^{18}F-FESB: Automated radiosynthesis, imaging and β-amyloid plaque studies of a novel ^{18}F-labeled PET tracer in transgenic mice with Alzheimer's Disease

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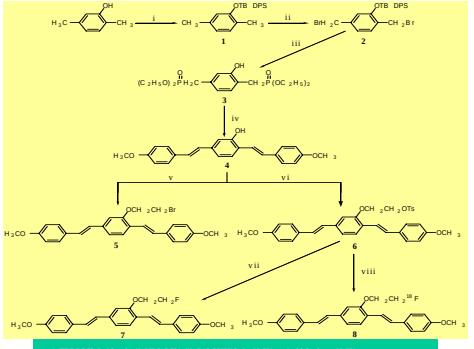
In spite of recent advances in new radiopharmaceuticals for the evaluation of Alzheimer's disease (AD), positron emission tomography (PET) and single photon emission tomography (SPECT) are not currently in routine use. The significant roles of amyloid cascades and neurofibrillary tangles (NFTs) in the pathogenesis of AD necessitate the development of a biomarker that facilitates early diagnosis of the disease, allows clinico-pathological correlations of amyloid deposition at an early stage and is receptor specific so that it serves as a true diagnostic tool for anti-amyloid therapies (1-3). A recent study suggests that anti-amyloid therapies, when co-investigated in combination with positron emission tomography (PET) or single photon emission computed tomography (SPECT) amyloid imaging tracers, could facilitate in vivo evaluation of the efficacy of therapy in the aging human brain. Earlier β-amyloid plaques related PET tracers for detecting the progression of Alzheimer's disorder include [11C]-labelled Congo Red and [99mTc]-Chrysamine-G analogs (4) and 11C-Methoxy XO4 and suffer from marginal brain entry and low radiochemical yields (5-8) which consequently makes the detection of the disease difficult. 11C-labeled radiopharmaceuticals also impose the risk of heavy total body radiation dose due to their short half life. Present work describes a structural modification of methoxy XO4 to overcome these problems where a radioactive fluorine is incorporated nucleophilically to produce a high specific activity radiopharmaceutical. Preliminary data on ionic fluid assisted radiofluorination of 18F-FESB and its PET imaging studies in transgenic Alzheimer's mouse.

EXPERIMENTAL

a) Chemistry:

The synthesis of 1-(2`-fluoroethoxy)-2,5-bis(4`-methoxystyryl)benzene **7** and its tosylate precursor started with 1-O-tert-butyldiphenylsilyl-2,5-dimethylphenol **1** (Scheme 1). 2,5-Dimethylphenol was silylated with tert-butyldiphenylsilane in anhydrous pyridine since it withstands the hard acidic conditions that exist during the bromination step. Bromination of the methyl groups at C-2 and C-5, using N-bromosuccinimide, proceeded through a free radical mechanism that was assisted by benzoyl peroxide, to afford 1-[(tert-butyldiphenylsilyl)oxy]-2,5-bis(bromomethyl)benzene **2** in 48 % yield. Reaction of the bromomethyl product **2** at 85°C, with triethylphosphite gave 1-[(tert-butyldiphenylsilyl)oxy]-2,5-bis(diethylphosphonatomethyl)benzene **3** in satisfactory yield (61%). The conversion of this diethylphosphonate derivative **3** to the corresponding 2,5-bis(4-methoxystyryl)phenol, **4**, progressed smoothly (74% yield). Tosylation of the free hydroxyl group using 1,2-bistoluenesulfonyloxyethane in anhydrous dimethylformamide afforded 1-(2`-toluenesulfonylethoxy)-2,5-bis(4`-methoxystyryl)benzene **6** as a yellow solid in 48% yield. The tosylate **6**, on reaction with tetrabutyl ammonium fluoride at 100°C, gave 1-(2`-fluoroethoxy)-2,5-bis(4`-methoxystyryl benzene), **7**, in 9% yield.



i=TBDPSCl, Imidazole; ii=NBS/CCl4; iii=P(OC2H5)3; iv=NaH/p-anisaldehyde; v=NaH/p-O2NC6H4O2SOCH2CH2Br; vi=(CH2OSO2C6H4CH3)2; vii=Emimtriflate/ Bu4NF/90°C/60 min and viii = ionic fluid/K10F/K-2.2.2./85°C/8-12 min

b) Radiofluorination:

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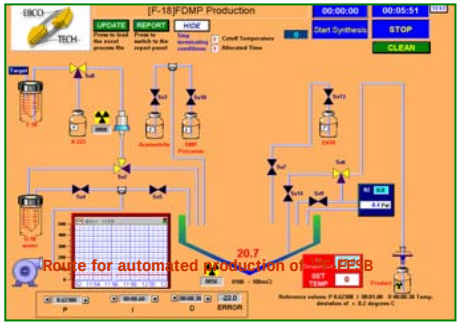
•The synthesis of ^{18}F labeled **8** was performed in an automated synthesis unit (ASU), initially using the conventional method of Kryptofix 2.2.2/K^{18}F complex assisted radiofluorination at elevated temperatures, but this provided low radiochemical yields (3-5%).

• Use of an appropriate ionic fluid in our radiofluorination reactions led to significant improvement in the labeling efficiency of ^{18}F-FESB (Table 1).

•A typical radiofluorination reaction involved reaction of tosylate precursor (4-6 mg) with K-2.2.2/radiofluoride complex (20 mg/60-70 GBq) in anhydrous acetonitrile (2 mL) and dimethylformamide (50 μL) at 110°C for 8-12 min in the presence of desired 'ionic fluid' (50 μL). The labeled product, after removal of the solvents was pushed out of the ASU with 1.5 mL of clinical grade ethanol and purified on a reversed phase HPLC to obtain pure ^{18}F-FESB.



Route for automated production of ^{18}F-FESB

c) HPLC Purification:

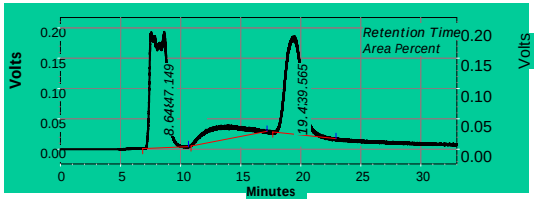
HPLC chromatography was performed to purify ^{18}F-FESB and determine it's specific activity. It involved the use of a Whatman reverse phase C-18 column (30 x 0.9 cm) as a stationary phase and EtOH:H$_2$O (75:25; v/v) as mobile phase. The elution was done at a flow rate of 1.5 mL/min. Auto-Scaled UV and radio chromatograms of ^{18}F-FESB were archived. A typical HPLC analysis of ^{18}F-FESB labeled mixture is as below.

Synthetic pathway for ^{18}F-FESB

• ^{18}F-FESB appears at 19.47 min (±5%) in its radio-chromatogram (it lags by ~0.4 min since the radio detector is attached after the UV detector).

•The retention times for emimtriflate and bmimtetrafluoroborate are 7.3 and 6.9 min respectively, therefore, the radioactive peaks appearing between 7-10 min in the radiochromatogram of the labeled ^{18}F-FESB mixture correspond to unreacted fluoride, radiofluorinated emimtriflate and bmimtetrafluoroborate (depending on the ionic solvent used in radiofluorination) salts formed due to the exchange of radioactive fluorine and unreacted ionic fluid.

• An uncharacterized radioactive hump appearing between 11-18 min is visible in the chromatogram (Fig. 1) but the purity of ^{18}F-FESB is not compromised during HPLC purification of the reaction mixture by collecting ^{18}F-FESB after 30 sec of the peak start.



HPLC Chromatogram of ^{18}F-FESB Reaction Mixture

RESULTS

Radiofluorination reaction parameters for ^{18}F-FESB

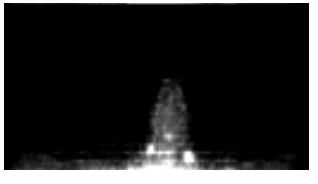
Reaction #	Ionic Fluid*	Amount (μL)	Reaction Time (min)	Purified RCY (%)	Specific Activity (Ci/μM)
1	BmimBF4	20	12	16.9	
2	BmimBF4	20	8	43.2	
3	BmimBF4	50	9	76.9	
4	Emimtriflate	50	8	39.6	>40
5	Emimtriflate	50	8	25.5	
6	Emimtriflate	50	8	42.5	
7	None	N/A	4	3.0	
8	None	N/A	8	7.0	

*Acetonitrile (2 mL) and anhydrous DMF (50 μL) were used as reaction solvent during the radiolabeling.

Digital Phosphor-imaging Studies:

In vitro binding of ^{18}FESB to mouse AD plaques (phosphor imaging): **15 min (right in each image) & 30 min (left in each image)** post-incubation with 0.2 MBq (left) and 0.6 MBq (right) of ^{18}FESB.

PET Imaging:



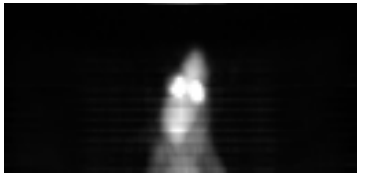


Figure a **Figure b**

PET scan of a transgenic AD mouse using a) ^{18}F-FESB and b) ^{18}F-FDG

CONCLUSION

The synthesis of 18F-FESB, a fluorinated Congo red derivative was developed using an ASU.
Use of 'ionic fluids' in the radiochemical synthesis significantly enhanced the labeling efficiency of the product.
An attempt to correlate radiochemical yield of 8 to varying amounts of the ionic fluids bmimBF4 or emimtriflate were inconclusive since correlations between these two parameters were not evident.
18F-FESB, being a fluorinated analog, has much longer half life than other 11C-labeled members of this class and, therefore, will have a direct impact on the patients radiation doses and delineation of β-amyloid plaques from other regions in the brain.
In vitro studies of mouse AD brain with 18F-FESB using digital phosphorimaging shows accumulation of radioactivity in the inner brain area indicating its uptake by β-amyloid plaques.
Congo Red staining of human AD brain tissues incubated with 18F-FESB shows its its specific binding to Aβ plaqques.
Initial PET imaging studies in AD mice with 18F-FESB show faint but specific uptake in the brain region.

ACKNOWLEDGEMENTS The authors gratefully acknowledge the Alberta Cancer Board and the University of Alberta Hospital Foundation for their financial support.