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Automated two-steps manufacturing of [¹¹C]glyburide radiopharmaceutical for PET imaging in Humans

Fabien Caillé^{a,*}, Philippe Gervais^a, Sylvain Auvity^b, Christine Coulon^a, Solène Marie^a, Nicolas Tournier^a, Bertrand Kuhnast^a

^a Biomaps, Service Hospitalier Frédéric Joliot, CEA, Inserm, Université Paris Sud, CNRS, Université Paris-Saclay, Orsay, France

^b Assistance Publique-Hôpitaux de Paris, Hôpital Necker – Enfants malades, Inserm, UMR-S 1144, Université de Paris, Optimisation thérapeutique en neuropsychopharmacologie, Paris, France.

*Corresponding author : fabien.caille@cea.fr (F. Caillé)

Keywords

Glyburide; carbon-11; radiosynthesis; PET imaging; radiopharmaceutical

Abstract

Glyburide is an approved anti-diabetes drug binding to the sulfonylurea receptors-1 (SUR-1) and substrate of solute carrier (SLC) transporters, which can be isotopically radiolabelled with carbon-11 for PET imaging. The aim of this work is to present an original and reproducible automated radiosynthesis of [¹¹C]glyburide and a full European Pharmacopeia 9.7 compliant quality control to use [¹¹C]glyburide in PET imaging clinical trials. Different conditions were explored to afford non-radioactive glyburide by one-step methylation. These experiments monitored by UPLC-MS revealed regioselectivity issues for this approach as methylation occurred preferentially on the sulfonylurea moiety. Therefore, a two-steps process was developed using sulfonamide **3** as precursor, synthesized in 79% yield. Methylation under non-radioactive conditions followed by reaction with cyclohexyl isocyanate in one pot afforded glyburide without formation of methylated side products. The optimized process was applied to the automated radiosynthesis of [¹¹C]glyburide from [¹¹C]MeOTf using a TRACERlab[®] FX C Pro. Ready-to-inject [¹¹C]glyburide was obtained in 5% non-decay corrected radiochemical yield and 110 ± 20 GBq/ μ mol molar activity within 40 minutes (n = 8). A full quality control according to the European Pharmacopeia 9.7 guidelines was realized including organoleptic test, pH measurement, chemical identification with chemical and radiochemical purity assessment by analytical HPLC, residual solvents determination by gas chromatography, radionuclide purity and half-life measurement by gamma-spectrometry, bubbling point filter integrity test, sterility check by incubation in growth media and bacterial endotoxins measurement using the limulus amoebocyte lysate test. [¹¹C]Glyburide was compliant with all requirements and can be used as a PET radiopharmaceutical in clinical trials.

INTRODUCTION

Glyburide, also named glibenclamide, is a second-generation sulfonylurea approved by the Food and Drug Administration and the European Medical Agency to treat type 2 diabetes mellitus. Glyburide increases the pancreatic production of insulin by binding to the ATP-sensitive potassium channels (K_{ATP}) subunit sulfonylurea receptor 1 (SUR-1, ABCC8) of β -cells, allowing the control of glucose levels in blood.¹ With widespread overexpression of SUR-1 in the central nervous system in co-association with transient receptor potential melastatin 4 (trpm4) after brain injury,² glyburide also proved to reduce edema, haemorrhage and tissue damage in preclinical models of numerous CNS injuries.^{3,4,5} More recently, the Glyburide Advantage in Malignant Edema and Stroke (GAMES-RP and GAMES-PILOT) clinical trials demonstrated that intravenous injection of glyburide was associated with improvements in midline shift, level of alertness, and NIH stroke scale in Humans.^{6,7} Glyburide is also known to be a substrate of several membrane transporters that are key players in the distribution and elimination of drugs. In particular, glyburide is an avid substrate of some organic anion-transporting polypeptide (OATP, SLCO) transporters such as OATP1A2, OATP2B1 and OATP1B1 (Figure 1)^{8,9,10} making an interesting probe to study the importance the OATP-mediated influx at the blood-tissue interface.¹¹ Glyburide is also carried by efflux ABC transporters such as the breast cancer resistance protein (BCRP, ABCG2), the P-glycoprotein (P-gp, ABCB1) and some multidrug resistance proteins (ABCC1 and 3).^{12,13}

Positron emission tomography (PET) is a powerful tool to quantitatively image at the molecular level and assess pharmacokinetic parameters of functional or pathological tissues. Therefore, PET imaging of SUR-1 using radiolabelled glyburide is an appealing approach supported by in vitro studies showing a saturable uptake of [³H]glyburide proportional to the number of β -cells.¹⁴ Unfortunately, PET imaging of SUR-1 at the pancreas level using [¹¹C]glyburide¹⁵ or fluorine-18 analogues^{16,17} only displayed a low radioactivity uptake in the pancreas and a rapid washout of the radiotracers through the liver and the small intestine. However, PET imaging of SUR-1 overexpression after CNS injuries remains promising to evaluate the recovery and detect possible residual damages. This approach is encouraged by recent results demonstrating a negligible brain penetration of [¹¹C]glyburide in a healthy human subject using PET imaging, allowing for a promising contrast between healthy/non-injured and blood-brain barrier (BBB)-disrupted brain regions.¹⁸ The use of [¹¹C]glyburide PET imaging is also a powerful tool to assess non-invasively the functional activity of the transporters for which glyburide is a substrate. The study of P-gp at the BBB level has already been demonstrated with [¹¹C]glyburide.¹¹

Glyburide can be radioisotopically labelled with carbon-11 (Figure 1), an extensively used positron emitter of 20.38 minutes half-life produced in cyclotron. Radioisotopic labelling does not modify the chemical structure and the radiotracer displays the exact same biological properties (binding to the receptors, selectivity, membrane permeation) as the original molecule. Radiolabelling of glyburide with carbon-11 has already been described by Hwand and co-workers in one step by methylation using *O*-desmethyl-glyburide as precursor and [¹¹C]methyl triflate in the presence of sodium hydroxide.¹⁵ In those conditions, the authors reported a non-decay corrected radiochemical yield (n.d.c. RCY) of 3%. In our hands, those conditions gave the same results¹¹ but were unfortunately not reproducible. Aiming at investigating the use of [¹¹C]glyburide in a clinical trial, we needed a more reliable automated radiolabelling of glyburide with carbon-11. Therefore, we have developed an original one pot

two-steps radiosynthesis of [^{11}C]glyburide by radiomethylation followed by urea formation inspired from the synthesis of the drug.¹⁹ We have finalized this radiosynthesis by a complete quality control compliant with to the European Pharmacopeia 9.7 guidelines for the manufacturing of [^{11}C]glyburide as a radiopharmaceutical preparation for Human injection.

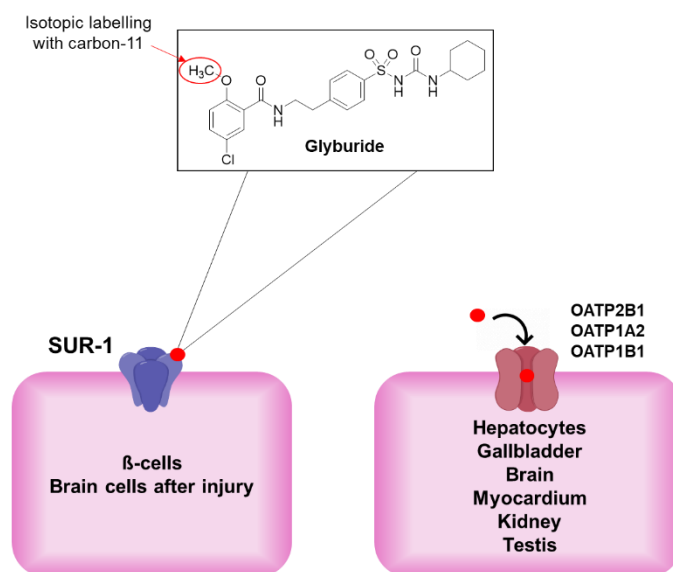


Figure 1. Glyburide is a substrate of SUR-1 and different transporters at the cell membrane which can be isotopically radiolabelled with carbon-11 at the methoxy position.

EXPERIMENTAL SECTION

Chemistry

Chemicals were purchased from Aldrich (France) and used as received. Compound **1** was purchased from Eras Labo (France). Unless otherwise stated, non-radioactive reactions were monitored by ultra-performance liquid chromatography - mass spectroscopy (UPLC-MS). Chromatography was realized on a Ultimate 3000 (Thermo Scientific, USA) device equipped with an Acquity BEH 2.1 x 50 mm, 1.7 μm column (Waters, USA). A gradient of water with 0.1% of formic acid and acetonitrile with 0.1% of formic acid (3% of $\text{CH}_3\text{CN}/\text{HCHO}$ for 2 minutes, then rising to 100% during 7 minutes then decreasing to 3% during 1 minute then keeping 3% for 2 minutes) at a flowrate of 0.3 mL/min was used. Mass spectroscopy was performed with a Linear Trap Quadrupole Orbitrap Velos (Thermo Scientific, USA) equipped with an electron spray ionization (ESI) chamber. Spectra were recorded between 100 and 1000 m/z . Melting point (M.p.) was measured with an Electrothermal IA9200 and is reported in $^{\circ}\text{C}$. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 MHz apparatus using $\text{DMSO-}d^6$ as solvent. The chemical shifts (δ) are reported in ppm, downfield from TMS (s, d, t, q and m for singlet, doublet, triplet, quadruplet and multiplet respectively) and referenced with the solvent residual chemical shift. Coupling constants (J) are expressed in Hertz (Hz). High resolution mass spectroscopy (HRMS) was performed by the Institut de Chimie Organique et Analytique of Orléans (France).

General procedure for the one-step non-radioactive methylation of 1.

To a solution of **1** (2 mg, 1.0 equiv.) in the appropriate solvent (DMF, DMA or butanone, 300 μ L) was added the appropriate base (sodium hydroxide, sodium hydride, DBU or potassium fluoride). The solution was stirred at room temperature for 2 minutes and a solution of methyl iodide in the corresponding solvent (1 M, 4 μ L, 1.0 equiv.) was added. The solution was stirred for 30 minutes at the appropriate temperature (70 $^{\circ}$ C or 110 $^{\circ}$ C). An aliquot (10 μ L) of the solution was withdrawn, diluted in CH₃CN/H₂O (1/1 v/v, 1 mL) and analysed qualitatively by UPLC-MS.

5-chloro-2-hydroxy-N-(4-sulfamoylphenethyl)benzamide (3).

To a cooled (-10 $^{\circ}$ C) suspension of compound **4** (200 mg, 1.0 equiv.) in anhydrous dichloromethane (2 mL) was added dropwise boron tribromide (1 M in CH₂Cl₂, 0.81 mL, 1.5 equiv.). The solution was stirred under argon for 1 h at 0 $^{\circ}$ C followed by 3 h at room temperature and monitored by thin layer chromatography on aluminum pre-coated plates of silica gel 60F₂₅₄ (VWR, France). The compounds were localized at 254 nm using a UV-lamp. Water (5 mL) was added at 0 $^{\circ}$ C and the precipitate formed was filtrated, washed with ethanol (2 x 5 mL) and diethyl ether (2 x 5 mL) to afford compound **3** (150 mg, 79%) as a white solid. M.p.: 187.2-187.5 $^{\circ}$ C. ¹H-NMR (DMSO-*d*⁶, 400 MHz) δ 12.50 (s, 1H, OH), 8.96 (t, *J* = 5.6 Hz, 1H, NH), 7.90 (d, *J* = 2.6 Hz, 1H, H_{Ar}), 7.75 (d, *J* = 8.3 Hz, 2H, H_{Ar}), 7.43 (m, 3H, H_{Ar}), 7.30 (s, 2H, NH₂), 6.93 (d, *J* = 8.8 Hz, 1H, H_{Ar}), 3.55 (q, *J* = 6.0 Hz, 2H, CH₂), 2.94 (t, *J* = 7.0 Hz, 2H, CH₂) ppm. ¹³C-NMR (DMSO-*d*⁶, 100 MHz) δ 167.4 [C], 158.5 [C], 143.5 [C], 142.2 [C], 133.3 [CH], 129.2 [2CH], 127.3 [CH], 125.8 [2CH], 122.4 [C], 119.4 [C], 116.9 [CH], 40.2 [CH₂], 34.5 [CH₂] ppm. HR-ESI(+)-MS *m/z* calcd for C₁₅H₁₆ClN₂O₄S: 355.0514 [M+H]⁺, found 355.0513.

Two-steps non-radioactive synthesis of glyburide from 3.

To a solution of **3** (2 mg, 1.0 equiv.) in butanone (300 μ L) was added an aqueous solution of sodium hydroxide (1 M, 7 μ L, 1.2 equiv.). The solution was stirred at room temperature for 2 minutes and a solution of methyl iodide in butanone (1 M, 6 μ L, 1.0 equiv.) was added. The solution was stirred at 70 $^{\circ}$ C for 30 minutes. An aliquot (10 μ L) of the solution was withdrawn, diluted into CH₃CN/H₂O (1/1 v/v, 1 mL) and analysed qualitatively by UPLC-MS. To the remaining solution, cyclohexyl isocyanate (7 μ L, 10.0 equiv.) and an aqueous solution of sodium hydroxide (3 M, 19 μ L, 10.0 equiv.) were added. The solution was stirred at 70 $^{\circ}$ C for 30 minutes. An aliquot (10 μ L) of the solution was withdrawn, diluted into CH₃CN/H₂O (1/1 v/v, 1 mL) and analysed qualitatively by UPLC-MS.

Radiochemistry

Radiosynthesis of [¹¹C]glyburide.

Automated radiosynthesis with carbon-11 was performed using a TRACERlab[®] FX C Pro (GE Healthcare, Uppsala, Sweden). No carrier-added [¹¹C]CO₂ (18-37 GBq) was produced via the ¹⁴N(p, α)¹¹C nuclear reaction by irradiation of a [¹⁴N]N₂ target containing 0.15 - 0.5% of O₂ on a cyclone 18/9 cyclotron (18 MeV, IBA, Belgium). [¹¹C]CO₂ was subsequently reduced to

[¹¹C]CH₄ and iodinated to [¹¹C]CH₃I following the process described by Larsen *et al.*²⁰ and finally converted to [¹¹C]CH₃OTf according to the method of Jewett.²¹ [¹¹C]CH₃OTf was bubbled into a solution of compound **3** (1 mg) and aqueous sodium hydroxide (1 M, 3.5 μL) in butanone (200 μL) at -20 °C for 3 min. The mixture was heated at 70 °C for 3 min and a solution of cyclohexyl isocyanate (4 μL) and aqueous sodium hydroxide (3 M, 7 μL) in butanone (200 μL) was added. The mixture was heated at 70 °C for 3 min under a stream of helium (7 mL/min). Upon cooling to 50 °C, a mixture of water / acetonitrile / trifluoroacetic acid (TFA) (45/55/0.1 v/v/v, 1 mL) was added. Purification was realized by reverse phase semi-preparative HPLC (Waters Symmetry[®] C18 7.8 x 300 mm, 7 μm) with a 515 HPLC Pump (Waters, USA) using a mixture of water / acetonitrile / TFA (45/55/0.1 v/v/v, 5 mL/min) as eluent. UV detection (K2501, Knauer, Germany) was performed at 220 nm. The purified compound was diluted with water (20 mL) and passed through a Sep-Pak[®] C18 cartridge (Waters, USA). The cartridge was rinsed with water (10 mL), eluted with ethanol (2 mL) and the final compound was diluted with saline (0.9% w/v, 18 mL) before filtration under a laminar flow hood using a 0.22 μm Millex[®] GV filter (Merck, USA). Ready-to-inject [¹¹C]glyburide (1.1 - 1.5 GBq) was obtained in 5% non-decay corrected radiochemical yield (n.d.c. RCY) within 40 minutes. Automated process with module synopsis are presented in supporting information (Figure S7).

Quality Control

The quality control was realized on three consecutive productions of [¹¹C]glyburide.

Organoleptic test

The final preparation was visually examined behind a lead-shielded glass window.

pH measurement

The pH value was determined using a pH paper.

Chemical identification, chemical and radiochemical purities determination and molar activity measurement.

Identification, purities and molar activity were assessed according to the “Liquid chromatography” monograph n°01/2019:20229 of the European Pharmacopeia 9.7 by analytical reverse phase HPLC equipped with a 717_{plus} Autosampler system, a 1525 binary pump, a 2996 photodiode array detector (Waters, USA) and a Flowstar LB 513 (Berthold, France) gamma detector. The system was operated with the Empower 3 (Waters) software. HPLC were realized on a reverse phase analytical Symmetry C18 (150 x 3.9 mm, 5 μm, Waters) column using a mixture of H₂O/CH₃CN/PicB7[®] (55/45/0.1 v/v/v, 2 mL/min) as eluent. UV detection was performed at 230 nm. The chemical identification of the peak was assessed by comparing the retention time of [¹¹C]glyburide with the retention time of the non-radioactive glyburide reference (t_R^{ref}). For acceptance, the retention time must be within the t_R^{ref} ± 10% range. Radiochemical and chemical purities were calculated as the ratio of the area under the

curve (AUC) of the glyburide peak over the sum of the AUCs of all other peaks on gamma and UV chromatograms respectively. Radiochemical and chemical purities are the mean values of three consecutive runs. Molar activity was calculated as the ratio of the activity of the collected peak of [¹¹C]glyburide measured in an ionization chamber (Capintec[®], Berthold) over the molar quantity of glyburide determined using calibration curves. Molar activity is calculated as the mean value of three consecutive runs.

Residual solvents

Content of ethanol and residual acetone, acetonitrile and butanone were determined according to the “Identification and control of residual solvents” monograph n°01/2017:20424 of the European Pharmacopeia 9.7. A gas chromatography system (Clarus 580, Perkin Elmer, USA) equipped with headspace injector (Turbomatrix 40, Perkin Elmer, USA), a woot fused silica column (CP select 624 CB 30 m x 0.53 mm 3µm, Varian, France) and a flame ionization detector was used. The system was monitored with the TotalChrom v6 (Pekin Elmer, USA) software. Chromatographic conditions were: headspace oven temp. 80°C, needle temp. 120°C, transfer line temp.: 180°C, oven temp. from 40 to 140°C, detector temp.: 250°C, gas flow (helium): 5 mL/min. Standard solutions of ethanol (5 mg/mL), acetone (5 mg/mL), acetonitrile (4.1 mg/mL) and butanone (5 mg/mL) were injected to calibrate the measured areas under the curve at known concentrations. The maximum authorized concentrations were calculated for a 10 mL injection and compared to the results observed on the chromatogram of the [¹¹C]glyburide preparation.

Radionuclide purity and half-life determination

Radionuclide purity and half-life determination were realized according to the “Radiopharmaceutical preparations” monograph n°07/2016:0125 of the European Pharmacopeia 9.7 by γ -ray spectrometry and radioactive decay measurement (Gamma ray spectrometry, gamma vision-32 V6.07 software, Ortec, France). Purity was assessed by the detection of the 511 keV γ -ray (> 99.9%) on the γ -spectrum (up to a total peak energy of 1022 keV) and half-life was calculated by decay measurement during a 2 minutes period.

Filter integrity

The filter integrity was determined by the “bubble point” test according to the “Radiopharmaceutical preparations” monograph n°07/2016:0125 of the European Pharmacopeia 9.7. The sterile filter was connected at the inlet side to a compressed air supply. A needle dipped in water was connected at the outlet side. The air pressure is gradually increased. The “bubble point” refers to the pressure producing a continuous stream of air bubbles in the water.

Sterility

The sterility was assessed according to the “Sterility” monograph n°04/2011:20601 corrected 7.7 of the European Pharmacopeia 9.7 by direct inoculation of resazurin thioglycolate, tryptic soy agar and sabouraud agar (Biomérieux, France) with an aliquot of the decayed preparation and incubation for 13 days at 32.5 °C for the first medium and 22.5 °C for the two others. Cultures were visually inspected and compared with positive and negative controls. Negative controls should be clear of any growth while positive cultures should present growth.

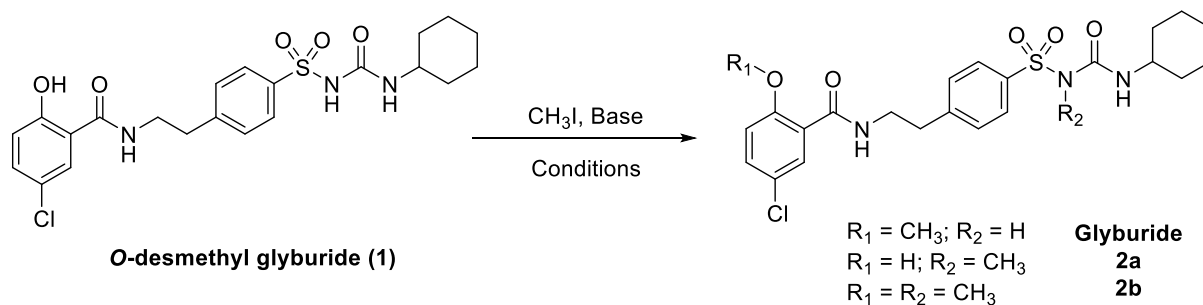
Bacterial endotoxins

The Endosafe® Portable Testing System™ (PTS™) LAL assay (Charles River, Wilmington, USA) is composed of four channels containing LAL reagent and a chromogenic substrate. Two of the four channels contain an endotoxin spike and serve as the positive control. Each channel ends in an optical cell where the samples are analysed. The sample (25 µL) is mixed with the LAL reagent alone or with the LAL reagent and positive control (spike channels). Each sample is then combined with the chromogenic substrate and incubated for 15 min. at 37 °C. The colour intensity directly related to the endotoxin concentration. Readouts between 50% and 200% spike recovery are considered to be acceptable. The sensitivity of the assay was 0.025 EU/mL. The samples were diluted at 1/100 before analysis.

RESULTS AND DISCUSSION

Chemistry

One-step radiosynthesis of [¹¹C]glyburide using *O*-desmethyl-glyburide **1** as precursor and sodium hydroxide showed poor n.d.c. RCY and reproducibility.^{15,11} Indeed, side products resulting from methylation reactions at other positions were observed. We have therefore decided to investigate different conditions (temperature, solvents, bases) for the one-step methylation of **1** under non-radioactive set up (Scheme 1 and Table 1). The crude reaction was analysed by UPLC-MS (see supporting information, Figures S1 to S5). A stronger base like sodium hydride resulted in the degradation of the starting material at high temperature (110 °C, entry 1) whereas lower temperature (70 °C, entry 2) afforded compound **2a** where the methylation occurs preferentially on the sulfonylurea moiety, as shown by MS² experiments (see Figure S2D). This result is consistent with the similar pK_a value of this moiety (~ 16.1 in DMSO²²) compared to the *p*-chlorophenol (~ 16.7 in DMSO²³). No reaction occurred with a softer base like 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU, entry 3). Cork and Hayashi reported the radiomethylation of phenols with [¹¹C]CH₃I using a large excess of potassium fluoride and kryptofix-222 (K₂₂₂) in dimethylacetamide (DMA) which afforded much higher yields compared to sodium hydroxide.²⁴ Following those experimental conditions, only the formation of the dimethylated product **2b** was observed (entry 4). Finally, reproducing the radiomethylation conditions using sodium hydroxide in butanone afforded a mixture of glyburide and compound **2a** (entry 5). It appeared from those experiments that selectively alkylating the phenol moiety of compound **1** from the sulfonylurea was difficult. These results can be explained by the high capacity of sulfonylurea to deprotonate at pH above 7.^{25,26}



Scheme 1. Non-radioactive methylation of *O*-desmethyl glyburide **1** under different conditions

Table 1. Different conditions tested for the non-radioactive methylation of **1**.

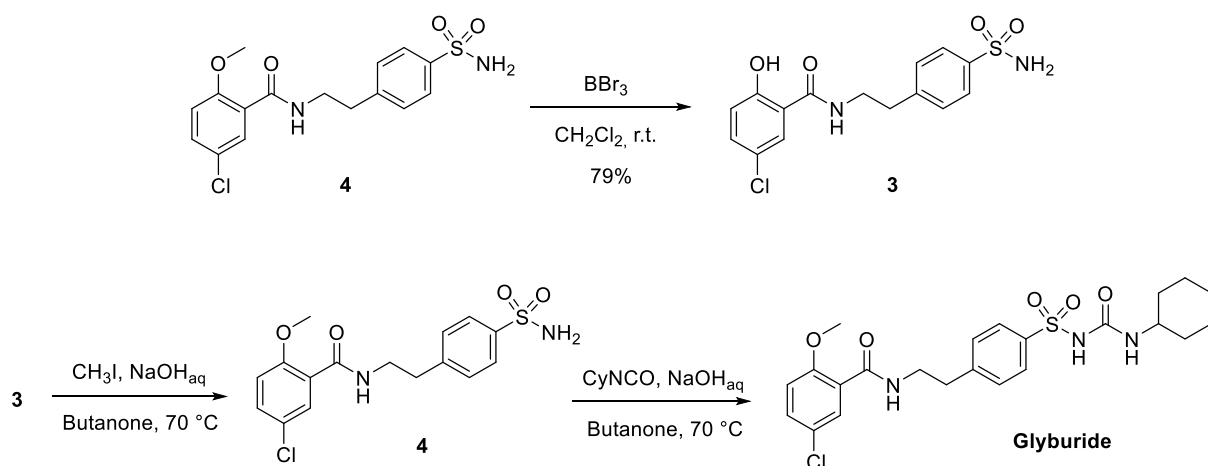
Entry	Base	Conditions ^a	Results ^b
1	NaH	DMF, 110 °C	Degradation of 1
2	NaH	DMF, 70 °C	Formation of 2a
3	DBU	Butanone, 70 °C	No reaction
4	KF + K ₂₂₂	DMA, 70 °C	Formation of 2b
5	NaOH _{aq} (3 M)	Butanone, 70 °C	Mixture of glyburide and 2a

^aAll reactions were carried out for 30 minutes with 1.0 equiv. of methyl iodide compared to **1**

^bThe crude reaction was analysed qualitatively by UPLC-MS

Given the non-orthogonality of a one-step methylation with the sulfonamide, a two-step approach was considered. Bormans and Verbruggen reported the radiomethylation of numerous phenol derivatives with sulfonamides using [¹¹C]CH₃I.²⁷ In this case, a phenol moiety could be methylated regioselectively regarding the sulfonamide. Inspired from this approach, non-radioactive glyburide was synthesized in two steps by methylation of the phenol **3** followed by reaction of the sulfonamide moiety with cyclohexyl isocyanate as described in the literature (Scheme 2).¹⁹ Firstly, compound **3** was synthesized from commercially available derivative **4** in one step and 79% yield by demethylation using boron tribromide. Methylation of compound **3** was realized using 1.0 equivalent of methyl iodide and a slight excess (1.2 equivalents) of sodium hydroxide in butanone at 70 °C for 30 minutes. The reaction was monitored by UPLC-MS (see Figure S6 in supporting information). In those conditions, methylation only occurred at the phenol moiety and no side product was observed. In parallel, reaction of sulfonamide **4** with cyclohexyl isocyanate in the presence of sodium hydroxide in butanone at 70 °C for 30 minutes was explored. The reaction afforded glyburide without formation of side products except for the hydrolysis of a part of the isocyanate into urea. Therefore, a large excess of cyclohexyl isocyanate and sodium hydroxide (10 equivalents each) were necessary to optimize the conversion into glyburide. Under those optimized conditions, the two steps were carried out sequentially in one pot (Scheme 2). At the end of the methylation reaction from **3**, cyclohexyl isocyanate and sodium hydroxide were directly added to the reaction mixture and reaction proceeded for 30 minutes at 70 °C. This two-steps one pot sequence afforded a mixture of 5 compounds, including glyburide (*t_R* = 7.52 min.) as observed by UPLC-MS (Figure 2). Some remaining starting material **3** was observed (*t_R* = 6.63 min.). Compound **4** (*t_R* = 6.79 min.) resulting from the methylation of **3** which was not fully converted into glyburide during the second step was also detected. Compound **1** (*t_R* = 7.71 min.) resulting from the reaction of

remaining starting material **3** with the excess of cyclohexyl isocyanate was also present in the mixture. Finally, di(cyclohexyl)urea ($t_R = 7.00$ min.) was observed as a side product resulting from the hydrolysis of the isocyanate. This one pot approach demonstrated the orthogonality of the sequence as no other methylated products were observed, which would be detrimental when moving to radiomethylation with carbon-11. In the course of translating this methodology to radiochemistry, the side products observed for the one pot sequence will not be problematic. Indeed, the conversion of **4** into glyburide is expected to be total thanks to the particular stoichiometry of carbon-11 reactions (typically 100 to 1000 fold excess of chemical precursors compared to the isotope). Remaining starting material **3**, compound **1** and di(cyclohexyl)urea will be present as non-radioactive side products which will not influence the radiochemical yield of [^{11}C]glyburide. They will be discarded during purification.



Scheme 2. Two-steps non-radioactive synthesis of glyburide by methylation of **3** followed by formation of the sulfonylurea moiety. Compound **3** was synthesized from commercially available derivative **4** by demethylation using boron tribromide.

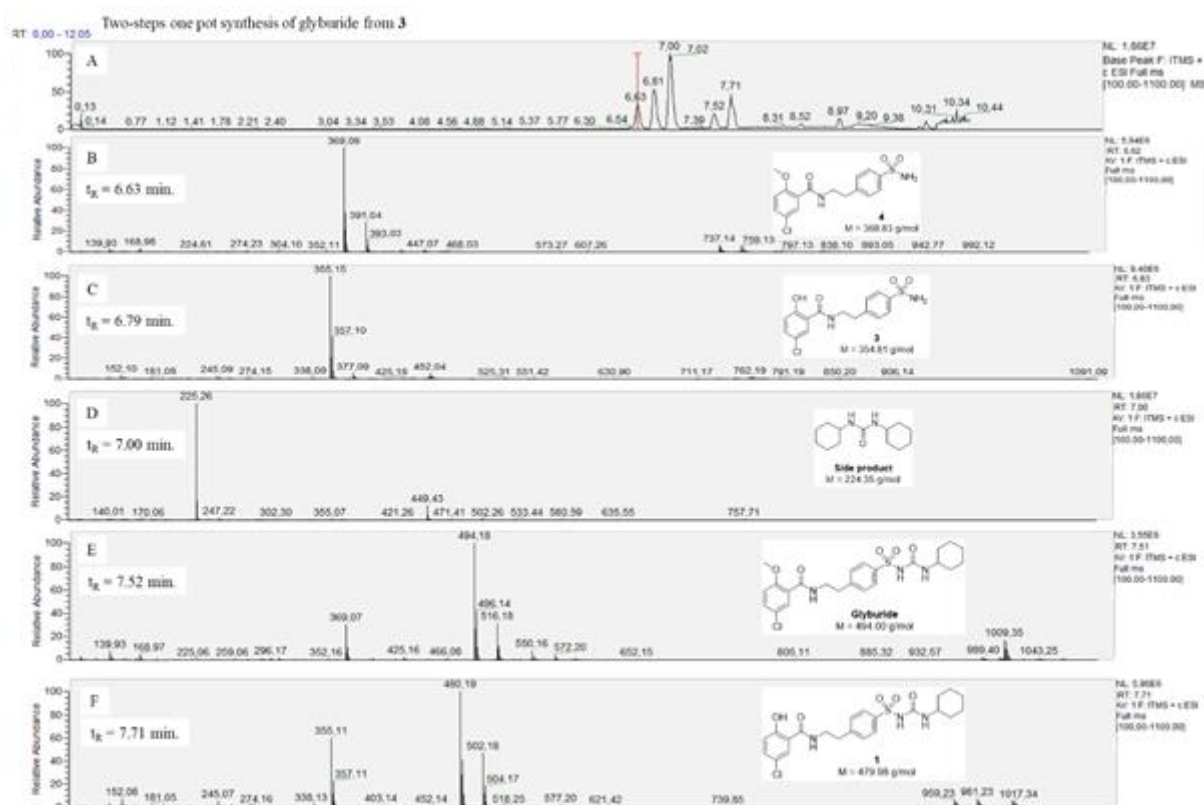
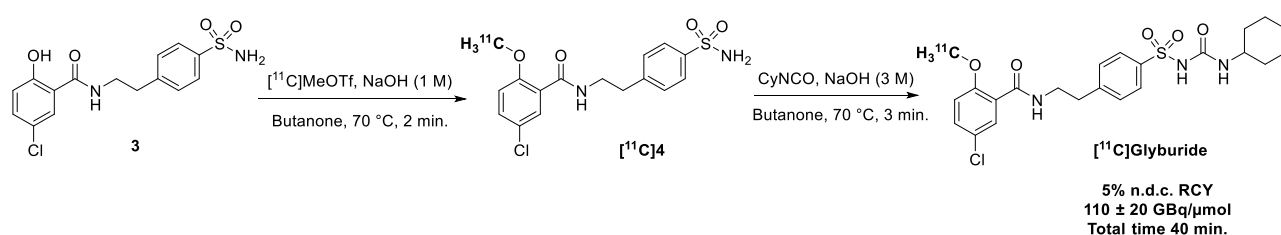


Figure 2. UPLC-MS analysis of the two-steps one pot non-radioactive synthesis of glyburide from **3** after 1 hour. A) Full chromatogram; B) Mass spectrum of the 6.63 min. peak; C) Mass spectrum of the 6.79 min. peak; D) Mass spectrum of the 7.00 min. peak; E) Mass spectrum of the 7.52 min. peak; F) Mass spectrum of the 7.71 min. peak.

Radiochemistry

The previous optimized conditions were applied to the one pot two-steps automated radiosynthesis of [^{11}C]glyburide from precursor **3** using a TRACERlab[®] FX C Pro module (Scheme 3). Automated process is depicted in supporting information (Figure S7). [^{11}C]Methyl triflate was prepared from cyclotron produced [^{11}C]CO₂ according to the gaseous method described by Larsen²⁰ and Jewett.²¹ [^{11}C]Methyl triflate afforded better RCY in a shorter reaction time than [^{11}C]methyl iodide for the synthesis of [^{11}C]**4** (28% in 2 minutes and 9% in 5 minutes respectively). In agreements with results obtained in non-radioactive conditions, no side product was observed during the methylation step, underlining the orthogonality of the method under radioactive conditions too. The large excess of precursor **3** compared to [^{11}C]MeOTf prevented from possible poly-methylation reactions and the phenol moiety proved to be more reactive than the sulfonamide in those conditions. Following the previous observations, a large excess of cyclohexyl isocyanate and sodium hydroxide (10 equivalents regarding **3** for both) were used to fully convert [^{11}C]**4** into [^{11}C]glyburide within 3 minutes at

70 °C. A stream of helium (7 mL/min) was used during to second step to ensure the homogeneity of the reaction mixture. Lower amounts of cyclohexyl isocyanate or sodium hydroxide during the second step resulted in a mixture of [¹¹C]4 and [¹¹C]glyburide, thus decreasing the radiochemical yield. Purification by reverse phase semi-preparative HPLC followed by reformulation by solid-phase extraction and sterilization afforded ready-to-inject [¹¹C]glyburide in 5% n.d.c. RCY (n = 8) and a molar activity of 110 ± 20 GBq/μmol within 40 minutes. This fully automated procedure afforded up to 1.5 GBq of [¹¹C]glyburide with high reproducibility. This amount of activity is sufficient enough to produce PET images in Humans as 300-400 MBq are typically injected. No radioactive side products were observed during the semi-preparative HPLC (see Figure S8 in supporting information), except for unreacted [¹¹C]methyl triflate, facilitating the purification of the radiotracer which was submitted to a complete quality control to aim at Human injection.



Scheme 3. Automated two-steps radiosynthesis of [¹¹C]glyburide.

Quality Control

Quality control of [¹¹C]glyburide was achieved on three consecutive batches of the radiopharmaceutical preparation following the European Pharmacopeia 9.7 general monograph on radiopharmaceutical preparations n° 07/2016:0125 (Table 2).²⁸ The representative analysis of the quality control of [¹¹C]glyburide are presented in supporting information (Figure S9 to S13). The final solution was considered limpid and colourless with a pH of 6.2. The identity of [¹¹C]glyburide confirmed by reverse phase analytical HPLC was within the acceptable range of retention time ($t_R \pm 10\%$) compared to the non-radioactive glyburide reference. The chemical and radiochemical purities also determined by HPLC with UV and gamma detections respectively were above 99.9%. Acetonitrile, acetone and butanone were investigated as residual solvents using a static headspace gas chromatography and the quantities detected were compliant with the specification of the European Pharmacopeia 9.7. The quantity of ethanol as excipient was also evaluated by static headspace gas chromatography at 0.21 g/inj., below the acceptable limit of 0.79 g/inj. A radionuclide purity above 99.9% (energy of photons at 511 keV) and a half-life of 20.38 minutes were measured by gamma spectrometry, within the acceptable range of 19.9 – 20.9 minutes for carbon-11. At the end of synthesis, the radiopharmaceutical preparation was sterilized by filtration through a 0.22 μm filter under a grade A laminar flow hood. The bubble point test showed that the integrity of the filter was conserved up to 60 psi. The sterility of the radiopharmaceutical preparation was checked by incubation of the final [¹¹C]glyburide preparation for 13 days in resazurin thioglycolate, tryptic soy agar and sabouraud agar for aerobic and anaerobic bacteria, aerobic bacteria and fungi respectively. The radiopharmaceutical preparation was considered sterile as no germs

aggregates could be observed. The bacterial endotoxins were measured quantitatively in the [¹¹C]glyburide preparation according to the Endosafe[®] test using limulus amoebocyte lysate (LAL). 3.5 EU/inj. of endotoxins were measured in the preparation, below the acceptable limit of 50 EU/inj.. Overall, the quality control of the [¹¹C]glyburide preparation proved to be compliant with the European Pharmacopeia 9.7 guidelines, making of [¹¹C]glyburide a radiopharmaceutical preparation suitable for human injection.

Table 2. Quality control on a [¹¹C]glyburide preparation according to the specifications of the European Pharmacopeia 9.7²⁸

Test	Specifications ²⁸	Results
Organoleptic test	Limpid colourless liquid	Compliant
pH	5.0 - 8.0	6.2
Chemical identification	$t_R \pm 10\%$	Compliant
Chemical purity	$\geq 95\%$	$\geq 99\%$
Radiochemical purity	$\geq 95\%$	$\geq 99\%$
Residual solvents	Ethanol ≤ 0.79 g/inj. Acetone ≤ 50.00 mg/inj. Acetonitrile ≤ 4.10 mg/inj. Butanone ≤ 50.00 mg/inj.	Compliant
Radionuclide purity	Photons energy : 511 keV Half-life : 19.9 – 20.9 min.	$\geq 99\%$ 20.3 min.
Filter integrity	≥ 50 psi	60 psi
Sterility	Sterile	Sterile
Bacterial endotoxins	≤ 50 EU/inj.	3.5 EU/inj.

CONCLUSION

We have described a highly reproducible and automated two-steps synthesis for the isotopic radiolabelling of glyburide with carbon-11. This original two-steps approach avoids the formation of methylated side products experienced in previously reported radiosynthesis to regioselectively radiolabel glyburide at the expected position. Optimized automated conditions afforded ready-to-inject [¹¹C]glyburide which was obtained in sufficient activity to perform PET images. A complete quality control was realized to qualify this tracer as a radiopharmaceutical for Human injection according to the European Pharmacopeia 9.7 guidelines. This whole manufacturing process is currently being integrated into an investigational medicinal procedure dossier (IMPD) aiming at conducting a clinical trial using [¹¹C]glyburide to elucidate the hepatic transport of drugs.

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