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SYNTHESIS OF A ^{11}C-LABELLED PROSTAGLANDIN $F_{2\alpha}$ ANALOGUE USING AN IMPROVED METHOD FOR STILLE REACTIONS WITH [^{11}C]METHYL IODIDE

Margareta Björkman,[1] Hisashi Doi,[2] Bahram Resul,[3] Masaaki Suzuki,[2,4] Ryoji Noyori,[2] Yasuyoshi Watanabe[5,6] and Bengt Långström[1]

[1]Uppsala University PET Centre, UAS, SE-751 85 Uppsala, Sweden.

[2]Department of Chemistry and Research Center for Materials Science, Nagoya University, Nagoya 464-8602, Japan.

[3] Department of Neuroscience, Unit of Pharmacology, Uppsala University, Uppsala, Sweden.

[4]Department of Biomolecular Science, Faculty of Engineering, Gifu University, Gifu 501-1193, Japan.

[5]Department of Neuroscience, Osaka Bioscience Institute, Osaka 565-0874, Japan.

[6]Department of Physiology, Osaka City University Graduate School of Medicine, Osaka 545-8585, Japan.

SUMMARY

17-(3-[^{11}C]methylphenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester **2** was synthesised using an improved method for cross-coupling reactions with [^{11}C]methyl iodide. The decay-corrected radiochemical yield of **2** was 34 % based on [^{11}C]methyl iodide in a synthesis time of 30 min from end of radionuclide production. The specific radioactivity was approximately 100 GBq/μmol and the radiochemical purity was higher than 95 % as determined by analytical LC. In a typical experiment 1.3 GBq of **2** was obtained from 11 GBq of [^{11}C]methyl iodide.

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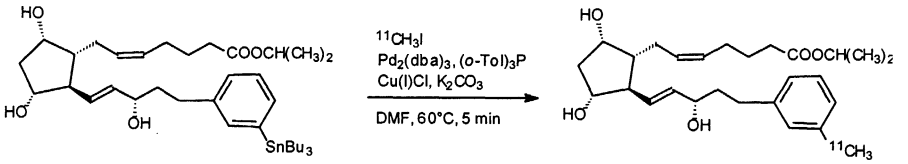
INTRODUCTION

Prostaglandins (PGs) are important lipid modulators in circulatory, digestive, reproductive, excretory, hormonal, immune and nervous system in the mammalian body, including humans. In order to study PG receptors in the brain with positron emission tomography (PET), PG receptor ligands labelled with short-lived β^+-emitting radionuclides have to be developed. As a part of this work a ^{11}C-labelled tracer for imaging of the PGI_2 receptor in the brain has been synthesised[1,2] and studied with PET.[3] $PGF_{2\alpha}$ exerts an antidiuretic effect[4] and an inhibitory effect on oxytocin release[5] and analogues of $PGF_{2\alpha}$ are known regulators in intestinal motility and therapeutic agents for glaucoma. Latanoprost, 13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester, the active principle in Xalatan®, is the most successful analogue for treatment of glaucoma to reduce intraocular pressure.[6] In the present study our aim was to develop a PET tracer for the $PGF_{2\alpha}$ receptor by introducing a [^{11}C]methyl group on the aromatic ring.

The cross-coupling between aryl or vinyl halides or triflates with organostannanes, commonly referred to as the Stille reaction,[7] has developed into a valuable synthetic tool for the preparation of carbon-carbon bonds. Cross-coupling reactions with [^{11}C]methyl iodide[8] have proved to be a reliable method for the synthesis of several [^{11}C]methylphenyl analogues.[8, 9] Compounds with complex chemical structures, such as prostaglandin and prostacyclin analogues,[1] have successfully been labelled with this approach without any use of protective groups. However, it was observed that unreacted [^{11}C]methyl iodide often was remaining after the reaction when analysing the reaction mixture. In order to increase the reactivity and to facilitate the oxidative addition, regarded as the first step in the catalytic mechanism in a Stille coupling, an unsaturated palladium(0) complex was used. Unsaturated palladium complexes are sensitive and they easily decompose at the high reaction temperatures that were required to obtain sufficient yield of the product. Thus, in the further development of the method it was desirable to

decrease the reaction temperature to improve the reproducibility of the reaction and to make it applicable to compounds that are thermally unstable. Improved results of the Stille coupling with methyl iodide using copper (I) salts as a co-catalyst have been published[10] and a modified method using copper (I) salts along with potassium carbonate and the palladium catalyst at 60 °C, were also recently published.[11] In this paper, these reaction conditions were investigated under labelling conditions with [^{11}C]methyl iodide. An improved method for the synthesis of [^{11}C]methylphenyl analogues is thus presented as well as the synthesis of 17-(3-[^{11}C]methylphenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester **2**, shown in Scheme 1.



1 **2**

Scheme 1. Synthesis of a ^{11}C-labelled $PGF_{2\alpha}$-analogue, using [^{11}C]methyl iodide in a Stille coupling.

RESULTS AND DISCUSSION

Chemistry

17-(3-[^{11}C]methylphenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester **2** was synthesised from [^{11}C]methyl iodide and 17-(3-(tri-n-butylstannyl)phenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester **1** in a palladium-promoted cross-coupling reaction. The [^{11}C]methyl iodide was produced from [^{11}C]CO_2, distilled and trapped in a solution containing tris(dibenzylideneacetone)dipalladium(0), [$Pd_2(dba)_3$], and tri(*o*-tolyl)phosphine, (*o*-Tol)$_3$P, in *N,N*-dimethylformamide, DMF. After trapping, the solution was transferred to a vial containing copper(I) chloride, tin precursor **1** and potassium carbonate in DMF and the final mixture was heated at 60°C. A solid phase extraction (SPE) was conducted to remove salts and palladium residues before injecting onto a semi-preparative liquid

chromatography (LC) column. The decay corrected radiochemical yield was 34 % calculated from the amount of [^{11}C]methyl iodide and the specific radioactivity was approximately 100 GBq/μmol. The radiochemical purity determined by analytical LC was >95% and the total synthesis time was 30 min. The identity of **2** was determined by analytical LC, after addition of authentic reference compound, and by LC-MS analysis where the positively charged protonated molecular ion was detected, m/z 445.

For an unknown reason the cross-coupling reaction was quenched when all reagents were present in the trapping vial, used to collect the distilled [^{11}C]methyl iodide. Several approaches were investigated to overcome this problem, such as additional drying of the [^{11}C]methyl iodide and attempts to trap iodine, that may co-distil with the [^{11}C]methyl iodide and quench the proposed copper-aryl complex formed from copper-tin transmetallation,[11] without success. The best results were achieved when two separate DMF solutions were prepared, one containing $Pd_2(dba)_3$ and (o-Tol)$_3$P used for trapping of [^{11}C]methyl iodide and one containing the copper salt, the tin precursor **1** and the potassium carbonate. The reagents in the latter solution were carefully flushed with argon and the DMF was added to the reagents just before use. A vigorous mixing of the two solutions prior to heating gave the best results.

Positron Emission Tomography

Uptake of **2** could be observed in temporal muscle and whole brain. The time activity curves showed longer retention time in temporal muscle than in whole brain. However, the faster disappearance of radioactivity from blood and plasma indicated some interaction of **2** in brain. These results indicate that **2** is not suitable as a tracer for imaging the $PGF_{2\alpha}$ receptor in the brain as intended, but might be useful in pharmacokinetic studies.

EXPERIMENTAL

General

[^{11}C]CO_2 was prepared by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in a nitrogen (AGA Nitrogen 6.0) gas target containing 0.05 % oxygen (AGA Oxygen 4.8), with 17 MeV protons produced by the Scanditronix MC-17 Cyclotron at the Uppsala University PET Centre. The [^{11}C]CO_2 was converted to [^{11}C]CH_3I via a reaction with 0.2 M lithium aluminium hydride and subsequent reaction with hydriodic acid,[12] using an automated system for production of radiopharmaceuticals.[13]

Chromatography

LC was performed using a Beckman 126 Pump and a Beckman 166 UV detector in a series with a β^+-flow detector. Data collections were performed using Beckman System Gold Chromatography Software Package for semi-preparative LC and Beckman System Gold Nouveau Chromatography Software Package for analytical LC. A C-18 Beckman Ultrasphere ODS 5μm (250 x 10 mm) column was used for semi-preparative LC and a Beckman Ultrasphere Octyl 5μm (250 x 4.5 mm) column was used for analytical LC. Mobile phases were 50 mM ammonium formate pH 3.5 (A), 10 mM potassium phosphate buffer pH 4.7 (B) and acetonitrile-water (50/7 v/v) (C). LC was performed at room temperature and the UV-detection wavelengths were 230 nm for semi-preparative LC and 220 nm for analytical LC. The semi-preparative LC purification of the reaction mixtures was performed at 5 mL/ min with isocratic elution 0-6 min (A:C) (45:65 v:v) a linear gradient 6-15 min to (A:C) (5:95 v:v). The analytical LC system used for identification and determination of radiochemical purity was performed at 1.5 mL/min with isocratic elution 0-6 min (B:C) (45:65 v:v) a linear gradient 6-12 min to (B:C) (10:90 v:v). The LC-MS equipment consisted of a Beckman 126 pump, a CMA 240 autosampler (CMA Microdialysis, Stockholm, Sweden) and a VG Quattro mass spectrometer (Micromass, Manchester, UK) equipped with pneumatically assisted electrospray. The column in the LC-MS system was a Beckman Ultrasphere C-18 ODS 5μm (250 x 4.5 mm). A post column 1:100 split was used, with 1% of the total flow

delivered to the electrospray probe and 99% delivery to a Bioscan Flow-count β^+-detector. Mobile phases were (D) 0.25 mM ammonium formate buffer pH 3.5 and (E) methanol. An isocratic elution 0-8 min with (D:E) (70:30 v:v) and a linear gradient 8-15 min to (D:E) (90:10 v:v) with a flow of 1 ml/min were employed.

Chemicals

17-(3-iodophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester and the reference compound 17-(3-methylphenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester were received from Pharmacia & Upjohn in Uppsala, Sweden. 17-(3-tributylstannylphenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester **1** was synthesised from 17-(3-iodophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ isopropyl ester using a palladium-catalysed cross-coupling reaction with hexa-*n*-butylditin.[14] A sterile solution containing 400 mg of propylene glycol and 100 mg of ethanol in an aqueous solution with a total volume of 10 ml, PPG-solution, was purchased from Apoteksbolaget, Sweden. $Pd_2(dba)_3$, (*o*-Tol)$_3$P, Cu(I)Cl, 57 % hydriodic acid, lithium aluminum hydride 1M and K_2CO_3 were purchased from Aldrich. All other chemicals and solvents were of analytical or gradient grade purity and used as received.

Synthesis of ^{11}C-labelled $PGF_{2\alpha}$ analogue **2**

A solution of $Pd_2(dba)_3$ (1.2 mg, 1.3 μmol) and (*o*-Tol)$_3$P (1.5 mg, 4.9 μmol) in 200 μL of DMF was prepared in a dry 0.8 mL septum equipped vial and purged with nitrogen gas for 10 min. $^{11}CH_3I$ was passed through a Sicapent drying tower and trapped in the solution at room temperature. After trapping the reaction mixture was transferred to a septum-equipped vial containing the tin precursor **1** (2 mg, 2.6 μmol), CuCl (1.2 mg, 12 μmol) and K_2CO_3 (1.4 mg, 10 μmol) dissolved in 100 μL of DMF. The vial containing the tin precursor, the copper salt and the potassium carbonate was flushed carefully with argon gas and the DMF was added just before mixing with the trapping solution. After the addition of the trapping solution the vial was shaken vigorously before heating.

The reaction vessel was heated at 60 °C for 5 min after which the reaction mixture was diluted with 8 mL of water and concentrated on a 100 mg Isolute® C-18 SPE. Washing of the SPE was conducted with 2 mL of water and the product fraction was eluted with 1 mL of acetonitrile. The SPE fraction was diluted with 2 mL of water and injected onto a semi-preparative LC column. The product fraction was collected after 9.5 min, except unreacted [^{11}C]methyl iodide (5.6 min), hydrophilic side-products could be detected (3.0-4.5 min). The organic solvent was evaporated and to the residue was added 4.5 mL of a sterile PPG-solution, (described under chemicals). Sterile filtration of the final product solution through a filter (Dynagard ME, 0.22 μm pore size) into a sterile vial was performed. Radiochemical purity was determined by analytical LC, the retention time for **2** was 6.7 min. The identity was determined by adding authentic reference compound to the solution and analysing with analytical LC.

Positron Emission Tomography

Two female rhesus monkeys with body weights of 6.9 kg (No.1) and 8.6 kg (No.2) were used for the PET study. The monkeys were anaesthetized by inhalation of nitrous oxide (60% v/v) and isoflurane (0.3% v/v) and throughout the study mechanically ventilated with 40% oxygen in air. Two consecutive PET scans with 100 min interval were performed in monkey No.1 with 107 and 52 MBq of **2** injected. In monkey No.2 one PET scan was performed with 52 MBq injected of the same tracer. The PET scan was started immediately after injection of the radiotracer and stopped at 60 min. The value of uptake in whole brain and temporal muscle was calculated from the following equation: Uptake = the radioactivity in the ROI / injected radioactivity x body weight / ROI volume (weight). The animal experiments were carried out with permission from Animal Ethics Committee, Uppsala University, approval No. C289/94.

CONCLUSION

An improvement in the Stille coupling reaction with [^{11}C]methyl iodide has been performed using copper as co-catalyst with an unsaturated palladium(0)

complex. The reaction was completed after 5 min at 60 °C, which is a reduction in both time and in temperature, compared to earlier reported methods. Under these reaction conditions the ^{11}C-labelled $PGF_{2\alpha}$ analogue **2** could be synthesised in high and more importantly in reproducible radiochemical yields. The ^{11}C-labelled tracer **2** was used for PET investigation with monkeys where it was evident that the tracer is not suitable as a receptor ligand for imaging the $F_{2\alpha}$ receptor *in vivo* in the brain.

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