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Synthesis of ApoSense compound [¹⁸F]2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid ([¹⁸F]NST732) by nucleophilic ring-opening of an aziridine precursor

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Abstract

Introduction—The small molecule 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid (NST732) is a member of the ApoSense[®] family of compounds, capable of selective targeting, binding and accumulation within cells undergoing apoptotic cell death. It has application in molecular imaging and blood clotting particularly for monitoring anti-apoptotic drug treatments. We are investigating a fluorine-18 radiolabeled analog of this compound for positron emission tomography studies.

Methods—We prepared the tosylate precursor methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(tosyloxymethyl)butanoate (**4**) to synthesize fluorine-18 labeled NST732. Fluorination reaction of the tosylate precursor in 1:1 acetonitrile, dimethylsulfoxide with tetrabutyl ammonium fluoride (TBAF) proceeds through an aziridine intermediate (**4A**) to afford two regioisomers 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-fluorobutanoate (**5**) and methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoate (**6**). Acid hydrolysis of the fluoromethylbutanoate (**6**) isomer produced NST732. As the fluorination reaction of the tosylate precursor proceeds through an aziridine intermediate (**4A**) and the fluorination conceivably could be done directly on the aziridine, we have separately prepared an aziridine precursor (**4A**). Fluorine-18 labeling of the aziridine precursor (**4A**) was performed with [¹⁸F]tetrabutyl ammonium fluoride to afford the same two regioisomers (**5** and **6**). The [¹⁸F]2-((5-(dimethylamino)naphthalene-1-sulfonamido)methyl)-2-fluorobutanoic acid (NST732) was then obtained by the hydrolysis of corresponding [¹⁸F]-labeled ester (**6**) with 6N hydrochloric acid.

Results—Two regioisomers obtained from the fluorination reaction of aziridine were easily separated by HPLC. The total radiochemical yield was 15 ± 3% (uncorrected, n = 18) from the aziridine precursor, in a 70 min synthesis time with a radiochemical purity > 99%.

Conclusion—Fluorine-18 labeled aposense compound [¹⁸F]NST732 is prepared in moderate yield by direct fluorination of an aziridine precursor.

1. Introduction

Apoptosis or programmed cell death is a normal biological phenomenon of multi-cellular organisms. Apoptosis produces cell fragments called apoptotic bodies that are engulfed by healthy surrounding cells and tissues [1, 2], without local inflammation from leakage of cell contents. Abnormal apoptosis plays a role in an extensive variety of diseases. Molecular imaging of this process *in vivo* is a potentially powerful tool for early diagnosis of disease [3–6] and monitoring the efficiency of treatments with apoptosis-inducing anticancer drugs [7–11]. Additionally, imaging of apoptosis may assist the early evaluation of organ transplant rejection [12–14].

There are several changes during the early stages of apoptosis which can be imaged by different kinds of probe [15–18]. Phosphatidylserine (PS) is a phospholipid component, usually present on the inner-leaflet, normally confined to the cytoplasmic face of cell membranes by an enzyme called flippase [19, 20]. When a cell undergoes apoptotic cell death phosphatidylserine is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell. Annexin V, a 35.8-kDa protein has nanomolar affinity for PS (21). Imaging of apoptosis using annexin V attached to either a fluorescent probe or a radionuclide has been studied extensively (22–26). However, certain drawbacks such as non specific binding, poor signal/noise ratio and slow clearance from the non targeted tissue limit further application of this imaging agent in clinical studies [21]. Moreover, annexin V is a relatively large protein, requiring complex procedures for synthesis and detectable marker attachment. Therefore, several other proteins, peptides, small molecules and nanoparticles have been investigated for the detection of apoptosis [17, 27–32].

ApoSense[®] compounds are a family of small molecules which can specifically identify apoptotic cells. These compounds accumulate within the cytoplasm [17, 33] of apoptotic cells from the early stages of the death process, unlike annexin V, which binds to the PS head groups exposed on the surface. Molecular imaging and therapy developer Aposense[®] is currently in Phase 2 clinical trials of its apoptosis molecular imaging agent, fluorine-18 labeled ML-10 [NCT00791063, NCT00696943, NCT00805636].

The performance of other members of the ApoSense[®] family (fluorescent compounds DCC, NST-732, NST-729, tritium-labeled ML-9) has been reported in various animal models [5, 33–38]. Recent reports demonstrated the performance of fluorine-18 labeled dansylhydrazine (DFNSH) in detecting paclitaxel-induced cancer cell death and ketamine-induced neuronal apoptosis [39, 40]. Fluorine-18 labeling of dansyl group-containing NST732 has been proposed previously by Ziv et al [36]. In the present study, we are reporting the first synthesis of [¹⁸F]NST732 from the easy-to-prepare aziridine precursor.

2. Materials and Methods

2.1. Materials

Methyl 2-aminobutyrate hydrochloride was purchased from TCI America (Portland, OR, USA) and used as received. Tetrabutylammonium hydrogen carbonate (0.075 M) for radiolabeling work was obtained from ABX (Radeberg, Germany). Whole human serum was obtained from MP Biomedicals, LLC (Solon, OH, USA). All other commercially available organic precursors and dry solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA), and used as received unless otherwise stated. Tetrahydro-7-methoxy-3,7,7a-trimethyl-5-oxo-2H-oxazolo-[3,2-c]oxazole-3-carboxylic acid ethyl ester (**1**) [41] and methyl 2-(benzylideneamino)butanoate (**8**) [42] were prepared by literature method. Lithium diisopropylamide (LDA) was freshly prepared every time as needed [43].

Fluorine-18 was purchased from PETNET Solutions (North Wales, PA, USA). Silica gel 60 with a mesh 70–230 (Sigma-Aldrich) was used for flash chromatography. Thin-layer chromatography (TLC) was performed on silica gel 60 F-254 plates (Sigma-Aldrich). All the Sep-Pak® cartridges used in this synthesis were obtained from Waters (Milford, MA, USA).

2.2. General methods

Flash chromatography was performed on an AnaLogix IntelliFlash 280 system, using Biotage® SNAP Cartridges. APCI mass spectrometry (MS) was performed on a 6130 Quadrupole LC/MS Agilent Technologies instrument equipped with a diode array detector. ^1H ^{13}C and ^{19}F NMR spectra were recorded on a Varian spectrometer (400 MHz). Chemical shifts (ppm) are reported relative to the solvent residual peaks of acetonitrile (δ ^1H , 2.50 ppm; ^{13}C 118.26, 1.79) and chloroform (δ ^1H , 7.26 ppm; ^{13}C 77.36). ^{19}F NMR spectra are reported with reference to the trifluoroacetic acid (δ ^{19}F , –76.55 ppm). High-resolution mass spectra (HRMS) were collected on an Agilent Time-Of-Flight Mass Spectrometer (TOF, Agilent Technologies). A 3 minute gradient from 4 to 100% Acetonitrile (0.1% formic acid) in water (0.1% formic acid) was used with a 4 minute run time at a flow rate of 1 mL/min. A Zorbax SB-C18 column (2.1 $\times$ 30 mm, 3.5 μm) was used at a temperature of 50°C. Molecular formula was confirmed using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02). Radiosynthesis was performed manually. Purification of the radiolabeled product was done by HPLC on a Beckman Coulter System Gold instrument equipped with a multi-wavelength detector using an Agilent Eclipse C18 9.4 $\times$ 250 mm, 5 μm column. Analytical HPLC analyses for radiochemical work were performed on an Agilent 1200 Series instrument equipped with multi-wavelength detectors using an Agilent Eclipse XDB C18 column (4.6 $\times$ 150 mm, 5 μm) with a flow rate of 1.0 mL/min. All the microwave reactions for fluorine-18 labeling were done in a Biotage Initiator 2.5 using Biotage 10 mL V-shaped vials at constant temperature mode.

2.3. Methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(hydroxymethyl)butanoate (3)

Compound **1** (1.05 g, 3.85 mmol) was suspended in a solution of aqueous 6N HCl (28 ml) and MeOH (28 ml). The mixture was refluxed for 15 h. The solvents were evaporated to give the crude product **2** (586 mg, 3.2 mmol). Compound **2** (330 mg, 2.25 mmol) and *N*-dansyl chloride (608 mg, 3.2 mmol) were dissolved in anhydrous pyridine (12 ml). The solution was stirred at room temperature for 15 h. After the solvent was evaporated, the residue was dissolved in EtOAc (200 ml), washed with water, brine and dried over anhydrous Na_2SO_4 . After evaporation of the solvents, the crude reaction mixture was purified by column chromatography to give a yellow oil (458 mg, 54% yield). ^1H NMR (CDCl_3 , 400 MHz) δ 8.56 (m, 1H), 8.32 (m, 1H), 8.29 (dd, J = 7.3, 1.3 Hz, 1H), 7.61 (dd, J = 8.6, 7.6 Hz, 1H), 7.52 (dd, J = 8.5, 7.3 Hz, 1H), 7.19 (dd, J = 7.5, 0.7 Hz, 1H), 5.85 (s, 1H), 4.01 (dd, J = 12.3, 5.4 Hz, 1H), 3.77 (dd, J = 12.2, 9.7 Hz, 1H), 3.63 (s, 3H), 2.88 (s, 6H), 2.32 (dd, 1H, J = 9.7, 5.5 Hz, 1H), 1.65–1.55 (m, 2H), 0.42 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CDCl_3 , 100 MHz): δ 172.1, 152.1, 136.2, 134.0, 129.8, 129.5, 129.1, 128.8, 123.2, 118.6, 115.4, 69.60, 65.08, 53.08, 45.14, 25.98, 7.45. MS (ESI, m/z) calculated for $\text{C}_{18}\text{H}_{25}\text{N}_2\text{O}_5\text{S}$, 381.15, found 381 ($\text{M}+\text{H}$) $^+$.

2.4. Methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2((tosyloxy)methyl) butanoate (4)

Compound **3** (248 mg, 0.75 mmol) and *p*-tosyl chloride (473 mg, 2.5 mmol) were dissolved in anhydrous pyridine (2 ml). The solution was stirred at room temperature for 15 h. The

solvent was evaporated and the residue was dissolved in EtOAc (50 ml), washed with water, brine and dried over anhydrous Na₂SO₄. The crude reaction mixture was purified by column chromatography to afford a yellow oil (311 mg, 90% yield). ¹H NMR (CDCl₃, 400 MHz) δ 8.52 (m, 1H), 8.21 (d, *J* = 8.8 Hz, 1H), 8.17 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.62-7.56 (m, 3H), 7.47 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.26-7.25 (m, 2H), 7.19 (d, *J* = 7.4 Hz, 1H), 5.70 (s, 1H), 4.43 (dd, *J* = 10.0 Hz, 1H), 4.24 (d, *J* = 10.0 Hz, 1H), 3.63 (s, 3H), 2.89 (s, 6H), 2.43 (s, 3H), 1.98 (m, 1H), 1.62 (m, 1H), 0.43 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.1, 151.9, 144.9, 137.2, 132.3, 130.6, 129.8, 129.7, 129.4, 128.5, 128.2, 127.8, 123.1, 118.6, 115.2, 70.01, 66.56, 53.22, 45.43, 26.12, 21.67, 7.22. HRMS (ESI-TOF) calculated for C₂₅H₃₁N₂O₇S₂, 536.1601, found 536.1598 (M + H)⁺.

2.5. Methyl 2-(benzylideneamino)-2-(bromomethyl)butanoate (9-Br)

To a freshly prepared solution of LDA (20 mmol) in THF (25 ml) under argon at -78 °C was added a solution of methyl 2-(benzylideneamino)butanoate (2.06 g, 10 mmol) in THF (25 ml) and hexamethyl phosphoramide (HMPA) (10 ml). The color of the reaction mixture turned brown. The mixture was stirred for 30 min at -78 °C and CH₂Br₂ (1.5 ml, 20 mmol) was added. The mixture was allowed to warm up to room temperature and the stirring was continued for 16 h. Solvent was evaporated under reduced pressure and water (100 ml) was added to the residue. The product was extracted with diethyl ether (2 × 100 ml), dried over Na₂SO₄. Solvent was evaporated to dryness and the residue was purified by chromatography (30% ethyl acetate in hexane) to afford title compound methyl 2-(benzylideneamino)-2-(bromomethyl)butanoate (2 g, 66%) as a yellow solid. ¹H NMR (CDCl₃, 400 MHz): δ 8.33 (s, 1H), 7.77 (dd, *J* = 7.6, 1.6 Hz, 2H), 7.45-7.40 (m, 3H), 3.87 (d, *J* = 10.3 Hz, 1H), 3.79 (d, *J* = 10.6 Hz, 1H), 3.77 (s, 3H), 2.09 (q, *J* = 7.4 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 171.6, 161.5, 136.0, 131.3, 128.6, 128.4, 71.67, 52.34, 36.89, 29.35, 8.07. MS (ESI, *m/z*) calculated for C₁₃H₁₇BrNO₂, 298.04, found 298 (M+H)⁺.

2.6. Methyl 1-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-2-ethylaziridine-2-carboxylate (4A)

To a solution of methyl 2-(benzylideneamino)-2-(bromomethyl)butanoate (**9-Br**, 1.2 g, mmol) in diethyl ether (30 ml), 2N HCl (15 ml) was added. After the reaction mixture was stirred for 3 h, the aqueous layer was separated and washed with diethyl ether (2 × 50 ml). Water was removed under reduced pressure to afford methyl 2-amino-2-(bromomethyl)butanoate hydrochloride (0.98 g, 3.98 mmol) as a white solid. To a mixture of methyl 2-amino-2-(bromomethyl)butanoate hydrochloride (1 g, 4.06 mmol) and triethyl amine (2.1 ml, 12.15 mmol) in acetonitrile (30 ml), a solution of 5-(dimethylamino)naphthalene-1-sulfonyl chloride (1.3 g, 4.82 mmol) in acetonitrile (10 ml) was added. The mixture was stirred for 24 h. A saturated solution of Na₂CO₃ (40 ml) was added and the mixture was stirred for 3 h. Acetonitrile was evaporated under reduced pressure and water (50 ml) was added to the residue. The product was extracted with dichloromethane (2 × 100 ml), dried over Na₂SO₄. Solvent was evaporated to dryness and the residue was purified by flash chromatography using chloroform eluent to produce title compound methyl 1-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-2-ethylaziridine-2-carboxylate (0.89 g, 59%) as yellow liquid. ¹H NMR (CDCl₃, 400 MHz): δ 8.75 (d, *J* = 8.5 Hz, 1H), 8.47 (d, *J* = 8.7 Hz, 1H), 8.20 (d, *J* = 8.2 Hz, 1H), 7.58-7.49 (m, 2H), 7.17 (d, *J* = 7.6 Hz, 1H), 3.77 (s, 3H), 3.02 (s, 1H), 2.86 (s, 6H), 2.62 (s, 1H), 2.39-2.30 (m, 1H), 2.03-1.94 (m, 1H), 1.04 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 168.0, 151.6, 135.2, 131.8, 130.1, 129.9, 129.1, 128.1, 122.9, 120.1, 115.2, 52.88, 52.57, 45.42, 37.85, 24.50, 10.48. HRMS (ESI-TOF) calculated for C₁₈H₂₃N₂O₄S, 364.1396, found 364.1403 (M + H)⁺.

2.7. 2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid (NST732) and 2-((5-(Dimethylamino)naphthalene-1-sulfonamido)methyl)-2-fluorobutanoic acid (7)

2.7.1. Method A: From the tosylate precursor—A tetrahydrofuran solution (1M) of tetrabutylammonium fluorine (0.55 mL, 0.55 mmol) was dried overnight under reduced pressure. To the solid was added a 5 mL solution of compound **4** (100 mg, 0.19 mmol) in 1:1 dimethylsulfoxide:acetonitrile and the reaction mixture was heated at 140 °C for 15 min. To this solution was added 100 mL of water and the mixture was extracted with dichloromethane. The dichloromethane solution was washed with 2N HCl and dried over Na₂SO₄. The solvent was evaporated under vacuum. To the crude reaction mixture in 1 mL of acetonitrile was added 2 mL of 6N HCl and the reaction mixture was heated at 160 °C for 20 min. Two isomers were separated by HPLC {method: 25–30% B for 30 min; solvents: A = water with 0.1% TFA, B = acetonitrile with 0.1% TFA; column: Acentis RP-Amide, 10 × 100 mm, 5 µm; Peaks were collected at 5.899 min (**7**) and 9.765 min (NST732)} to obtain NST732 (11 mg, 0.03 mmol, 15%) and compound **7** (17 mg, 0.04 mmol, 24%).

¹H NMR of NST732 (CD₃CN, 400 MHz) δ 8.55 (d, *J* = 8.6 Hz, 1H), 8.49 (d, *J* = 8.6 Hz, 1H), 8.29 (dd, *J* = 1.2, 7.2 Hz, 1H), 7.73–7.64 (m, *J* = 7.7 Hz, 2H), 7.53 (d, *J* = 7.4 Hz, 1H), 6.29 (s, 1H), 4.70 (dd, *J* = 9.8, 4.7 Hz, 1H), 4.59 (dd, *J* = 8.6, 46.6 Hz, 1H), 3.06 (s, 6H), 1.86–1.65 (m, 2H), 0.57 (t, *J* = 7.4 Hz, 3H). ¹⁹F NMR (CD₃CN, 376 MHz): –227.9 (t, *J* = 46.4 Hz). ¹³C NMR (CD₃CN, 100 MHz): δ 171.2 (*J* = 4.7 Hz), 147.9, 138.7, 129.5, 129, 128.8, 128.6, 128.3, 124.8, 121.9, 117.0, 83.26 (*J* = 175 Hz), 66.78 (*J* = 18 Hz), 45.66, 25.56 (*J* = 4.7 Hz), 6.91. HRMS (ESI-TOF) calculated for C₁₇H₂₂FN₂O₄S, 369.1286, found 369.1279 (M + H)⁺.

¹H NMR of Compound **7** (CD₃CN, 400 MHz) δ 8.54 (d, *J* = 9.0 Hz, 2H), 8.24 (dd, *J* = 1.2, 8.2 Hz, 1H), 7.74–7.68 (m, 2H), 7.61 (d, *J* = 8.2 Hz, 1H), 6.24 (t, *J* = 6.3 Hz, 1H), 3.48–3.19 (m, 2H), 3.12 (s, 6H), 1.78–1.66 (m, 2H), 0.81 (t, *J* = 7.4 Hz, 3H). ¹⁹F NMR (CD₃CN, 376 MHz): –169.8 – 170.1 (m). ¹³C NMR (CD₃CN, 100 MHz): δ 170.0 (*J* = 27 Hz), 146.9, 136, 129.6, 129.4, 128.8, 128.4, 127.9, 124.8, 122.4, 117.1, 96.96 (*J* = 187 Hz), 47.96 (*J* = 22 Hz), 45.53, 27.45 (*J* = 22 Hz), 6.59. HRMS (ESI-TOF) calculated for C₁₇H₂₂FN₂O₄S, 369.1279, found 369.1279 (M + H)⁺.

2.7.2. Method B: From Compound **8** (independent synthesis)

2.7.2.1. Methyl 2-(benzylideneamino)-2-(fluoromethyl)butanoate (9-F): The compound was prepared following the same procedure of **9-Br** using fluorochloromethane instead of dibromomethane.

¹H NMR (CDCl₃, 400 MHz): δ 8.38 (s, 1H), 7.78–7.40 (m, 5H), 4.81 (dd, *J* = 47, 9.4 Hz, 1H), 4.69 (dd, *J* = 47, 9.2 Hz, 1H), 3.78 (s, 3H), 2.05–1.98 (m, 2H), 0.98 (t, *J* = 7.8 Hz, 3H). ¹⁹F NMR (CDCl₃, 376 MHz): –227.16 (t, *J* = 47.09 Hz). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.8 (*J* = 3.9 Hz), 162.3, 136.2, 131.2, 128.6, 128.4, 84.57 (*J* = 177 Hz), 71.59 (*J* = 18 Hz), 52.29, 27.39 (*J* = 4.7 Hz), 8.13. MS (ESI, *m/z*) calculated for C₁₃H₁₇FNO₂ 238.12, found 238 (M+H)⁺.

2.7.2.2. Methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoate (**6**):

The compound was prepared following the same procedure of the aziridine precursor (**4A**) from **10-F**.

¹H NMR (CDCl₃, 400 MHz): δ 8.52 (dt, *J* = 8.5, 1.08 Hz, 1H), 8.27 (dt, *J* = 8.7, 1 Hz, 1H), 8.25 (dd, *J* = 7.4, 1.1 Hz, 1H), 7.63–7.47 (m, 2H), 7.17 (dd, *J* = 7.5 Hz, 1H), 5.83 (s, 1H), 4.79 (d, *J* = 46.9, 9.7 Hz, 1H), 4.55 (d, *J* = 46.2, 9.6 Hz, 1H), 3.67 (s, 3H), 2.86 (s, 6H),

2.04-1.63 (m, 2H), 0.66 (t, $J = 7.4$ Hz, 3H). ^{19}F NMR (CDCl_3 , 376 MHz): -227.6 (t, $J = 47.7$ Hz). ^{13}C NMR (CDCl_3 , 100 MHz): δ 170.7 ($J = 4$ Hz), 151.9, 137.4, 130.4, 129.8, 129.5, 128.5, 128.1, 123.0, 118.7, 115.2, 83.78 ($J = 180$ Hz), 67.46 ($J = 17$ Hz), 53.25, 45.39, 25.32, 7.27. MS (ESI, m/z) calculated for $\text{C}_{18}\text{H}_{24}\text{FN}_2\text{O}_4\text{S}$, 383.14, found 383 ($\text{M} + \text{H}$) $^+$.

2.7.2.3. 2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid (NST732): To the acetonitrile solution (0.3 mL) of compound **6** prepared from compound **10-F** was added 6N HCl and the mixture was heated at 130 °C in a microwave for 1 h. The solution was neutralized with K_2CO_3 , the NST732 was extracted with acetonitrile and the solvent was evaporated. Mass and NMR spectra were matched with the authentic NST732 prepared from the tosylate precursor (**4**).

2.9. Synthesis of [^{18}F]2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid (NST732)

Fluorine-18 (2 – 5 mCi) was eluted from a cartridge (PETNET) into a 0.5 – 2 mL Biotage V-shaped vial with 50 μl TBAHCO₃ mixed with 300 μl of water followed by 1 ml of acetonitrile and dried under nitrogen at 120°C. The residue was further azeotropically dried with (3 $\times$ 1 mL) anhydrous acetonitrile at the same temperature under nitrogen. To the dried activity was added methyl 1-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-2-ethylaziridine-2-carboxylate (2–3 mg) in anhydrous acetonitrile (300 μl) and the reaction mixture was heated at 100°C in a microwave for 10 min. The reaction mixture was passed through a silica plus Sep-Pak[®] cartridge and the compound was eluted with dichloromethane (3 ml) into a microwave vial. The solvent was evaporated at 60°C under nitrogen. To the residue was added 6N HCl (0.6 ml) and the mixture was heated at 160°C for 20 min in a microwave. After cooling down the reaction mixture to room temperature the solution was partially neutralized with 6N NaOH (0.5 ml) and injected to a semi-preparative HPLC {Agilent Eclipse C18 9.4 $\times$ 250 mm, 5 μm column, eluent: 35% CH_3CN (0.1% TFA), 65% H_2O (0.1% TFA), flow rate = 3 mL/min}. The fraction containing NST732 ($t_R = 7.7$ min) was collected. The collected fraction was diluted with 10 mL water and passed through a Sep-Pak[®] light C18 cartridge (pre-conditioned with 5 mL of ethanol, 10 mL of water, 10 mL of air). The trapped [^{18}F]NST732 was eluted with 1 mL of ethanol. The compound was formulated for further use by evaporating the ethanol at 70°C under nitrogen and the [^{18}F]NST732 was dissolved in 2 mL of 10% ethanol in PBS 1X.

2.10. Distribution coefficient

The organic phase n-octanol and the aqueous phase PBS (pH = 7.4) were mixed together for 24 h and separated prior to the experiment. Fluorine-18 labeled NST732 (1 mCi) was added to the octanol (2.5mL)/PBS (2.5mL) mixture, vibrated for 3 min and centrifuged for 5 min. The amount of activity in each phase (1 mL) was measured to determine the partition coefficient ($\log D = \text{activity in 1-octanol}/\text{activity in buffer}$).

2.11. Stability test

To test the serum stability 1 mCi of [^{18}F]NST732 in 100 μL of ethanol was added to human serum (2 mL), kept in an water bath at 37 °C and monitored by RP-HPLC up to 6 h (supporting information).

Results and Discussion

3. 1. Synthesis of precursor and standard compounds

To prepare [^{18}F]2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid ([^{18}F]NST732) we synthesized the following precursors: the tosylate precursor, methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-((tosyloxy)methyl)butanoate (**4**) and the aziridine precursor, methyl 1-((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-2-ethylaziridine-2-carboxylate (**4A**).

Synthesis of the tosylate precursor methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-((tosyloxy)methyl)butanoate (**4**) is summarized in Scheme 1. Compound **1** was prepared according to the literature method [41] without regard to stereochemistry. Hydrolysis of compound **1** in methanol produced compound **2**. The amine group of **2** was subsequently functionalized with the dansyl fluorophore and provided **3** in 54% yield. Finally, the primary hydroxyl group of **3** was tosylated to produce tosylate precursor (**4**) in 90% yield.

The fluorination reaction of the tosylate precursor was done first with tetrabutylammonium fluoride (TBAF) to prepare the nonradioactive standard of [^{18}F]NST732 (Scheme 2). The progress of the reaction was monitored by LC/MS, observing two major peaks (m/z 383, compound **5** and **6**). Fluorination of the tosylate precursor was tested at different temperatures, solvents and fluorinating agents (TBAF, CsF, KF/K_{222}) and it was found that the best yield was obtained at 140°C in a 1:1 dimethylsulfoxide:acetonitrile mixture with TBAF. Formation of the two isomers can be explained by the formation of the aziridine intermediate (**4A**) followed by ring opening of the aziridine with fluoride ion. Formation of the aziridine intermediate was indeed confirmed by LC/MS from the reaction of tosylate precursor (**4**) with TBAF at room temperature in a 1:1 dimethylsulfoxide:acetonitrile mixture. Finally, by hydrolysing these esters with 6N HCl at 160°C for 20 min and HPLC purification we were able to isolate nonradioactive standard NST732 in 15% and its isomer in 24% (Scheme 2) yield, respectively.

As reaction of the tosylate precursor proceeded through an aziridine intermediate (**4A**) we prepared the aziridine precursor separately (Scheme 3) from commercially available methyl 2-aminobutanoate. Protection of the amine group was done by the formation of Schiff's base according to the previously published method [42]. The reaction of compound (**8**) with dibromomethane in the presence of freshly prepared lithium diisopropylamide (LDA) afforded bromo derivative (**9-Br**). Deprotection of the amine protecting group with 2N hydrochloric acid followed by dansylation produced the aziridine precursor (**4A**). In order to determine the fluorination efficiency, the aziridine precursor was reacted with tetrabutylammonium fluoride (TBAF). The yield of the reaction was comparable to that seen with the tosylate precursor.

3. 2. Radiosynthesis of [^{18}F]NST732

Fluorine-18 labeling of the tosylate precursor with [^{18}F]TBAF in 1:1 DMSO/ACN at 140 °C was not clean and only a minor amount of the desired product (**6**) was obtained. No attempts were made to improve the yield by changing the solvent and temperature. However, reaction of the aziridine with [^{18}F]TBAF proceeded cleanly. The fluorination efficiency was tested at different temperatures (Table 1) and the progress of the reaction was monitored by analytical HPLC (Fig. 2).

Table 1 shows that the best conversion ratio (50:38) of ester mixture (**5** and **6**) was obtained when the reaction was done at 100°C in acetonitrile. Hydrolysis of ester mixtures (**5** and **6**)

with 6N HCl (0.3 ml) at 160°C produced [^{18}F]NST732 (Fig. 3) along with its regioisomer (7). Nucleophilic ring openings of aziridines by fluorine-18 are rare [44–48] although this could be a feasible route to prepare various fluorine-18 labeled radiopharmaceuticals. In 2009 Vasdev et al. [47] reported a highly regioselective ring-opening of *N*-benzyloxycarbonyl-protected 2-methylaziridine by fluorine-18 and noted that the ratio of the two isomers could be controlled by the solvent and by modifying the substituent on the nitrogen. In this synthesis we did not have the option of modifying the substituent on the nitrogen but we did explore the effects of solvent. Here, electronic properties play a major role and fluorine-18 was seen to attack preferably at the quaternary carbon of the aziridine ring rather than the secondary carbon. Partial positive charge on nitrogen of the aziridine ring weakens the nitrogen-to-quaternary carbon bond [49] and the resulting partial positive charge on the quaternary carbon may be further stabilized by the lone pair of the carboxylate oxygen. We believe that these factors facilitate nucleophilic attack preferentially at the quaternary carbon [50]. The cold standard NST732 was also independently synthesized following the same procedure used to prepare the aziridine precursor, by using fluorochloromethane instead of dibromothethane (Scheme 3). Characterization data for the independently synthesized molecule is a match with the minor product from the aziridine ring cleavage reaction with fluorine-19.

The initial aim of this study was to prepare [^{18}F]NST732 but the fluorination of the aziridine precursors produced two regioisomers in moderate to high yields. As isomers were separable in HPLC, this could be a useful method to prepare fluorine-18 labeled NST732. Moreover the other regioisomer 7 contains a dansyl amino group and could also potentially be an apoptosis imaging agent.

The total radiochemical yield was $15 \pm 3\%$ (uncorrected, $n = 18$) from the aziridine precursor (4A) in a 70-min synthesis time with very high radiochemical (> 99%) and chemical purity (>98%) (Fig. 4a). The specific activity of the product was 170 ± 90 Ci/mmol ($n = 6$). The specific activity is low due to the low amount [51] of starting [^{18}F]fluoride ion used in this synthesis. The identity of the [^{18}F]NST732 was further confirmed by comparing the HPLC retention time with co-injected, authentic nonradioactive NST732 (Fig. 4b). Distribution coefficient (logD) was measured (-0.18 ± 0.04 , $n = 3$) using 1-octanol and phosphate buffer to get an idea of the lipophilicity of the compound. The compound demonstrated excellent serum stability over 6 h at 37 °C. No defluorination or decomposition of the product was observed (supporting information).

Conclusion

We have synthesized two different precursors and performed fluorine-18 labeling reactions with [^{18}F]TBAF to prepare [^{18}F]NST732. An uncorrected radiochemical yield of $15 \pm 3\%$ (uncorrected, $n = 18$) was obtained using aziridine precursor within a 70 min synthesis time. The described procedure would be easily adaptable to the commercially available automatic synthesis units. Additionally, fluorination of the aziridine precursor also produced a regioisomer of [^{18}F]NST732 which could also represent a potential PET tracer for apoptosis imaging.

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Fig. 1.
Structure of NST732.

Fig. 2. HPLC analysis of the crude reaction mixture of compound **5** ($t_R = 7.3$ min) and its regioisomer (**6**, $t_R = 8.7$ min). HPLC conditions: Agilent Eclipse XDB C18 5 μ m, 4.6 $\times$ 150 mm column, 35–50% acetonitrile in water (0.1% TFA) in 10 min, flow: 1 ml/min; solid line, in-line radiodetector; dotted line, UV detector at 254 nm.

Fig. 3. HPLC analysis of the crude reaction mixture of [^{18}F]NST732 ($t_R = 7.4$ min) and its regioisomer (**7**, $t_R = 6.0$ min). HPLC conditions: Agilent Eclipse XDB C18 4.6×150 mm, $5 \mu\text{m}$ column, 20–40% acetonitrile in water (0.1% TFA) for 10 min, flow: 1 ml/min; solid line, in-line radiodetector; dotted line, UV detector at 254 nm.

Fig. 4. HPLC analysis of a) [^{18}F]NST732 after formulation; b) [^{18}F]NST732 co-injected with the non-radioactive standard. HPLC conditions: Agilent Eclipse XDB C18 4.6×150 mm, $5\ \mu\text{m}$ column, 20–40% acetonitrile in water (0.1% TFA) for 10 min, flow: 1 ml/min; solid line, in-line radiodetector; dotted line, UV detector at 254 nm.

Scheme 1.

Synthesis of the tosylate precursor methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-((tosyloxy)methyl)butanoate (**4**).

- i) TBAF, DMSO/CH₃CN (1:1), 140 °C, 15 min
 ii) 6N HCl, Microwave, 160 °C, 20 min

Scheme 2.
 Fluorination reaction of the tosylate precursor.

Scheme 3.

Preparation of the aziridine precursor and the non-radioactive standard NST732.

Table 1

Fluorine-18 labeling of aziridine precursor

Entry	Solvent ^a (400 μ L)	Temperature ($^{\circ}$ C)	RCY ^b (%; n = 6)	
			Compound 5	Compound 6
1	DMSO/ACN	140	25 $\pm$ 5	16 $\pm$ 4
2	DMSO/ACN	120	35 $\pm$ 10	23 $\pm$ 7
3	DMSO/ACN	100	51 $\pm$ 8	32 $\pm$ 5
4	DMSO/ACN	90	40 $\pm$ 5	30 $\pm$ 10
5	Acetonitrile	100	50 $\pm$ 4	38 $\pm$ 6
6	DMSO	100	30 $\pm$ 5	20 $\pm$ 7

^a 1:1 DMSO/ACN for entry 1–4; RCY = radiochemical yield;^b radiolabeling was carried out with 2–5 mCi of activity, 1–2 mg of precursor, 10 min. Yield was determined by radio-HPLC.