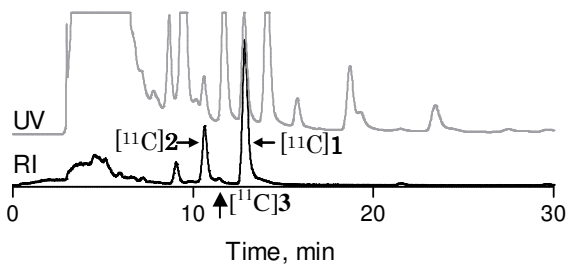
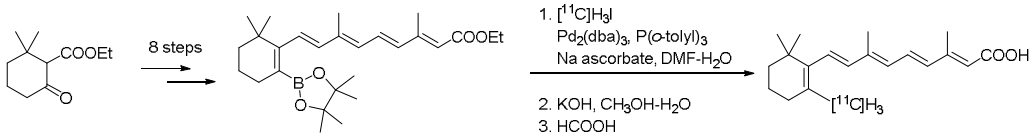
5

Figure 2. Representative chromatogram of [^{11}C]**1** purification. UV absorption: 360 nm



1 Graphical abstract



2 **7** **15** **[^{11}C]ATRA ([^{11}C]1)**

3