TABLE OF CONTENTS

PAGE

List of Tables

PAGE

List of Figures

PAGE

1 GENERAL INFORMATION

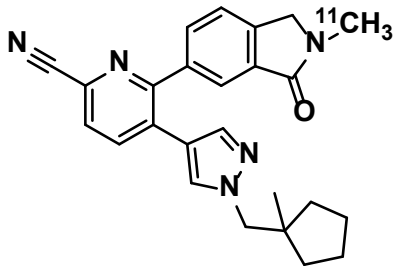
1.1 Nomenclature

Chemical Name: 6-(2-(^{11}C)methyl-3-oxo-2,3 dihydro-1H-isoindol-5-yl)-5-{1-[1-methylcyclopentyl)methyl]-1H-pyrazol-4-yl}pyridine-2-carbonitrile

Laboratory Code: [^{11}C]MK-6884 or [^{11}C]L-005385924

1.2 Structure

Figure 1 Structural Formula of [^{11}C]MK-6884



Molecular Formula: $C_{25}H_{25}N_5O$

Molecular Weight: 411.51 for MK-6884 and 410.50 for [^{11}C]MK-6884

Stereochemistry: [^{11}C]MK-6884 contains no asymmetric centers

1.2.1 General Properties

Appearance: [^{11}C]MK-6884 is isolated as a clear, colorless solution.

2 MANUFACTURE

2.1 Manufacturers

Preparation of the radiolabeled tracer will be conducted at:

Cyclotron cGMP Radiochemistry

Houston Methodist Research Institute

6670 Bertner Avenue, Houston, TX 77030

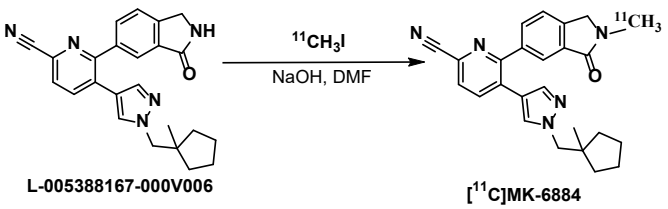
FDA ID: 185641052
FEI: 3008674916
Cyclotron RAM License number: L06331
X-ray License number: R34530

2.2 Description of Manufacturing Process and Process Controls

The information below describes the production procedures for [^{11}C]MK-6884. The parameters and the data of the synthetic procedures are recorded in the batch protocol for the production of [^{11}C]MK-6884.

2.2.1 Process Flow Diagram

Figure 2 Flow Diagram of the Manufacturing Process of [^{11}C]MK-6884

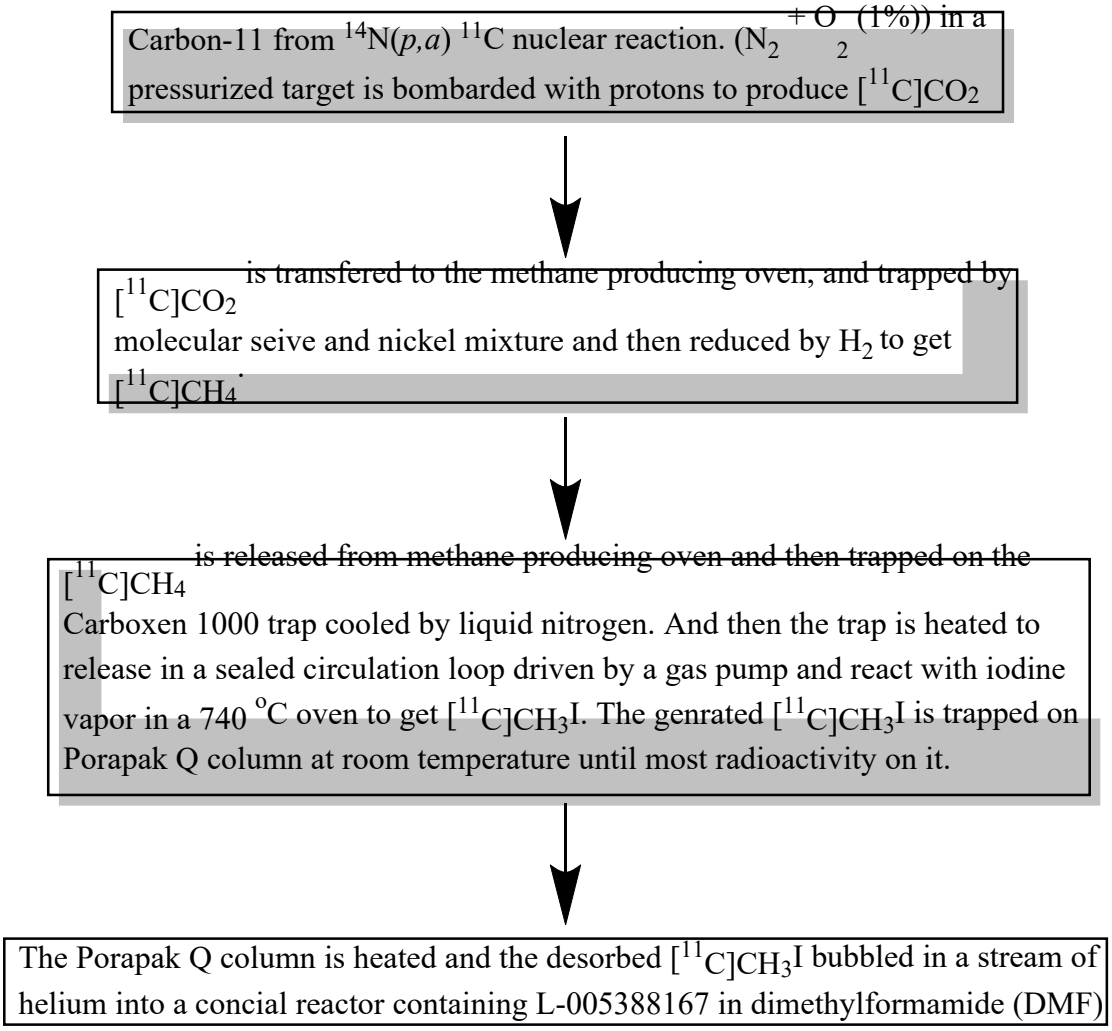


2.2.2 L-005388167 Component Synthesis

The synthesis begins with a palladium mediated coupling under standard conditions with commercially available 5-bromo-6-chloropicolinonitrile and pinacol borate to provide intermediate **3** in high yield. L-005388167 is produced under palladium mediated conditions with pinacol borates **4** in good yield.

2.2.3 Radionuclide production

Figure 3 Flow Diagram of Radionuclide Production



The [^{11}C]CO_2 is produced from the $^{14}N_2$ (p, α) ^{11}C reaction in target containing 1% oxygen. The target nitrogen gas is bombarded by proton beam (30~80 μA) for 10~40 min with trace of oxygen to form [^{11}C]CO_2. The [^{11}C]CO_2 is then transferred to the methane producing oven where [^{11}C]CH_4 is produced by reducing [^{11}C]CO_2 with hydrogen on nickel catalyst at 350 ºC. The [^{11}C]CH_4 released from the methane oven, is then trapped in Carboxen 1000 at -75 ºC, which is later released at 80 ºC in a sealed and cycled gas reaction loop. The circulation is driven with a small gas pump, and the [^{11}C] CH_3I is produced by gas reaction of [^{11}C]CH_4 and iodine gas (generated from heating iodine crystal at 100 ºC) at 740 ºC for 5 min. The generated [^{11}C]CH_3I is trapped in Porapak Q, which is later released at 200 ºC with a stream of helium to the conical reactor containing L-005388167 for the alkylation reaction.

2.2.4 Radiochemical synthesis of [^{11}C]MK-6884

The [^{11}C]CH_3I is incubated with the component L-005388167-000V006 (250 μL, 0.8mg/mL) and NaOH (5 μL 1.0M sodium hydroxide solution) in DMF at 0 ºC for 3 min. The reaction mixture is diluted with 1.0 mL of preparative mobile phase and then purified by preparative HPLC using a mobile phase of acetonitrile : 40 mM aqueous ammonium

acetate (45:55, v/v) at a flow rate of 8.0 mL/min. The HPLC fraction containing [^{11}C]MK-6884 is collected in a reservoir which is pre-filled with 30 mL sterile water, then the mixture is passed through a light C18 cartridge where [^{11}C]MK-6884 is trapped. The cartridge is dried with helium for 45 second passing through, then risend with 10 mL sterile water, followed by helium drying for 45 second. This [^{11}C]MK-6884 drug substance trapped in cartridge is eluted to the final product vial through a sterile filter (Millex FG filter, 0.20µm) with 1 mL ethanol for injection followed by 14 mL saline.

2.2.5 Pre-production procedures for the radiochemical synthesis

Preparation of the synthesis apparatus

Before each production run, the device is cleaned and prepared for synthesis.

Component stock solution

Dissolve the component L-005388167 in anhydrous DMF at a concentration of 0.8 mg/ml.

Storage: freezer

Expiry: 1 month

Reactor

Add 5 µl of 1M NaOH and 250 µl component stock solution.

Storage: 0 °C

Expiry: 1 hour

2.2.6 Pre-production procedures for the preparative HPLC

Eluents for prep HPLC

Acetonitrile/40 mM NH_4OAc 45:55 V/V

Storage: room temperature

Expiry: 1 week after preparation

Preparative HPLC system

Column: ACE 5 C18-HL 250 × 10mm mm

UV: 220 nm

Flow rate: 8 ml/min

Condition the column with the HPLC eluent for at least 10X column volume at room temperature.

Flush the injection loop with 5-10 ml acetone and sweep dry with helium flow.

2.2.7 Pre-Production Procedures for the Formulation of the Sample

Filter and sterile vial

Sterile vials are assembled in the critical zone of the laminar flow hood. The septa of the vial is wiped using alcohol swabs before the membrane filter and filter vent needle are inserted into the septum of the vial. The final product vial is stored in a sterile bag after assembly.

2.2.8 Post-production procedures

All of the devices used for the production are either switched off or set on their idle status. The HPLC solvent is switched to 70% MeCN to rinse the preparative column, and the pump is switched off.

2.2.9 Rinsing procedure

Target and target transfer lines

Before the irradiation, the target is loaded (184~188 psi) and short time bombarded and emptied twice with the mixture N_2-O_2 1%. The target is then loaded with the same mixture.

CH_3I gas phase synthesis

Prior to the synthesis, the unit is leak check by He gas. The transfer line from the CH_3I unit to the alkylation reactor is rinsed with 14 second 50ml/min Helium.

Alkylation module and transfer line from alkylation reactor to HPLC injector

Every individual Teflon transfer tube is rinsed with appropriate solvents to remove any residual contaminant.

Reactor/vials The glass alkylation reactor and vials are first cleaned with distilled water (5~10 ml × 2) and rinsed with acetone (5~10 ml × 2). The reactor/vials are dried under vacuum at 120°C for at least 10 min.

Preparation of HPLC column and lines

After every synthesis, the prep column is immediately rinsed with MeCN:H_2O (70/30, V/V) with flow rate 5ml/min for 10 min and then 7ml/min for another 20 min.

The HPLC column is stored on MeCN:H_2O 70/30 V/V

2.3 Control of Starting Materials

The components and other reagents and solvents are typically purchased as reagent grade. Materials are tested for assay and/or identity, as appropriate, or accepted on the basis of supplier's guarantee.

Materials Contributing to the Molecular Structure

L-005388167-000V006 (component for [^{11}C]MK-6884) was prepared by Merck Sharp & Dohme Corp. from MK-6884, which was manufactured utilizing the following facility:

Pharmaron Beijing, Co. Ltd.
6 Taihe Road
BDA, Beijing, 100176
P.R. China

2.3.1 Specification and Batch Analysis for L-005388167-000V006

The specifications and typical batch analysis data for L-005388167-000V006 are presented in Table 1 below.

Table 1 Specification and Batch Analysis for L-005388167-000V006

Tests	Tentative Acceptance Criteria	L-005388167-000V006
Appearance	Clean, white to off-white powder	Clean, white powder
Impurities by HPLC Individual impurity Total impurities	 Max. 1.0 area% Max. 3.0 area%	 ImpA (RRT 0.90) 0.26 area% ImpB(RRT 0.96) 0.21 area% ImpC(RRT 1.15) 0.06 area% 0.52 area%
Water and Residual Organic Solvents by TGA	Max. 1.0 wt%	0.01 wt%
Identity by IR	Spectrum conforms to authentic sample	Spectrum conforms to authentic sample

L-005388167-000V006 is stored at -20°C. Retest will be conducted if stored more than 1 year.

2.3.2 Other Reagents, Solvents, and Catalysts

Table 2 Other Reagents, Solvents and Catalysts Used in the Manufacture of the Drug Substance

	Grade	Identification
Acetonitrile	HPLC grade	CoA
Ammonium Acetate	99.99%	CoA
DMF dry	HPLC grade.	CoA
Ethanol (200 proof)	USP	CoA
NaOH	Semiconductor grade	CoA
1%O_2+N_2 gases	1% O_2 + 99% N_2	CoA
He gas	99.9999%	CoA
Iodine	99.5%	CoA
saline (0.9% NaCl for injection)	USP	CoA
Sterile water for injection	USP	CoA

2.4 Controls of Critical Steps and Intermediates

Several in-process controls are monitored and documented. This includes continuous monitoring of radioactivity, reaction temperatures, and visual verification of addition of reagents to the reactors:

Check pressure ^{11}C target during irradiation (pressure transducer)

Check arrival of $^{11}CH_4$ to Porapak N column (radiometric)

Check arrival of $^{11}CH_3I$ to Porapak column (radiometric)

Check arrival of $^{11}CH_3I$ into reaction mixture (radiometric)

Check temperature of alkylation (thermocouple)

Check addition of water to reaction (visual)

Check transfer to injection loop (visual)

Check retention time and separation of L-005388167 starting material from radioactive end product (prep HPLC chromatogram)

2.5 Process Validation and/or Evaluation

The process and analysis parameters are monitored by the production chemist and all deviations from default values are recorded in the batch protocol. The documentation of the synthesis and analysis of the preparation, including the batch protocol, will be checked by an independent second person. This person (responsible person) performs the conditional release of the final drug product, which is indicated as a signature in the batch protocol.

The production process of [^{11}C]CH_3I occurs in the gas phase (in helium environment) and will have a low bioburden. Alkylation is performed in a module with lines that are rinsed with water p.i. and dried by rinsing with acetone and helium. The alkylation reaction is done inDMF. The alkylation mixture is diluted with HPLC mobile phase solution, and preparative HPLC is performed using acetonitrile-ammonium acetate buffer.

2.6 Manufacturing Process Development

Not Applicable.

3 CHARACTERIZATION

3.1 Elucidation of the structure and other characteristics

Characterization of the analogous non-radioactive MK-6884 is provided in Sec S.5 Reference Standard. Based on retention time on HPLC, radioactive [^{11}C]MK-6884 is considered to be identical to non-radioactive MK-6884.

3.2 Impurities

[^{11}C]MK-6884 drug substance is used to manufacture the drug product without intermediate quality control in view of the short half-life of carbon-11 (20.3 min). The impurities are, therefore, assayed on the drug product.

3.2.1 Radiochemical Purity

The radiochemical purity of [^{11}C]MK-6884 was determined on the drug product and was found to be ≥ 95% (radio-RP-HPLC) during the validation runs.

3.2.2 Radionuclidic Identity

The radionuclidic identity is determined by a half-life calculation on the drug product. The observed half-life must be between 18.4 and 22.4 minutes.

A gamma spectrum is recorded and shows the 511 keV peak.

3.2.3 Radionuclide purity

The radionuclidic purity is assayed by gamma-spectrometry multichannel analyzer (MCA) 3-7 days after formulation of carbon-11. The amount of long-lived radionuclides should be lower than 0.1% of the activity of ^{11}C at time of injection.

A 2-hour blank spectrum using a 1 mL sterile water was generated before doing a 2 hour spectrum check of a 1mL sample. The ROI of any notable impurity peaks is identified and computed as activity. The corresponding computed activity is back calculated to the time of injection and its corresponding contribution to the strength at the time of injection.

3.2.4 Chemical Purity

The chemical purity is determined on the drug product.

The amount of L-005388167 is ≤ 1.5 µg per administered dose.

The total amount of unidentified impurities is ≤ 3.0 µg per administered dose (calculated with response factor for MK-6884).

3.2.5 Residual Solvents

The solvent, DMF and acetonitrile, are used in the preparation of [^{11}C]MK-6884 and is controlled by gas chromatographic analysis on the drug product. If injected into a single subject, the theoretical maximum level of DMF and acetonitrile is below the class 2 solvent limit of ? mg for DMF, 4.1 mg for acetonitrile per day stated in the EMEA is this US requirement? note for guidance on residual solvents (CPMP/ICH/283/95).

4 CONTROL OF DRUG SUBSTANCE

4.1 Specification

The specifications are presented in Drug ProductSpecifications – Control of Drug Product [^{11}C]MK-6884.

	Specification	Method
Identification	Retention time (RT) deviation between UV and gamma peak of co-injection of [^{11}C]MK-6884 and reference standard ≤ 2%	HPLC with radio-detector and UV/VIS detector
Radiochemical purity	≥ 95% of total radioactivity	Radio-HPLC
Individual chemical purity, and total unidentified impurities†	Individual unidentified chemical impurity ≤ 1.5 µg/injected volume† Total of unidentified chemical impurities ≤ 3.0 µg/injected volume†	HPLC with UV/VIS detector

Amount of MK-6884 in total volume (µg)	≤ 4.9 µg per injected volume	HPLC with UV/VIS detector
Amount of component L-005388167 (precursor) in total volume (µg) †	≤1.5 µg per injected volume	HPLC with UV/VIS detector
pH	pH of the finished product is 4.5-7.5	pH strip
Integrity of the sterile membrane	Bubble point ≥ 13 psi	Bubble point determination
Residual solvent	Conform USP EtOH < 10% V/V Acetonitrile ≤ 0.04% (w/w) DMF <	GC
Radionuclide identity- half life ($T_{1/2}$)	T1/2 = 18.4 – 22.4 min	Two time point radioactivity measurement in dose calibrator
Radionuclide identity – gamma spectrometry	Gamma energy is 511keV	MCA
Sterility	No growth after 14 days incubation at 37 °C conform USP	Incubation with growth medium
Bacterial Endotoxins	< 175 IU per injected volume	LAL test
† Calculated using the method for MK-6884.		

4.2 Analytical Procedures

No intermediate analysis on the drug substance is performed. Analytical procedures for the drug product are presented in section P.5.2- Analytical Procedures – Drug Product [^{11}C]MK-6884.

4.3 Validation of Analytical Procedures

No intermediate analysis on the drug substance is performed. Validation of analytical procedures for the drug product are presented in section P.5.3 Validation of Analytical Procedures – Drug Product [^{11}C]MK-6884.

4.4 Batch Analysis

Radiolabeled drug substance is used *in situ* to produce the drug product formulation and is, therefore, not isolated. Batch analysis data of the drug product are presented in section P.5.4 Batch Analysis - Drug Product [^{11}C]MK-6884.