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Synthesis of 2,6-Dideoxy-2-Fluoro-6-[^{18}F]-Fluoro-β-D-Glucopyranosyl Fluoride (2,6FGF) as a Potential Imaging Probe for Glucocerebrosidase

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SUMMARY

We have previously synthesized 2-deoxy-2-[^{18}F]-fluoro-β-mannosyl [^{18}F]-fluoride and shown that it behaves as a mechanism-based inhibitor of *Agrobacterium* sp. β-glucosidase. *In-vivo* experiments indicate that this compound undergoes partial hydrolysis to produce 2-deoxy-2-fluoro-mannose, which can become phosphorylated and trapped within the cell. We now report the synthesis of another 2-fluoro glycoside which is ^{18}F-labelled at the 6 position so that the label cannot be lost during such glycoside hydrolysis and which, further, cannot be phosphorylated. The mechanism-based glycosidase inhibitor 2,6-dideoxy-2-fluoro-6-[^{18}F]-fluoro-β-D-glucopyranosyl fluoride (2,6FGF) was synthesized in 69% overall chemical yield and in 9% radiochemical yield (decay corrected) as a potential imaging probe for glycosidase.

Key Words: PET, Glycosidase, 2-fluoro-glucose, fluorine-18, Gaucher's Disease

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INTRODUCTION

A number of relatively commonly-occuring genetic diseases are the result of deficiencies in lysosomal enzymes (glycosidases) responsible for the hydrolysis of glycosides. The most common such ailment is Gaucher's disease, which is caused by an inherited deficiency in glucocerebrosidase (1). This results in the accumulation of glucocerebrosides, mainly in the cells of the mononuclear phagocyte system. Other lysosomal diseases include Tay-Sachs disease, which is due to a deficiency in hexosaminidase A, and Hurler's syndrome, which is due to a lack of iduronidase (2).

Gaucher's disease can now be treated by enzyme replacement therapy, and good progress has been made in the development of other such therapies. Both diagnosis of the presence and the severity of the disease, as well as the monitoring of treatment would benefit from new non-invasive methods of quantitating and localizing enzyme *in vivo*. Selective covalent inhibitors of these glycosidases that can be detected by positron emission tomography (PET) would be valuable in this regard. Excellent candidates for this task are 2-deoxy-2-fluoro-β-D-glycopyranosyl fluorides (3,4). These compounds have been shown to function as specific mechanism-based inactivators of 'retaining' β-glycosidases via formation of a relatively stable 2-deoxy-2-fluoro-α-glycopyranosyl-enzyme intermediate. Therefore, by use of a 2-deoxy-2-[^{18}F]-fluoroglycopyranosyl fluoride it should be possible to radiolabel all functional glycosidases of that class and then localize them with PET.

In an earlier approach to this problem we synthesized 2-deoxy-2-[^{18}F]-fluoro-β-D-mannopyranosyl [^{18}F]-fluoride and showed that it covalently derivatized *Agrobacterium* sp. β-glucosidase (5). We further showed that the trapped species was catalytically competent since it was capable of 'turnover' with release of 2-deoxy-2-fluoro-mannose and native enzyme. Unfortunately tests of this derivative *in vivo* revealed that hydrolysis occurred with formation of 2-deoxy-2-fluoro-mannose (6).

The choice of the synthesized 2-deoxy-2-fluoro-β-D-mannopyranosyl fluoride was a consequence of the relative simplicity and speed of its synthesis in radiolabelled form via electrophilic addition of [^{18}F]-fluorine gas to D-glucal. A

better, more specific, analogue would appear to be synthesized 2-deoxy-2-[^{18}F]-fluoro-β-D-glucopyranosyl fluoride. However, rapid synthesis of this derivative in the desired β-anomeric form is non-trivial. Further, should spontaneous hydrolysis occur, the released of 2-deoxy-2-fluoro-glucose would become rapidly phosphorylated and trapped in the usual manner, obliterating any imaging from labelled glycosidase. A possible solution to both problems was offered by the title compound since the replacement of the 6-hydroxyl group with fluorine prohibits possible phosphorylation and offers a simpler means of radiolabelling using higher specific activity ^{18}F-fluoride. Earlier studies had demonstrated the tolerance of such enzymes for modifications of the substrate at the 6-position.

Therefore, we report herein the synthesis and radiolabelling of 2,6-dideoxy-2,6-difluoro-β-D-glucopyranosyl fluoride (2,6FGF) **5** as a potential imaging agent for glucocerebrosidase, and the enzyme kinetic data obtained with *Agrobacterium* sp. β-glucosidase (Abg) and human glucocerebrosidase (GCase).

EXPERIMENTAL

General

Analytical methods

^{1}H NMR spectra were recorded on the following instruments: a Bruker AC-200 at 200 MHz or a WH-400 at 400 MHz. ^{19}F NMR spectra were recorded on a Bruker AC-200 multinuclear spectrometer operating at a frequency of 188 MHz. ^{1}H NMR spectra chemical shifts are externally referenced to tetramethylsilane and for samples dissolved in D_2O, the spectra are externally referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (δ = 0.015 ppm). ^{19}F NMR shifts are referenced to $CFCl_3$, although the samples were externally referenced to trifluoroacetic acid (δ = -76.53 ppm).

Thin layer chromatography (TLC) was performed using plates (silica gel 60 F_{254}, Merck) with visualization under UV light or after charring with 10% H_2SO_4 in MeOH or ammonium molybdate-H_2SO_4 solution in MeOH. Radio-TLC was performed on a Bio-Scan QC-scan. Column chromatography was performed under elevated pressure using Kieselgel 60 (230-400 mesh).

Microanalyses were performed by Mr. Peter Borda in the microanalytical laboratory at the Department of Chemistry, University of British Columbia.

Solvents and reagents

Solvents and reagents were either of reagent, certified, spectral, or HPLC grade. Dry solvents were prepared as follows. Dichloromethane and pyridine were distilled over calcium hydride. 1,4-Dioxane was predried over 4 Å molecular sieves overnight and stored over 4 Å molecular sieves. Methanol was distilled over magnesium with iodine.

Synthesis of 2,6-dideoxy-2,6-difluoro-β-D-glucopyranosyl fluoride (2,6FGF) 5

3,4-Di-O-acetyl-6-O-triphenylmethyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride 1

2-Deoxy-2-fluoro-β-D-glucopyranosyl fluoride (1.19 g, 6.45 mmol) was dissolved in 20 mL dry pyridine, and triphenylmethyl chloride (1.98 g, 1.1 eq) then added. After stirring at room temperature overnight, acetic anhydride (4 mL, 42 mmol, 6.6 eq) was added and the reaction mixture was allowed to stir at room temperature for 2 hours. The mixture was evaporated *in vacuo* and the resulting syrup diluted with methylene chloride. The resultant solution was washed with 1 N HCl, saturated sodium bicarbonate; dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. The crude material was purified by flash chromatography using 1:2 ethyl acetate:petroleum ether to yield a white foam (2.54 g, 77%). The foam was crystallized by dissolving in minimal dichloromethane and adding petroleum ether, yielding white crystals (2.06 g, 63%). **^{1}H NMR data** (400 MHz, $CDCl_3$): δ 7.35-7.45 (m, 6 H, H-2', H-6'), 7.15-7.35 (m, 9 H, H-3', H-4', H-5'), 5.40 (ddd, 1 H, $J_{1,2}$ = 6.2 Hz, $J_{1,F1}$ = 52.3 Hz, $J_{1,F2}$ = 3.8 Hz, H-1), 5.18-5.30 (m, 2 H, H-3, H-4), 4.50 (dddd, 1 H, $J_{2,1}$ = 6.2 Hz, $J_{2,F1}$ = 11.5 Hz, $J_{2,F2}$ = 50.5 Hz, $J_{2,3}$ = 8.2 Hz, H-2), 3.72 (ddd, 1 H, $J_{5,4}$ = 9.4 Hz, $J_{5,6a}$ = 2.7 Hz, $J_{5,6b}$ = 4.3 Hz, H-5), 3.36 (dd, 1 H, $J_{6a,5}$ = 2.7 Hz, $J_{6a,6b}$ = 10.7 Hz, H-6a), 3.11 (dd, 1 H, $J_{6b,5}$ = 4.3 Hz, $J_{6b,6a}$ = 10.7 Hz, H-6b), 2.15 (s, 3 H, OAc), 1.75 (s, 3 H, OAc). **^{19}F-NMR data** ($CDCl_3$, 188 MHz): δ -64.27 (ddd, 1 F, $J_{F1,H1}$ = 52.3 Hz, $J_{F1,H2}$ = 11.5 Hz, $J_{F1,F2}$ = 16.1 Hz, F-1), -124.68 (ddd, 1 F, $J_{F2,H1}$ = 3.8 Hz, $J_{F2,H2}$ = 50.5 Hz, $J_{F1,F2}$ = 16.1 Hz, F-2).

3,4-Di-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride 2

3,4-Di-O-acetyl-6-O-triphenylmethyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (2.00 g, 3.91 mmol) was dissolved in 40 mL anhydrous methylene chloride,

then dry methanol (1.6 mL, 10 eq) and boron trifluoride-diethyl etherate (0.48 mL, 1 eq) were added. After stirring at room temperature for 15 minutes, the solution was diluted with methylene chloride, washed twice with water, washed once with saturated sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. The crude material was purified by flash chromatography using 1:1 ethyl acetate:petroleum ether to yield a white foam (0.99 g, 95%). **^{1}H NMR data** (400 MHz, $CDCl_3$): δ 5.42 (ddd, 1 H, $J_{1,2}$ = 6.3 Hz, $J_{1,F1}$ = 52.2 Hz, $J_{1,F2}$ = 3.8 Hz, H-1), 5.30-5.42 (m, 1 H, H-3), 5.09 (t, 1 H, $J_{4,3}$ = 9.4 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.45 (dddd, 1 H, $J_{2,1}$ = 6.3 Hz, $J_{2,F1}$ = 11.5 Hz, $J_{2,F2}$ = 50.3 Hz, $J_{2,3}$ = 8.3 Hz, H-2), 3.74-3.82 (m, 1 H, H-6a), 3.69 (ddd, 1 H, $J_{5,4}$ = 9.6 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.3 Hz, H-5), 3.58-3.65 (m, 1 H, H-6b), 2.00-2.10 (2 s, 6 H, OAc). **^{19}F-NMR data** ($CDCl_3$, 188 MHz): δ -64.31 (dt, 1 F, $J_{F1,H1}$ = 52.2 Hz, $J_{F1,H2}$ = 11.5 Hz, $J_{F1,F2}$ = 15.6 Hz, F-1), -124.83 (ddd, 1 F, $J_{F2,H1}$ = 3.8 Hz, $J_{F2,H2}$ = 50.3 Hz, $J_{F1,F2}$ = 15.6 Hz, F-2).

3,4-Di-O-acetyl-6-O-trifluoromethanesulfonyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride **3**

3,4-Di-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (0.63 g, 2.34 mmol) was dissolved in 5 mL anhydrous dichloromethane followed by a solution of 2,6-di-*t*-butylpyridine (0.74 mL, 1.4 eq) and trifluoromethanesulfonic anhydride (0.55 mL, 1.4 eq) in 5 mL anhydrous dichloromethane. After stirring at room temperature for 1 hour under nitrogen, the solution was diluted with dichloromethane and washed with ice-cold saturated sodium bicarbonate. The aqueous phase was re-extracted with dichloromethane. The combined organic layers were washed twice with cold water, dried over anhydrous magnesium sulfate, filtered and evaporated *in vacuo*. The crude material was purified by flash chromatography using 1:3 ethyl acetate:petroleum ether to yield a faint yellow solid (0.66 g, 70%). **^{1}H NMR data** (400 MHz, $CDCl_3$): δ 5.49 (ddd, 1 H, $J_{1,2}$ = 5.4 Hz, $J_{1,F1}$ = 51.3 Hz, $J_{1,F2}$ = 4.3 Hz, H-1), 5.29-5.39 (m, 1 H, H-3), 5.10 (t, 1 H, $J_{4,3}$ = 9.0 Hz, $J_{4,5}$ = 9.1 Hz, H-4), 4.51-4.60 (m, 2 H, H-6a, H-6b), 4.50 (dddd, 1 H, $J_{2,1}$ = 5.4 Hz, $J_{2,F1}$ = 9.6 Hz, $J_{2,F2}$ = 48.9 Hz, $J_{2,3}$ = 7.1 Hz, H-2), 4.00-4.10 (m, 1 H, H-5), 2.00-2.10 (2 s, 6 H, OAc). **^{19}F-NMR data** ($CDCl_3$, 188 MHz): δ 1.78 (s, 3 F, $-CF_3$), -61.60 (ddd, 1 F, $J_{F1,H1}$ = 51.3 Hz, $J_{F1,H2}$ = 9.6 Hz, $J_{F1,F2}$ = 15.6 Hz, F-1), -122.90 (dt, 1 F, $J_{F2,H1}$ = 4.3 Hz, J_{F2H2} = 48.9 Hz, $J_{F1,F2}$ = 15.6 Hz, F-2).

3,4-Di-O-acetyl-2,6-dideoxy-2,6-difluoro-β-D-glucopyranosyl fluoride **4**

3,4-Di-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (0.202 g, 0.75 mmol) was dissolved in 3 mL anhydrous 1,4-dioxane followed by diethylaminosulfur trifluoride (0.30 mL, 3 eq). The solution was stirred at 100°C for 9 minutes, then cooled to 4°C and 1 mL MeOH added dropwise. The mixture was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate until the washings remained basic, then washed with brine. The organic layer was dried over magnesium sulfate, suction-filtered, and the solvents evaporated *in vacuo*. The crude material was purified by flash chromatography using 1:2 ethyl acetate:petroleum ether to yield a yellowish solid (0.198 g, 97%). **^{1}H NMR data** (400 MHz, $CDCl_3$): δ 5.42 (ddd, 1 H, $J_{1,2}$ = 5.8 Hz, $J_{1,F1}$ = 51.9 Hz, $J_{1,F2}$ = 4.3 Hz, H-1), 5.31 (dd, 1 H, $J_{3,2}$ = 7.8 Hz, $J_{3,4}$ = 8.9 Hz, $J_{3,F2}$ = 15.8 Hz, H-3), 5.12 (t, 1 H, $J_{4,3}$ = 8.9 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.36-4.61 (m, 3 H, H-2, H-6a, H-6b), 3.90 (dddd, 1 H, $J_{5,4}$ = 9.6 Hz, $J_{5,6a}$ = 2.9 Hz, $J_{5,6b}$ = 4.5 Hz, $J_{5,6F}$ = 21.0 Hz, H-5), 2.00-2.14 (2 s, 6 H, OAc). **^{19}F NMR** (188 MHz, D_2O): δ -63.30 (1 F, dt, $J_{F,H1}$ = 51.9 Hz, $J_{F,H2}$ = 13.7 Hz, F-1), -124.21 (dt, 1 F, $J_{F,H1}$ = 4.3 Hz, $J_{F,H2}$ = 49.9 Hz, $J_{F,H3}$ = 15.8 Hz, F-2), -156.01 (dt, 1 F, $J_{F,H5}$ = 21.0 Hz, $J_{F,H6}$ = 46.8 Hz, F-6).

2,6-Dideoxy-2,6-difluoro-β-D-glucopyranosyl fluoride **5**

3,4-Di-O-acetyl-2,6-dideoxy-2,6-difluoro-β-D-glucopyranosyl fluoride (0.40 g, 1.5 mmol) was dissolved in 20 mL anhydrous MeOH followed by 1 M NaOMe in MeOH (1 mL, 0.67 eq). After stirring at room temperature for 15 minutes, the solution was neutralized with Rexyn 101 (H^+) resin, filtered and evaporated *in vacuo*. The crude material was purified by flash chromatography using 2:1 ethyl acetate:petroleum ether to yield a white solid (0.271 g, 97%). **^{1}H NMR data** (400 MHz, D_2O): δ 5.55 (ddd, 1 H, $J_{1,2}$ = 7.0 Hz, $J_{1,F1}$ = 52.8 Hz, $J_{1,F2}$ = 3.4 Hz, H-1), 5.31 (dd, $J_{3,2}$ = 7.8 Hz, $J_{3,4}$ = 8.9 Hz, $J_{3,F2}$ = 15.8 Hz, H-3), 5.12 (t, 1 H, $J_{4,3}$ = 8.9 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 4.36-4.61 (m, 3 H, H-2, H-6a, H-6b), 4.65-4.85 (m, 2 H, H-6a, H-6b), 4.38 (dddd, 1 H, $J_{2,1}$ = 7.0 Hz, $J_{2,F1}$ = 13.6 Hz, $J_{2,F2}$ = 51.3 Hz, $J_{2,3}$ = 8.9 Hz, H-2), 3.88 (dt, 1 H, $J_{3,2}$ = 8.9 Hz, $J_{3,4}$ = 9.3 Hz, $J_{3,F2}$ = 15.5 Hz, H-3), 3.80 (ddt, 1 H, $J_{5,4}$ = 9.9 Hz, $J_{5,6}$ = 2.7 Hz, $J_{5,F6}$ = 25.8 Hz, H-5), 3.86 (t, $J_{4,3}$ = 9.3 Hz, $J_{4,5}$ = 9.9 Hz, H-4).

^{19}F NMR (188 MHz, D_2O): δ -67.44 (1 F, dt, $J_{F,H1}$ = 52.8 Hz, $J_{F,H2}$ = 13.6 Hz, $J_{F1,F2}$ = 15.8 Hz, F-1), -126.14 (1 F, dt, $J_{F,H2}$ = 51.3 Hz, $J_{F,H3}$ = 15.8 Hz, $J_{F2,F1}$ = 15.8 Hz, F-2), -159.00 (dt, 1 F, $J_{F,H5}$ = 25.8 Hz, $J_{F,H6}$ = 47.2 Hz, F-6). **Anal.** Calcd. for $C_6H_9O_3F_3$: C, 38.72; H, 4.87; Found: C, 38.75; H, 4.85.

Radiosynthesis of 2,6-dideoxy-2-fluoro-6-[^{18}F]-fluoro-β-D-glucopyranosyl fluoride 5

Using the facilities at TRIUMF, 1 mL of $H_2{}^{18}O$ was irradiated with a 13 MeV proton beam for 15 minutes. The water was then passed through a QMA Sep-Pak conditioned with 5 mL deionized water followed by 5 mL saturated sodium bicarbonate. The radioactivity content using a Capintec, Inc. CRC-35R dose calibrator was determined to be 131 mCi. A solution of Kryptofix 2.2.2 (4 mg) and potassium carbonate (1.8 mg) dissolved in 0.7 mL acetonitrile and 1.5 mL deionized water was used to elute the fluoride-18 off the Sep-Pak into a conical glass tube. The solvent was boiled off and acetonitrile (2x1.5 mL) was used to remove residual water via azeotropic distillation. To the ^{18}F-fluoride residue was added a solution of 3,4-di-O-acetyl-6-O-trifluoromethanesulfonyl-2-deoxy-2-fluoro--D-glucop y ranosyl fluoride **3** (10 mg) in 2 mL acetonitrile and the resulting mixture boiled at 80°C. Additional volumes of acetonitrile were added as the solvent decreased in volume during this period. After 15 minutes, the solvent was boiled off and the residue cooled to room temperature, then 0.5 mL of 0.1 M NaOMe/MeOH was added and the solution allowed to sit at room temperature for 15 minutes. The solution was loaded directly onto a silica gel column using 1:1 ethyl acetate:hexanes as the eluant. Fractions containing the desired product) were pooled and concentrated *in vacuo*. The radioactivity content was 4.15 mCi (after 2 hours 43 minutes). The product was identified by radio-TLC (R_f=0.35, 2:1 ethyl acetate:hexanes) and had an R_f identical to the authentic, non-radioactive standard. After correcting for radioactive decay, the radiochemical yield was 9%. The specific activity was 1 Ci/μmole.

Enzymology

Enzyme kinetic studies using the cold compound **5** were performed on Abg and GCase using a continuous spectrophotometric assay as described previously (3,7). Values for the inactivation rate constant (k_i), the reversible dissociation constant (K_i),

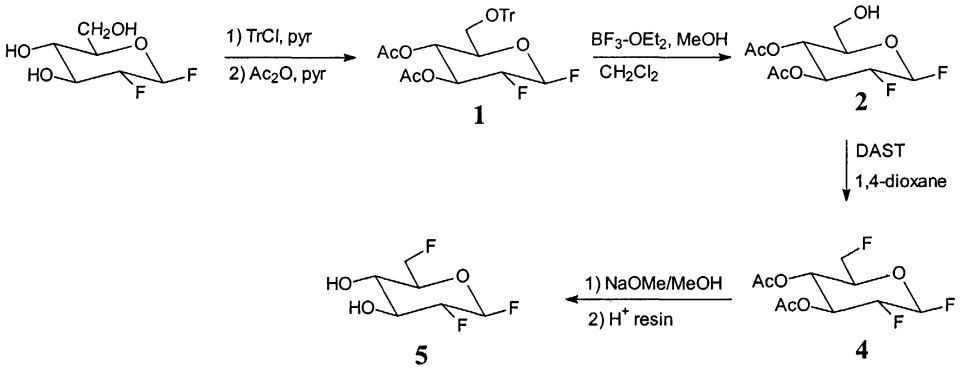
and the second order rate constant are shown in the following table. For comparison, values obtained using 2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride **6** and 2-deoxy-2-fluoro-β-D-mannopyranosyl fluoride **7** are also shown (8).

Table 1: Kinetic parameters determined for 2,6FGF **5** using Abg and GCase

Kinetic parameters	Abg			GCase		
	5	**6**	**7**	**5**	**6**	**7**
k_I(min^{-1})	3.8 ± 0.5	5.9	5.6	0.17 ± 0.01	-	-
K_i (mM)	58.8 ± 8.4	0.4	1.2	101 ± 17	-	-
k_i / K_i(min^{-1}mM^{-1})	0.065 ± 0.018	14.75	4.7	0.0017 ± 0.0004	0.0227	0.0019

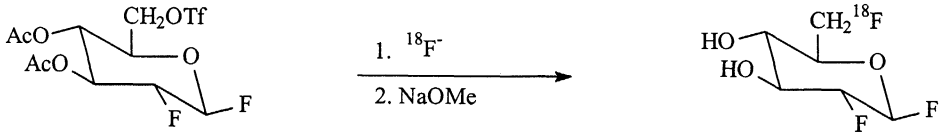
RESULTS AND DISCUSSION

The synthesis of the unlabelled 2,6FGF was achieved in a four step synthesis in an overall chemical yield of 69%. Briefly, the synthesis was achieved by the tritylation of 2-fluoro-glucosyl fluoride followed by acetylation of the 3,4-hydroxyl



groups and subsequent de-tritylation to give the protected 6 hydroxy derivative. The 6-hydroxy compound was then fluorinated with DAST followed by conventional deacetylation with sodium methoxide.

Synthesis of the 6-triflate derivative required as the precursor for ^{18}F labelling was achieved in 70% chemical yield by treating the 6-OH sugar **2** with trifluoromethanesulfonic anhydride in dichloromethane followed by flash chromatography.



3 5

The radiolabelling was achieved by the standard Kryptofix 2.2.2/potassium carbonate assisted ^{18}F-fluoride reaction followed by deprotection with catalytic sodium methoxide with subsequent silica gel column chromatography. The radiochemical yield of 2,6FGF **5** was 9% and the overall time for synthesis, deprotection and silica column purification was 2 hours 43 minutes. Careful purification of the product was essential since the by-product resulting from triflate hydrolysis of the precursor would form 2-fluoro-glucosyl fluoride that would compete more favourably for binding to the enzyme.

Enzyme kinetic data showed that 2,6FGF has a similar second order rate constant compared to the 2-deoxy-2-fluoro-β-D-mannopyranosyl fluoride used previously with GCase (0.0017 vs 0.0019 $min^{-1}mM^{-1}$). This indicates that substitution of the 6-OH of 2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride with fluorine has the same overall effect as changing the 2-F from the *gluco* epimer to the *manno* epimer. Thus, radiolabelling at the 6-position using ^{18}F-fluoride would yield a comparable inactivator to 2-deoxy-2-[^{18}F]-fluoro-β-D-mannopyranosyl [^{18}F]-fluoride.

CONCLUSIONS

A new glucocerebrosidase inactivator (2,6FGF) has been synthesized, in 69% overall chemical yield and in 9% (decay corrected) radiochemical yield, as a possible imaging agent for glucocerebrosidase. Enzyme studies on the cold compound suggest that this inactivator is an equivalent inactivator to the 2-fluoro-mannosyl fluoride inactivator previously synthesized. Enzyme binding and animal studies with ^{18}F-2,6FGF are underway and will be reported elsewhere.

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