

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

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Background

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 without local inflammation from leakage of cell contents.^{1,2} Abnormal apoptosis plays a role in an extensive variety of diseases. Molecular imaging of this process *in vivo* is potentially a powerful tool for early diagnosis of disease³⁻⁶ and monitoring the efficiency of treatments with apoptosis-inducing anticancer drugs.⁷⁻¹¹ Additionally, imaging of apoptosis may assist with early evaluation of organ transplant rejection.¹²⁻¹⁴

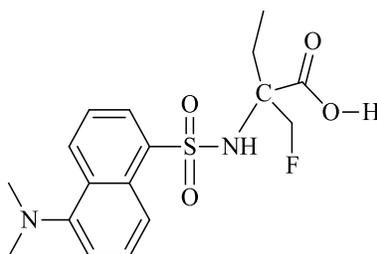


Figure 1. Structure of NST732

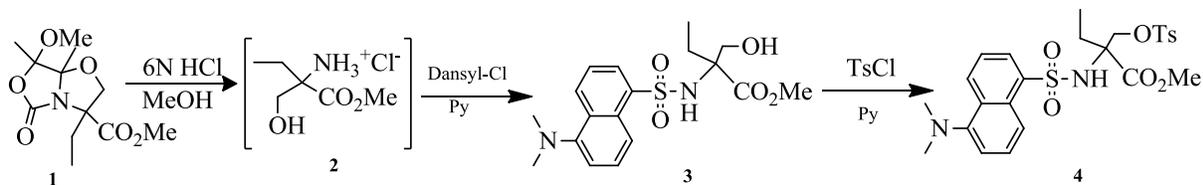
ApoSense[®] compounds are a family of small molecules which can specifically identify apoptotic cells. These compounds accumulate within the cytoplasm^{15,16} of apoptotic cells from the early stages of the death process, unlike annexin V, which binds to the phosphatidylserine head groups exposed on the surface. Fluorine-18 labeled ML-10 [NCT00791063, NCT00696943, NCT00805636] developed by Aposense[®] is currently undergoing Phase 2 clinical trials. 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, 16-21} Recent

reports demonstrated the performance of fluorine-18 labeled dansylhydrazone (DFNSH) in detecting paclitaxel-induced cancer cell death and ketamine-induced neuronal apoptosis.^{22, 23}

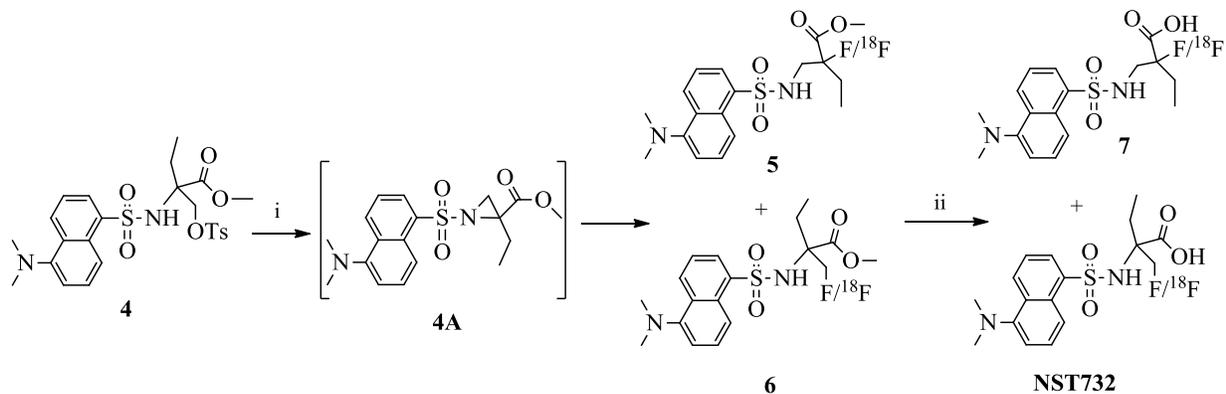
Fluorine-18 labeling of dansyl group-containing NST732 has been proposed previously by Ziv et al.¹⁹ In the present study, the first synthesis of [¹⁸F]NST732 from the easy-to-prepare aziridine precursor is reported.

Chemistry

To prepare [¹⁸F]2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid ([¹⁸F]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. The fluorination reaction of the tosylate precursor was done with tetrabutylammonium fluoride (TBAF), first to prepare the nonradioactive standard of [¹⁸F]NST732 (Scheme 2). The nonradioactive standard of [¹⁸F]NST732 was also prepared independently according to the Scheme 3. 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. Fluorine-18 labeling of the tosylate precursor with [¹⁸F]TBAF in 1:1 DMSO/ACN at 140 °C was not clean and only a minor amount of the desired product was obtained. However, reaction of the aziridine with [¹⁸F]TBAF proceeded cleanly. **Scheme 1.** Synthesis of the tosylate precursor methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-((tosyloxy)methyl)butanoate (**4**)



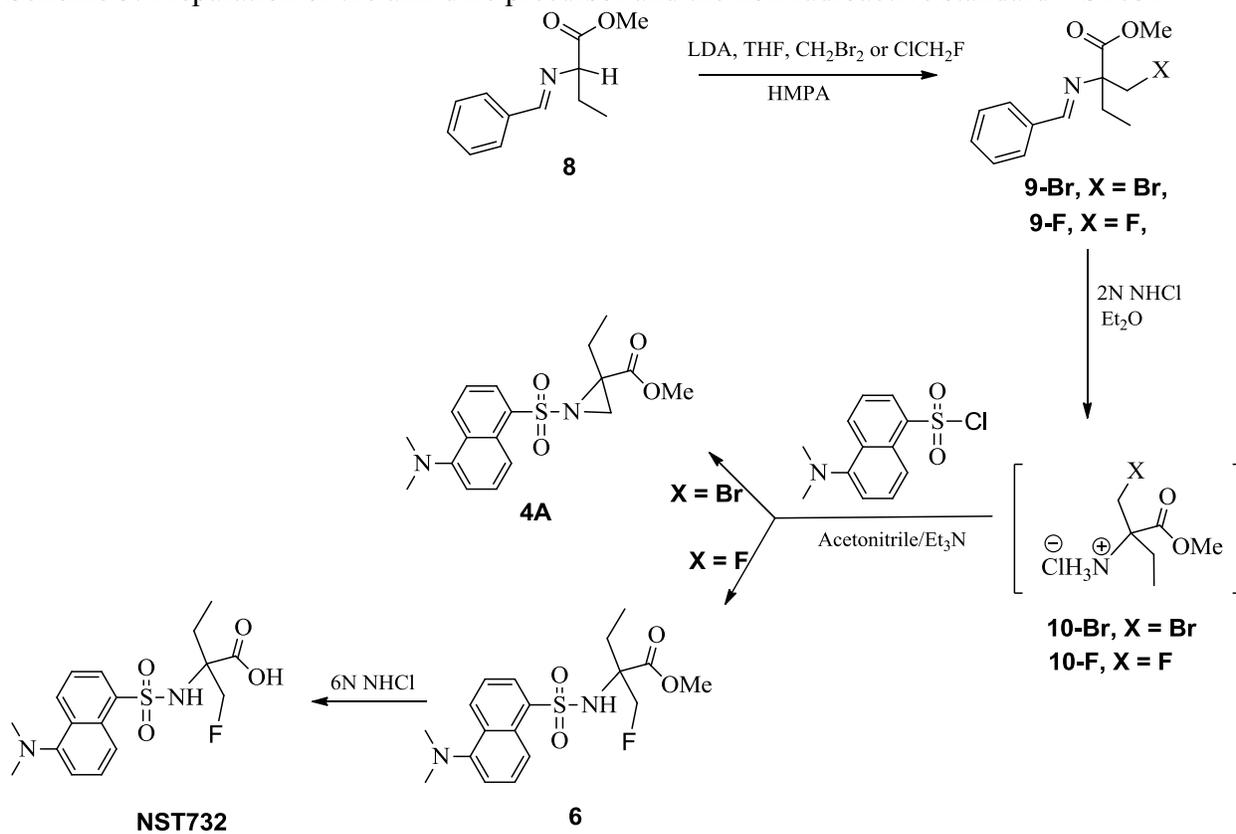
Scheme 2. Fluorination reaction of the tosylate precursor



i) TBAF, DMSO/CH₃CN (1:1), 140 °C, 15 min

ii) 6N HCl, Microwave, 160 °C, 20 min

Scheme 3. Preparation of the aziridine precursor and the non-radioactive standard NST732



Experimental

General. 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). 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**)²⁴ and (E)-methyl 2-(benzylideneamino)butanoate (**8**)²⁵ were prepared by the literature method. Lithium diisopropylamide (LDA) was freshly prepared every time, as needed.²⁶ Fluorine-18 was purchased from PETNET Solutions (North Wales, PA,). Silica gel 60 70-230 mesh (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,).

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 and ¹⁹F 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) and chloroform (δ ¹H, 7.26 ppm). ¹⁹F NMR spectra are reported with reference to the trifluoroacetic acid (δ ¹⁹F, -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 (3.5 micron, 2.1 x 30 mm) was used at a temperature of 50°C. Confirmation of 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 5 μ m, 9.4 x 250 mm 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 x 150 mm, 5 μ m) 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.

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 Na₂SO₄. After evaporation of the solvents, the crude reaction mixture was purified by column chromatography (ethyl acetate/hexane 1:3) to give a yellow oil (458 mg, 54% yield). ¹H NMR (CDCl₃, 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). MS (ESI, *m/z*) calculated for C₁₈H₂₅N₂O₅S, 381.15, found 381 (M+H)⁺.

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, 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 (ethyl acetate/hexane 1:5) 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). HRMS (ESI-TOF) calculated for C₂₅H₃₁N₂O₇S₂, 536.1601, found 536.1598 (M + H)⁺.

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

hexamethylphosphoramide (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. The 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. The 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). MS (ESI, *m/z*) calculated for C₁₃H₁₇BrNO₂, 298.04, found 298 (M+H)⁺.

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) was added 2N HCl (15 ml). 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) was added a solution of 5-(dimethylamino)naphthalene-1-sulfonyl chloride (1.3 g, 4.82 mmol) in acetonitrile (10 ml). 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. The solvent was evaporated to dryness and the residue was purified by flash chromatography using chloroform eluent to produce the 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). HRMS (ESI-TOF) calculated for C₁₈H₂₃N₂O₄S, 364.1396, found 364.1403 (M + H)⁺.

2-(5-(Dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid (NST732) and 2-((5-(Dimethylamino)naphthalene-1-sulfonamido)methyl)-2-fluorobutanoic acid (7). Method A: From the tosylate precursor. A tetrahydrofuran solution (1M) of tetrabutylammonium fluoride (0.55 mL, 0.55 mmol) was dried overnight under reduced pressure. To the solid was added a 5 mL solution of compound **4** in 1:1 dimethylsulfoxide:acetonitrile (100 mg, 0.19 mmol) 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 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: Ascentis 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, 47 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). 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). HRMS (ESI-TOF) calculated for C₁₇H₂₂FN₂O₄S, 369.1279, found 369.1279 (M + H)⁺.

Method B: From Compound **8 (independent synthesis) 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). ^{19}F NMR (CDCl_3 , 376 MHz): -227.16 (t, $J = 47.09$ Hz). MS (ESI, m/z) calculated for $\text{C}_{13}\text{H}_{17}\text{FNO}_2$ 238.12, found 238 (M+H) $^+$.

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 (CDCl_3 , 400 MHz): δ 8.52 (dt, $J = 8.5$ Hz, 1H), 8.27 (dt, $J = 8.7$ Hz, 1H), 8.25 (dd, $J = 8.2$ Hz, 1H), 7.58 (dd, 1H), 7.49 (dd, $J = 7.6$ Hz, 1H), 7.17 (dd, $J = 7.6$ Hz, 1H), 5.83 (s, 1H), 4.79 (d, $J = 46.9$ Hz, 1H), 4.55 (d, $J = 46.2$ 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). MS (ESI, m/z) calculated for $\text{C}_{18}\text{H}_{24}\text{FN}_2\text{O}_4\text{S}$, 383.14, found 383 (M+H) $^+$.

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**).

Synthesis of [^{18}F]2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-

(fluoromethyl)butanoic acid (NST732). Fluorine-18 (3 – 5 mCi) was eluted from a cartridge (PETNET) into a 10-mL Biotage V-shaped vial with 50 μl TBAHCO_3 , 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 5 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) with subsequent heating at 160°C for 20 min in a microwave. After cooling 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 5 μ , 9.4 x 250 mm 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 [¹⁸F]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 [¹⁸F]NST732 was dissolved in 2 mL of 10% ethanol in PBS 1X. The total radiochemical yield was 11-18% (uncorrected, n = 8) in a 70-min synthesis time with a radiochemical purity > 99%.

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