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A novel synthesis of 6''-[¹⁸F]-fluoromaltotriose as a PET tracer for imaging bacterial infection

Mohammad Namavari, Gayatri Gowrishankar, Ananth Srinivasan, Sanjiv S. Gambhir, Thomas Haywood, Corinne Beinat

Molecular Imaging Program at Stanford (MIPS), Department of Radiology and Bioengineering Bio-X Program, Stanford University, Stanford, California, USA

Abstract

The aim of this study was to develop a positron emission tomography (PET) tracer to visualize and monitor therapeutic response to bacterial infections. In our continued efforts to find maltose based PET tracers that can image bacterial infections, we have designed and prepared 6''-[¹⁸F]fluoromaltotriose as a second generation PET imaging tracer targeting the maltodextrin transporter of bacteria. We have developed methods to synthesize 6''-deoxy-6''-[¹⁸F]fluoro- α -D-glucopyranosyl-(1-4)-O- α -D-glucopyranosyl-(1-4)-O-D-glucopyranose (6''-[¹⁸F]-fluoromaltotriose) as a bacterial infection PET imaging agent. 6''-[¹⁸F] fluoromaltotriose was prepared from precursor, 2'',3'',4''-tri-O-acetyl-6''-O-nosyl- α -D-glucopyranosyl-(1-4)-O-2',3',6'-tri-O-acetyl- α -D-glucopyranosyl-(1-4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (per-O-acetyl-6''-O-nosyl-maltotriose **4**). This method utilizes the reaction between precursor **4** and anhydrous [¹⁸F] KF/Kryptofix 2.2.2 in dimethylformamide (DMF) at 85°C for 10 minutes to yield per-O-acetyl-6''-deoxy-6'' [¹⁸F]-fluoromaltotriose (**7**). Successive acidic and basic hydrolysis of the acetyl protecting groups in **7** produced 6''-[¹⁸F] fluoromaltotriose (**8**). Also, cold 6''-[¹⁹F]fluoromaltotriose was prepared from per-O-acetyl-6''-hydroxymaltotriose via a diethylaminosulfur trifluoride reaction followed by a basic hydrolysis. A successful synthesis of 6''-[¹⁸F]-fluoromaltotriose has been accomplished in 8 $\pm$ 1.2% radiochemical yield (decay corrected). Total synthesis time was 120 minutes. Serum stability of 6''-[¹⁸F]fluoromaltotriose at 37°C indicated that 6''-[¹⁸F]-fluoromaltotriose remained intact up to 2 hours. In conclusion, we have successfully synthesized 6''-[¹⁸F]-fluoromaltotriose via direct fluorination of an appropriate precursor of a protected maltotriose.

1 | INTRODUCTION

Due to the inability to accurately diagnose bacterial infections and the development of antibiotic resistance, bacterial infections cause significant mortality and morbidity in the world.¹ Currently, in the clinic, a bacterial infection is diagnosed by physical examination and microbial cultures from sites of infection. These methods, although accurate, require time leading to a delay in effective treatment strategies.² A major limitation preventing the

Correspondence: Sanjiv Sam Gambhir MD, PhD, Molecular Imaging Program at Stanford Departments of Radiology and Bioengineering, Bio-X Program, 318 Campus Dr., Clark Center, E-150, Stanford University, Stanford, CA 94305, USA. sgambhir@stanford.edu.

effective treatment of bacterial infection is the inability to image the location and spread of bacterial infection. Common imaging modalities such as computed tomography and magnetic resonance imaging offer excellent structural resolution, but they are unable to distinguish bacterial infection from non-bacterial inflammation.³ Modern imaging modalities such as single-photon emission computer tomography or positron emission tomography (PET) with suitable radiotracers allow bacterial infection to be detected at early stages. Diaz et al⁴ reported that [¹²⁴I]-1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodouracil ([¹²⁴I]FIAU) is a very promising bacterial infection imaging tracer. ([¹²⁴I]FIAU) is a substrate for bacterial thymidine kinase. ([¹²⁴I]FIAU) is also a substrate for mammalian mitochondrial thymidine kinase and results in non-specific uptake in certain tissues. Using PET with radiotracer 2-deoxy-2-[¹⁸F]fluoro-D-glucose (¹⁸FDG) for imaging bacterial infection via glucose metabolism of activated white blood cell mechanism has been reported by Sasser et al⁵ and Peruzzi et al.⁶ However ¹⁸FDG imaging cannot distinguish infection from inflammation. Recently, other PET contrast agents for imaging bacterial infection have been reported. Weinstein et al⁷ reported the use of 2-¹⁸F-fluorodeoxysorbitol (¹⁸FDS) as a PET probe to image certain class of bacteria (enterobacteriaceae, Gram negative). It has been known that enterobacteriaceae express the enzyme sorbitol-6-phosphate dehydrogenase which is responsible for sorbitol metabolism. We showed the use of 6-[¹⁸F]fluoromaltose,^{8,9} and Ning et al¹⁰ reported the application of ¹⁸F- labeled fluoromaltohexose as PET probes targeting the bacterial maltodextrin transporter, to image all bacterial infections caused by both Gram positive and Gram negative bacteria. Both of these tracers demonstrated great specificity for distinguishing bacterial infection from inflammation but had poor signal to noise ratios due to possible metabolism in the hepatobiliary system. It has been known that maltose and maltodextrins are energy sources for bacteria, and they are taken up by bacteria via the maltodextrin transporter system which is not present in mammalian cells. Shelburne et al¹¹ studied the transport of glucose, maltose, and higher maltodextrin in *Escherichia coli*. *Streptococcus* strain, which is one of the bacteria responsible for many infections in human, possesses an efficient transport system for linear maltodextrins and had the highest affinity for maltotriose. In addition, based on V_{max} values, they also concluded that the uptake of maltotriose is also faster than that of maltose and glucose. Hence, we postulated that introduction of an F-18 label in maltotriose would lead to a second generation imaging agent perhaps with better pharmacokinetic properties than the previously published maltose tracer.

In our continuing effort to design PET tracers for imaging bacterial infection, we report the synthesis of 6''-deoxy-6''-[¹⁸F]fluoro-α-D-glucopyranosyl-(1-4)-α-D-glucopyranosyl-(1-4)-D-glucopyranose (6''-[¹⁸F]fluoromaltotriose) as a potential bacterial infection PET imaging agent.

2 | MATERIALS AND METHODS

2.1 | General

Chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI) and Fisher Scientific (Hanover Park, IL). 6''-[¹⁸F]Fluoromaltotriose was prepared from precursor 2',3'',4''-tri-O-acetyl-6''-deoxy-6''-O-nosyl-α-D-glucopyranosyl-(1-4)-O-(2',3',6'-tri-O-

acetyl- α -D-glucopyranosyl-(1-4)-1,2,3,6-tetra-O-acetyl-D-glucopyranose (per-O-acetyl-6''-O-nosyl-maltotriose **4**) (Scheme 1).

6''-[^{18}F]fluoromaltotriose purification was accomplished on a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA) equipped with a Dionex P680 quaternary gradient pump and Knauer K-2001 UV detector (Berlin Germany) set at 254 nm and radioactivity detector (Carroll & Ramsey Associates, model 105S, Berkeley, CA). Semi preparative HPLC reverse phase column (Phenomenex, Gemini, Hesperia, CA, C18, 5 μ , 10 mm $\times$ 250 mm) with the mobile phase water/acetonitrile (99/1) and flow rate of 3 mL/min under isocratic condition was used for purification of 6''-[^{18}F] fluoromaltotriose. Radioactivity measurements were performed by A CRC-15R PET dose calibrator (Capintec Inc., Ramsey, NJ). Radio TLC chromatographs were done on a Bioscan AR-2000 model. Analytical HPLC was carried out using Dionex Ultimate 3000 system with diode array detector equipped with Ultimate 3000 auto sampler with a radioactivity detector (Carroll & Ramsey mentioned previously) and a refractive index detector (Refracto MAX 521, Thermo Fisher). Analytical HPLC reverse phase column (Phenomenex, Gemini, Hesperia, CA, C18, 5 μ , 4.6 mm $\times$ 250 mm) with the mobile phase $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (99/1) and flow rate of 1 mL/min under isocratic condition was used for analysis of 6''-[^{18}F] fluoromaltotriose.

^1H , ^{13}C , and ^{19}F NMR spectra were done on Mercury 400 MHz spectrometer. Electron spray ionization (ESI) mass spectrometry was performed by Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University.

No carrier-added [^{18}F]fluoride was prepared by the $^{18}\text{O}(\text{p}, \text{n})^{18}\text{F}$ nuclear reaction on a GE PET tracer cyclotron. [^{18}F]Fluoride processing and synthesis of 2'',3'',4''-tri-O-acetyl-6'-deoxy-6''-[^{18}F]fluoro- α -D-glucopyranosyl-(1-4)-O-(2',3',6'-tri-O-acetyl- α -D-glucopyranosyl-(1-4)-1,2,3,6-tetra-O-acetyl-D-glucopyranoside (per-O-acetyl-6''-[^{18}F]fluoromaltotriose **7** Scheme 3) were completed in the GE TRACER lab FX-FN synthesis module (GE Medical System, Milwaukee, WI).

2.2 | 2'',3'',4''-Tri-O-acetyl-6''-O-Trityl- α -D-glucopyranosyl-(1-4)-O-2',3',6'-tri-O-acetyl- α -D-glucopyranosyl-(1-4)-1,2,3,6-tetra-O-acetyl-D glucopyranose (per-O-acetyl-6''-O-Trityl-maltotriose **2**)

A solution of maltotriose (441 mg, 0.87 mmol) and trityl chloride (366 mg, 1.27 mmol) in dry pyridine (8 mL) (Scheme 1) was stirred at 45°C for 24 hours. After cooling to room temperature, acetic anhydride (8 mL) was added, and the reaction mixture was stirred for another 24 hours at room temperature. Finally, the reaction mixture was evaporated under vacuum, and the residue was co-evaporated with toluene. The crude product was purified by column chromatography (silica gel) using 50/50 ethyl acetate and hexane as the eluent to afford 205 mg (21%) of **2** as a foam. ^1H NMR (400 MHz, CDCl_3) δ ppm: 6.22 (d, J = 3.66 Hz, α -H-1) 5.72 (d, J = 8.08 Hz, β -H-1) 3.30 (d, J = 10.4 Hz, H-6a), 2.94 (dd, J = 10.4 and J = 2.7 Hz, H-6b). ^{13}C NMR (400 MHz, CDCl_3) δ ppm: 168.7–171.1 (10(C=O)), 126–143 (phenyl ring), 95.9, 95.8, 95.7, 95.4 ($\text{C}_{1''}$, $\text{C}_{1'}$), 91.1 (C_{1-} β), 88.7 (C_{1-} α), 68.8–76.4 (C_2 - C_5 , C_2' - C_5' , C_2'' - C_5''), 60.3–62.4 (C_6 , C_6' , C_6''), 86.3 ($\text{Ph}_3\text{-C}$), 20.4–21.0 (10 CH_3). MS: Calcd for [$\text{C}_{57}\text{H}_{66}\text{O}_{26}$]: 1167.12; ESIMS found: [$\text{M} + \text{Na}$] $^+$ 1190.6.

2.3 | 2'',3'',4''-tri-O-acetyl- α -D-glucopyranosyl-(1-4)-O-(2',3',6'-tri-O-acetyl- α -D-glucopyranosyl-(1-4))-1,2,3,6 tetra-O-acetyl-D-glucopyranose (3)

Compound **2** (141 mg, 0.121 mmol) in 2 mL of aqueous acetic acid (80% in water) (Scheme 2) was stirred for 3.5 hours at 50°C.¹² The mixture was concentrated under vacuum, and the crude protected triose **3** was purified by column chromatography (silica gel) using 70/30 ethyl acetate and hexane as the eluent to afford 56 mg (50%) of **3** as a foam. ¹H NMR (400 MHz, CDCl₃) δ ppm: 6.22 (d, J = 3.5 Hz, α -H-1) 5.73 (d, J = 8.1 Hz, β -H-1). ¹³C NMR (400 MHz, CDCl₃) δ ppm: 168.8–170.6 (10(C=O)), 95.9, 95.8, 95.6, 95.3 (C₁'', C₁'), 91.2 (C₁- β), 88.8(C₁- α), 68.53–75.1(C₂-C₅, C₂'-C₅', C₂''-C₅''), 60.8–62.6 (C₆, C₆', C₆''), 20.3–21.1 (10 CH₃). MS: Calcd for [C₃₈H₅₂O₂₆]: 924.80; ESIMS found: [M + Na]⁺ 946.70.

2.4 | 2'',3'',4''-tri-O-acetyl-6''-O-nosyl- α -D-glucopyranosyl-(1-4)-O-(2',3',6'-tri-O-acetyl- α -D-glucopyranosyl-(1-4))-1,2,3,6 tetra-O-acetyl-D-glucopyranose (per-O-acetyl-6''-O-nosyl-maltotriose 4)

4-Nitrophenylsulfonyl chloride (78.4 mg, 0.354 mmol) was added to a solution of **3** (56 mg, 0.061 mmol) (Scheme 1) in CH₂Cl₂ (1.0 mL) containing triethylamine (70 μ L). ¹³ The mixture was stirred for 1.5 hours at 0°C to 5°C and concentrated under vacuum. The crude nosylate **4** was purified by column chromatography (silica gel) using 70/30 ethyl acetate and hexane as the eluent to afford 38 mg (56.1%) of **4** as colorless viscous oil with α / β = 1.1/1. ¹H NMR (400 MHz, CDCl₃) δ ppm: 8.41 (d, J = 9.0 Hz, 2H, H-nitrophenyl), 8.14 (d, J = 8.8 Hz, 2H, H-nitrophenyl), 6.24 (d, J = 3.8 Hz, 1H, α -H-1) 5.74 (d, J = 8.1 Hz, 1H, β -H-1). ¹³C NMR (400 MHz, CDCl₃) δ ppm: 168.8–170.6 (10(C=O)), 124.3–150.9 (nitrophenyl ring), 95.9, 95.8, 95.6, 95.4 (C₁'', C₁'), 94.4(C₆''), 91.2 (C₁- β), 88.8 (C₁- α), 67.6–75.1 (C₂-C₅, C₂'-C₅', C₂''-C₅''), 60.3–62.4 (C₆, C₆'), 20.4–21.0 (10 CH₃). HRMS: Calcd for [C₄₄H₅₅O₃₀NNaS][M + Na]: 1132.2422; ESIMS found: [M + Na]⁺ 1132.2423.

2.5 | 2'',3'',4''-tri-O-acetyl-6''-deoxy-6''-fluoro- α -D-glucopyranosyl-(1-4)-O-(2',3',6'-tri-O-acetyl- α -D-glucopyranosyl-(1-4))-1,2,3,6 tetra-O-acetyl-D-glucopyranose (per-O-acetyl-6''-deoxy-6''-fluoromaltotriose 5)

Compound **3** (48 mg, 0.052 mmol) in dry CH₂Cl₂ (1.3 mL) was added to a solution of diethylaminosulfur trifluoride (42 μ L, 0.3 mmole, 6 equivalent) (Scheme 2) in dry pyridine (42 μ L). The mixture was stirred at room temperature overnight and diluted with CH₂Cl₂ (30 mL). The solution was subsequently washed with 5% NaHCO₃ (6 mL) and H₂O (6 mL). After drying the organic fraction with sodium sulfate, the solvent was evaporated under vacuum. Crude product was purified by column chromatography (silica gel) using 4:1 chloroform and ethyl acetate as the eluent to afford 15.3 mg (31%) of **5** as foam.

¹⁹F NMR (CDCl₃) δ ppm: -232.9 (td, J_{F6''} = 47.3 Hz, J_{F5''} = 23 Hz, α -anomer), -233.0 (td, β -anomer). ¹³C NMR (400 MHz, CDCl₃) δ ppm: 168.8–170.6 (10(C=O)), 95.3–95.9 (C₁'', C₁'), 91.2 (C₁, β -anomer), 88.8 (C₁, α -anomer), 81.06 (d, J_{C6''F} = 176.2 Hz, C₆''), 71.75, (d, J_{C5''F} = 12.2 Hz, C₅'', β -anomer.), 70.42 (d, J_{C5''F} = 6.9 Hz, C₅'', α -anomer) 67.6–75.1 (C₂-C₅, C₂'-C₅', C₂''-C₄'), 60.3–62.4 (C₆, C₆'), 20.4–21.0 (10 CH₃).

2.6 | 6''-Deoxy-6''-fluoro- α -D-glucopyranosyl-(1-4)-O- α -D-glucopyranosyl-(1-4)-O-D-glucopyranose (6''-fluoromaltotriose 6)

Compound **5** (15 mg, 0.016 mmol) in 1-mL methanol and 0.6 mL of 0.5 M of NaOCH₃ in methanol (Scheme 2) was stirred for 1.5 hours at room temperature. The mixture was concentrated under vacuum, and the crude triose **6** was purified by column chromatography (silica gel) using 50/20/10 ethyl acetate /methanol/ water as the eluent to afford 3.5 mg (43%) of **6** as a foam.

¹⁹F NMR (D₂O) δ ppm: -235.90 (td, $J_{F6''} = 47.3$ Hz, $J_{F5''} = 27.5$ Hz, α -anomer), -235.60 (td, β -anomer). MS: Calcd for [C₁₈H₃₁O₁₅F]: 506.44; ESIMS found: [M + Na]⁺ 529.44.

2.7 | 6''-Deoxy-6''-[¹⁸F]-fluoro- α -D-glucopyranosyl-(1-4)-O- α -D-glucopyranosyl-(1-4)-O-D-glucopyranose (6''-[¹⁸F]fluoromaltotriose 8)

No carrier-added [¹⁸F]fluoride trapped on a QMA cartridge was eluted with a solution of K₂CO₃ (3.5 mg) and kryptofix 2.2.2 (15 mg) in water (0.1 mL) and acetonitrile (0.9 mL). The solvent was removed under vacuum at 65°C and to the anhydrous residue was added a solution of nosylate precursor (5–6 mg) (Scheme 3) in DMF (1 mL). The mixture was heated for 10 minutes at 85°C. After cooling to room temperature, 10 mL of water was added, and the solution passed through a light C-18 Sep-pack cartridge (Water) and the crude protected 6''-[¹⁸F] fluoromaltotriose (**7**) removed by passing 3 mL of acetonitrile through the cartridge. The crude **7** was concentrated and de-protected first by 1 N HCl (1 mL) at 110°C for 10 minutes then by 2 N NaOH (0.5 mL) at room temperature for 5 minutes to afford crude 6''-[¹⁸F] fluoromaltotriose **8**. The neutral solution of **8** was injected into a C-18 reverse phase semipreparative HPLC column (Phenomenex Gemini, C-18, 5 μ , 10 mm $\times$ 250 mm), flow rate of 3 mL/min and 2-mL loop. The mobile phase was 1% acetonitrile in water and HPLC performed under isocratic condition. The product 6''-[¹⁸F]fluoromaltotriose (**8**) was collected at 9 minutes into a collection flask. After evaporation of solvent, it was dissolved in saline and transferred into a sterile receiving vial through sterile Millipore GP-filter (0.2 μ m). The chemical and radiochemical purities of **8** were determined by reverse phase analytical HPLC method (Phenomenex Gemini C- 18, 5 μ , 4.6 $\times$ 250 mm) and was more than 95% pure. Also, radio TLC, of [¹⁸F]**8** (silica gel plate, CH₃CN/H₂O; 70/30, Figure 1) gave an R_f value of 0.48 which is identical to the R_f value of cold 6''-fluoromaltotriose under the same TLC condition. The radio synthesis time was 120 minutes with the radiochemical yield of $8 \pm 1.2\%$ (decay corrected, $N = 6$). The average specific activity was 0.701 ± 0.036 Ci/ μ mole ($N = 6$).

2.8 | Stability of 6''-[¹⁸F]fluoromaltotriose in serum

Serum (human or mouse) was centrifuged at 4°C, maximum speed (13 000 rpm) for 10 minutes; 330 μ L of supernatant was transferred to an Eppendorf vial containing 20 μ L of formulated radiolabelled 6''-[¹⁸F] fluoromaltotriose (50–100 μ Ci minimum). The same volume of radiolabelled compound in 330 μ L PBS was used as control. After vortexing the radiolabelled mixtures, 70- μ L aliquots were transferred to Eppendorf tubes and incubated at 37°C. At different time points (eg, 0, 5, 15, 30, 60 minutes), 140 μ L of ice-cold CH₃CN was added to corresponding samples to stop metabolism. After vortexing and centrifuging

samples (10-minutes max speed), the supernatants were transferred to vials prior to injection on an HPLC column.

2.8.1 | Ex vivo determination of radiometabolites: Plasma and liver—At 60-minutes post iv injection of 1000–1200 μCi , [^{18}F] fluoromaltotriose blood, brain, and muscle from female Nu/Nu mice ($n = 4$) were obtained. Blood samples were collected via cardiac puncture and were immediately centrifuged at 1800 g for 4 minutes at room temperature to separate plasma. Each plasma sample was added to cold acetonitrile (300 μL), mixed thoroughly, and centrifuged at 9400 g for 4 minutes. The resulting supernatants were collected and analyzed via the same HPLC method used for quality control of [^{18}F]fluoromaltotriose. Liver samples were homogenized in 400- μL cold acetonitrile, followed by centrifugation at 9400 g for 4 minutes. The resulting supernatants were analyzed by HPLC as described earlier. The percent ratio of intact [^{18}F] fluoromaltotriose on the HPLC chromatogram was determined as peak area for [^{18}F] fluoromaltotriose /total peak area. The radioactivity in samples (100 μL) from each supernatant were counted to determine the extraction efficiency. The activity in the supernatants were compared with that of the corresponding pellets to determine the amount of tracer bound to serum proteins.

3 | RESULTS AND DISCUSSION

Scheme 1 shows the synthesis of per-O-acetyl-6''-O-trityl-maltotriose **2**. Compound **2** was characterized by mass spectrometry (ESI-MS) and NMR. The NMR pattern is similar to the NMR pattern of per-O-acetyl-6''-O-Tr-1,6- anhydromaltotriose.¹⁴ Also, Scheme 1 shows the synthesis of 6''-[^{18}F]-fluoromaltotriose precursor **4**. De-protection of **2** with aqueous acetic acid at 50°C produced compound **3** which was nosylated [13] at 0°C to 5°C to afford per-O-acetyl-6''-O-nosyl-maltotriose **4**, 6''-[^{18}F]- fluoromaltotriose precursor). Compound **4** was confirmed by high-resolution mass spectrometry and NMR. 6''-Fluoromaltotriose (**6**) was synthesized as shown in Scheme 2. Compound **3** was reacted with diethylaminosulfur trifluoride reagent to produce per-O-acetyl-fluoromaltotriose derivative **5**. ^{19}F -NMR of **5** shows a triplet of a doublet (td, $J_{\text{F6''}} = 47.3 \text{ Hz}$, $J_{\text{F5''}} = 23 \text{ Hz}$), at -232.9 ppm belongs to $\text{F6''}-\alpha$ anomer. Also, a triplet of a doublet at -233.0 ppm belongs to $\text{F6''}-\beta$ anomer. This NMR pattern is similar to the NMR pattern of per-O-acetyl-6'-fluoromaltose -234.8 ppm for $\text{F6'}-\alpha$ anomer and -234.9 ppm for $\text{F6'}-\beta$ anomer.¹⁵ Derivative **5** was de-protected with CH_3ONa to afford 6''-fluoromaltotriose **6**. Compound **6** was characterized by mass spectrometry (ESI-MS) and NMR. MS showed mass peak of 529.44 ($\text{M} + \text{Na}$). ^{19}F -NMR of **6** shows a triplet of a doublet (td, $J_{\text{F6''}} = 47.3 \text{ Hz}$, $J_{\text{F5''}} = 27.5 \text{ Hz}$), at -235.9 belongs to $\text{F6''}-\alpha$ anomer. Also, a triplet of a doublet at -235.6 ppm belongs to $\text{F6''}-\beta$ anomer. These NMR patterns are similar to the NMR patterns of 6'-fluoromaltose (-235.8 ppm for $\text{F6'}-\alpha$ anomer and -235.8 ppm for $\text{F6'}-\beta$ anomer.¹⁵

[^{18}F]-labeled maltotriose derivative **7** (Scheme 3) was prepared by nucleophilic reaction of the nosylate group in **4** with anhydrous [^{18}F]KF/Kryptofix 2.2.2 in DMF at 85°C for 10 minutes. Initial purification of [^{18}F]**7** was performed via a light C-18 Sep-pack cartridge. After passing a solution of [^{18}F]**7** in acetonitrile through a light neutral alumina Sep-pack, it was concentrated and hydrolyzed first by acid (1 N HCl) at 110°C for 10 minutes and then

by base (2 N NaOH) at room temperature for 5 minutes to yield 6''-[¹⁸F]fluoromaltotriose, **8**.

To check whether acidic and basic conditions used in de-protection of the intermediate 6''-[¹⁸F]-fluoromaltotriose (**7**, Scheme 3) have any effect on α and β anomers, we applied the same conditions to de-protection of **5**. It appeared that using 1 N HCl at 110°C and the subsequent treatment with 2 N NaOH during the de-protection of the intermediate 6''-[¹⁸F]-fluoromaltotriose (**7**) did not destroy α and β anomers. It might interconvert α and β anomers, and perhaps no aldose-ketose interconversion condensation took place. ¹⁹F-NMR suggested only α and β anomers formed. Also, N. Thiebault et al.¹⁶ have prepared several maltotriose derivatives. In order to prepare various derivatives by regioselective displacement reactions of protected maltotriose derivatives, the compounds were subjected to reaction conditions (acidic and basic) similar to the one we employed in our studies. They did not report any isomerizations under these conditions.

The radiochemical yields were obtained in DMF, DMSO, THF, or acetonitrile as a solvent at temperature range of 65°C to 120°C (Table 1). We found that with 6''-nosyl-maltotriose precursor and DMF as a solvent at 85°C for 10 minutes, one can obtain 6''-[¹⁸F]fluoromaltotriose in radiochemical yield of $8 \pm 1.2\%$ (decay corrected, $N = 6$). Our refractive index detector (Refracto MAX 521, Thermo Fisher) has low sensitivity and cannot measure concentration of fluoromaltotriose if it is less than 1 μg in 100 μL of carrier solvent. To determine specific activity, we measured the activity of 100 μL of final product 6''-[¹⁸F]-fluoromaltotriose, and it was injected onto the analytical HPLC column (Phenomenex Luna-NH2, C-18, 5 μ , 4.6 $\times$ 250 mm). The area under the peak of refractive index detector of this sample was compared with standard calibration curve and determined the number of moles of fluoromaltotriose of injected sample. Finally, the injected activity divided by the number of moles afforded the specific activity. The average specific activity was $0.701 \pm 0.036 \text{ Ci}/\mu\text{mole}$ ($N = 6$). Analytical HPLC profile of co-injection of 6''-[¹⁸F]-fluoromaltotriose, **8** with fluoro standard, 6''-[¹⁹F]-fluoromaltotriose, **6** is shown in Figure 2.

Finally, stability of 6''-[¹⁸F]fluoromaltotriose in serum was evaluated. The percentage of intact 6''-[¹⁸F]fluoromaltotriose in PBS, human, and mouse serum at 37°C from 0 to 2 hours was determined by HPLC and indicates no changes in percentage of intact of the tracer up to 2 hours. The only known enzymes that could metabolize maltotriose are the amylases and maltase which are present in the hepatobiliary system. However, our in vivo metabolite study, where we injected the tracer and analyzed various tissues for metabolites, indicates that the tracer is not being metabolized and is very stable when administered intravenously.

4 | CONCLUSION

We have successfully synthesized 6''-[¹⁸F]fluoromaltotriose via a direct fluorination of an appropriate precursor of a protected maltotriose. This methodology can be also used to synthesize 1-[¹⁸F]fluoromaltotriose, 2-[¹⁸F]fluoromaltotriose, and 6-[¹⁸F]fluoromaltotriose.

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SCHEME 1.

Synthesis of (per-O-acetyl-6''-trityl-maltotriose **2**) and (per-O-acetyl-6''-deoxy-6''-nosyl-maltotriose **4**, 6''-[¹⁸F]fluoromaltotriose precursor)

SCHEME 2.

Synthesis of 6''-deoxy-6''-fluoro-α-D-glucopyranosyl-(1-4)-O-α-D-glucopyranosyl-(1-4)-O-D-glucopyranose (6''-fluoromaltotriose **6**)

SCHEME 3.

Synthesis of 6''-deoxy-6''-[¹⁸F]fluoro-α-D-glucopyranosyl-(1-4)-O-α-D-glucopyranosyl-(1-4)-O-D-glucopyranoside (6''-[¹⁸F]fluoromaltotriose **8**)

FIGURE 1.

Radio TLC of 6''-[^{18}F]fluoromaltotriose **8** (silica gel plate, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$; 70/30) gave an R_f value of 0.48 which is identical to the R_f value of cold 6''-fluoromaltotriose standard

FIGURE 2.
Analytical HPLC profile of co-injection of ^{18}F -fluoromaltotriose, 8 with cold fluoro standard, ^{19}F -fluoromaltotriose, 6 (Phenomenex Luna-NH₂ column, C-18, 4.6 $\times$ 250 mm, eluent; acetonitrile/ H₂O: 65/35)

TABLE 1Radiochemical yields of [^{18}F]fluoromaltotriose with respect to solvent and temperature

Solvent	Temperature °C	% radiochemical yield (decay corrected.)
THF	65	3
DMSO	120	5.5
Acetonitrile	85	7
DMF	85	9
DMF	75	6.5