GMP Compliant Synthesis of $[^{18}F]$Canagliflozin, a Novel PET Tracer for the Sodium–Glucose Cotransporter 2

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ABSTRACT: Inhibition of the sodium–glucose cotransporter 2 (SGLT2) by canagliflozin in type 2 diabetes mellitus results in large between-patient variability in clinical response. To better understand this variability, the positron emission tomography (PET) tracer $[^{18}F]$canagliflozin was developed via a Cu-mediated $^{18}$F-fluorination of its boronic ester precursor with a radiochemical yield of 2.0 ± 1.9% and a purity of >95%. The GMP automated synthesis originated $[^{18}F]$canagliflozin with a yield of 0.5−3% ($n$= 4) and a purity of >95%. Autoradiography showed $[^{18}F]$canagliflozin binding in human kidney sections containing SGLT2. Since $[^{18}F]$canagliflozin is the isotopologue of the extensively characterized drug canagliflozin and thus shares its toxicological and pharmacological characteristics, it enables its immediate use in patients.

INTRODUCTION

Patients with type 2 diabetes mellitus (T2DM) remain at an increased risk of cardiovascular diseases and chronic kidney diseases despite currently available treatment options. Sodium–glucose cotransporter 2 (SGLT2) inhibitors are clinically available to optimize glycemic control. The SGLT2 is located in the proximal tubules of the kidneys where it reabsorbs filtered glucose. Through inhibition of this transporter, glucose reabsorption is diminished, urinary glucose excretion increases, and consequently plasma glucose levels will decrease. In addition, SGLT2 inhibitors decrease the body weight, blood pressure, and urinary excretion of albumin, a surrogate marker for the progression of kidney disease. Large clinical trials have shown that SGLT2 inhibitors reduce the risks of heart failure and end-stage kidney disease.

Despite these clinical benefits at a population level, there is a large variation in drug response. Clinical studies showed that in 20% of patients albuminuria does not improve in response to SGLT2 inhibition. The underlying mechanisms of this individual variation in response to SGLT2 inhibition are unknown.

We hypothesize that differences in drug response can, among others, be explained by variations in the SGLT2 inhibitor tissue distribution and/or changes in the SGLT2 density due to the pathology of the disease. To quantitatively, and preferably noninvasively, investigate the tissue distribution of SGLT2 inhibitors and SGLT2 density in patients, there is a need for a radiolabeled SGLT2 positron emission tomography (PET) tracer. α-Methyl-4-$[^{18}F]$fluoro-4-deoxy-D-glucopyranoside (Me-4FDG) and $[^{11}C]$methyl-D-glucoside have been suggested as PET tracers for imaging SGLT. However, these tracers are not SGLT2-specific; both are also substrates for SGLT1, and Me-4FDG additionally possesses affinity toward GLUT2. The marketed drugs from the SGLT2 inhibitor class, gliflozins, have been proven to be more selective toward SGLT2. Recently, 4-$[^{18}F]$fluoro-dapagliflozin (F-Dapa) was synthesized and subsequently shown to exhibit subtype selectivity toward SGLT2. However, due to the addition of an $^{18}$F-fluoro group to dapagliflozin, F-dapa does not retain the original molecular structure of the marketed drug dapagliflozin. It may, as a result, exhibit different pharmacokinetic and pharmacodynamic properties.

Therefore, our aim was to synthesize a PET tracer with a molecular structure identical to one of the currently marketed SGLT2 inhibitors, as this would also retain the biochemical properties of the drug. An additional benefit is that the PET tracer can be used immediately in human studies without extensive toxicity testing. We preferred an $^{18}$F-labeled tracer because of its favorable physical and nuclear characteristics for...
PET imaging. Within the drug class of (marketed) SGLT2 inhibitors, only canagliflozin contains a fluorine atom that can be replaced by an $^{18}$F-radiolabel. In this study, we describe the synthesis of $[^{18}$F$]$canagliflozin and its precursor, as well as its in vitro affinity toward SGLT2.

## RESULTS AND DISCUSSION

### Chemistry

Canagliflozin precursor equipped with a pinacol aryl boronic ester moiety has been successfully prepared and its synthesis is depicted in Scheme 1. The route is inspired by the work of Meng et al. and Nomura et al. Whereas Nomura used 2-substituted thiophenes in the "Friedel–Crafts" acylation reaction, we started from the unsubstituted thiophene. In the final steps, canagliflozin precursor was prepared by a Pd-catalyzed thiophene−aryl coupling reaction via the C−H bond activation followed by a Miyaura borylation reaction by cross-coupling of bis(pinacolato)diboron (B$_2$pin$_2$) with the aryl halide.

Briefly, the synthesis started with the coupling of thiophene 1 with acid chloride 2 (prepared by the reaction of 5-bromo-2-methylbenzoic acid with oxalyl chloride) through a Friedel–Crafts reaction in an 86% yield. Initial attempts to reduce the ketone functionality in compound 3 with triethylsilane and BF$_3$·OEt$_2$ at 0 °C gave 4 albeit in a low yield (25%, data not shown). At this temperature, a dimeric byproduct was formed. This was circumvented by warming the reaction mixture at 35 °C after addition of the reducing agents (triethylsilane/BF$_3$·OEt$_2$), forming intermediate 4 with an improved yield of 77%. Delta-gluconolactone was protected with trimethylsilyl chloride to yield 5 in 92%. To prevent the organolithium intermediate to be quenched by the moisture present in compound 5, it is of utmost importance that compound 5 is dry. This was achieved by stripping the product a few times with toluene. Silylated gluconolactone 5 was then reacted with 4 to afford 6 as a mixture of α- and β-anomers in a 49% yield. The anomers were not separated at this stage.

When the hydrosilylation on 6 was performed on a small scale (200 mg) at 0 °C, a selectivity α/β of 1:5 was obtained. However, when the reaction was scaled up to 18 g, the selectivity dropped to 1:2. This drop in selectivity is most likely due to the much larger exothermic effect observed on this scale during the addition of boron trifluoride to the reaction mixture. Nevertheless, after acetylation of the crude product and purification by column chromatography, pure β-7 anomer was isolated in a 39% yield.

Pure β-7 anomer was then reacted with an excess of dibromobenzene (8) via a Pd-catalyzed thiophene−aryl coupling reaction via the C−H bond activation using (Cy$_3$P)$_2$Pd(0) as catalyst (20%) to afford intermediate 9 in a 31% yield. Finally, the canagliflozin precursor was synthesized by borylation of 9 using bispinacolatodiboron and Pd(dppf)Cl$_2$ (5%) as catalysts in an 86% yield after purification. Thus, this multistep procedure proved to be successful and gave the desired borylated-canagliflozin precursor in multigram amounts.
Radiochemistry. The production of $^{18}$F-aranes through the copper-mediated oxidative $^{18}$F-fluorination from pinacol-derived aryl boronic esters (arylBPin), upon treatment with $[^{18}$F]KF/K2.2.2 and Cu(OTf)$_2$ (py)$_4$, has shown to be a robust synthesis method. This method tolerates electron-poor and electron-rich aranes and various functional groups. Therefore, this strategy would be most suitable for the radiosynthesis of $[^{18}$F]canagliflozin from the borylated precursor.

Figure 1. UPLC chromatogram from the final product $[^{18}$F]canagliflozin (black: $\gamma$ detector, green: UV detector). The final product had the same retention time compared to the commercially available nonradioactive-labeled reference standard.

Radiochemistry. The production of $^{18}$F-aranes through the copper-mediated oxidative $^{18}$F-fluorination from pinacol-derived aryl boronic esters (arylBPin), upon treatment with $[^{18}$F]KF/K2.2.2 and Cu(OTf)$_2$ (py)$_4$, has shown to be a robust synthesis method. This method tolerates electron-poor and electron-rich aranes and various functional groups. Therefore, this strategy would be most suitable for the radiosynthesis of $[^{18}$F]canagliflozin from the borylated precursor.

Table 1. Results of Production Process and Quality Control

| $[^{18}$F]canagliflozin specification | validation 1 | validation 2 | validation 3 |
|-------------------------------------|-------------|-------------|-------------|
| appearance clear, colorless         | yes         | yes         | yes         |
| pH 5–8                              | 6.5         | 7.0         | 6.5         |
| filter integrity: pressure hold test | >1.8 bar    | >1.8        | >1.8        |
| bubble point test$^a$               | <10% reduction of pressure | <10     | <10        |
| identity retention time ≤ 0.4 min compared to standard | yes         | yes         | yes         |
| radiochemical purity ≥95%           | 100         | 100         | 100         |
| unknown impurities <1 mg/L          | <1.0        | <1.0        | <1.0        |
| -canagliflozin LOQ$^b$ = 0.4 mg/L    | <0.4        | <0.4        | <0.4        |
| -canagliflozin precursor <1 mg/L    | <0.1        | <0.1        | 0.6         |
| molar activity EOS: $[^{18}$F]canagliflozin ≥10 000 GBq/mmol | >23 000 | >47 000 | >52 000 |
| activity yield >200 MBq (standard dose) | 232       | 466         | 524         |
| sterility sterile                   | sterile     | sterile     | sterile     |
| osmomolarity <2250 mosmol/kg        | 1690        | 1820        | 1760        |
| DMA <410 mg/L                       | 17.7        | 14.8        | 10.1        |
| acetonitrile <1090 mg/L             | <50         | <50         | <50         |
| ethanol <1090 mg/L                  | 62.8        | 68.7        | 65.8        |
| propanol <5000 mg/L                 | <100        | <100        | <100        |
| copper <50 mg/L                     | <1          | <1          | <1          |
| TEAHC$^c$ <2.5 EU/mL                | <0.05       | <0.05       | <0.05       |
| endotoxins <260 mg/L                | <260        | <260        | <260        |

$^a$With a failing pressure hold test, the bubble point test needs to be done after radioactive decay. $^b$Limit of quantification. $^c$Tetraethylammonium hydrogen carbonate.
Typically, in copper-mediated oxidative $^{18}$F-fluorinations of aryl boronic esters, the most common Cu-catalyst used is Cu(OTf)$_2$(py)$_4$. Where most of the reactions were found to be optimal at 120 ± 10 °C, either in dimethylacetamide or in DMF, the former solvent gave a better conversion efficacy after 20−30 min of reaction time. This method was applied in the synthesis of [18F]canagliflatin with a final radiochemical yield of 2.0 ± 1.9% (n = 12) within 90 min, as depicted in Scheme 2.

An automated program was created to synthesize [18F]-canagliflatin in a Modular-Lab PharmTracer Eckert and Ziegler cassette-based synthesis module, which is suitable for the GMP compliant production of PET tracers (Supporting Information Figure S3). For automated synthesis, some small adjustments were made. n-Butanol being highly viscous was mostly lost in the plastic tubes of the automated synthesizer. This led to very low yields to no product. Thus, this was replaced with a less viscous solvent, 2-propanol, and [18F]canagliflatin could be produced with a final yield of 0.5−3.0% (n = 4) and a consistent quality complying with all predefined specifications, as described in Table 1 (see the Supporting Information for optimization of the reaction conditions).

Despite the relatively low yield, the automated synthesis was found to be robust and stable under different conditions (Supporting Information "Pilot experiments"), yielding at least 200 MBq of formulated pure [18F]canagliflatin with a molar activity higher than 10 GBq/μmol and a radiochemical purity of >95%, with a starting activity of 100 GBq (Figure 1), ready for patient injection.

In Vitro Binding Experiments with Autoradiography.

The advantage of our labeling strategy is that [18F]canagliflatin retains the chemical structure of canagliflatin and therefore also its high selectivity for the SGLT2 (in vitro IC$_{50}$ for hSGLT1, hSGLT2, and GLUT1, respectively, at 910, 2.2, and >1000 nM[11]). To confirm the binding of [18F]canagliflatin to SGLT2, human kidney samples taken from cortical areas, corresponding with the known anatomical localization of the SGLT2, were used and compared to staining of the SGLT2 with immunohistochemistry (IHC). As depicted in Figure 2, autoradiography showed tracer binding diffusely over the slices corresponding with the pattern of SGLT2 distribution by IHC. By combining autoradiography with IHC, we were also able to demonstrate that the areas with extreme [18F]canagliflatin binding (yellow areas in Figure 2B) were caused by the folded tissue on the slice, whereas areas that completely lack [18F]canagliflatin binding were the result of the missing tissue on the slice.

In addition, in the presence of an excess of canagliflatin, the binding of [18F]canagliflatin was significantly reduced (52.1 ± 8.1%, p < 0.0001, n = 6; Figure 3A) and there was a trend toward reduced binding of [18F]canagliflatin when the nonspecific biological substrate of the SGLT2 glucose (22.6 ± 25.1%) (p = 0.2598, n = 3; Figure 3B) was added (in vitro K$_{0.5}$ = 4.9 mM[23]).

Together, these experiments confirm that [18F]canagliflatin binds to the SGLT2. Without the requirement for toxicity testing, [18F]canagliflatin is directly suitable for clinical studies. The "Canagliflatin Renal Distribution Intervention Trial" (CREDIT) is planned to assess the feasibility of [18F]-canagliflatin and quantify, noninvasively, individual differences in target-site exposure in patients with T2DM (Netherlands Trial Register; NL7707).

CONCLUSIONS

We showed the successful automated synthesis of the SGLT2 inhibitor [18F]canagliflatin. This tracer showed specificity to the SGLT2 in autoradiography experiments with human kidney...
2-(5-Bromo-2-methylbenzyl)thiophene (4). Triethylsilane (115.80 g, 0.16 L, 4 equiv, 995.84 mmol) was added at 0 °C to a solution of 3 (70.00 g, 1 equiv, 248.96 mmol) in a 1/1 mixture of DCM/acetonitrile (ACN) (700 mL). Boron trifluoride diethyl etherate (BF3·OEt2, 106.00 g, 94.6 mL, 3 equiv, 746.88 mmol) was added over 10–15 min, and the reaction mixture was stirred at 35 °C. At this temperature, the reaction was quite fast and turned from dark to clear orange (heterogeneous). After 45 min, the starting material was fully converted (according to HPLC analysis) and the reaction mixture was cooled down to 0 °C. The reaction was carefully quenched with 2 M potassium hydroxide solution (gas evolution and exothermic). The aqueous layer was extracted with MTBE (3×), the organic layers were washed successively with potassium hydroxide (1×) and brine (2×), dried with sodium sulfate, filtered, and concentrated to afford a crude orange oil. The latter was diluted in a minimum of heptane and purified by automated silica gel chromatography eluted with heptane. The first fractions (bottles 1–10) were concentrated to dryness to afford 4 (51.2 g, 77.0%) as a colorless oil.

1H NMR (300 MHz, chloroform-d) δ 6.30 (q, J = 2.1 Hz, 1H), 7.28–7.24 (m, 1H), 7.15 (dd, J = 5.1, 1.3 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.96–6.87 (m, 1H), 6.72 (dq, J = 3.6, 1.2 Hz, 1H), 4.09 (s, 2H), 2.25 (s, 3H).

13C NMR (75 MHz, chloroform-d) δ 142.47, 140.71, 135.28, 132.17, 132.00, 129.79, 126.90, 125.39, 120.03, 119.64, 33.54, 19.03. Mass (HPLC-MS): 268.9 (M + 1).

26 Trimethylchlorosilane (82.3 g, 97 mL, 6 equiv, 758 mmol) was slowly added (over 40 min) via an addition funnel to a cold (−10 °C, salty iced water) mixture of (3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-one (22.5 g, 1 equiv, 126 mmol) and N-methylmorpholine (102 g, 0.11 L, 8 equiv, 1.01 mol) in tetrahydrofuran (200 mL). The heterogeneous reaction mixture was stirred overnight from −10 to 25 °C. Tolueno (300 mL) was added, and the white heterogeneous mixture was cooled down to 0 °C and brine was slowly added. The organic layer was washed successively with aqueous sodium phosphate (monobasic) and brine, then dried over sodium sulfate, filtered, and concentrated to afford a crude oil, which was spiked with toluene (3×) and heptane (1×). Intermediate 5 (61.0 g, 103%) was isolated as a yellow oil.

1H NMR (300 MHz, chloroform-d) δ 6.41 (dt, J = 7.7, 2.5 Hz, 1H), 3.99 (d, J = 9 Hz, 1H), 3.90 (t, J = 9 Hz, 1H), 3.84–3.72 (m, 3H), 0.28–0.07 (m, 36H).

13C NMR (75 MHz, chloroform-d) δ 170.94, 81.25, 75.81, 73.02, 70.73, 61.30, 0.73, 0.52, 0.23, −0.46. Mass (HPLC-MS): 282.9 (M + 1).

Figure 3. Representative autoradiography images of [18F]canagliozin binding in human kidney slices incubated with [18F]canagliozin alone (A), together with canagliozin or with glucose (B). Bar graph showing the binding of [18F]canagliozin in the three different groups presented as a percentage relative to incubation with [18F]canagliozin alone (B).
compound 6 as a mixture of diastereoisomers. The sugar part and methoxy group are clearly visible in the NMR spectrum, and the mixture was used as such in the next step.

\(2R,3R,4S,5R\)-2-(Acetoxymethyl)-6-(4-methyl-3-(thiophen-2-yl)methyl)-4-methylphenyl)tetrahydro-2H-pyran-3,4,5-triyl Triacetate (7). BF3·OEt2 (20 g, 18 mL, 3 equiv, 0.14 mol) was slowly added (during 20 min) at 0 °C to a solution of 6 (18 g, 1 equiv, 47 mmol) and triethylsilane (17 g, 23 mL, 3 equiv, 0.14 mol) in a 1/1 mixture of DCM/ACN (180 mL) resulting in a brown homogeneous mixture. The latter was stirred at 0 °C for 1 h and then for 2 h at room temperature. The reaction mixture was cooled to 0 °C and quenched with saturated aqueous sodium bicarbonate. The aqueous layer was extracted with AcOEt (2×), organic layers were washed with brine (2×), dried over sodium sulfate, filtered, and concentrated to afford a light brown foam (147.5 g, 42.09 mmol, 89% yield, many impurities present). The intermediate was then dissolved in DCM (150 mL), and 4-dimethylaminopyridine (514.2 mg, 0.1 equiv, 4.21 mmol), pyridine (3.32 g, 34 mL, 10 equiv, 420.9 mmol), and acetic anhydride (49.72 g, 39.8 mL, 10 equiv, 420.9 mmol) were successively added. The reaction mixture was stirred overnight at room temperature. The reaction mixture was partitioned between DCM and water. The aqueous layer was extracted with DCM (2×), and the organic layers were washed with 1 M hydrochloric acid (1×) and water (1×). The combined organic layers were dried over sodium sulfate, filtered, and concentrated to a brown oil. The residue was dissolved in a minimum amount of DCM and was purified by silica gel chromatography, eluting with a gradient from 10 to 25% AcOEt in heptane. The first fraction that contained α-anomer was discarded. The second fraction (4.5 g) that contained a mixture of α- and β-anomers was isolated to be repurified. The third fraction (6.5 g) that contained pure β-isomer was isolated. The second fraction was repurified by automated silica gel chromatography to gel afford another crop of pure β-anomer. Intermediate 7 was isolated as a white solid (8.5 g, 39% yield).

\[1^1C\] NMR (300 MHz, chloroform-d) δ 7.73–7.08 (m, 4H), 6.89 (ddt, J = 5.1, 3.3, 1.5 Hz, 1H), 6.66 (dt, J = 3.2, 1.5 Hz, 1H), 5.37–5.15 (m, 2H), 5.19–5.05 (m, 1H), 4.39–4.21 (m, 2H), 2.41–2.02 (m, 3H), 3.82 (ddd, J = 10.0, 4.8, 2.4 Hz, 1H), 2.30–2.23 (m, 3H), 2.11–1.96 (m, 9H), 1.79–1.71 (m, 3H).

\[1^3C\] NMR (75 MHz, chloroform-d) δ 170.72, 169.49, 138.38, 137.09, 134.13, 130.70, 128.51, 125.75, 124.95, 80.03, 76.06, 74.33, 72.64, 68.65, 60.40, 33.68, 20.76, 20.62, 20.38, 19.23.

\[2^1C\] NMR (300 MHz, chloroform-d) δ 87.48–7.37 (m, 4H), 7.17 (tq, J = 5.4, 3.9, 2.7 Hz, 3H), 7.09 (dd, J = 3.6 Hz, 1H), 6.62 (dd, J = 3.6 Hz, 1H), 5.21 (ddd, J = 37.2, 18.2, 9.3 Hz, 3H), 4.36 (ddd, J = 9.8 Hz, 1H), 4.28 (dd, J = 12.3, 4.7 Hz, 1H), 4.15 (dd, J = 12.3, 2.3 Hz, 1H), 4.09 (s, 2H), 3.82 (ddd, J = 9.8, 4.8, 2.3 Hz, 1H), 2.29 (s, 3H), 2.02 (2H s, 6H), 1.99 (s, 3H); 1.75 (s, 3H).
and glucose were performed on slices incubated with $^{[18F]}$canagliflozin. Validation of the production method was performed before the validation of the production method was started.

**Validation Production Method.** To assure that the production method is robust and results in a product with consistent quality, validation of the production method was performed. Validation of $^{[18F]}$canagliflozin consisted of three independent productions, including complete quality control. All batch productions had to comply with the predefined acceptance criteria summarized in Table 1.

**In Vitro Competition Experiments with Autoradiography.**

The use of human kidney tissues and the procedures for the in vitro experiments were performed according to the Dutch national ethical guidelines. The Medical Ethics Review Board of the University Medical Center Groningen judged that our study was exempt from Medical Research Involving Human Subjects Act (WMO). Visually healthy cortical parts of kidneys, removed because of renal cell carcinoma, were frozen and cut into 4 $\mu$m slices using a Leica CM 3050 cryostat (Leica Microsystems, Nussloch, Germany).

To assess specific binding, blocking experiments with canagliflozin and glucose were performed on slices incubated with $^{[18F]}$canagliflozin. To correlate $^{[18F]}$canagliflozin binding with SGLT2 distribution, IHC was performed on slices adjacent to the autoradiography slices incubated with the tracer.

**Autoradiography.** Kidney slices were incubated in $^{[18F]}$-canagliflozin (5 MBq in 50 mL) containing either alone phosphate-buffered saline (PBS) or PBS and canagliflozin (0.02 mM) or glucose (0.025 mM) for 60 min and then washed 3 times with cold PBS and 1 time with cold water. After drying by exposure to air for 20 min, the sections were exposed to a phosphor storage screen for 30 min. The exposed screens were then scanned, and images were analyzed.

**Immunohistochemistry.** The kidney slices were dried for 20 min using a cold blower and fixed with cold acetone for 10 min. After rinsing in PBS, the endogenous peroxidase was blocked with 0.015% H2O2 in PBS, the endogenous peroxidase was blocked with 0.015% H2O2 in

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UPLC, ultraperformance liquid chromatography

Abbreviations

ACN, acetonitrile; AcOEt, ethyl acetate; GMP, Good Manufacturing Practice; SGLT, sodium-glucose cotransporter; GLUT, glucose transporter; MTBE, methyl tert-butyl ether; UPLC, ultraperformance liquid chromatography

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