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

3 Abbreviated Title: Manufacturing of [¹¹C]glyburide for PET

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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 [¹¹C]glyburide and a full European
29 Pharmacopeia 9.7 compliant quality control to use [¹¹C]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 [¹¹C]glyburide using a TRACERlab[®]
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 [¹¹C]glyburide was obtained in 5% non-decay corrected
39 radiochemical yield and 110 ± 20 GBq/μmol molar activity within 40 minutes (n = 8).
40 [¹¹C]Glyburide quality control was compliant with the Pharmacopeia requirements.

41 *Conclusions:* We have described a highly reproducible and automated two-step radiosynthesis
42 of [¹¹C]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 [¹¹C]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 [¹¹C]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 β -
58 cells, allowing the control of glucose levels in blood.¹ 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,² glyburide also proved to reduce edema, haemorrhage
61 and tissue damage in preclinical models of numerous CNS injuries.^{3,4,5} 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.^{6,7}
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)^{8,9,10} making an interesting probe to study the
69 importance the OATP-mediated influx at the blood-tissue interface.¹¹ 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).^{12,13}

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 [³H]glyburide proportional to the
77 number of β -cells.¹⁴ Unfortunately, PET imaging of SUR-1 at the pancreas level using
78 [¹¹C]glyburide¹⁵ or fluorine-18 analogues^{16,17} 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 [¹¹C]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.¹⁸ The use of [¹¹C]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 [¹¹C]glyburide.¹¹

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 [¹¹C]methyl triflate in the presence of sodium hydroxide.¹⁵ 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 results¹¹ but were unfortunately not reproducible. Aiming at
97 investigating the use of [¹¹C]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 [¹¹C]glyburide by radiomethylation followed by urea formation
100 inspired from the synthesis of the drug.¹⁹ 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 [¹¹C]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 CH₃CN/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. ¹H and ¹³C NMR spectra were recorded on a Bruker Advance 400 MHz apparatus
120 using DMSO-*d*⁶ 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 μL) 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 μL , 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 μL) 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 CH_2Cl_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 60F₂₅₄ (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. ¹H-NMR (DMSO- d_6 , 400 MHz) δ 12.50 (s, 1H, OH), 8.96 (t, J = 5.6
144 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}),
145 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

146 Hz, 2H, CH₂) ppm. ¹³C-NMR (DMSO-*d*⁶, 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 [CH₂], 34.5 [CH₂] ppm. HR-ESI(+)-MS m/z calcd for C₁₅H₁₆ClN₂O₄S: 355.0514
149 [M+H]⁺, 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 CH₃CN/H₂O (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 CH₃CN/H₂O (1/1 v/v, 1 mL) and analysed qualitatively by UPLC-MS.

161

162 **Radiochemistry**

163 *Radiosynthesis of [¹¹C]glyburide.*

164 Automated radiosynthesis with carbon-11 was performed using a TRACERlab[®] FX C Pro
165 (GE Healthcare, Uppsala, Sweden). No carrier-added [¹¹C]CO₂ (18-37 GBq) was produced
166 via the ¹⁴N(p, α)¹¹C nuclear reaction by irradiation of a [¹⁴N]N₂ target containing 0.15 - 0.5%
167 of O₂ on a cyclone 18/9 cyclotron (18 MeV, IBA, Belgium). [¹¹C]CO₂ was subsequently
168 reduced to [¹¹C]CH₄ and iodinated to [¹¹C]CH₃I following the process described by Larsen *et*

169 *al.*²⁰ and finally converted to [¹¹C]CH₃OTf according to the method of Jewett.²¹ [¹¹C]CH₃OTf
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[®] 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[®] 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[®] GV filter (Merck, USA). Ready-to-inject
183 [¹¹C]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 [¹¹C]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_{plus} 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 H₂O/CH₃CN/PicB7[®] (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 [¹¹C]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 [¹¹C]glyburide measured in an ionization chamber
211 (Capintec[®], 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 [¹¹C]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 [¹¹C]glyburide using *O*-desmethyl-glyburide **1** as precursor and
269 sodium hydroxide showed poor n.d.c. RCY and reproducibility.^{15,11} 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 MS²
277 experiments (see Figure S2D). This result is consistent with the similar pKa value of this
278 moiety (~ 16.1 in DMSO²²) compared to the *p*-chlorophenol (~ 16.7 in DMSO²³). 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 [¹¹C]CH₃I using a large excess of
281 potassium fluoride and kryptofix-222 (K₂₂₂) in dimethylacetamide (DMA) which afforded
282 much higher yields compared to sodium hydroxide.²⁴ 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.^{25,26}

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 [¹¹C]CH₃I.²⁷ 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).¹⁹ 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}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 [¹¹C]glyburide from precursor **3** using a TRACERlab[®] FX C Pro module
337 (Scheme 3). Automated process is depicted in supporting information (Figure S7).
338 [¹¹C]Methyl triflate was prepared from cyclotron produced [¹¹C]CO₂ according to the gaseous
339 method described by Larsen²⁰ and Jewett.²¹ [¹¹C]Methyl triflate afforded better RCY in a
340 shorter reaction time than [¹¹C]methyl iodide for the synthesis of [¹¹C]**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 [¹¹C]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 [¹¹C]**4** into [¹¹C]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 [¹¹C]**4** and [¹¹C]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 [¹¹C]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_d) is in the nanomolar range.²⁸ Expected in vivo concentrations
356 of [¹¹C]glyburide are far below the transport capacity of OATP transporters, which substrate
357 concentration at half-maximal uptake rate (K_m) is in the micromolar range.²⁹ This fully
358 automated procedure afforded up to 1.5 GBq of [¹¹C]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 [¹¹C]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 [¹¹C]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).³⁰ The
372 representative analysis of the quality control of [¹¹C]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 [¹¹C]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/ μ mol at the time of injection, which corresponds to a
379 mass of 42 μ 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/ μ mol at the
382 time of injection, [¹¹C]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 [¹¹C]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 [¹¹C]glyburide
397 preparation according to the Endosafe[®] 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 [¹¹C]glyburide preparation proved to be compliant
400 with the European Pharmacopeia 9.7 guidelines, making of [¹¹C]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 [¹¹C]glyburide for injection. This amount of activity is sufficient
405 enough to produce PET images in humans as 300-400 MBq are typically injected.

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407

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Table 2

409

Figure 3

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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 [¹¹C]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 [¹¹C]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 ^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

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

514 ^bThe crude reaction was analysed qualitatively by UPLC-MS

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527 **Table 2.** Quality control on a [¹¹C]glyburide preparation according to the specifications of the
 528 European Pharmacopeia 9.7³⁰

Test	Specifications ³⁰	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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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 [¹¹C]glyburide.

548

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

551

552 **Figure 3.** Analytical radio-HPLC for the quality control of [¹¹C]glyburide. A) HPLC with UV
553 detection of the glyburide reference compound; B) HPLC with UV detection of the
554 preparation of [¹¹C]glyburide; C) HPLC with gamma detection of the preparation of
555 [¹¹C]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.)





