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Optimization of precursor synthesis, formulation and stability of 1'-[¹⁸F] fluoroethyl-β-D-lactose ([¹⁸F]FEL) for preclinical studies in detection of pancreatic cancer

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Abstract

Introduction—1'-[¹⁸F]Fluoroethyl-β-D-lactose ([¹⁸F]FEL) is a new PET imaging agent for early detection of pancreatic cancer and hepatocellular carcinoma. We previously reported the syntheses of [¹⁸F]FEL using a bromo- and a tosyl- precursor, followed by an improved method using a nosyl-precursor. However, some steps in the synthesis of the precursor appeared to be problematic producing low yields. Here, we report on an optimized method for synthesis of the precursor and production of [¹⁸F]FEL; we also describe [¹⁸F]FEL's formulation and stability.

Methods—Acetylation of D-lactose **1** was performed following a literature procedure to obtain 1', 2', 3', 6', 2, 3, 4, 6-D-lactose octa-acetate **2a/2b**. Bromination of **2a/2b** was performed using HBr/acetic acid to produce 1'-bromo-2', 3', 6', 2, 3, 4, 6-hepta-O-acetyl-α-D-lactose **3**. Coupling of **3** with ethylene glycol was performed in the presence of Ag-tosylate and an excess of ethylene glycol to produce **4a**. Compound **4a** was reacted with *p*-nitrophenylsulfonyl chloride to produce the nosyl derivative **5**. Radiofluorination of **5** was performed using K[¹⁸F]fluoride/kryptofix to obtain **6**, which was purified by HPLC and hydrolyzed with Na-methoxide to produce **7**.

Results—Compound **2** (**2a/2b**) was obtained in 83% yield as a mixture of two anomeric products. Compound **3** was obtained from the **2a/2b** mixture in 80% yield as one product. Coupling of **3** with ethylene glycol produced **4a** in 90% yield. Compound **5** was obtained in 64% yield, and radiofluorination of **5** produced **6** in 62.5% ± 7.5% yields (n = 8). Hydrolysis of **6** with Na-methoxide produced **7** in 42.0% ± 7.0% yield (n = 8) from the end of bombardment.

Conclusions—A simple 4-step synthesis of the precursor, compound **5**, has been achieved with improved yields. A new formulation of [¹⁸F]FEL has been developed that allows the product to remain stable at ambient temperature for use in animal studies. This improved synthesis of the precursor and stable formulation of [¹⁸F]FEL should be useful for routine production of the radiotracer and its preclinical and, possibly, clinical applications.

Keywords

Pancreatic cancer; Fluorine-18; Lactose; PET

1. Introduction

Pancreatic ductal adenocarcinoma, also known as pancreatic cancer, is the fourth leading cause of cancer-related deaths in the western world [1]. During its early stages of formation and progression, small tumor foci (2–3 mm) are not easily detected by computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET) with [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) [2]. Therefore, 80%–90% of pancreatic cancer patients have unresectable tumors at the time of detection [3]. The early detection of pancreatic cancer may save thousands of lives. Currently, there is no suitable modality that is sensitive enough to detect early stage disease. PET as an imaging modality may be promising for early detection of this cancer but a suitable radiotracer that can be used in place of [^{18}F]FDG is not available. The main limitation of [^{18}F]FDG/PET is resolution of the PET scanner (0.5 mm); because [^{18}F]FDG targets hexokinase in the tumors cells, small pancreatic tumor (2–3 mm) are less than the scanner's resolution. On the other hand, radiotracer targeting of tissues in the peritumoral region may amplify the lesion and overcome the limitation of the scanner's resolution.

We have an ongoing effort in developing and testing novel PET radiotracers for early detection of pancreatic cancer and hepatocellular carcinoma [4–7]. Previously, we developed and reported on the synthesis of ethyl- β -D-galactopyranosyl-(1,4')-2'-deoxy-2'-[^{18}F] fluoro- β -D-glucopyranoside (Et-[^{18}F]FDL) [4] and its PET imaging results in orthotopically implanted pancreatic tumor xenografts in nude mice [5]. Et-[^{18}F]FDL demonstrated good localization properties with regard to detection of pancreatic cancer in this xenograft model [5]. However, synthesis of the precursor for radiosynthesis of Et-[^{18}F]FDL was a lengthy process that required more than 12 steps and resulted in over-all low yields [4]. Because of the lengthy synthesis, we sought to minimize the number of steps needed to obtain a suitable precursor for Et-[^{18}F]FDL.

Lactose binds with the hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein (HIP/PAP) through 3- and 4-hydroxyl groups of the galactose moiety [8]; therefore, structural modification to the glucose moiety should not interfere with this binding. With this in mind, we designed, synthesized and radiolabeled another analogue of lactose, namely 1'-[^{18}F]fluoroethyl- β -D-lactose ([^{18}F]FEL) [6], for PET imaging of pancreatic carcinoma and hepatocellular carcinoma. Its precursor synthesis was much shorter and required only four steps; therefore, it is more advantageous to produce [^{18}F]FEL rather than Et-[^{18}F]FDL. The precursors have either a bromo- or a tosyl moiety as the leaving group for nucleophilic substitution reaction with a fluoride ion.

Although synthesis of the precursors for [^{18}F]FEL was much shorter compared to that for Et-[^{18}F]FDL, these precursors produced only 10% radiochemical yield of [^{18}F]FEL [6]. In order to improve the radio-chemical yield of [^{18}F]FEL, we designed and synthesized two additional precursors, mesyl- and nosyl-derivatives. The nosyl-derivative resulted [^{18}F]FEL in greater than 60% yield [7]. Thus, the precursor with nosyl as a leaving group appears to be an ideal compound for the routine and high-yield production of [^{18}F]FEL and its possible automated synthesis. It should be noted that the nosyl-derivative also has a short shelf life; after 1.5 months it is degraded and produces either low yields or no product. Therefore, it is suggested that the precursor be freshly purified on a silica gel column a couple of days prior to synthesis.

Both Et-[^{18}F]FDL and [^{18}F]FEL were developed on the basis that D-lactose and its analogues bind to the HIP/PAP [8–10]. It has been reported that HIP/PAP is overexpressed (130-fold) in the peritumoral region of pancreatic acinar cells compared with its expression in normal pancreatic cells [11,12]. In addition, HIP/PAP is known to be overexpressed (13-

fold) in pancreatitis, an inflammatory disease of the pancreas [11]. Furthermore, it is overexpressed in hepatocellular carcinoma cells and continues to be expressed with cell growth [13–17]. Therefore, not only do the Et-[^{18}F]FDL and [^{18}F]FEL PET imaging agents have high potential in the early detection of pancreatic cancer, pancreatitis and hepatocellular carcinoma, they may also be useful in differentiating between pancreatic cancer and pancreatitis based on quantitative differences in expression levels of HIP/PAP. Preliminary studies on orthotopic pancreatic cancer in nude mice revealed that [^{18}F]FEL has high efficacy in detecting the peritumoral region using PET (unpublished). It is also effective in imaging of chemically induced hepatocellular carcinoma in swine liver by PET (unpublished).

Currently, we are attempting to further improve the high-yield production of [^{18}F]FEL as a preclinical and (possibly) clinical grade probe. In the precursor synthesis, we experienced difficulties obtaining a high yield of an intermediate compound **4a**, which is produced by coupling ethylene glycol and 1'-bromo-heptaacetyl lactose. The yield in this step was drastically reduced to 30% as compared to the previously reported yield of 70% [6]. To synthesize the precursor in high yield for the routine production of [^{18}F]FEL and for possible preclinical and clinical applications, we have repeated and optimized each step, improving the synthesis of the precursor and radiosynthesis of [^{18}F]FEL. We have also improved the quality control analytical techniques for [^{18}F]FEL and performed an *in vitro* stability study. In this article, we report on the optimization of precursor synthesis, production of [^{18}F]FEL, and its formulation and *in vitro* stability.

2. Materials and methods

2.1. Reagents and Instrumentation

Reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. K[^{18}F]kryptofix 2.2.2. was obtained from the Center for Advanced Biomedical Imaging (CABI) at The University of Texas MD Anderson Cancer Center (Houston, TX) as an aqueous solution. Silica gel solid-phase extraction cartridges (Sep-Pak, 900 mg) were purchased from Alltech Associates (Deerfield, IL).

Thin layer chromatography (TLC) was performed on pre-coated Kieselgel 60 F254 glass plates (Merck, Darmstadt, Germany). Proton, ^{13}C and ^{19}F NMR spectra were recorded at the University of Texas MD Anderson Cancer Center using a Bruker 300-MHz or 600-MHz spectrometer, with tetramethylsilane as an internal reference or hexafluorobenzene as an external reference. High-resolution mass spectrum (HRMS) was obtained on a Bruker BioTOF II mass spectrometer at the University of Minnesota using electrospray ionization.

High Performance Liquid Chromatography (HPLC) was performed using an 1100 series pump (Agilent Technologies, Stuttgart, Germany), with a built-in UV detector operated at 210 nm and 254 nm, and a radioactivity detector with a single-channel analyzer (Bioscan, Washington, D C). Crude products were purified on a semipreparative C_{18} reverse-phase column (Alltech, Econosil, 10×250 mm) and quality control analyses were performed on an analytical C_{18} column (Alltech, Econosil, 4.6×250 mm). An acetonitrile/water solvent system (45% MeCN/ H_2O) was used to purify the radiolabeled product at a flow of 4 mL/min. Quality control analyses were performed using 55% MeCN/ H_2O solvents at a flow of 1 mL/min. Radio-TLC plates were scanned on an AR-2000 Imaging Scanner (Bioscan, Washington, DC).

2.2. Preparation of 1',2',3',6',2,3,4,6-octa-O-acetyl- β -D-lactose: **2a** and **2b**

This compound was prepared according to a previously reported method [18]. In brief, β -D-lactose (1.0 g, 2.92 mmol) was refluxed with excess acetic anhydride (20.0 mL, 211.97 mmol) in the presence of sodium acetate (1.0 g, 12.19 mmol) for 4 h, followed by cooling and stirring at ambient temperature overnight. After cooling, the product was solidified, filtered, and purified by re-crystallization or flash chromatography on a silica gel column using 50% ethyl acetate in hexane. The product that was obtained from re-crystallization (1.31 g) showed a single anomer **2b** (as observed by ^1H NMR) in 70% yield. The product obtained from flash chromatography (1.65 g) was a mixture of **2a** and **2b**, (α and β) in the ratio 27:73, respectively, in 83% yield.

2.3. Preparation of 1'-bromo-2',3',6',2,3,4,6-hepta-O-acetyl- α -D-lactose: **3**

This compound was prepared according to previously published methods [6,7,18]. Briefly, a solution of the mixture of **2a** and **2b** (2 g, 2.94 mmol) in anhydrous dichloromethane (DCM, 10 mL) was treated with 30% HBr in acetic acid (4 mL) at ambient temperature for 30 min, when TLC revealed no starting material remained. The solution was cooled in an ice bath, diluted with DCM (50 mL), washed with ice-cold water (2×25 mL) and ice-cold saturated sodium bicarbonate (2×25 mL), dried under Na_2SO_4 , and evaporated to produce a colorless oil. The crude product was then stirred in diethyl ether (15 mL) to produce a white crystalline solid, which was filtered to obtain **3** (1.7 g) in 80% yield.

2.4. Preparation of 1'-hydroxyethyl-2',3',6',2,3,4,6-hepta-O-acetyl- β -D-lactose: **4a**

Compound **4a** was prepared following a previously published method [6], with modifications.

2.4.1. Method 1—A mixture of **3** (2.0 g, 2.86 mmol), ethylene glycol (5 mL, 25 equiv.), and molecular sieves (4 Å, 5 g) in dry acetonitrile (MeCN, 20 mL) was stirred at ambient temperature under argon for 15 min. Silver *p*-toluenesulfonate (Ag-OTs, 1.6 g, 5.8 mmol) was added and the reaction mixture was stirred for 3 h at ambient temperature. The reaction mixture was filtered, the solvent was evaporated, and the crude product was dissolved in ethyl acetate (EtOAc, 30 mL). The solution was washed with water (3×30 mL), and the aqueous layer was back extracted with EtOAc (30 mL). The combined EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography on a silica gel column, and eluted with 40% acetone in hexane. Fractions with different R_f values were separated and combined. The solvent of the combined fractions was evaporated to obtain **4a** (600 mg) in 30% yield. The ^1H NMR spectrum of **4a** was consistent with that reported earlier [6].

Another compound, **4b**, was isolated from this reaction (640 mg) in 58% yield as a white solid. ^1H NMR **4b**: (CDCl_3) δ : 6.185 (d, $J = 3.6$ Hz, 1H), 5.543 (t, $J = 9.9$ Hz, 1H), 5.37–5.40 (m, 3H), 5.24–5.29 (m, 1H), 5.11–5.17 (m, 2H), 4.96–5.00 (m, 2H), 4.73–4.87 (m, 2H), 4.49–4.54 (m, 4H), 4.40 (dd, $J = 12$ Hz and 2 Hz, 1H), 4.08–4.23 (m, 8H), 3.89–3.95 (m, 3H), 3.75–3.83 (m, 3H), 3.61–3.69 (m, 1H), 2.22 (s, 3H, acetate), 2.19 (s, 3H, acetate), 2.18 (s, 3H, acetate), 2.17 (s, 3H, acetate), 2.16 (s, 3H, acetate), 2.14 (s, 3H, acetate), 2.11 (s, 3H, acetate), 2.09 (s, 3H, acetate), 2.087 (s, 3H, acetate), 2.08 (s, 6H, acetate), 2.07 (s, 3H, acetate), 1.998 (s, 3H, acetate), 1.992 (s, 3H, acetate). ^{13}C NMR (CDCl_3) δ : 171.35, 170.53, 170.40, 170.34, 170.24, 170.16, 170.10, 169.68, 169.29, 169.24, 169.08, 101.18, 100.97, 91.20, 90.08, 76.29, 76.21, 75.59, 73.43, 73.30, 72.94, 72.18, 71.33, 71.05, 70.98, 70.68, 70.65, 70.62, 70.35, 70.20, 69.59, 69.16, 69.13, 68.20, 66.67, 66.63, 66.59, 62.04, 61.89, 61.79, 60.85, 60.78, 21.03, 20.98, 20.89, 20.88, 20.81, 20.74, 20.62, 20.49. HRMS: calculated for $\text{C}_{26}\text{H}_{36}\text{O}_{18}\text{Na}$ ($M + \text{Na}$, fragment) 659.1794, found 659.1784.

2.4.2. Method 2—A solution of **3** (0.2 g, 0.29 mmol) and ethylene glycol (4.9 mL, 88.36 mmol, 300 equiv.) in dry acetonitrile (MeCN, 4 mL) was stirred at ambient temperature, in the presence of molecular sieves (4 Å, 0.5 g) under argon for 15 min. Ag-OTs (0.16 g, 0.58 mmol) was added and the reaction mixture was stirred for an additional 3 h at ambient temperature. The reaction mixture was filtered, the solvent was evaporated, and the crude product was purified by flash chromatography on a silica gel column. The product was eluted with 40% acetone in hexane. The solvent of the combined fractions was evaporated to afford **4a** (0.18 mg) in 90% yield.

2.5. Preparation of ethyl-di-[2',3',6',2,3,4,6-hepta-O-acetyl-β-D-lactose]: **4b**

A solution of **3** (0.1 g, 0.15 mmol, 2 equiv.) and ethylene glycol (4.1 μL, 0.075 mmol, 1 equiv.) in dry acetonitrile (MeCN, 4 mL) was stirred at ambient temperature in the presence of molecular sieves (4 Å, 0.25 g) under argon for 15 min. Ag-OTs (0.08 g, 0.29 mmol) was added, and the reaction mixture was stirred for an additional 3 h at ambient temperature, when TLC revealed that no starting material remained. The reaction mixture was filtered, the solvent was evaporated, and the crude product was purified by flash chromatography on a silica gel column. The product was eluted with 40% acetone in hexane. The solvent of the combined fractions was evaporated to produce **4b** (0.18 mg) in 92% yield. The ¹H NMR spectrum was identical to that described in Section 2.4.

2.6. Preparation of 1'-(p-nitrophenylsulfonyl)ethyl-2',3',6',2,3,4,6-hepta-O-acetyl-β-D-lactose: **5**

Compound **5** was prepared as previously described [6]. Briefly, 1'-(hydroxyethyl)-2',3',6',2,3,4,6-hepta-O-acetyl-β-D-lactose **4a** (68.0 mg, 0.1 mmol) was dissolved in dry pyridine (3.0 mL) in a small flask and cooled to 0 °C under argon. *p*-Nitrophenylsulfonyl chloride (55 mg, 2.5 equiv.) was dissolved in dry DCM (1.0 mL), added slowly to the reaction mixture and stirred for 1 h at 0 °C, when TLC showed no starting material remained. The reaction mixture was evaporated on a high vacuum pump and the crude product was purified by flash chromatography on a silica gel column and eluted with 40% acetone in hexane. The solvent was evaporated to obtain **5** (55 mg), as a white solid, in 64% yield. The ¹H NMR spectrum was identical to that previously reported [6].

2.7. Preparation of 1'-[¹⁸F]fluoroethyl-2',3',6',2,3,4,6-hepta-O-acetyl-β-D-lactose: **6**

Compound **6** was also prepared as previously reported [6]. In brief, an aqueous solution of K[¹⁸F]/kryptofix [2.2.2], obtained from CABI, at MD Anderson Cancer Center, was evaporated by the azeotropic removal of water with acetonitrile (1.0 mL) at 100 °C under a flow of argon. A solution of **5** (4–5 mg) in dry acetonitrile (0.4 mL) was added to the dry K¹⁸F/kryptofix [2.2.2]. The reaction mixture was heated at 80 °C for 20 min. The crude reaction mixture was passed through a silica Sep-Pak cartridge and eluted with EtOAc (2.0 mL). The solvent from the eluent was evaporated, and the residue was dissolved in 60% acetonitrile/water (1.0 mL) before being injected into the HPLC system for purification. Product **6** was collected in a two-necked pear-shaped flask in 55%–70% yields, and an aliquot was analyzed on an analytical HPLC column to verify its identity and purity by being coinjected with the authentic nonradioactive sample **6**.

2.8. Preparation of 1'-[¹⁸F]fluoroethyl-β-D-lactose: **7**

The HPLC solvent from the purified product **6** was removed under reduced pressure using a high vacuum pump. The residue was dissolved in DCM (2–3 mL), transferred to a V-vial, and crimped. DCM was evaporated by blowing argon at ambient temperature. The residue was dissolved in methanol (0.4 mL), followed by addition of NaOMe/MeOH solution (0.5 M, 0.1 mL), and heated at 80 °C for 7 min. An aliquot was removed from the V-vial,

evaporated, neutralized with HCl, re-dissolved in MeCN, and analyzed by analytical HPLC, which revealed that **6** was completely hydrolyzed. Radioactivity of the crude product was measured in a dose calibrator and obtained in 80% yield (d. c.). The product was then applied for dose preparation and formulation.

2.9. Formulation of [¹⁸F]-FEL: **7**

The solvent (MeOH) from the V-vial containing **7** was evaporated by blowing argon at 40 °C; the residue was neutralized with aqueous HCl (1 M, 0.05 mL), and diluted with either saline or phosphate buffered saline (PBS) that contained 1% ethanol. The product solutions were filtered through an anion exchange cartridge (ABX). The pH values of both solutions were checked using pH paper (Aldrich Chem. Co.) and found to be slightly acidic (pH 4.5–5.0). A drop (20 µL) of NaHCO₃ solution (1 M) was added to the product solution in PBS with 1% ethanol to adjust the pH to 7.0–7.5. Both solutions were analyzed by radio-TLC and maintained at ambient temperature for stability studies.

2.10. Quality control analysis

2.10.1. TLC—Compound **7** was analyzed by radio-TLC, as previously reported [6], with a minor modification on the composition of the TLC developing solvent. Briefly, 1 µL of **7** was spotted on a silica gel TLC plate, and the plate was developed in a 70% MeOH and 30% EtOAc solvent system. The TLC plate was scanned over a radio-TLC scanner.

2.10.2. Ion exchange cartridge—Compound **7** was analyzed on an ion exchange cartridge as previously reported [19]; in brief, anion-exchange cartridge (ABX, Radeberg, Germany) was activated by passing 1 ml of ethanol, followed by 1 ml of water. Excess solvent was removed by pushing air. A 20-µl solution of [¹⁸F]-FEL (10–15 × 10⁶ cpm/mL) was loaded onto the ion exchange cartridge. The radioactivity was eluted from the cartridge using a solution of 20% MeCN in water, and fractions of 1 mL were collected in scintillation vials. Radioactivity in each fraction and cartridge was counted using a gamma counter (Cobra II, Perkin Elmer). Combined radioactivity in the washing was expressed as the percent activity eluted, and radioactivity in the cartridge was considered the percent retained. The combined radioactivity in the cartridge and washings was considered as the total activity (100%).

2.11. Stability study

Both solutions of product **7** (in saline and in PBS with 1% ethanol), as formulated above, were analyzed by radio-TLC at an interval of 2 h time up to 6 h. The TLC plates were developed in 70% MeOH/EtOAc, scanned on a TLC scanner, and the radioactive peaks were recorded. The compound in saline, with an acidic pH, was found to be unstable at ambient temperature, as determined by TLC.

3. Results and discussion

Scheme 1 represents the synthesis of precursor **5** and radio-synthesis of [¹⁸F]FEL **7**. Acetylation of β-D-lactose **1** produced **2** as a mixture of anomeric compounds: the less favorable α anomer (**2a**) and more favorable β anomer (**2b**) in the ratio of 27:73, respectively, as observed by ¹H NMR spectroscopy. The crude yield of **2** (**2a** & **2b**) was 95% and purified yield was 83% after chromatography.

Compound **2b** was previously purified by recrystallization [18] and the yield was approximately 70%. Our attempt to separate this mixture using flash chromatography was not successful; as a result, we also purified the compound by recrystallization to obtain the pure β-anomer, **2b**. In addition, we recovered the mother liquor, which contained α-

anomeric compound as the major product; we attempted to further recrystallize it to isolate the α -anomer but failed.

Compound **3** was prepared from **2b** as reported earlier [6]. To investigate bromination on the α -anomer, we brominated the mixture (**2a/2b**) obtained from the mother liquor by reacting it with HBr/acetic acid and obtained a single anomeric product, **3**. The ^1H NMR spectrum of the bromo-lactose obtained from the mixture was identical to that obtained from the pure β -anomer, **2b**. This result suggests that both **2a** and **2b** produced the same compound, **3**; therefore, purification of the β -D-lactose octa-acetate **2b** from the anomeric mixture **2a** and **2b** is not necessary. The crude products **2a** and **2b** can be converted to **3** giving an overall 15%–20% higher yield, avoiding the crystallization step.

Coupling ethylene glycol with the bromolactose **3** in the presence of Ag-OTs produced a mixture of two compounds, **4a** and **4b** (Scheme 1). Although it was previously reported that **4a** is the only product [6,7], the formation of **4b** was not reported. When we repeated the coupling experiment under identical conditions, the isolated yield of **4a** was 30%–42% in several repeated runs. Then we focused on the by-product and isolated compound **4b** in 50%–60% yields. We fully characterized compound **4b** by ^1H NMR spectroscopy. The ^1H NMR spectrum of **4b** revealed 32 protons between 3.6 and 6.1 ppm (δ), where the backbone protons and methylene protons of lactose resonate. There were 14 peaks (singlet) between 1.99 and 2.22 ppm, corresponding to the acetate protons. This suggested that two lactose moieties were present in the compound. The product appeared to be a di-substituted ethylene glycol, which means two molecules of lactose were connected by an ethylene bridge through oxygen atoms, producing a product with 32 protons in the downfield and 14 acetate peaks in the up-field. ^{13}C NMR spectrum showed the number of carbons consistent to be an ethylene glycol di-substituted with lactose.

For further characterization, we attempted to obtain a high-resolution mass spectrum of **4b**. The compound did not produce a stable molecular ion; instead, a fragment ion was observed, with an $M + \text{Na}$ ion of 659.1784 (due to the loss of one lactose moiety from the parent compound). Because we did not find the molecular ion of **4b**, we synthesized the compound (Scheme 2) by reacting two equivalents of the bromo-lactose **3** with one equivalent of ethylene glycol. The compound **4b** was isolated in a quantitative yield. This chemical synthesis further confirmed the formation of **4b**, a di-substituted ethylene glycol lactose derivative.

To improve the yield of the desired product **4a**, we increased the amount of ethylene glycol in much higher quantities than those reported earlier. In 200-fold excess of ethylene glycol the product ratio was improved to approximately 80:20 (**4a:4b**), as observed by TLC. When the amount of ethylene glycol was further increased by 300-fold, the product ratio was approximately 98:2 (TLC), and the isolated yield of **4a** was almost quantitative (92%). We attempted to quantitate the product ratio between mono- and di-substituted compounds by HPLC, but it was not adequate to separate the mixture in our system. However, it is reasonable to believe that the probability of di-substitution is diminished in high concentrations of ethylene glycol, because compound **3** is the limiting reagent.

Compound **5** was obtained with yields, as reported earlier; therefore, no further optimization was necessary at this step. Similarly, radiofluorination of **5** with K^{18}F /kryptofix at 80 °C produced **6** in 62.5% $\pm$ 7.5% yields ($n = 8$) and no further optimization was performed. However, compound **6** was found to be unstable at a slightly high temperature; for example, when the solvent EtOAc from the crude product **6** was evaporated at 80 °C, some product was lost, and unidentified radioactive peaks (broad) were observed during HPLC purification. When a crude product **6** was analyzed on an analytical HPLC column after

evaporating EtOAc at ambient temperature, a single radioactive peak (desired product) was obtained in contrast to the semipreparative HPLC chromatogram, which produced additional unknown peaks. Thus, a high yield can be maintained if the temperature remains at 40–41 °C during evaporation of the solvent. Product **6** had the same HPLC retention time (t_R) as was previously reported [7] and co-eluted with a nonradioactive authentic sample **6** (Fig. 1).

Compound **7** was prepared in a V-vial after transferring product **6** from the evaporating flask and performing hydrolysis with a strong base, NaOMe. During evaporation of the HPLC solvent from the purified product **6** and transfer from the flask to the V-vial, a significant amount of activity was lost, particularly, when the temperature during evaporation was higher than 40 °C. This indicates that not only is the product unstable, but the byproduct is also volatile. After deacetylation of peracetyl-1'-[18 F]-fluoroethyl- β -D-lactose **6** by reacting with 0.5 M sodium methoxide, we expected [18 F]FEL **7** to be in a quantitative yield. However, the yield was lower (80%) than expected. This loss may be due to decomposition of the product and evaporation of the decomposed byproduct at elevated temperatures, which was demonstrated in additional tests (described later in this report).

In our previous study [6,7], compound **7** was assumed to be neutral, which was diluted with saline, and filtered to obtain the final dose; however, an accurate pH measurement of the final product was not determined. In the current study, the pH of the product in saline was tested and found to be 4.5–5.0. An analysis of the product on TLC revealed a small amount of radioactivity (**a**, 5%–8%) at the point of application (Fig. 2A). TLC of free fluoride revealed that it remained at the point of application (origin, Fig. 2B). When a TLC plate was developed after co-spotting with free [18 F]-fluoride, the small unknown activity stayed at the origin with free fluoride (Fig. 2C). An analysis of the product on an ion-exchange cartridge [19] also revealed that 5%–8% of the activity was retained in the cartridge, suggesting the presence of free fluoride in the product. Further analysis of the product **7** using TLC showed that the peak at the origin increased with time to 70% of the total activity after 4 h (Fig. 2D). The TLC plate for 2D was developed in 40% MeOH/EtOAc instead of 70% MeOH/EtOAc and, as a result, the R_f value of **7** was reduced from 0.6 to 0.4. Although the mechanism of decomposition is not known, the desired product appeared to be unstable at ambient temperature under this acidic condition.

To address instability of the product, we took the following two measures: 1) diluted the product with PBS instead of normal saline and adjusted the pH to 7.0–7.5 and 2) added 1% ethanol to the product in PBS at pH 7.0–7.5. It was reported that the presence of ethanol reduces the radiolysis of radioactive compounds [20–22]; therefore, our diluent was formulated to contain 1% ethanol. Using this formulation, the product was found to be stable at ambient temperature for up to 6 h. Fig. 3 shows radio-TLC data that demonstrate the product's stability at ambient temperature. TLC was taken after 15 min of dose preparation (Fig. 3A), 2 h (**3B**), 4 h (**3C**) and 6 h (**3D**). No significant change in the product ratio was observed at 2 h, 4 h and 6 h, suggesting that under this condition, product stability is maintained.

To better understand the stability of the product, [18 F]FEL was spotted on a TLC plate and heated for 1 min at 100 °C; the plate was developed in a jar with 20% MeOH/EtOAc solvent system and scanned. Fig. 4 depicts the effect of temperature on [18 F]FEL as monitored by radio-TLC. Fig. 4A is the normal radio-TLC of [18 F]FEL developed in 70% MeOH/EtOAc with a single peak **b**. Fig. 4B is the radio-TLC of [18 F]FEL developed in 20% MeOH/EtOAc with a single peak **b**. Fig. 4C is a separate TLC plate (spotted with [18 F]FEL, heated for 1 min then developed in 20% MeOH/EtOAc and scanned), which showed a new spot **c** near the solvent front, in addition to a small amount of [18 F]FEL **b**. Fig. 4D is the same TLC plate (Fig. 4C), further heated and rescanned.

It should be noted that the R_f value of [^{18}F]FEL was reduced from 0.6 to 0.25 when developed in 20% MeOH/EtOAc; this solvent was used to differentiate between [^{18}F]FEL and any decomposition product. Another shift of the R_f value was observed in a previous preparation (Fig. 2D) when 40% MeOH/EtOAc was used for TLC plate development.

As shown in Fig. 4, the new unknown compound **c** was formed from compound **b** when the TLC plate was heated and then developed (Fig. 4C). When the developed plate (Fig. 4C) was further heated, a significant amount of radioactivity was lost (Fig. 4D) from both **b** and **c** (cf counts changed from 6000 to 100). This suggests that [^{18}F]FEL decomposes upon heating and the decomposed radioactive product is volatile. This observation supports the loss of activity during evaporation of the solvent from the purified product **6** and possibly some loss may take place during hydrolysis of the protecting groups from **6** at 80 °C. To better understand this decomposition and loss of activity, additional studies will be necessary. In a recent preparation, we did not observe any byproduct (at the origin of TLC) when all solvent evaporation steps were performed at around 40 °C (Fig. 4A).

The radiochemical yield of **7** was $42.0\% \pm 7.0\%$ ($n = 8$) with $> 99\%$ radiochemical purity; and the estimated specific activity was 50–55 GBq/ μmol at the end of HPLC purification. The synthesis time was 105–110 min from the end of bombardment.

We have modified the radio-TLC analytical method by changing the developing solvents because the previous method was not suitable for separating other species that may form due to decomposition of the compound *in vitro* and *in vivo*. Our current TLC condition differentiates between the product, free fluoride, and other unknown species, which may form *in vitro* or *in vivo*. However, two TLC developing solvents will be required: 1) 70% MeOH/EtOAc for separation between fluoride and product; 2) 20% MeOH/EtOAc to separate the product from decomposition product. These TLC conditions should be suitable for performing metabolite analyses in the plasma samples of animals injected with the probe.

4. Conclusions

An optimized synthesis process for the precursor has been achieved that results in high yield production of the precursor without the need for purification of an intermediate compound. The precursor is suitable for the routine production of 1'-[^{18}F]fluoroethyl- β -D-lactose **7** and its automated synthesis. The product is temperature and pH sensitive; with the appropriate precautions, [^{18}F]FEL can be produced in high yields. The product with appropriate formulation is suitable for PET imaging of early pancreatic carcinoma and hepatocellular carcinoma in preclinical and, possibly, clinical applications.

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Fig. 1. HPLC chromatogram of 1'-[^{18}F]fluoroethyl-2',3',6',2,3,4,6-hepta-*O*-acetyl- β -D-lactose, **6**, co-injected with standard 1'-fluoroethyl-2',3',6',2,3,4,6-hepta-*O*-acetyl- β -D-lactose, **6**. Analytical C_{18} Column; 55% MeCN and 45% H_2O ; flow, 1.0 mL/min.

Fig. 2. TLC analysis of $[^{18}\text{F}]$ FEL (developing solvent 70% MeOH/EtOAc); A: $[^{18}\text{F}]$ FEL; B: $[^{18}\text{F}]$ fluoride; C: $[^{18}\text{F}]$ FEL co-spotting with $[^{18}\text{F}]$ fluoride; D: TLC of $[^{18}\text{F}]$ FEL after 4 h (developed in 40% MeOH/EtOAc). Note: a = fluoride; b = $[^{18}\text{F}]$ FEL.

Fig. 3.

In vitro stability study of [^{18}F]FEL at room temperature (developing solvent 70% MeOH/EtOAc); A: within 15 min of dose preparation, B: after 2 h, C: after 4 h, and D: after 6 h.

Fig. 4.

Stability study of [^{18}F]FEL (developing solvent 70% MeOH/EtOAc); A: [^{18}F]FEL. TLC Plate developed in 20% MeOH/EtOAc; B: [^{18}F]FEL; C: TLC plate spotted with **7**, heated for 1 min and developed in 20% MeOH/EtOAc; D: TLC Plate C was further heated and rescanned. Note: c = unknown product produced by thermal decomposition of **7**.

Scheme 1.

Scheme for synthesis of the precursor and radiosynthesis of 1'-[^{18}F]fluoroethyl- β -D-lactose 7.

Scheme 2.

Scheme for synthesis of ethyl-di-[2',3',6',2,3,4,6-hepta-*O*-acetyl-β-D-lactose], **4b**.