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Fluorine-18 labeling of S100 proteins for small animal positron emission tomography

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i. Running Head

^{18}F-labeling of S100 proteins for small animal PET

ii. Abstract

The interaction of S100 proteins (S100s), a multigenic family of Ca^{2+}-binding and -modulated proteins, with pattern recognition receptors, e.g., Toll-like receptors (TLRs), the receptor for advanced glycation endproducts (RAGE), or scavenger receptors (SR), is hypothesized to be of high relevance in the pathogenesis of various diseases. This includes chronic inflammatory conditions, atherosclerosis, cardiomyopathies, neurodegeneration, and progression of cancers. However, data concerning the role of circulating S100s in these pathologies are scarce. One reason for this is the shortage of suitable radiolabeling methods for direct assessment of the metabolic fate of circulating S100s in vivo. We report a radiotracer approach using radiolabeling of recombinant human S100s with the positron emitter fluorine-18 (^{18}F) by conjugation with N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB). The methodological radiochemical part focuses on an optimized and automated synthesis of [^{18}F]SFB comprising HPLC purification to achieve higher chemical purity. The respective radioligands, [^{18}F]fluorobenzoylated S100s ([^{18}F]FB-S100s), were obtained with appropriate radiochemical purities, yields, and effective molar activities. Biological applications comprise cell and tissue binding experiments in vitro, biodistribution and metabolite studies in rodents in vivo/ex vivo, and dynamic positron emission tomography studies using dedicated small animal PET systems. Radiolabeling of S100s with ^{18}F and, particularly, the use of small animal PET provide novel probes to delineate both their metabolic fate and the functional expression of their specific receptors under normal and pathophysiological conditions in rodent models of disease.

iii. Key words

Bolton-Hunter-type reagent; *in vivo* imaging; radiopharmacological characterization; ^{18}F building block; module-assisted radiosynthesis; S100 proteins; calcium; EF-hand

1. Introduction

The multigenic S100 protein family comprises to date at least 25 distinct members of Ca^{2+}-binding and -modulated proteins of the EF-hand type, differentially expressed in a wide variety of, exclusively, vertebrate cells [1,2]. In human and in rodents, S100 genes and proteins are strongly conserved [3]. S100 proteins (S100s) have been implicated in Ca^{2+}-dependent regulation of intracellular activities such as protein phosphorylation, enzyme activity, cell growth and differentiation, cell motility, dynamics of cytoskeletal constituents, structural organization of membranes, Ca^{2+}-homeostasis, as well as inflammatory stimulation and differentiation. Recent experimental and clinical evidence indicates that S100s play important regulatory roles not only within cells but also exert effects in a cytokine-like manner on definite target cells once released into the extracellular space or the circulating blood [1,2,4]. After cellular activation, some S100s are actively secreted following various pathways [4-9]. This potentially qualifies various S100s to be used as biomarkers [4,10-12] or as potential pharmacological/therapeutic targets [11 and references therein]. Functional complexity and diversity of S100s is determined at several levels, including a*)* expression in a cell-, tissue- and organ-specific manner, b*)* intracellular localization to both cytoplasm and nucleus, c*)* action in both an autocrine and paracrine manner, d*)* different affinity to calcium followed by various degrees of conformational changes, e*)* binding of other divalent metal ions, such as copper and zinc, and f*)* interaction with various, particularly, pattern recognition (multi-ligand) receptors. Target cells of extracellular S100s comprise granulocytes, monocytes, macrophages, mast cells, lymphocytes, dendritic cells, endothelial cells, neurons, astrocytes, microglia, cardiomyocytes, alveolar epithelial cells, and a plethora of tumor cells. Interaction of S100s with these cells both in receptor-dependent and –independent manner results, e.g., in pro- and anti-inflammatory modulation of monocytes, stimulation of angiogenesis, induction of endothelial dysfunction, trophic and pro-survival effects on neurons, stimulation of astrocyte proliferation, stimulation of NO secretion by astrocytes and microglia, as well as pro- and anti-tumorigenic modulation of tumor cells or cells of the tumor microenvironment. Accordingly, increased levels of S100 proteins in the circulating blood have been associated with a number of disease states, e.g., ischemic, infectious or traumatic injury of cerebral tissue, neurodegenerative disorders, ischemic myocardial injury, cardiomyopathies, diabetes, atherosclerosis, chronic inflammatory disorders, cancer and metastasis [2 *and references therein*]. An emerging concept of pattern recognition involves certain multi-ligand receptors, particularly, Toll-like receptors (TLRs), the receptor for advanced glycation end products (RAGE), and scavenger receptors (SR), as those transducing the biological effects of most S100s [2]. This all results in a tremendous spectrum of pleiotropic intra- and extracellular functions and determines the modes of interaction with numerous protein and peptide partners. Finally, the fact that multi-ligand receptors and multi-receptor ligands hit each other forms an intricate network of S100-associated pathways. This fact further supports the potential pathophysiological role of S100s and disclosed new routes of investigation in the overall S100s field. However, the diagnostic significance of different circulating S100s in blood and tissue specific S100-receptor interaction during initiation, progression and manifestation of several human pathologies is still poorly understood. This also applies to a deeper understanding of the effects of pharmacological intervention using compounds targeting S100s. One reason for this is the shortage of suitable radiolabeling methods for direct assessment of the metabolic fate of circulating S100 proteins and, on the other hand, the functional expression of known and putative S100 receptors *in vivo*.

In order to address this question, the methodical development focused on a novel sensitive and specific radiotracer approach using *no-carrier-added* (*n.c.a.*) radiolabeling of recombinant human S100s. This is exemplified by radiolabeling S100A12 (common aliases calgranulin C and ENRAGE) and S100A4 (Mts1, metastasin) with the positron-emitting nuclide fluorine-18 (^{18}F) and the application of radiolabeled ^{18}F-S100s in dynamic small animal positron emission tomography (PET) studies in rodent models [13-15]. On the one hand, this approach provides novel probes to delineate the metabolic fate in terms of S100s' blood retention, elimination, and interaction with membrane and soluble binding molecules. On the other hand, the functional expression of specific receptors under normal and pathophysiological conditions can be investigated *in vivo* [13-15]. The selected examples require consideration of specific marginal conditions for the choice of the most appropriate radiolabeling method. It has to be taken into account that physiological formation of S100 dimers or oligomers and interaction with binding molecules should not be affected. Therefore, space-consuming prosthetic groups, e.g., chelating units for metal radionuclides, were excluded. Furthermore, especially in case of S100A4, adverse oxidation of cysteine residues should be avoided [16]. This essentially precludes classical radiolabeling approaches like oxidative iodination, transition metal-catalyzed reactions, or cysteine-targeted labeling via maleimide-functionalized prosthetic groups [17 *and references therein,* 18,19]. S100A12, on the other hand, does not contain cysteine residues, *a priori* favouring a NH_2-targeted labeling approach. Consequently, radiolabeling of recombinant S100s with the Bolton-Hunter-type reagent *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) [20] was preferred.

Please insert figure 1.

Figure 1. Synthesis of **[^{18}F]SFB**

N-succinimidyl-4-[^{18}F]fluorobenzoate [^{18}F]SFB is an active ester suitable for the labeling of peptides and proteins which has been used in PET chemistry for years [21-24]. The radiosynthesis of [^{18}F]SFB was at first described by Vaidyanathan and Zalutsky [20] comprising a three-step synthesis starting with the [^{18}F]fluorination of *N,N,N*-trimethylammoniumbenzaldehyde triflate, followed by oxidation with MnO_2 to 4-[^{18}F]fluorobenzoic acid and final conversion to [^{18}F]SFB using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide. Beside this synthetic route and optimization thereof [25,26], [^{18}F]SFB was also synthesized by three-step procedures comprising the ^{18}F-labeling of an *O*-protected *N,N,N*-trimethylammoniumbenzoic acid ester triflate, followed by a deprotection step and the conversion into the final *N*-succinimidyl derivative with TSTU or HSTU. For example, Wester et al. introduced the radiosynthesis starting from the respective ethyl ester [27] which was adopted for automatization [28-33] as well as optimized for microfluidic [34,35] or microwave based [36] methods. In modifications of this route, the pentamethylbenzyl [37,38] and, as reported herein, the *tert*-butyl [39] ester was utilized. Recently, the copper-mediated nucleophilic [^{18}F]fluorination of aryl boronic esters was reported for an one-step synthesis of [^{18}F]SFB [40]. Within this report, the methodological part focusses on the radiosynthesis of [^{18}F]SFB starting from the *tert*-butyl protected *N,N,N*-trimethylammoniumbenzoic acid ester triflate **1** (Figure 1). The radiosynthesis follows the synthetic route [39] and automated procedure [41,22,42] described by our group but was optimized by application of a final HPLC purification step to increase the chemical purity of the product in order to raise the labeling yields of S100 proteins with [^{18}F]SFB. The latter can then be used for the ^{18}F-labeling of a multitude of different peptides, polypeptides, and proteins, but in the context of this article especially for the radiolabeling of different S100s.

2. Materials

2.1. Chemicals provided or synthesized

All commercial available chemicals and solvents were purchased in high quality and used without further purification. The precursor, *tert*-butyl protected *N,N,N*-trimethylammoniumbenzoic acid ester triflate **1**, was synthesized as previously reported [39]. The non-radioactive reference compound SFB was prepared as described by Wester et *al*. [27]. No-carrier-added aqueous [^{18}F]fluoride was produced in a CYCLONE 18/9® cyclotron (IBA, Belgium) by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction.

2.2. Preparation of reagents and eluents

1. 1 M $NaHCO_3$ solution: Weigh 84.01 g $NaHCO_3$ and dissolve in ca. 300 mL water. Dilute solution to 1 L in a volumetric flask.
2. K_2CO_3 /K_{222} solution: Weigh 500 mg 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclohexacosane (K_{222}) and dissolve in 50 mL MeCN to prepare K_{222} solution. Weigh 92.3 mg K_2CO_3 and dissolve in 7 mL water. Transfer K_2CO_3 solution into a 50 mL volumetric flask and add K222 solution to the 50 mL graduation mark. Store the stock solution in a closed bottle in the fridge.
3. 1 M HCl solution: Dilute 1 mol HCl (concentrated volumetric solution in an ampoule, PanReac Applichem) with water to 1 L in a volumetric flask.
4. Precursor solution: Store *tert*-Butyl-(4-*N*,*N*,*N*-trimethylammonium)benzoate triflate (precursor for radiolabeling) in the fridge until use. Let the precursor warm up to RT, weigh 7 mg *tert*-butyl-(4-*N*,*N*,*N*-trimethylammonium)benzoate triflate in an HPLC vial and dissolve in 1 mL MeCN. Close the vial until preparation of the synthesis module.
5. Me_4NOH solution: Store 25% Me_4NOH in MeOH (Sigma) in the fridge until use. Let the solution warm up to RT and add 20 µL of this solution to 0.5 mL MeCN in an HPLC vial to prepare the final Me_4NOH solution. Close the vial until preparation of the synthesizer module.
6. TSTU solution: Store *O*-(*N*-succinimidyl)-*N,N,N',N'*-tetramethyluroniumtetrafluoroborat (TSTU) in the fridge until use. Let TSTU warm up to RT, weigh 15 mg into a glass vial and dissolve it in 0.5 mL MeCN. Close the vial until preparation of the synthesizer module.
7. HPLC eluent: Add 1 mL glacial acetic acid to approx. 200 mL of ultrapure water and dilute to 1 L in a volumetric flask to prepare 0.1 % acetic acid . Mix 650 mL 0.1 % acetic acid in ultrapure water with 350 mL MeCN and degas by ultrasonification for 15 min to prepare MeCN / 0.1 % acetic acid in water 35/65 (v/v) as HPLC eluent.

2.3.1. Equipment for [^{18}F]SFB synthesis

1. [^{18}F]SFB synthesis is performed in a lead-shielded hot cell with adequate underpressure regulation, inlet and exhaust filters, and radiation monitoring systems by using the commercially available synthesizer TRACERlab Fx_{FDG} for nucleophilic fluorination (GE Medical Systems, Münster, Germany). The system set-up and initial position of all valves at the beginning of the synthesis is shown in Figure 2. Nitrogen 5.0 was used as auxiliary gas (AUX GAS).
2. For reasons of clarity and since program structure of commercially available synthesizers differs, explicit time information and program codes have been omitted. Valve positions are highlighted in bold and italic, e.g. ***V1c***, in the text indicating valve number and valve position with the following codes: o = open, c = closed, a = position a (Figure 2), b = position b (Figure 2). Unless otherwise stated, the time between each command for switching valves to the given state should be 0.1-1 s. Longer time intervals between two commands, e.g. X seconds, have been indicated by ***,(X s),*** or *wait for* commands.

3. For solid phase extraction the following cartridges are used: Sep-Pak light Accell Plus QMA® (130 mg, Part Nr. WAT023525), Lichrolut® RP-18E (500 mg, 3 mL PP, Part Nr. 1.19849.0001), Chromafix® C18ec(S) (270 mg, Part Nr. 731804) , Lichrolut® SCX (500 mg, 3 mL PP, Part Nr. 1.02022.0001), Oasis HLB Plus Short (225 mg, Part Nr. 186000132).
4. Semi-preparative HPLC is integrated in the synthesizer module and performed with an JASCO® system: pump PU-980, degasser DG-1580-53, gradient unit LG-980-02 and UV-detector Knaur K2001; semi-preparative C18 precolumn; column Discovery HS F5-5 (5 µm, 250 x 10 mm, Supelco®), isocratic mode, HPLC eluent (see 2.2.7), 4 mL/min flow rate. The products are monitored at λ = 220 nm and with a radioactivity detector integrated in the synthesizer module.
5. ^{18}F activity of [^{18}F]fluoride, [^{18}F]SFB, and [^{18}F]FB-S100s is measured with an acitvimeter (Isomed 2000 dose calibrator, raytest, Germany).
6. Manual handling of ^{18}F activity like labeling of S100s with [^{18}F]SFB is performed in a lead-shielded and well ventilated hood.
7. Analytical HPLC is performed on a Kinetex PFP 100 Å column (5 µm 150 x 4.6 mm, phenomenex) equipped with an analytical C18 precolumn using Agilent 1100 HPLC: binary pump G1312A, auto sampler G1313A, column oven G1316A, degasser G1322A, UV detector G1314A, γ detector Gabi Star® (raytest, Straubenhardt, Germany); column temperature: 25°C, isocratic eluent: MeCN/H_2O 35/65 (v/v), flow rate = 0.5 mL/min, run time 20 min. The products are monitored at λ = 235 nm.

2.3.2. Preparation of the synthesis module for [^{18}F]SFB synthesis

1. Fill dewar with liquid nitrogen and immerse cooling trap.
2. Open pressurized air and nitrogen gas supply.
3. Activate QMA cartridge by rinsing with 1 M $NaHCO_3$ solution (10 mL) and then water (10 mL) and install in the activimeter at **position A** (for positions A-E see figure 2).
4. Activate both RP18e (500 mg) and Chromafix C18 by rinsing each with ethanol (10 mL) and then water (10 mL). Connect RP18e and Chromafix C18 in a row and install cartridges at **position B**.
5. Rinse PTFE filter (0.2 µm, diameter 2 cm) with HPLC eluent and install at **position C**.
6. Activate Lichrolut SCX cartridge with 20 mL water and install at **position D**.
7. Activate HLB cartridge with 10 mL ethanol and then 10 mL water and install at the product line (**position E**). For radiation safety reasons it is recommended to place the HLB cartridge within an activimeter in a neighboring hot cell.
8. Install cleaned injection **vial X**.
9. Fill 30 mL water into the cleaned **HPLC product vial P**.
10. Equilibrate analytical and semi-preparative HPLC column. Turn UV detector on and make sure that gamma and UV detector work properly and the fluidic system has no leaks.
11. Heat an aluminium block placed on a magnetic stirrer with heating plate to 75°C. The heating block should be equipped with an oil-filled glass vial for temperature measurement and cavities suitable for placing a V-shaped 7 mL glass vial in it.
12. Fill the following reagents and solvents into the respective vials: 1.5 mL K_2CO_3 /K_{222} solution (vial 1), 3 mL MeCN *HPLC grade* (vial 2), 1 mL precursor solution (vial 3), 0.5 mL 1 M HCl (vial 4), 12 mL water (vial 5), 3 mL MeCN *HPLC grade* (vial 6), 0.5 mL Me_4NOH solution (vial 7), 0.5 mL TSTU solution (vial 9), 1.5 mL HPLC eluent (vial 10)
13. Perform in cooperation with the cyclotron staff and under respective safety measures a pressure test before radiosynthesis to ensure that tubings are not clogged and reaction

vessel is tightly closed. ***V13b, V17o*** – Switch valves towards the ^{18}F transportation system. Pressurize the transportation system with nitrogen starting from a suitable point of the transport system. Check that pressure rises to a stable end point. ***V13a, (4s), V17c -*** Close connection of reaction vessel towards transportation system. Observe if pressure is stable or only slowly falling to ensure that reaction vessel is tightly closed. ***V33b, V31o*** – Open reaction vessel towards exhaust and wait for pressure release. ***V31c, V33a*** – Close reaction vessel 1.

Please insert figure 2.

Figure 2: Schematic representation of the modified TRACERlab Fx_{FDG} synthesis module used for [^{18}F]SFB synthesis. Initial positions of remote controlled valves are colored grey if closed and green if opened.

3.1. Methods

3.1.1. Synthesis of [^{18}F]SFB

Caution – Handling of ^{18}F activity should be performed considering institutional, local, and governmental regulations. Safety procedures should include but not be restricted to, e.g., the use of adequate lead shielding (lead walls or hot cells), wearing gloves during manual operations, minimizing time for handling radioactive substances, and increasing distance to the radioactive probe by distance tools. If unfamiliar with handling ^{18}F activity contact your institutional radiation safety officer to define appropriate training and safety measures as well as personal and/or local radiation monitoring procedures.

Start automated procedure comprising the following steps:

1. ***V13b, V15b, V29o*** - Pass [^{18}F]Fluoride through the QMA cartridge (**A**). Monitor adsorbed activity and wait until activity has reached a maximum.
2. Heat reaction vessel to 65°C during steps 3-6.
3. ***V15a, V29c, V13a*** - Close connection of QMA cartridge towards target.
4. Note [^{18}F]fluoride activity (A_{start}) and the time.
5. ***V25o, V1o*** - Open valves towards reaction vessel 1. ***V33b, V17o, V31o*** - Elute [^{18}F]fluoride from the QMA cartridge. Wait 40 s for [^{18}F]fluoride transfer to the reaction vessel 1.
6. Note [^{18}F]fluoride activity (A_{rest}) and the time.
7. Start automated azeotropic drying procedure. ***V33a, (20 s), V17c, V1c, V27o -*** Close Vial 1 and wait for reaction vessel 1 to reach a temperature of 65°C while purging the reaction vessel under constant nitrogen stream. Evaporate solvent for 2 min.
8. Increase temperature to 95°C and wait for temperature. Evaporate solvent for 2 min. ***V27c*** - Close nitrogen.
9. ***V33b, V2o*** - Add acetonitrile from vial 2 at ambient pressure. Wait 15 seconds for liquid transfer. ***V2c, V25c*** – Close connection to vial 2. ***V27o, V33a*** – Evaporate solvent under constant nitrogen flow and reduced pressure at 95°C for 2 min. ***V27c*** - Close nitrogen and remove residual acetonitrile for 6 min under reduced pressure. ***V31c*** - Close reaction vial. Cool reaction vessel to 50°C. ***V27o, (4 s), V27c,V33b,V31o,(5 s),V31c*** – Pressurize reaction vessel with nitrogen and equalize to ambient pressure.
10. ***V25o, V31o, V3o*** - Add precursor solution from vial 3 to the reaction vessel. Wait 10 seconds for liquid transfer. ***V3c***, ***V31c*** - Close Vial and reaction vessel 1. Heat to 90°C and wait for temperature. Hold temperature for 10 min for [^{18}F]fluorination. Cool reactor to 45°C and wait for temperature.

11. ***V31o,(10s),V31c*** - Equalize Pressure in reaction vessel 1. ***V4o*** - Add HCl from vial 4 and wait 10 seconds for liquid transfer. ***V4c*** – Close Vial.
12. Heat reaction vessel to 100°C and wait for temperature. Hold temperature for 5 min for saponification. Cool reaction vessel to 40°C.
13. ***V31o, V5o*** - Add water from vial 5 and wait 15 seconds for liquid transfer. ***V31c, V5c*** – Close vial 5 and reaction vessel 1.
14. Stir reaction mixture for 1 min.
15. ***Move needle 1 down***. ***V21b, V27o, V18o*** - Pressurize the reaction vessel 1 with nitrogen and transfer reaction mixture via SCX cartridge (**D**) to RP18E/C18 cartridge (**B**) and route liquid to waste equipped with ventilation needle. Wait 3 minutes for liquid transfer. ***V18c, V21a, V27c*** - Close nitrogen gas and reaction vessel 1.
16. ***V6o, V23o, V32o*** - Elute adsorbed 4-[^{18}F]fluorobenzoic acid from the SPE cartridges with acetonitrile from vial 6 into reaction vessel 2. Wait 50 seconds for liquid transfer. ***V6c, V23c, V21b, V32c -*** Close Vial 6, connection between SPE and reaction vessel 2, and ventilation valve.
17. ***V30b, V32o, V7o*** - Add Me_4NOH solution. Wait 7 seconds for liquid transfer. ***V7c*** - Stop Addition from vial 7. ***V33a, V28o*** - Evaporate solvent under constant nitrogen stream and reduced pressure while heating reaction vessel 2 to 90°C. Wait for temperature. Hold temperature for 2 min. ***V28c*** - Close nitrogen and evaporate residual solvent for 2 min under reduced pressure.
18. ***V32c*** – Close reaction vessel 2. Cool reaction vessel 2 to 70°C and wait for temperature. ***V33b, V32o, (4s),V32c*** - Equalize pressure in the reaction vessel 2. Set temperature to 70°C in reaction vessel 2 and wait for temperature.
19. ***V32o, V9o*** - Add TSTU solution from vial 9. Wait 7 seconds for liquid transfer. ***V9c, (3s), V32c*** - Close reactor. Heat reaction vessel 2 to 90°C and wait for temperature. Hold temperature for 2 min to convert 4-[^{18}F]fluorobenzoate into [^{18}F]SFB. Cool reaction vessel 2 to 50°C and wait for temperature.
20. ***V32o, V10o*** - Add eluent from vial 10. Wait 17 seconds for liquid transfer.***V10c, V32c*** – Close reaction vessel 2. ***V22b, V20o, V24c*** - Stir for 10 seconds while switching valves for liquid transfer.
21. ***Move needle 2 down, V28o*** - Transfer reaction mixture to injection vial by pressurizing reaction vessel 2 with nitrogen. Wait 1 min for liquid transfer. ***V28c, V20c, move needle 2 up*** - Stop transfer. ***V30a, V21a, V22a, V33a*** - Reset module to initial values***.***
22. Note activity level in the injection vial (A_{inj}) and the time.
23. Check that HPLC pump is turned on at a flow rate of 4 mL/min, pressure is stable and within the normal range and HPLC load/inject valve is in ***load*** position.
24. ***V12o, V24o*** - Transfer reaction mixture to the sample loop of the semi-preparative HPLC system via fluid detector. Wait for liquid ON signal. Wait for liquid detector OFF signal.
25. Turn valve immediately to ***inject*** position. Perform auto zero for UV detector signal and observe radioactivity signal.
26. ***V12c, V24c, V25c*** – Close valves between vial 12 and HPLC load/inject valve.
27. [^{18}F]SFB elutes with the given chromatographic system at approximately t_R =21-23 min. ***$VA_{product}$*** - Start collection of [^{18}F]SFB fraction into HPLC-product vial when radioactivity signal exceeds significantly background levels. Consider delay volume between radioactivity detector and valve A. ***VA_{waste}*** – Stop collection of [^{18}F]SFB fraction when radioactivity signal has decreased approximately to background levels. Turn HPLC load/inject valve to ***load*** position.
28. ***V24a-b*** - Transfer product solution to HLB cartridge to adsorb [^{18}F]SFB on an HLB cartridge and route liquid to waste vial equipped with a ventilation needle. Wait for liquid transfer,

observe activity level on HLB cartridge. ***V24a-a*** - Stop liquid transfer. Wait 30 seconds for pressure equalization. For radiation safety reasons it is recommended to place HLB cartridge and activimeter in a neighbored hot cell and transfer product solution through a connection between these two hot cells.

29. Measure radioactivity on HLB cartridge (A_{HLB}) and note the time.

The following steps are performed manually.

30. Take the HLB cartridge out of the activimeter and disconnect luer lock connections at both sides of the cartridge. Connect a disposable 5 mL syringe filled with 2 mL MeCN to the female side of the cartridge and place the cartridge upon a V-shaped glass vial in a lead shielded container. Pass MeCN through the HLB cartridge to elute [^{18}F]SFB into the glass vial (*Caution! Push gently to elute [^{18}F]SFB but avoid too high pressure to prevent loosening of the connection*).
31. Measure activity in the eluate (A_{SFB}) and on the HLB cartridge (HLB_{rest}) and note the time t_{SFB} / $_{HLB}$.
32. Take a sample of 50-100 µL with a 200 µL (V_{HPLC}) Eppendorf pipette and transfer liquid into an HPLC vial. Measure activity of the sample (A_{HPLC}) and note the time (t_{HPLC}). Inject 5 µL (V_{inject}) of the product solution into analytical HPLC. Perform quality control as described in 3.1.2.
33. Close the glass vial with a screw cap having a septum punctured with argon inlet and ventilation exhaust tubing. Route exhaust gas to a well ventilated waste vial.
34. Place the product vial in the heating block preheated to 75°C. Close hot cell. *Caution! For radiation safety reasons it is recommended to perform the evaporation while the hot cell is closed because [^{18}F]SFB can evaporate off if product solution is evaporated to dryness. Observe radiation levels in the hood, and stop evaporation immediately if activity levels rise.*
35. Open argon gas stream with a pressure set at 0.5 bar so that a gentle stream of argon is applied. Make sure that argon tubing is placed about 1 cm above the initial liquid level and does not touch the surface. Gently evaporate acetonitrile with visual inspection of the product solution in 30-60s intervals. Slowly increase argon pressure to 1 bar with advancing evaporation. Concentrate product solution to a volume of 100-200 µL.
36. Stop argon gas flow. Place product vial in a lead shielded container and wait for 3 min to cool down product vessel. Replace screw cap septum with a closed cap. Final [^{18}F]SFB solution is now ready and suitable for further processing, e.g. labeling of S100 proteins.

3.1.2. Quality control for [^{18}F]SFB

1. Determine decay corrected radiochemical yield by using the formula (1):

$$RCY\ [\%] = \frac{A_{SFB}}{A_{start}} \times e^{\frac{(t_{SFB}-t_{start})\ min}{109{,}7\ min} \times \ln(2)} \times 100. \qquad (1)$$

2. Determine radiochemical purity and molar activity by injecting an analytical sample as described in 3.1.1-32 three times into the analytical HPLC (see 2.3.1.).

2.1. For radiochemical purity, determine the area under the curve from the radioactivity signal of each peak and the respective percentage for the [^{18}F]SFB peak. Under the specified HPLC conditions, SFB elutes with a retention time t_R of 11.2 min. If it is unclear which peak corresponds to [^{18}F]SFB, an HPLC run either with the non-radioactive reference SFB or with a SFB spiked product solution has to be performed.

2.2 Calculate the molar activity at the time of sample taking by formula (2):

$$A_m(t_{HPLC}) = \frac{A_{[^{18}F]SFB}}{n_{SFB}} = \frac{A_{HPLC} \cdot \frac{V_{inject}}{V_{HPLC}}}{n_{SFB(HPLC,inject)}}. \quad (2)$$

For this, the amount of substance in the injected volume $n_{SFB(HPLC,inject)}$ is calculated from the integrated UV signal corresponding to SFB and comparison with a calibration curve, which is determined beforehand (see Figure 3). In brief, SFB standards are prepared by dissolving a definite amount of SFB in MeCN in a volumetric flask followed by serial 1:1 dilution with a 1 mL Eppendorf pipette. A calibration curve is determined by injection of standard solutions in duplicate into the analytical HPLC system under the HPLC specifications given in 2.3.1, integration of the UV signal corresponding to SFB, and application of a linear fit to the data set.

3.1.3. Cleaning procedure for [^{18}F]SFB synthesizer module

An automated cleaning procedure is performed twice after each synthesis, the first time by application of the procedure with solvent and second as a dry clean without liquids to remove any solvent residues. *Caution* – Depending on the time between synthesis and clean the synthesizer module may still be contaminated with ^{18}F. We usually performed the clean 24-48h after the radiosynthesis. In brief, SPE cartridges **A**-**E** and the **PTFE filter** are removed and the respective tubings are short-circuited. The [^{18}O]water vial is emptied. Both reaction vessels **R1** and **R2**, the **injection vial X** and the **HPLC product vial P** are dissembled, carefully cleaned, dried, and reinstalled into the synthesis module. For the first clean, 20 mL water is filled into **HPLC product vial P** and the given amount of acetone is filled into each given vial: 2 mL in vials 1, 4, and 7; 1 mL into vials 2, 3, 8, and 9; 5 mL into vials 5 and 10, and 3 mL into vial 6. The cleaning procedure comprises *a)* use a., b. instead of i. consecutive addition of solvent from vial 1 and vial 2 to **R1** and vial 7 and vial 8 to **R2** while exhaust is opened via V33, stirring for 90 s, and emptying of **R1** via V18 and V21 to the waste and **R2** via V20, **injection vial X**, and HPLC loop to the waste, *b)* similar rinsing procedure like *a)* for V3/V4 and V9/V10, and after heating of both reaction vessels to 85°C for V5 and V11, *c)* rinsing lines between **R1** and **R2** via V18, V21, and V23 at 40°C with solvent from V6 followed by routing this and solvent from V12 via **injection vial X** and V24 to the waste, *d)* consecutive purging each pair of lines (V1/V7, V2/V8,…) via the reaction vessels with nitrogen for 3 min at 120°C and vacuum suction, *e)* drying of lines between **R1**, **R2**, **HPLC injection vial X**, V22, and the waste in a stream of nitrogen, *f)* cooling down of both reaction vessels under vacuum for 5 min and meanwhile emptying of **HPLC product vial P** to clean tubings towards HLB cartridge and waste, *g)* pressure equalization in vials and reaction vessels.

3.1.4. Anticipated Results

A typical experiment started from 15 GBq [^{18}F]fluoride and yielded about 2.9 GBq [^{18}F]SFB after 89 min synthesis time. The described synthetic procedure was reproducible and gave [^{18}F]SFB in isolated radiochemical yields of 28 ± 3%, molar activity of 52 ± 3%, and chemical purity of 77 ± 12% (n=12). The radiochemical purity exceeded 99% (see Figures 4 and 5).

Please insert figure 3.

Figure 3. Representative calibration curve and linear fit obtained from a dilution series of SFB and HPLC analyses.

Please insert figure 4.

Figure 4. Representative semi-preparative HPLC chromatogram of [^{18}F]SFB purification. Time points for starting collection of [^{18}F]SFB by switching valve $VA_{product}$ and ending collection of [^{18}F]SFB by

switching valve VA_{waste} are labeled with start and end, respectively, and the collected [^{18}F]SFB fraction is colored in pale grey.

Please insert figure 5.

Figure 5. Representative analytical HPLC chromatogram of 5 µL [^{18}F]SFB solution obtained after isolation of [^{18}F]SFB from the HLB cartridge. The major non-radioactive component in the mixture can be assigned to non-radioactive SFB, the time delay between UV and radioactivity signals is based on the consecutive arrangement of both detectors.

3.1.5. Radiolabeling of S100 proteins with [^{18}F]SFB

1. In principle, ^{18}F-radiolabeling of either mature or tagged S100s can be performed through acylation of both the chemically accessible N-terminal amino acid residues and lysine side chain residues with [^{18}F]SFB. In the present approach, [^{18}F]fluorobenzoylation of lysine side chain residues (e.g., 10 lysines in S100A12 and 11 lysines in S100A4) should be avoided.

2. Therefore, labeling reactions are carried out at pH 7.4. Below this pH value nearly all lysine side chain residues are supposed to be protonated, hence being not available for [^{18}F]fluorobenzoylation with [^{18}F]SFB. As a consequence, the chemically accessible *N*-terminal residues of S100s are the most privileged site for conjugation. Lysine residues become the preferred site of conjugation with [^{18}F]SFB only under basic conditions (e.g. pH 8.4). For peptide labeling with [^{18}F]SFB the pH is usually adjusted to 8.2 to 8.4.

3. The obtained [^{18}F]SFB (500-1000 MBq) diluted in acetonitrile then is directly added to 100-400 µg S100 protein dissolved in PBS and incubated at pH 7.4 for 30 min at 37°C with continuous and gentle shaking in a thermal mixer.

4. The radiolabeled S100 proteins are purified using a HiTrap desalting column (GE Healthcare; equilibrated with 20 mM HEPES, 150 mM NaCl, 1.2 mM $MgCl_2$, and 1.3 mM $CaCl_2$, pH 7.4) with an ÄKTAprime® plus (GE Healthcare) chromatography system. Gel filtration is carried out at a flow rate of 0.5 mL/min and fractionated in 0.5 mL steps. The radioactivity is determined with an activimeter. Fractions containing the purified ^{18}F-labeled S100 proteins, separated from unreacted [^{18}F]SFB and the by-product [^{18}F]fluorobenzoic acid, will be combined and used for further experiments.

5. The activity of the product is measured using the ISOMED 2000 calibrator (Nuklear-Medizintechnik Dresden GmbH). Because of the short half-life of ^{18}F ($t_{1/2}$ = 109.77 min), it is necessary to adjust radioactivity data in order to compare all the time points. Accordingly, all radioactivity measurements *in vitro* and *in vivo* should be adjusted and expressed as decay-corrected values [13,15].

4. Additional Notes and Applications

Purified fluorine-18 labeled S100s ([^{18}F]FB-S100s) can be used as probes, e.g., in receptor binding assays and cell uptake/association experiments *in vitro* as well as in biodistribution, metabolism, and dynamic PET (imaging) experiments *in vivo*. The following is an excerpt of essential aspects of the basic methodical approaches employed so far. Special aspects, e.g., for cell-based investigations, were excluded here, since for example, cell passages, cell numbers, cell density, and protein concentrations depend on the individual cell type. For this and other detailed descriptions, e.g., regarding blocking experiments *in vitro* and *in vivo* aiming at binding specificity and differentiation of several binding partners, we refer to the work published elsewhere [13-15,43].

4.1. In vitro experiments

1. Binding of [^{18}F]FB-S100s to certain receptors can be studied in cell-free binding assays. Exemplarily, suitable 96-well plates (Maxisorb TM 96-well plates; Nunc, Langenselbold, Germany) are coated with recombinant sRAGE or albumin (each 2.5 µg/well) in bicarbonate/carbonate buffer (50 mM, pH 9.6) and are incubated overnight at 4°C. After washing three times with 250 µL phosphate-buffered saline (PBS) containing 0.05% Tween 20, wells are blocked with PBS containing 2% bovine serum albumin for 2 h. Then, wells with adsorbed sRAGE, albumin, or without protein are incubated with 100 µL of radiolabeled [^{18}F]FB-S100s (approximately 300–400 kBq; in 20 mM HEPES buffer with 150 mM NaCl, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$, pH 7.5 supplemented with 2% bovine serum albumin) for 1 h at 37°C with gentle agitation. For this and the following approaches molar activity and radiochemical yield (>95% minimum) of [^{18}F]FB-S100s must be specified in each case. Subsequently, wells are washed three times with ice cold PBS on ice. Bound protein is dissolved in 100 µL of 0.1 M NaOH containing 1% SDS at 37°C for 30 min under shaking conditions. Fluorine-18 activity (in terms of radioactivity concentration) is measured in a gamma counter (Cobra II; Canberra-Packard, Meriden, USA).

2. For cellular association experiments, cells are seeded in 96-well plates at definite time points (24 h - 72 h) and cell numbers (10^3 – 10^5 cells/well) prior to the experiment. The assay is performed in the presence or absence of non-radioactive S100s. [^{18}F]FB-S100s probes (approximately 100–200 kBq; in PBS) are added and cells are incubated for 1 h at 37°C. Subsequently, cells are washed three times with ice-cold PBS and dissolved in 0.1 M NaOH containing 1% SDS. Fluorine-18 activity is measured in a gamma counter, and afterwards protein concentration is determined using an appropriate protein assay (Pierce® BCA Protein Assay; Thermo Fisher Scientific, Waltham, USA).

4.2. In vivo experiments

1. All animal experiments are carried out according to local guidelines of the national regulations for animal welfare. The protocols must be approved by the local ethical committees for animal experiments. In vivo stability, biodistribution, and dynamic small animal PET studies are assessed, exemplarily, in rats (e.g., Kyoto-Wistar strain; aged 9–12 weeks; 200–230 g).

2. For the investigation of metabolic stability, animals are anesthetized with desflurane (10% and 30% oxygen/air; suprane®; Baxter, Unterschleißheim, Germany) and a catheter is placed into the right common carotid artery or right femoral artery. A volume of 0.5 mL [^{18}F]FB-S100s probes containing 5-20 MBq (PBS, pH 7.4) is injected into the tail vein. Arterial blood samples (0.5 mL) are taken at definite time points (e.g., 5, 10, 20, 40, 60, 120 min post injection). The depleted blood volume is compensated by the injection of saline. Blood samples were immediately centrifuged for 5 min at 10,000 × *g*. Arterial plasma samples were heated using SDS-sample buffer containing β-mercaptoethanol at 95°C for 5 min, analyzed by SDS–PAGE (radioluminography using e.g. a BioImaging Analyzer BAS-5000, Fuji Photo Film, Düsseldorf, Germany) and, thereafter, are deproteinated with the twofold volume of water/methanol/TFA (50/45/5; v/v/v). After centrifugation for 2 min at 10,000 × *g*, supernatants and subnatants were measured

using an activimeter for fluorine-18 (dose calibrator Isomed 2000 dose calibrator; Nuklear-Medizintechnik Dresden GmbH, Dresden, Germany). Radioluminographic intensities of the electrophoretic bands from SDS-PAGE are quantified using an appropriate software version (AIDA; Raytest, Straubenhardt, Deutschland) and expressed as percentage of total activity amount.

3. For biodistribution experiments, groups of rats (n≥4) are administered with 500 µL [^{18}F]FB-S100s (1.2–1.5 MBq, PBS, pH 7.4) into the tail vein and sacrificed at 5 and 60 min post injection (p.i.) by heart puncture under desflurane anaesthesia. Organs and tissues of interest are rapidly excised, weighed, and the fluorine-18 activity is determined using a gamma counter cross calibrated with an activimeter for fluorine-18 by aliquots of the injected activity. The radioactivity concentration in organs and selected tissues is calculated as the percentage of the injected dose per gram tissue (%ID/g) or standard uptake value (SUV, g/g) of the tissue. SUV is defined as the tracer concentration at a certain time point normalized to injected dose per unit body weight and is used for better comparison within animals of different size and weight and with other species.

4. For dynamic small animal PET studies rats under desflurane anesthesia are positioned prone with thorax and abdominal region (organs of interest: heart, lung, liver, kidneys, large vessels) in the center of field of view of a dedicated PET scanner for small animals (microPET P4; CTI Concorde Microsystems, Knoxville, USA or NanoScan PET/CT scanner, Mediso, Budapest, Hungary). A bolus injection of 500 µL of [^{18}F]FB-S100s (10–20 MBq, PBS, pH 7.4) is administered via the tail vein within 30 s. Simultaneously with tracer injection, dynamic PET scanning was started for 120 min. Sinogram generation and image reconstruction followed the protocol given by us elsewhere [44]. Images were analyzed by assigning three-dimensional regions-of-interest (ROI) over the heart region, the liver, and the kidneys using ROVER software (ABX GmbH, Radeberg, Germany). From these ROIs time-activity-curves (TACs) representing the total (decay-corrected) fluorine-18 activity in a defined volume and expressed as radioactivity concentration, percent of maximum (or SUV) were obtained in each rat.

5. TACs from ROIs over the heart region, majorly representing the cardiac blood pool, are used to estimate, e.g., the pharmacokinetic parameter mean residence time. Therefore, the numerical module of SAAM II program (University of Washington, Seattle, USA) using a derivative-free nonlinear regression analysis is used to fit fluorine-18 activity data from ROIs over the heart region from each animal to multiexponential equations. The individual responses are quite consistent, showing a three-exponential equation $A(t) = A1e^{-k1t} + A2e^{-k2t} + A3e^{-k3t}$, where A(t) = % of injected dose per cm^3, to provide the best fit. The distribution/tissue association ($t_{1/2}{}^{d/a}$; $t_{1/2}k1 + t_{1/2}k2$) and elimination ($t_{1/2}{}^{e}$, $t_{1/2}k3$) half-lifes were calculated as 0.693/k. For estimation of mean residence time the area under the heart region TAC and the area under the momentum curve are calculated using standard equations according to Wolfe [45].

5. Concluding remarks

5.1. Radiopharmacological studies on [^{18}F]S100A12

Both *in vivo* and *in vitro* experiments using [^{18}F]FB-S100A12 have indicated that S100A12 is a physiologically highly affine ligand for the receptor for advanced glycation endproducts (RAGE). In this regard, the use of fluorine-18 is of particular interest since ^{18}F-labeled radiotracers, including protein based radioligands, can usually be obtained in high molar activities. By using dedicated small animal PET systems, images of ^{18}F-radiolabeled probes showing their time-dependent organ/tissue distribution and elimination exhibit the highest resolution [46,44,47]. In a first study [^{18}F]FB-S100A12 served as probe for the functional characterization of RAGE [13]. The study showed in a rat model strong interaction of [^{18}F]FB-S100A12 with organs and tissues showing high expression of RAGE, in particular, lung and large blood vessels. The *in vivo* kinetics of [^{18}F]FB-S100A12 was characterized by its high metabolic stability and a relatively long residence time (mean plasma residence time was approximately 5 hours) in the circulating blood as well as a sustained tissue-associated temporary retention in the lung and the whole vascular system. Considering an overall faster metabolism in rodents, it can be expected that terminal half-life of S100A12 in human circulation is much more prolonged. In situations where homeostasis of S100A12 secretion and elimination is disturbed, this should have pathophysiological consequences. Of importance, high plasma levels of S100A12 have been described in various inflammatory disorders [4,48-50]. Interaction of S100A12 with membrane-anchored RAGE in a cytokine-like manner has been proposed to be the key process in initiation or perpetuation of a concerted inflammatory action in various cells and tissues. Continuing secretion of S100A12 by activated granulocytes and macrophages into the blood, and, on the other hand, this prolonged terminal half-life of S100A12 would consecutively trigger this process. The PET data in terms of tissue-associated temporary retention correlated well with the observed organ-specific expression of the non-internalizing RAGE in the rat. Highest RAGE expression both on mRNA and protein level was found in the lung. The retention in the vascular system can be explained by the presence of RAGE in endothelial cells and medial smooth cells. Systemic clearance of ^{18}F-radioactivity after intravenous injection of [^{18}F]S100A12 was almost completely explained by uptake in the kidneys and subsequent renal elimination, and to a minor part, by uptake in the liver and hepatobiliary excretion. This can be explained majorily by glomerular filtration of the small acidic S100s and their degradation in the proximal tubuli in the kidney. At this point, a hypothesis on formation of complexes between S100A12 and circulating soluble isoforms of RAGE (sRAGE), which are also present in rats, could be deduced. Potential uptake of these complexes by the liver or the kidneys should further contribute to the systemic elimination of the highly affine S100A12 [13]. In a more detailed study, including cell binding experiments in human aortic endothelial cells and macrophages exhibiting, beside membrane RAGE, also various SR, we demonstrated substantial SR binding of [^{18}F]FB-S100A12. This finding was also confirmed in subsequent dynamic PET experiments in rats *in vivo* [15]. At this point we already hypothesized that the possible interaction of S100A12 with SR like CD36, which has been reported to mediate pro-inflammatory signaling, is likely to further contribute to the scenarios discussed above. Very recently, by using both fluorescent and radiolabeled derivatives of S100A12, the class-B scavenger receptor CD36 actually was identified as a highly affine binding partner of S100A12. Here, cell binding experiments showed a specific S100A12-CD36 interaction that was blocked by anti-CD36 antibody. The experiments also revealed that S100A12 shares the same binding site (between amino acids 93 and 120) on CD36 as do other CD36 ligands like thrombospondin and collagen. A further finding of this study was that S100A12 upregulates CD36 expression and recruits CD36 to the cell membrane, indicating a potential pathophysiological link between high circulating S100A12 levels and the onset of atherosclerosis, particularly, in chronic inflammatory disorders [43,51]. Furthermore, there are initial considerations hypothesizing possible interaction of S100A12, RAGE, and CD36 in chronic kidney disease [52-54].

5.2. Radiopharmacological studies on [^{18}F]S100A4

In an *in vivo* study using rats and a comparable experimental setting, the interaction of [^{18}F]FB-S100A4 with organs and tissues showing high RAGE expression also could be demonstrated. However, from the data we argued that under physiological conditions in the rat interaction of extracellular S100A4 with RAGE is weak to moderate when compared to [^{18}F]S100A12 and other S100s investigated by radiotracer approaches ([^{18}F]FB-S100A1, [^{18}F]FB-S100B [13]; [^{18}F]FB-S100B-oligomers, unpublished data). Besides its lower affinity to the receptor, as shown in surface plasmon resonance analyses, a substantially faster metabolic degradation of S100A4 contributes to this observation. Of importance, like for [^{18}F]FB-S100A12, the recognition of [^{18}F]FB-S100A4 by other receptors, e.g., SR, cannot be excluded. Indicative for this assumption was the high uptake of [^{18}F]FB-S100A4 observed in liver, spleen, and adrenals, organs comprising cells that overexpress SR, e.g., CD36 and SRBI. Furthermore, the cellular binding studies in both an endothelial cell (human aortic endothelial cells) and a RAGE-expressing human melanoma cell (A375) model revealed a substantially higher [^{18}F]FB-S100A4 binding to putative binding sites in endothelial cells than in melanoma cells. This makes it likely that scavenger receptors and other endothelial surface structures, e.g, heparin sulfate moieties on the cell surface of endothelial cells or annexins, contribute to the higher [^{18}F]FB-S100A4 association [55,56]. Nevertheless, the significant higher association of [^{18}F]FB-S100A4 to A375 cells overexpressing RAGE compared with wild type A375 cells and mock-transfected cells demonstrated the direct interaction of [^{18}F]FB-S100A4 and RAGE. Taking into account physiological situations with various competing RAGE ligands available, which exhibit higher concentrations and higher affinity to the receptor, the systemic interaction between extracellular S100A4 and RAGE thus is likely to be of minor importance. On the other hand, a typical attribute of RAGE is that it is expressed at relatively low levels in homeostasis but in situations characterized by enhanced cellular activation or stress, the expression of RAGE is strikingly enhanced, particularly in certain tissue compartments. Under these circumstances, S100A4 could substantially contribute to the overall complex interaction with RAGE. We were later able to elucidate some possible physiological consequences, especially for the tumor microenvironment. First we could demonstrate that macrophage-derived S100A4 interacted with RAGE in melanoma cells in a paracrine manner potentiating tumor invasion and metastatic lung colonization [57]. Subsequent experiments indicated that interaction of S100A4 with RAGE driving a pro-metastatic phenotype is not limited to paracrine signaling. Involvement of an autocrine S100A4-RAGE signaling has been recognized when it was observed that A375 melanoma cells actively secrete S100A4 in the extracellular space via an endoplasmic reticulum-Golgi-dependent pathway. We were able to demonstrate that pro-metastatic properties of melanoma cells like cell motility were diminished by inhibition of this secretory pathway [8]. We also could demonstrate a role for high levels of extracellular S100A4 in decreasing inter-endothelial tight junction integrity by using the A375 melanoma cell model and an *in vitro* blood-brain barrier model. Paracrine-mediated signaling of S100A4 secreted from melanoma cells with RAGE expressed on endothelial cells enabled melanoma cell transmigration. The study also revealed that brain metastasis formation by A375 cells injected intracardially into athymic mice was increased with overexpression of S100A4 and RAGE [58,59]. It has to be clarified whether S100A4 induced endothelial dysfunction is specific for melanoma brain metastasis or rather a general mechanism in S100A4 expressing cancers [60].

5.3. The radiopharmacologist's perspective

S100-based radioligand approaches have high potential to contribute to better understanding of the role of extracellular S100s in specific pathophysiological situations. Gentle radiolabeling under physiological conditions is an essential prerequisite for this. The presented protocol gives a detailed description for the radiosynthesis of [^{18}F]SFB using an automated synthesis module. The synthesis is based on the ^{18}F-labeling of the *tert*-butyl protected *N,N,N*-trimethylammoniumbenzoic acid ester

triflate **1**, followed by a hydrolysis step forming 4-[^{18}F]fluorobenzoate and the final conversion to [^{18}F]SFB. High chemical and radiochemical purity have been achieved by applying a semi-preparative HPLC purification of [^{18}F]SFB presented in this protocol as a further optimization of the previously reported method. Ongoing work aims at investigating in more detail the role of S100-RAGE- and S100-SR axes and their therapeutic targeting in animal models of inflammatory or neoplastic disease. This also includes research to clarify the role of soluble RAGE or soluble SR as decoy receptors and modulators of circulating S100s levels in certain pathophysiological situations. Furthermore, the importance of S100 proteins as a therapeutic target will be better underlined.

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Conflict of Interests

The authors declare that they have no conflicts of interest with respect to research, authorship, and/or publication of this chapter.

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