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Research Article

Synthesis of a mGluR5 antagonist using [^{11}C]copper(I) cyanide

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Summary

5-(2-Phenylethynyl)pyridine-3-[^{11}C]carbonitrile ([^{11}C]LY2232645), a metabotropic glutamate 5 receptor (mGluR5) antagonist, was synthesized by a no-carrier-added nucleophilic halogen displacement with [^{11}C]copper(I) cyanide. The average radiochemical yield was 2.5%, and the average specific activity was 1365 mCi/μmol at end-of-synthesis. The total time of synthesis, purification, and formulation was 26 min. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: ^{11}C; copper cyanide; glutamate; mGluR5; PET

Introduction

Metabotropic glutamate receptors (mGluRs) are a heterogeneous family of G-protein-coupled receptors that are divided into three main groups based on sequence homology and their common effects on secondary messenger systems.[1] Across these three groups, eight receptor subtypes have been identified to date.[2] The group I mGluR5 subtype is distributed throughout the central nervous system (CNS) and has become a potential target for a number of CNS disorders including anxiety,[3–8] depression,[6] amyotrophic lateral sclerosis,[9,10] Parkinson's disease,[11,12] psychosis,[13] pain,[14] and addiction.[15] The mGlu5 receptor is, therefore, an attractive target for drug development.

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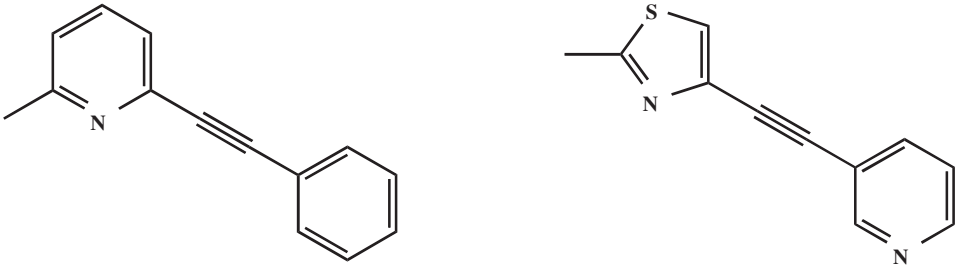
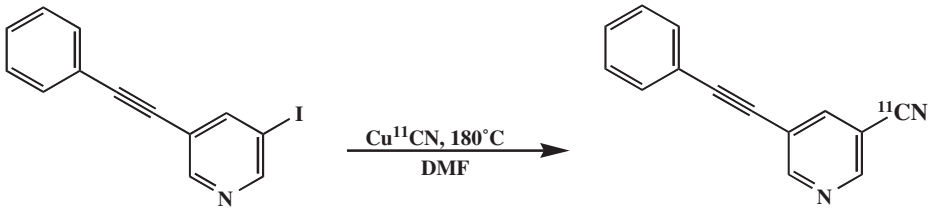
Diaryl alkynes such as 2-methyl-6-(phenylethynyl)pyridine (MPEP)[16] and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP)[17] have formed the parent structures (Figure 1) for a number of potent and selective ligands for mGluR5.

While a number of analogs[18–27] have been labeled with carbon-11, fluorine-18, or iodine-123, few have resulted in successful radioligands for imaging mGluR5 *in vivo*. Therefore, a radiotracer for mGluR5 with better imaging properties is still needed. In order to find a tracer that would provide a longer-lived specific signal for mGluR5, 5-(2-phenylethynyl)pyridine-3-carbonitrile (LY2232645) was synthesized. If labeled with [^{11}C]cyanide, this diaryl alkyne would have a direct carbon–carbon bond between the ^{11}C label and the pyridine ring, thus being less prone to metabolic loss of the radiolabel *in vivo*. This compound exhibited a 2.5 nM *in vitro* binding affinity for mGluR5, which is suitable for positron emission tomography (PET). Here we describe the introduction of [^{11}C]cyanide into this molecule by a no-carrier-added nucleophilic halogen displacement using [^{11}C]copper(I) cyanide (Figure 2).



MPEP MTEP

Figure 1. Structures of MPEP and MTEP



[^{11}C]LY2232645

K_i = 2.5 nM

Figure 2. Synthesis and *in vitro* binding affinity of [^{11}C]LY2232645

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Results and discussion

The use of [^{11}C]cyanide in the synthesis of biologically active radioligands has been hampered by the presence of ammonia carrier gas. However, relatively simple halogenated aromatic compounds have been successfully used as precursors to [^{11}C]cyano-products. Known methods for the introduction of [^{11}C]cyanide include palladium-mediated cyanation[28] and the use of [^{11}C]copper(I) cyanide.[29] The latter method has an advantage since it can be adapted to work in a one-pot reaction that is not sensitive to water or the presence of ammonia.

In this case, [^{11}C]hydrogen cyanide was trapped in an aqueous solution of copper(II) sulfate and sodium metabisulfite and converted to [^{11}C]copper(I) cyanide in 2 min by heating the aqueous solution to 80°C. It is important that there be a slight excess of sodium metabisulfite in the trapping solution and that ample time (5–10 min) is given for the reduction of copper(II) to copper(I) before bubbling in [^{11}C]hydrogen cyanide. The iodo precursor was added to the aqueous solution, and the aqueous/dimethylformamide mixture was heated in a sealed vial for 5 min at 180°C. After cooling to 80°C, the reaction mixture was diluted with HPLC solvent and the product purified by semi-preparative HPLC (Figure 3). Solid phase extraction was used to isolate the purified product because it was noted that no-carrier-added [^{11}C]LY2232645 is somewhat volatile under normal rotary evaporation conditions. The total time of synthesis, purification, and formulation was 26 min from end-of-bombardment ($n = 8$). The average radiochemical yield was 2.5%, and the average specific activity was 1365 mCi/μmol at end-of-synthesis. The radiochemical

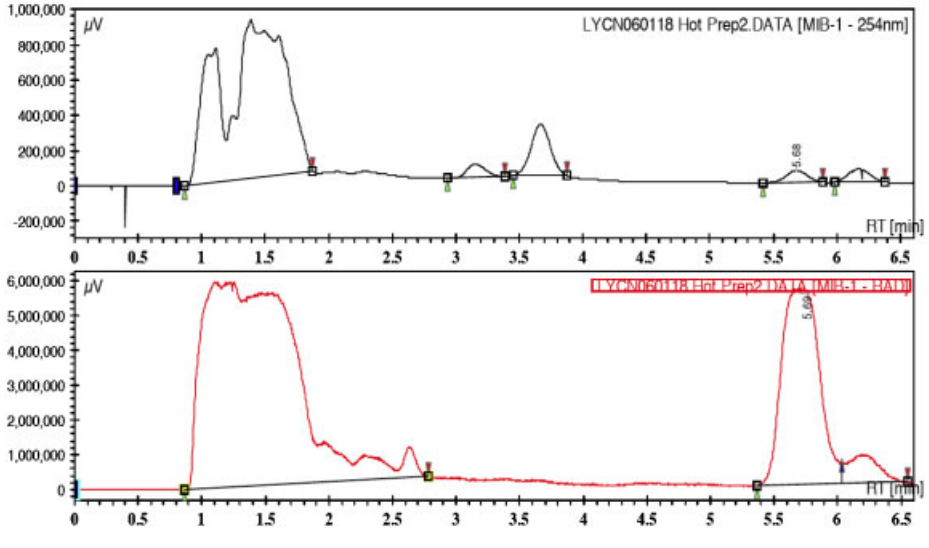

Figure 3. Semi-preparative HPLC purification of [^{11}C]LY2232645

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purity of the final product was greater than 99% by analytical HPLC. The identity of [^{11}C]LY2232645 was confirmed by co-elution with an authentic standard.

Experimental

Chemicals were purchased from Aldrich Chemical Co. Dimethylformamide (DMF) was purified by stirring overnight with barium oxide and distilled prior to use. 3-Iodo-5-(2-phenylethynyl)pyridine was provided by Lilly Research Laboratories (Indianapolis, IN). HPLC analysis and purification were performed with two Waters model 590EF pumps, two VICI model ETC6UV injectors, an in-line Waters model 441 UV-detector (254 nm), and a single 2-in NaI crystal flow-count radioactivity detector. HPLC chromatograms were recorded by a dual channel control/interface module connected to a PC with Varian Galaxie software. Radioactivity measurements were made using a Capintec CRC-15R dose calibrator. The carbon-11 isotope was made by a General Electric PETtrace cyclotron. [^{11}C]Carbon dioxide was converted to [^{11}C]hydrogen cyanide by an automated General Electric PETtrace chemistry system, which reduced [^{11}C]CO_2 to [^{11}C]methane at 400°C and then reacted with ammonia at 1000°C.

Synthesis of [^{11}C]LY2232645

A 3 ml v-vial was charged with 50 µl of 22 mM copper(II) sulfate and 50 µl of 24 mM sodium metabisulfite 5–10 min prior to end-of-bombardment. The trapping solution was cooled to 0°C, and [^{11}C]hydrogen cyanide was bubbled in at a flow rate of 300 ml/min until the radioactivity reached a plateau. The aqueous solution was then heated to 80°C for 2 min. A solution of 1 mg iodo precursor in 200 µl DMF was added to the aqueous solution, and the reaction mixture was heated in a sealed vial for 5 min at 180°C. After cooling back to 80°C, the solution was diluted with 200 µl of 60:40 acetonitrile:water (0.1 M ammonium formate) and injected into the semi-preparative HPLC system described above. The column (Alltech C_{18} Econosil 10 mm × 250 mm) was eluted with 60:40 acetonitrile:water (0.1 M ammonium formate) at a flow rate of 11 ml/min. The radioactive peak corresponding to [^{11}C]LY2232645 (t_R = 5.7 min.) was collected, diluted with 60 ml of water, and applied to a Waters C_{18} Plus Sep-Pak. The product was eluted from the Sep-Pak with 1.0 ml of 200 proof ethanol followed by 9.0 ml of sterile normal saline through a 0.2 micron Millex GV (Millipore) sterile filter into a sterile, pyrogen-free bottle.

A 100 µl aliquot of the final product was injected onto an analytical Alltech C_{18} Econosil HPLC column (4.6 mm × 250 mm) and eluted with 70:30 acetonitrile:water (0.1 M ammonium formate) at a flow rate of 4 ml/min. The area of the UV absorbance peak at 254 nm corresponding to carrier

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product was measured and compared to that of a calibrated authentic standard. The radioactive peak corresponding to [^{11}C]LY2232645 ($t_R = 2.0$ min.) co-eluted with an authentic standard.

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