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1 **Automated two-step manufacturing of [<sup>11</sup>C]glyburide radiopharmaceutical**  
2 **for PET imaging in humans**

3 Abbreviated Title: Manufacturing of [<sup>11</sup>C]glyburide for PET

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6  
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12  
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20  
21 **Keywords**

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

23

24 **Abstract**

25 *Introduction:* Glyburide is an approved anti-diabetes drug binding to the sulfonylurea  
26 receptors-1 (SUR-1) and substrate of solute carrier (SLC) transporters, which can be  
27 isotopically radiolabelled with carbon-11 for PET imaging. The aim of this work is to present  
28 an original and reproducible automated radiosynthesis of [<sup>11</sup>C]glyburide and a full European  
29 Pharmacopeia 9.7 compliant quality control to use [<sup>11</sup>C]glyburide in PET imaging clinical  
30 trials.

31 *Methods:* Different conditions were explored to afford non-radioactive glyburide by one or  
32 two-step methylation. These experiments were monitored by UPLC-MS. The optimized  
33 process was applied to the automated radiosynthesis of [<sup>11</sup>C]glyburide using a TRACERlab<sup>®</sup>  
34 FX C Pro. A complete quality control according to Pharmacopeia guidelines was realized.

35 *Results:* One-step methylation revealed regioselectivity issues as methylation occurred  
36 preferentially on the sulfonylurea moiety. Two-step approach by methylation followed by  
37 reaction with cyclohexyl isocyanate afforded glyburide without formation of methylated side  
38 products. Ready-to-inject [<sup>11</sup>C]glyburide was obtained in 5% non-decay corrected  
39 radiochemical yield and  $110 \pm 20$  GBq/ $\mu$ mol molar activity within 40 minutes (n = 8).  
40 [<sup>11</sup>C]Glyburide quality control was compliant with the Pharmacopeia requirements.

41 *Conclusions:* We have described a highly reproducible and automated two-step radiosynthesis  
42 of [<sup>11</sup>C]glyburide which was qualified as a radiopharmaceutical for human injection. This  
43 whole manufacturing process is currently being used to conduct a clinical trial to elucidate the  
44 hepatic transport of drugs.

45 *Advances in knowledge:* Compared to previously reported radiosynthesis of [<sup>11</sup>C]glyburide,  
46 this work provides an original and reproducible approach which can be transferred to any PET  
47 centre interested in using this radiotracer for preclinical or clinical imaging.

48 *Implication for patient care:* This work provides a method to manufacture [<sup>11</sup>C]glyburide for  
49 human PET imaging. This radiopharmaceutical could be used to elucidate the role of  
50 transporters in drug exposure of different organs or to monitor brain recovery after central  
51 nervous system (CNS) injuries.

52

## 53 **INTRODUCTION**

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

73 Positron emission tomography (PET) is a powerful tool to quantitatively image at the  
74 molecular level and assess pharmacokinetic parameters of functional or pathological tissues.  
75 Therefore, PET imaging of SUR-1 using radiolabelled glyburide is an appealing approach  
76 supported by in vitro studies showing a saturable uptake of [<sup>3</sup>H]glyburide proportional to the  
77 number of β-cells.<sup>14</sup> Unfortunately, PET imaging of SUR-1 at the pancreas level using  
78 [<sup>11</sup>C]glyburide<sup>15</sup> or fluorine-18 analogues<sup>16,17</sup> only displayed a low radioactivity uptake in the  
79 pancreas and a rapid washout of the radiotracers through the liver and the small intestine.  
80 However, PET imaging of SUR-1 overexpression after CNS injuries remains promising to  
81 evaluate the recovery and detect possible residual damages. This approach is encouraged by  
82 recent results demonstrating a negligible brain penetration of [<sup>11</sup>C]glyburide in a healthy  
83 human subject using PET imaging, allowing for a promising contrast between healthy/non-  
84 injured and blood-brain barrier (BBB)-disrupted brain regions.<sup>18</sup> The use of [<sup>11</sup>C]glyburide  
85 PET imaging is also a powerful tool to assess non-invasively the functional activity of the  
86 transporters for which glyburide is a substrate. The study of P-gp at the BBB level has already  
87 been demonstrated with [<sup>11</sup>C]glyburide.<sup>11</sup>

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

98 radiolabelling of glyburide with carbon-11. Therefore, we have developed an original one pot  
99 two-step radiosynthesis of [<sup>11</sup>C]glyburide by radiomethylation followed by urea formation  
100 inspired from the synthesis of the drug.<sup>19</sup> We have finalized this radiosynthesis by a complete  
101 quality control compliant with to the European Pharmacopeia 9.7 guidelines for the  
102 manufacturing of [<sup>11</sup>C]glyburide as a radiopharmaceutical preparation for human injection.

103

104 **Figure 1**

105

## 106 **EXPERIMENTAL SECTION**

### 107 **Chemistry**

108 Chemicals were purchased from Aldrich (France) and used as received. Compound **1** was  
109 purchased from Eras Labo (France). Unless otherwise stated, non-radioactive reactions were  
110 monitored by ultra-performance liquid chromatography - mass spectroscopy (UPLC-MS).  
111 Chromatography was realized on a Ultimate 3000 (Thermo Scientific, USA) device equipped  
112 with an Acquity BEH 2.1 x 50 mm, 1.7 μm column (Waters, USA). A gradient of water with  
113 0.1% of formic acid and acetonitrile with 0.1% of formic acid (3% of CH<sub>3</sub>CN/HCHO for 2  
114 minutes, then rising to 100% during 7 minutes then decreasing to 3% during 1 minute then  
115 keeping 3% for 2 minutes) at a flowrate of 0.3 mL/min was used. Mass spectroscopy was  
116 performed with a Linear Trap Quadrupole Orbitrap Velos (Thermo Scientific, USA) equipped  
117 with an electron spray ionization (ESI) chamber. Spectra were recorded between 100 and  
118 1000 m/z. Melting point (M.p.) was measured with an Electrothermal IA9200 and is reported  
119 in °C. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker Advance 400 MHz apparatus  
120 using DMSO-*d*<sup>6</sup> as solvent. The chemical shifts (δ) are reported in ppm, downfield from TMS  
121 (s, d, t, q and m for singlet, doublet, triplet, quadruplet and multiplet respectively) and

122 referenced with the solvent residual chemical shift. Coupling constants ( $J$ ) are expressed in  
123 Hertz (Hz). High resolution mass spectroscopy (HRMS) was performed by the Institut de  
124 Chimie Organique et Analytique of Orléans (France).

125

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

127 To a solution of **1** (2 mg, 1.0 equiv.) in the appropriate solvent (DMF, DMA or butanone, 300  
128  $\mu\text{L}$ ) was added the appropriate base (sodium hydroxide, sodium hydride, DBU or potassium  
129 fluoride). The solution was stirred at room temperature for 2 minutes and a solution of methyl  
130 iodide in the corresponding solvent (1 M, 4  $\mu\text{L}$ , 1.0 equiv.) was added. The solution was  
131 stirred for 30 minutes at the appropriate temperature (70 °C or 110 °C). An aliquot (10  $\mu\text{L}$ ) of  
132 the solution was withdrawn, diluted in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1/1 v/v, 1 mL) and analysed qualitatively  
133 by UPLC-MS.

134

135 *5-chloro-2-hydroxy- $N$ -(4-sulfamoylphenethyl)benzamide (3).*

136 To a cooled (-10 °C) suspension of compound **4** (200 mg, 1.0 equiv.) in anhydrous  
137 dichloromethane (2 mL) was added dropwise boron tribromide (1 M in  $\text{CH}_2\text{Cl}_2$ , 0.81 mL, 1.5  
138 equiv.). The solution was stirred under argon for 1 h at 0 °C followed by 3 h at room  
139 temperature and monitored by thin layer chromatography on aluminum pre-coated plates of  
140 silica gel 60F<sub>254</sub> (VWR, France). The compounds were localized at 254 nm using a UV-lamp.  
141 Water (5 mL) was added at 0 °C and the precipitate formed was filtrated, washed with ethanol  
142 (2 x 5 mL) and diethyl ether (2 x 5 mL) to afford compound **3** (150 mg, 79%) as a white solid.  
143 M.p.: 187.2-187.5 °C. <sup>1</sup>H-NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  12.50 (s, 1H, OH), 8.96 (t,  $J$  = 5.6  
144 Hz, 1H, NH), 7.90 (d,  $J$  = 2.6 Hz, 1H, H<sub>Ar</sub>), 7.75 (d,  $J$  = 8.3 Hz, 2H, H<sub>Ar</sub>), 7.43 (m, 3H, H<sub>Ar</sub>),  
145 7.30 (s, 2H, NH<sub>2</sub>), 6.93 (d,  $J$  = 8.8 Hz, 1H, H<sub>Ar</sub>), 3.55 (q,  $J$  = 6.0 Hz, 2H, CH<sub>2</sub>), 2.94 (t,  $J$  = 7.0

146 Hz, 2H, CH<sub>2</sub>) ppm. <sup>13</sup>C-NMR (DMSO-*d*<sup>6</sup>, 100 MHz) δ 167.4 [C], 158.5 [C], 143.5 [C], 142.2  
147 [C], 133.3 [CH], 129.2 [2CH], 127.3 [CH], 125.8 [2CH], 122.4 [C], 119.4 [C], 116.9 [CH],  
148 40.2 [CH<sub>2</sub>], 34.5 [CH<sub>2</sub>] ppm. HR-ESI(+)-MS m/z calcd for C<sub>15</sub>H<sub>16</sub>ClN<sub>2</sub>O<sub>4</sub>S: 355.0514  
149 [M+H]<sup>+</sup>, found 355.0513.

150

151 *Two-step non-radioactive synthesis of glyburide from 3.*

152 To a solution of **3** (2 mg, 1.0 equiv.) in butanone (300 μL) was added an aqueous solution of  
153 sodium hydroxide (1 M, 7 μL, 1.2 equiv.). The solution was stirred at room temperature for 2  
154 minutes and a solution of methyl iodide in butanone (1 M, 6 μL, 1.0 equiv.) was added. The  
155 solution was stirred at 70 °C for 30 minutes. An aliquot (10 μL) of the solution was  
156 withdrawn, diluted into CH<sub>3</sub>CN/H<sub>2</sub>O (1/1 v/v, 1 mL) and analysed qualitatively by UPLC-  
157 MS. To the remaining solution, cyclohexyl isocyanate (7 μL, 10.0 equiv.) and an aqueous  
158 solution of sodium hydroxide (3 M, 19 μL, 10.0 equiv.) were added. The solution was stirred  
159 at 70 °C for 30 minutes. An aliquot (10 μL) of the solution was withdrawn, diluted into  
160 CH<sub>3</sub>CN/H<sub>2</sub>O (1/1 v/v, 1 mL) and analysed qualitatively by UPLC-MS.

161

## 162 **Radiochemistry**

163 *Radiosynthesis of [<sup>11</sup>C]glyburide.*

164 Automated radiosynthesis with carbon-11 was performed using a TRACERlab<sup>®</sup> FX C Pro  
165 (GE Healthcare, Uppsala, Sweden). No carrier-added [<sup>11</sup>C]CO<sub>2</sub> (18-37 GBq) was produced  
166 via the <sup>14</sup>N(p, α)<sup>11</sup>C nuclear reaction by irradiation of a [<sup>14</sup>N]N<sub>2</sub> target containing 0.15 - 0.5%  
167 of O<sub>2</sub> on a cyclone 18/9 cyclotron (18 MeV, IBA, Belgium). [<sup>11</sup>C]CO<sub>2</sub> was subsequently  
168 reduced to [<sup>11</sup>C]CH<sub>4</sub> and iodinated to [<sup>11</sup>C]CH<sub>3</sub>I following the process described by Larsen *et*



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

186

## 187 **Quality Control**

188 The quality control was realized on three consecutive productions of [<sup>11</sup>C]glyburide.

## 189 *Organoleptic test*

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

191

192 *pH measurement*

193 The pH value was determined using a pH paper.

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

196 Identification, purities and molar activity were assessed according to the “Liquid  
197 chromatography” monograph n°01/2019:20229 of the European Pharmacopeia 9.7 by  
198 analytical reverse phase HPLC equipped with a 717<sub>plus</sub> Autosampler system, a 1525 binary  
199 pump, a 2996 photodiode array detector (Waters, USA) and a Flowstar LB 513 (Berthold,  
200 France) gamma detector. The system was operated with the Empower 3 (Waters) software.  
201 HPLC were realized on a reverse phase analytical Symmetry C18 (150 x 3.9 mm, 5 µm,  
202 Waters) column using a mixture of H<sub>2</sub>O/CH<sub>3</sub>CN/PicB7<sup>®</sup> (55/45/0.1 v/v/v, 2 mL/min) as  
203 eluent. UV detection was performed at 230 nm. The chemical identification of the peak was  
204 assessed by comparing the retention time of [<sup>11</sup>C]glyburide with the retention time of the non-  
205 radioactive glyburide reference ( $t_{R}^{ref}$ ). For acceptance, the retention time must be within the  
206  $t_{R}^{ref} \pm 10\%$  range. Radiochemical and chemical purities were calculated as the ratio of the area  
207 under the curve (AUC) of the glyburide peak over the sum of the AUCs of all other peaks on  
208 gamma and UV chromatograms respectively. Radiochemical and chemical purities are the  
209 mean values of three consecutive runs. Molar activity was calculated as the ratio of the  
210 activity of the collected peak of [<sup>11</sup>C]glyburide measured in an ionization chamber  
211 (Capintec<sup>®</sup>, Berthold) over the molar quantity of glyburide determined using calibration  
212 curves. Molar activity is calculated as the mean value of three consecutive runs.

213

214 *Residual solvents*

215 Content of ethanol and residual acetone, acetonitrile and butanone were determined according  
216 to the “Identification and control of residual solvents” monograph n°01/2017:20424 of the  
217 European Pharmacopeia 9.7. A gas chromatography system (Clarus 580, Perkin Elmer, USA)  
218 equipped with headspace injector (Turbomatrix 40, Perkin Elmer, USA), a woot fused silica  
219 column (CP select 624 CB 30 m x 0.53 mm 3µm, Varian, France) and a flame ionization  
220 detector was used. The system was monitored with the TotalChrom v6 (Pekin Elmer, USA)  
221 software. Chromatographic conditions were: headspace oven temp. 80°C, needle temp.  
222 120°C, transfer line temp.: 180°C, oven temp. from 40 to 140°C, detector temp.: 250°C, gas  
223 flow (helium): 5 mL/min. Standard solutions of ethanol (5 mg/mL), acetone (5 mg/mL),  
224 acetonitrile (4.1 mg/mL) and butanone (5 mg/mL) were injected to calibrate the measured  
225 areas under the curve at known concentrations. The maximum authorized concentrations were  
226 calculated for a 10 mL injection and compared to the results observed on the chromatogram of  
227 the [<sup>11</sup>C]glyburide preparation.

228

#### 229 *Radionuclide purity and half-life determination*

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

236

#### 237 *Filter integrity*

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

244

#### 245 *Sterility*

246 The sterility was assessed according to the “Sterility” monograph n°04/2011:20601 corrected  
247 7.7 of the European Pharmacopeia 9.7 by direct inoculation of resazurin thioglycolate,  
248 trypticase soy agar and sabouraud agar (Biomérieux, France) with an aliquot of the decayed  
249 preparation and incubation for 13 days at 32.5 °C for the first medium and 22.5 °C for the two  
250 others. Cultures were visually inspected and compared with positive and negative controls.  
251 Negative controls should be clear of any growth while positive cultures should present  
252 growth.

253

#### 254 *Bacterial endotoxins*

255 The Endosafe® Portable Testing System™ (PTS™) LAL assay (Charles River, Wilmington,  
256 USA) is composed of four channels containing LAL reagent and a chromogenic substrate.  
257 Two of the four channels contain an endotoxin spike and serve as the positive control. Each  
258 channel ends in an optical cell where the samples are analysed. The sample (25 µL) is mixed  
259 with the LAL reagent alone or with the LAL reagent and positive control (spike channels).  
260 Each sample is then combined with the chromogenic substrate and incubated for 15 min. at 37  
261 °C. The colour intensity directly related to the endotoxin concentration. Readouts between

262 50% and 200% spike recovery are considered to be acceptable. The sensitivity of the assay  
263 was 0.025 EU/mL. The samples were diluted at 1/100 before analysis.

264

265

## 266 RESULTS AND DISCUSSION

### 267 Chemistry

268 One-step radiosynthesis of [<sup>11</sup>C]glyburide using *O*-desmethyl-glyburide **1** as precursor and  
269 sodium hydroxide showed poor n.d.c. RCY and reproducibility.<sup>15,11</sup> Indeed, side products  
270 resulting from methylation reactions at other positions were observed. We have therefore  
271 decided to investigate different conditions (temperature, solvents, bases) for the one-step  
272 methylation of **1** under non-radioactive set up (Scheme 1 and Table 1). The crude reaction  
273 was analysed by UPLC-MS (see supporting information, Figures S1 to S5). A stronger base  
274 like sodium hydride resulted in the degradation of the starting material at high temperature  
275 (110 °C, entry 1) whereas lower temperature (70 °C, entry 2) afforded compound **2a** where  
276 the methylation occurs preferentially on the sulfonylurea moiety, as shown by MS<sup>2</sup>  
277 experiments (see Figure S2D). This result is consistent with the similar pKa value of this  
278 moiety (~ 16.1 in DMSO<sup>22</sup>) compared to the *p*-chlorophenol (~ 16.7 in DMSO<sup>23</sup>). No reaction  
279 occurred with a softer base like 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU, entry 3). Cork and  
280 Hayashi reported the radiomethylation of phenols with [<sup>11</sup>C]CH<sub>3</sub>I using a large excess of  
281 potassium fluoride and kryptofix-222 (K<sub>222</sub>) in dimethylacetamide (DMA) which afforded  
282 much higher yields compared to sodium hydroxide.<sup>24</sup> Following those experimental  
283 conditions, only the formation of the dimethylated product **2b** was observed (entry 4). Finally,  
284 reproducing the radiomethylation conditions using sodium hydroxide in butanone afforded a  
285 mixture of glyburide and compound **2a** (entry 5). It appeared from those experiments that

286 selectively alkylating the phenol moiety of compound **1** from the sulfonylurea was difficult.  
287 These results can be explained by the high capacity of sulfonylurea to deprotonate at pH  
288 above 7.<sup>25,26</sup>

289

290

### Scheme 1

291

### Table1

292

293 Given the non-orthogonality of a one-step methylation with the sulfonylurea, a two-step  
294 approach was considered. Bormans and Verbruggen reported the radiomethylation of  
295 numerous phenol derivatives with sulfonamides using [<sup>11</sup>C]CH<sub>3</sub>I.<sup>27</sup> In this case, a phenol  
296 moiety could be methylated regioselectively regarding the sulfonamide. Inspired from this  
297 approach, non-radioactive glyburide was synthesized in two steps by methylation of the  
298 phenol **3** followed by reaction of the sulfonamide moiety with cyclohexyl isocyanate as  
299 described in the literature (Scheme 2).<sup>19</sup> Firstly, compound **3** was synthesized from  
300 commercially available derivative **4** in one step and 79% yield by demethylation using boron  
301 tribromide. Methylation of compound **3** was realized using 1.0 equivalent of methyl iodide  
302 and a slight excess (1.2 equivalents) of sodium hydroxide in butanone at 70 °C for 30  
303 minutes. The reaction was monitored by UPLC-MS (see Figure S6 in supporting  
304 information). In those conditions, methylation only occurred at the phenol moiety and no side  
305 product was observed. In parallel, reaction of sulfonamide **4** with cyclohexyl isocyanate in the  
306 presence of sodium hydroxide in butanone at 70 °C for 30 minutes was explored. The reaction  
307 afforded glyburide without formation of side products except for the hydrolysis of a part of  
308 the isocyanate into urea. Therefore, a large excess of cyclohexyl isocyanate and sodium  
309 hydroxide (10 equivalents each) were necessary to optimize the conversion into glyburide.

310 Under those optimized conditions, the two steps were carried out sequentially in one pot  
311 (Scheme 2). At the end of the methylation reaction from **3**, cyclohexyl isocyanate and sodium  
312 hydroxide were directly added to the reaction mixture and reaction proceeded for 30 minutes  
313 at 70 °C. This two-step one pot sequence afforded a mixture of 5 compounds, including  
314 glyburide ( $t_R = 7.52$  min.) as observed by UPLC-MS (Figure 1). Some remaining starting  
315 material **3** was observed ( $t_R = 6.63$  min.). Compound **4** ( $t_R = 6.79$  min.) resulting from the  
316 methylation of **3** which was not fully converted into glyburide during the second step was also  
317 detected. Compound **1** ( $t_R = 7.71$  min.) resulting from the reaction of remaining starting  
318 material **3** with the excess of cyclohexyl isocyanate was also present in the mixture. Finally,  
319 di(cyclohexyl)urea ( $t_R = 7.00$  min.) was observed as a side product resulting from the  
320 hydrolysis of the isocyanate. This one pot approach demonstrated the orthogonality of the  
321 sequence as no other methylated products were observed, which would be detrimental when  
322 moving to radiomethylation with carbon-11. In the course of translating this methodology to  
323 radiochemistry, the side products observed for the one pot sequence will not be problematic.  
324 Indeed, the conversion of **4** into glyburide is expected to be total thanks to the particular  
325 stoichiometry of carbon-11 reactions (typically 100 to 1000 fold excess of chemical  
326 precursors compared to the isotope). Remaining starting material **3**, compound **1** and  
327 di(cyclohexyl)urea will be present as non-radioactive side products which will not influence  
328 the radiochemical yield of [ $^{11}\text{C}$ ]glyburide. They will be discarded during purification.

329

330

## Scheme 2

331

332

## Figure 1

333

## 334 **Radiochemistry**

335 The previous optimized conditions were applied to the one pot two-step automated  
336 radiosynthesis of [<sup>11</sup>C]glyburide from precursor **3** using a TRACERlab<sup>®</sup> FX C Pro module  
337 (Scheme 3). Automated process is depicted in supporting information (Figure S7).  
338 [<sup>11</sup>C]Methyl triflate was prepared from cyclotron produced [<sup>11</sup>C]CO<sub>2</sub> according to the gaseous  
339 method described by Larsen<sup>20</sup> and Jewett.<sup>21</sup> [<sup>11</sup>C]Methyl triflate afforded better RCY in a  
340 shorter reaction time than [<sup>11</sup>C]methyl iodide for the synthesis of [<sup>11</sup>C]**4** (28% in 2 minutes  
341 and 9% in 5 minutes respectively). In agreements with results obtained in non-radioactive  
342 conditions, no side product was observed during the methylation step, underlining the  
343 orthogonality of the method under radioactive conditions too. The large excess of precursor **3**  
344 compared to [<sup>11</sup>C]MeOTf prevented from possible poly-methylation reactions and the phenol  
345 moiety proved to be more reactive than the sulfonamide in those conditions. Following the  
346 previous observations, a large excess of cyclohexyl isocyanate and sodium hydroxide (10  
347 equivalents regarding **3** for both) were used to fully convert [<sup>11</sup>C]**4** into [<sup>11</sup>C]glyburide within  
348 3 minutes at 70 °C. A stream of helium (7 mL/min) was used during to second step to ensure  
349 the homogeneity of the reaction mixture. Lower amounts of cyclohexyl isocyanate or sodium  
350 hydroxide during the second step resulted in a mixture of [<sup>11</sup>C]**4** and [<sup>11</sup>C]glyburide, thus  
351 decreasing the radiochemical yield. Purification by reverse phase semi-preparative HPLC  
352 followed by reformulation by solid-phase extraction and sterilization afforded ready-to-inject  
353 [<sup>11</sup>C]glyburide in 5% n.d.c. RCY (n = 8) and a molar activity of 110 ± 20 GBq/μmol within  
354 40 minutes. The molar activity is consistent with the high affinity of glyburide for SUR-1,  
355 which dissociation constant (K<sub>d</sub>) is in the nanomolar range.<sup>28</sup> Expected in vivo concentrations  
356 of [<sup>11</sup>C]glyburide are far below the transport capacity of OATP transporters, which substrate  
357 concentration at half-maximal uptake rate (K<sub>m</sub>) is in the micromolar range.<sup>29</sup> This fully  
358 automated procedure afforded up to 1.5 GBq of [<sup>11</sup>C]glyburide at the end of the synthesis with



359 high reproducibility. This amount of activity is sufficient enough to produce PET images in  
360 Humans as 300-400 MBq are typically injected. No radioactive side products were observed  
361 during the semi-preparative HPLC (Figure 2), except for unreacted [<sup>11</sup>C]methyl triflate,  
362 facilitating the purification of the radiotracer which was submitted to a complete quality  
363 control to aim at human injection.

364

365 **Scheme 3**

366

367 **Figure 2**

### 368 **Quality Control**

369 Quality control of [<sup>11</sup>C]glyburide was achieved on three consecutive batches of the  
370 radiopharmaceutical preparation following the European Pharmacopeia 9.7 general  
371 monograph on radiopharmaceutical preparations n° 07/2016:0125 (Table 2).<sup>30</sup> The  
372 representative analysis of the quality control of [<sup>11</sup>C]glyburide are presented in supporting  
373 information (Figure S8 to S11). The final solution was considered limpid and colourless with  
374 a pH of 6.2. The identity of [<sup>11</sup>C]glyburide confirmed by reverse phase analytical HPLC was  
375 within the acceptable range of retention time ( $t_R \pm 10\%$ ) compared to the non-radioactive  
376 glyburide reference (Figure 3). The chemical and radiochemical purities also determined by  
377 HPLC with UV and gamma detections respectively were above 99.9%. A lower limit for  
378 molar activity has been set up at 4 GBq/ $\mu$ mol at the time of injection, which corresponds to a  
379 mass of 42  $\mu$ g of glyburide for a 350 MBq average injection in humans. This mass is 100  
380 times lower than the pharmacological dose prescribed (5 mg) to stay in the lower dose  
381 specification for PET radiotracers. With an average molar activity of 14 GBq/ $\mu$ mol at the  
382 time of injection, [<sup>11</sup>C]glyburide was still considered in the tracer dose range. Acetonitrile,

383 acetone and butanone were investigated as residual solvents using a static headspace gas  
384 chromatography and the quantities detected were compliant with the specification of the  
385 European Pharmacopeia 9.7. The quantity of ethanol as excipient was also evaluated by static  
386 headspace gas chromatography at 0.21 g/inj., below the acceptable limit of 0.79 g/inj. A  
387 radionuclide purity above 99.9% (energy of photons at 511 keV) and a half-life of 20.38  
388 minutes were measured by gamma spectrometry, within the acceptable range of 19.9 – 20.9  
389 minutes for carbon-11. At the end of synthesis, the radiopharmaceutical preparation was  
390 sterilized by filtration through a 0.22 µm filter under a grade A laminar flow hood. The  
391 bubble point test showed that the integrity of the filter was conserved up to 60 psi. The  
392 sterility of the radiopharmaceutical preparation was checked by incubation of the final  
393 [<sup>11</sup>C]glyburide preparation for 13 days in resazurin thioglycolate, tryptic soy agar and  
394 sabouraud agar for aerobic and anaerobic bacteria, aerobic bacteria and fungi respectively.  
395 The radiopharmaceutical preparation was considered sterile as no germs aggregates could be  
396 observed. The bacterial endotoxins were measured quantitatively in the [<sup>11</sup>C]glyburide  
397 preparation according to the Endosafe<sup>®</sup> test using limulus amoebocyte lysate (LAL). 3.5  
398 EU/inj. of endotoxins were measured in the preparation, below the acceptable limit of 50  
399 EU/inj.. Overall, the quality control of the [<sup>11</sup>C]glyburide preparation proved to be compliant  
400 with the European Pharmacopeia 9.7 guidelines, making of [<sup>11</sup>C]glyburide a  
401 radiopharmaceutical preparation suitable for human injection. The pre-release quality control  
402 operations, which include the organoleptic test, the pH measurement, the chemical  
403 identification and purity assessment and the filter integrity test, was realized within 15  
404 minutes, leaving 900 MBq of [<sup>11</sup>C]glyburide for injection. This amount of activity is sufficient  
405 enough to produce PET images in humans as 300-400 MBq are typically injected.

406

407

408

## **Table 2**

409

## **Figure 3**

410

411

412

## **413 CONCLUSION**

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

424

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- 511

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

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

513 <sup>a</sup>All reactions were carried out for 30 minutes with 1.0 equiv. of methyl iodide compared to **1**

514 <sup>b</sup>The crude reaction was analysed qualitatively by UPLC-MS

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527 **Table 2.** Quality control on a [<sup>11</sup>C]glyburide preparation according to the specifications of the  
 528 European Pharmacopeia 9.7<sup>30</sup>

Test	Specifications <sup>30</sup>	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	$\geq 95\%$	$\geq 99\%$
Molar activity at the time of injection	$\geq 4 \text{ GBq}/\mu\text{mol}$	$14 \pm 2 \text{ GBq}/\mu\text{mol}$
Residual solvents	Ethanol $\leq 0.79 \text{ g/inj.}$ Acetone $\leq 50.00 \text{ mg/inj.}$ Acetonitrile $\leq 4.10 \text{ mg/inj.}$ Butanone $\leq 50.00 \text{ mg/inj.}$	Compliant
Radionuclide purity	Photons energy : 511 keV Half-life : 19.9 – 20.9 min.	$\geq 99\%$ 20.3 min.
Filter integrity	$\geq 50 \text{ psi}$	60 psi
Sterility	Sterile	Sterile
Bacterial endotoxins	$\leq 50 \text{ EU/inj.}$	3.5 EU/inj.

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531

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

534

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

537

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

541

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

546

547 **Scheme 3.** Automated two-step radiosynthesis of [<sup>11</sup>C]glyburide.

548

549 **Figure 2.** UV (A) and radioactive (B) chromatograms of the semi-preparative HPLC  
550 purification for the preparation of [<sup>11</sup>C]glyburide.

551

552 **Figure 3.** Analytical radio-HPLC for the quality control of [<sup>11</sup>C]glyburide. A) HPLC with UV  
553 detection of the glyburide reference compound; B) HPLC with UV detection of the  
554 preparation of [<sup>11</sup>C]glyburide; C) HPLC with gamma detection of the preparation of  
555 [<sup>11</sup>C]glyburide. The peaks in red on B and C correspond to glyburide according to their  
556 retention times ( $t_R = 3.00$  min. and 3.16 min.) compared to the retention time of the reference  
557 on chromatogram A ( $t_R^{\text{ref}} = 2.96$  min.)







