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Synthesis of new $^{18}$F-radiolabeled silicon-based nitroimidazole compounds

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Abstract
The syntheses of new nitroimidazole compounds using silicon-$^{18}$F-fluorine chemistry for the potential detection of tumor hypoxia are described. $^{18}$F-based compounds were synthesized by coupling 2-nitroimidazole with silyldinaphtyl or silylphenyldi-tert-butyl groups and labeled by fluorolysis or isotopic exchange. Dinaphtyl compounds (6, 10) were labeled in 56–71% yield with a specific activity of 45 GBq/μmol, however these compounds [$^{18}$F] and [$^{18}$F]11 were not stable in plasma. Phenyl-di-tert-butyl compounds were labeled in 70% yield with a specific activity of 3 GBq/μmol by isotopic exchange, or in 81% yield by fluorolysis of siloxanes with a specific activity of 45 GBq/μmol. The labeled compound [$^{18}$F]18 was stable in plasma and excreted by the liver and kidneys in vivo. In conclusion, the fluorosilylphenyldi-tert-butyl (SiFA) group is more stable in plasma than fluorosilyldiphenyl moiety. Thus, compound [$^{18}$F]18 is suitable for further in vivo assessments.

1. Introduction
Molecular imaging is a fast growing research area, including the development of new tools, reagents and methods for imaging the human body. In particular, positron emission tomography (PET) is a powerful non-invasive molecular-imaging technique providing physiological and biological information about the distribution of radiolabeled molecules by 180° coincidence detection of two simultaneously-emitted photons from positron–electron annihilation. $^{18}$F is among the most widely used positron emitters due to its almost ideal physical properties. With a half-life of 110 min and a low-energy positron of 640 KeV, $^{18}$F can yield PET images with high resolution. $^{18}$F is often incorporated into organic molecules by electrophilic or nucleophilic reactions forming a carbon–$^{18}$F bond. Thus, the development of PET followed the need of new techniques for incorporation of radionuclides into biologically active molecules, such as the use of silicon as a fluorine-accepting agent.

Traditional $^{18}$F-labeling requires azeotropically dried $^{18}$F-fluoride under basic reaction conditions at high temperature with the use of a cation-complexing agent, generally Kryptofix [2.2.2], in order to increase the reactivity of fluoride. An alternative method to conventional $^{18}$F-labeling consists of the creation of Si–$^{18}$F, B–$^{18}$F and Al–$^{18}$F bonds1–3 instead of a C–$^{18}$F bond. Herein, we report on the synthesis of organofluorosilane compounds. The use of fluorosilanes was primarily introduced by Rosenthal4 and first in vivo images showed fast bone uptake. Despite the higher thermodynamic bond energy of Si–F compared to the C–F bond, the kinetic stability of the Si–F bond against hydrolysis is very low due to strong bond polarization.5 More recently, Ametamey and Schirrmacher6–10 independently reported efficient labeling of organosilicon compounds. Schirrmacher et al. described a rapid and versatile approach toward the synthesis of a $^{18}$F-labeled peptide6 based on simple $^{18}$F–$^{18}$F isotopic exchange from di-tert-butylphenylfluorosilane. This work confirmed the low in vivo stability of the Si–F bond and overcame this problem by the introduction of sterically-hindered substituents around the silicon atom. Almost parallel to these findings, Ametamey et al. described the synthesis of a 18F-silicon-based building block and employed an approach using the displacement of a leaving group such as an alkoxo moiety, hydroxy group or hydrogen atom under slightly acidic conditions.8 Again, it was shown that the presence of a bulky substituent was a key factor to prevent the hydrolysis of fluorosilane.

In recent years, the research activity in our group focused on the development of new fluorinated tracers for the imaging of hypoxia by means of positron emitting tomography.11 The presence of hypoxic cells in tumors has long been recognized as a major problem in radiotherapy and is also a potential problem in the chemotherapy of cancer. Thus, both identification and quantitative estimation of tumor hypoxia are important issues in the therapeutic strategy

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for a better clinical outcome. Nitroimidazoles are known to be involved in metabolic processes which appear under low oxygen concentration in hypoxic tissues. Various hypoxic tracers containing nitroimidazole moieties have been synthesized such as 1-α-o-(5-[18F]fluoro-5-deoxyarabinofuranosyl)-2-nitroimidazole ([18F]fluorozymycin arabinoside) and 1-(2-nitroimidazolyl)-3-[18F]fluoro-2-hydroxypropanol ([18F]fluoromisonidazole; [18F]F-MISO). However, the interpretation of images is often rendered difficult due to the low signal to noise ratio depending on the tracer itself, and is also influenced by the intensity of hypoxia in the cells.12,13

Clinically, we observed low SUVmax (Standardized Uptake Value) for [18F]F-MISO in NSCLC (non-small-cell lung carcinoma) unlike FDG uptake, where FDG is a tracer of tumor metabolism.14 These low values may be explained by the heterogeneous distribution of hypoxia at the cellular level, far below the spatial resolution of the PET/CT.15 In this case, we looked for a compound with a rapid clearance from healthy tissues and higher retention in hypoxic cells in order to improve the signal-to-noise ratio independently of the hypoxia heterogeneity. Whereas the outline of the hypoxic tumor will remain blurred due to limitations of the PET/CT, the overall signal will be more intense thus providing easier interpretation of the images.

The special specifications of these molecules involve striking a fine balance between the bioreductive trapping in hypoxic cells on the one hand, and on the other hand assessing a selective delivery into hypoxic cells by the hypoxic cells-selective delivery is mainly ensured by its lipophilicity. Commonly, lipophilicity should be between 0.1 ([18F]FETA) and 5.7 ([18F]EF5); lower values leading to rapid clearance from the organism without hypoxic cells uptake; higher values leading to a poor clearance from healthy cells, resulting in a bad signal-to-noise ratio.12,13

However, the development of a new radiotracer allowing a better monitoring of hypoxia is challenging. In previous work, our group reported on the synthesis of new silicon-based analogues of [18F]fluoromisonidazole (Fig. 1).11 The labeling step was improved owing to the better affinity of fluoride for silicon than for carbon. The hydrolytic stability of these new fluorosilanes is, not surprising, correlated to the steric hindrance at the silicon center. The best in vivo stability towards hydrolytic cleavage was observed for a compound having a dinaphtyl substituent. However, the biodistribution of this molecule showed that it was mainly retained in pulmonary capillaries.

In this preliminary work, we report on improvements to the water solubility of previous dinaphtyl silicon-based compounds. Moreover, the stability towards hydrolysis of these new labeled fluorosilanes by comparison with the di-tert-butylphenyfluorosilane prosthetic groups (SiFA) described in the literature16–23 (Fig. 2) will be discussed.

2. Results

2.1. Chemistry

The design of new silicon-based compounds having a 2-nitroimidazole moiety to identify hypoxic cells is reported. In order to improve their solubilities in water, either an amide function (Scheme 1) or a polyethylene glycol linker with a 1,2,3-triazole group was used (Scheme 2).

To prepare the first silicon derivative with an amide group (Scheme 1), (chloropropyl)dinapth-1-ylsilane 1 was synthesized according to a procedure reported for the introduction of bulky substituents onto alkoxychlorosilanes.24 The so-obtained chloro derivative 2 was then reacted with sodium azide to provide the expected compound 3 in 88% yield. The subsequent reduction of the azide function to the corresponding amine 3 was achieved by using hydrogen on 10% Pd/C in 73% yield. Then, the 2-nitroimidazole acid derivative 5, obtained by a classical alkylation reaction of 2-nitroimidazole with tert-butyl-2-bromoacetate followed by the hydrolysis of the tert-butyl group, was coupled with amine 3 to afford precursor 6 in 63% yield.

The second silicon derivative having a polyethylene moiety with a 1,2,3-triazole group was prepared from 2-(2-bromoethoxy)ethanol (Scheme 2). This compound was reacted with propargyl bromide and sodium hydride in THF to afford compound 7 in 94% yield. The so-obtained chloro derivative 8 was then reacted with a solution of 2-nitroimidazole in DMF to give the expected compound 9 in 60% yield. Subsequently, the previously prepared azide 2 was reacted with alkyne 9 in presence of copper(II) to give precursor 10 in a moderate 44% yield.

In parallel to the generation of the Si–F bond via siloxane fluororolysis, we also developed a new nitroimidazole-containing compound 18, using di-tert-butylphenyfluorosilane as a building block. This approach was firstly developed by Schirrmacher et al. in 2006 and provided superior stability to hydrolysis towards silyl ether.6 Labeling can be achieved from the precursor 17a via an isotopic exchange reaction. Finally, radiolabeling can also be performed from 17b using the Ametamey approach based on exchange with a hydrogen atom under slightly acidic conditions.9 Precursor 17a and 17b were prepared as reported by Kostikov (Scheme 3).25,26 Compounds 12a and 12b were synthesized by nucleophilic substitution of di-tert-butylidifluorosilane and di-tert-butylchlorosilane respectively, starting from the reaction between ((3-bromobenzyl)oxy)(tert-butyl)dimethylsilane and tert-BuLi. Acidic deprotection afforded compounds 13a and 13b in good yields. Following oxidation of benzyl alcohol moiety (13a) to benzaldehyde (15), activation with N-hydroxysuccinimide (16) then coupling with 2-(2-nitro-1H-imidazol-1-yl)ethyamine, precursor 17a was obtained. After oxidation of benzyl alcohol moiety (13b) leading benzoic acid (14) then coupling with 2-(2-nitro-1H-imidazol-1-yl)ethyamine27 precursor 17b was obtained.

2.2. Radiochemistry

Radiolabeling of 6 and 10 initially proceeded with a common nucleophilic substitution reaction using CH3CN as solvent, and a Kryptofix 2.2.2/[k2CO3 system to produce naked, highly reactive [18F] fluoride anion even in the presence of acetic acid.9 Running this reaction at room temperature gave a poor conversion. However, at 75 °C, high [18F] incorporation was obtained. An excess of acetic acid (50 μL) is needed for a better radiochemical yield.
(RCY). Under these conditions (75 °C, KF/K222, CH3CN, AcOH), 71% and 56% 18F-incorporation can be reached respectively for [18F]7 and [18F]11, after a 30 min reaction time. Following solid phase extraction (through Waters SE Pack silica), [18F]7 and [18F]11 were easily isolated and re-dissolved in a 0.9% NaCl solution for injection (Schemes 1 and 2). This solution, suitable for injection contains ethanol at a concentration of 5%.

Radiolabeling of 17a was first carried out under classical conditions of isotopic exchange (room temperature, K18F/Kryptofix K2.2.2, AcOH, CH3CN, 70 °C). When gradually increasing the temperature from 25 °C to 100 °C, the yield rose from 0% to 70%. The results concerning this optimization are listed in Table 1, entries 4–9. We observed a rapid degradation of the precursor 17a in presence of K18F. In order to avoid it, we used acetic acid on K18F/Kryptofix 2.2.2 before adding 17a in the reaction mixture. This result highlighted the requirement for slightly acidic conditions to obtain good RCY (glacial acetic acid: 83 μmol and sodium carbonate: 25 μmol; acid/base molar ratio was approximately 3.5). High temperature was also necessary to allow rapid incorporation of the [18F] fluoride anion. The crude reaction mixture was purified by means of preparative reverse phase-HPLC. Radiolabeled compound was then isolated in 65% RCY and >98% radiochemical purity. [18F]18 compound was also obtained from 17b by direct nucleophilic substitution of the hydroxyl leaving group.28,29 Previous conditions were applied (0.1 mg; 100 °C; K18F/Kryptofix 2.2.2; CH3CN), and led to the formation of [18F]18 in 24% RCY. A better yield (66%) can be obtained by increasing the amount of precursor 17b from 0.1 mg to 1 mg. The yield can also be improved to 81% when conducting the reaction at 100 °C in DMSO instead of CH3CN. Temperature is an important parameter, since in DMSO at 60 °C the RCY was only 29%. The amount of acetic acid also has a dramatic influence on the RCY, since adding a large quantity decreased the yield (Table 1, entries 10–15).


After purification, radiolabeled compounds were reformulated in saline (solution for injection). Hydrolytic stabilities were evaluated by radio-HPLC and radio-TLC analysis of [18F]7, [18F]11 and [18F]18 aqueous samples at different times (5, 15, 30, 60, 120 min). Dinaphthyl compounds [18F]7, [18F]11 have a half-life of about 60 min whereas no hydrolysis occurred after 180 min for compound [18F]18. In vitro stability was also evaluated by incubating a solution of [18F]7, [18F]11 and [18F]18 in human plasma. Dinaphthyl compounds [18F]7 and [18F]11 were found to be unstable and rapid radiolysis occurred after a few minutes whereas the SiFA compound [18F]18 was stable for at least 120 min. After this period of time, less than 10% of decomposition of the compound [18F]18 occurred in water at pH 7.4. When compounds [18F]7 and [18F]11 were injected in mice, fast bone uptake was observed, demonstrating a rapid in vivo release of 18F whereas in the case of the di-tert-butyl compound, [18F]18, only a small amount of bone uptake was measured after a 90 min acquisition (Table 3).

2.4. Lipophilicities of compounds [18F]7, [18F]11 and [18F]18

The lipophilicities of compounds [18F]7, [18F]11 and [18F]18 were determined under physiological conditions (logD) or by clogP calculations using ChemDraw 11.0 software. Compound [18F]7 was insufficiently stable at pH 7.4, thus the lipophilicity was measured at pH 4. The low logD value found (0.12) seems unreliable and might be explained by a fluoride release from the native compound. The clogP value of [18F]18 is 5.01. Compound [18F]18 was sufficiently stable at pH 7.4 to estimate the logD (2.12), and the clogP of compound [18F]11 was estimated by calculation because of its instability. These results are summarized in Table 2.
2.5. Preliminary biological evaluation of compound [18F]18

In vivo assessment of [18F]18 was achieved by injection of a Wistar rat. Dynamic acquisitions under a Mosaic PET camera were performed until 90 min after tracer injection. Fluorosilane [18F]18 was distributed in all compartments of the organism, but predominantly in liver, intestine and bladder after 90 min post injection. These results indicate an extensive hepatic extraction paired with renal extraction. A small amount of uptake in bones was measured. No significant uptake in heart or muscle was observed (Table 3).

3. Discussion

The aim of this work was to synthesize new fluorinated nitroimidazole compounds in order to further explore their potential for their in vivo imaging of hypoxia by means of PET. The most used compound in nuclear medicine for this indication is
[18F]-F-MISO. The drawback of this compound is its bad signal-to-background ratio. We synthesized nitroimidazole compounds with a silicon core in order to improve the radiochemical yield. Indeed, the typical radiochemical yield of [18F]-F-MISO is close to 60% as previously reported in literature with 5 mg of precursor. Our method afforded yields ranging from 56% to 71% with 1 mg of precursor for dinaphtyl compounds and up to 82% for SiFA compound.

In a previous study we observed that dimethyl, diisopropyl and diphenyl fluorosilanes were unstable in water, when dinaphtylfluorosilane (See Fig. 1; R = Napht) revealed a good stability. However, we also noted that the labeling from precursor [18F] was retained in the pulmonary capillaries maybe due to its lipophilicity (clogP = 6.47). This lipophilicity is outside the limits (0.1–5.7) set by other hypoxic tracers such as EF5 (clogP = 5.7). However all attempts to increase the hydrophilicity of this compound by introducing polar substituents at the naphthalene moiety failed. Thus, we undertook to tune the hydrophilicity of the linker between the silicon core and the nitroimidazole.

Compounds [18F]7 and [18F]11 were not stable in saline (pH 5.5), exhibiting half-lives around 60 min, whereas the first dinaphtylfluorosilane synthesized had a half-life of 130 min. In basic conditions, compounds [18F]7 and [18F]11 were more prone to nucleophilic attacks by hydroxyl ion. At 37 °C in plasma, half-lives of both compounds were below 5 min. We then refocused our efforts to the SiFA (Silicon–fluoride-acceptor) compounds developed by Schirrmacher et al.6–8,16–23 In this case, silicon is substituted by a phenyl and two -butyl groups.

Two different radiolabeling pathways can provide access to compound [18F]18. The first one involves the stable compound [18F]17a where fluorine is isotopically exchanged by fluorine-18. The second way consists of the addition of fluorine-18 on the silane [18F]17b. We used glacial acetic acid as an additive for this purpose. Obviously, the specific activity was better when using the second procedure (respectively, for the first and the second way: 3 GBq/μmol and ~50 GBq/μmol), the radiochemical yields being similar. The specific activity was calculated from a concentration range of cold fluorinated compound and activities obtained after labeling. The second way involving the precursor [18F]17b can be performed both in DMSO and CH3CN and does not require a defined amount of acetic acid since the precursor is not sensitive to hydrolysis in basic conditions. We also noticed that the labeling from precursor [18F]17a may be achieved directly in CH3CN/H2O (9:1), with a good RCY (65–75%). Moreover, the mechanism of action of hypoxic tracers does not require a high specific activity. Indeed, the uptake of a nitroimidazole compound is not a saturable process and does not involve a specific receptor but only a redox reaction involving the nitro group in the hypoxic cell.

Hydrolytic stability of compound [18F]18 is promising (100% in saline after two hours, and 85% in plasma after two hours). Since hydrolysis of fluorosilanes can be avoided with bulky groups at the silicon atom, it is likely that the -butyl groups provide better steric hindrance than the naphtyl groups. The silicon atom is thus protected from nucleophilic attack, and the stability of the silicon–fluorine bond is improved in water. Nevertheless, in plasma, we noticed a slight degradation of the labeled compound [18F]18 after two hours, which might be due to the presence of enzymes such as hydrolase, or peptidase.

When compounds [18F]7 or [18F]11 were injected in rat, extensive uptake of [18F] occurred in bones, demonstrating a rapid release of fluoride in vivo. These results were in agreement with the in vitro experiments. When compound [18F]18 was injected in rat, after 90 min, we observed a biodistribution of the tracer throughout the organism, including bones (Fig. 3).

The stability of compound [18F]18 is within the range found with other SiFA labeling procedures. Further studies will be needed to evaluate this radiolabeled compound [18F]18 in mice bearing hypoxic tumors. Indeed, this tracer has a lipophilicity (logD = 2.12) that could hamper its clearance from healthy tissues, but is favorable with hypoxic tumor uptake. Nevertheless, this study confirms that -butyl groups stabilize the silicon–fluorine bond more than naphtyl groups in vitro and in vivo.

4. Conclusion

We have described the preparation of various [18F]silamisonidazoles derivatives. Only the SiFA derivatized misonidazole is sufficiently stable in vivo. This compound may be directly labeled by isotopic exchange or by fluorine addition on the corresponding silane in DMSO or acetonitrile. These mild reaction conditions make the synthesis of F-MISO analogous a key for the development of new 18F-radiopharmaceuticals dedicated to the detection of tumoral hypoxic domains. Compound [18F]18 will be assessed as a hypoxic tumor tracer in upcoming studies.
5. Experimental

5.1. Reagents and instrumentation

All commercially available reagents were purchased from Sigma/Aldrich or Alfa Aesar and used without further purification. Di-tert-butyldifluorosilane was purchased from Fluorochem. Unless otherwise stated, reactions were performed under nitrogen atmosphere using freshly distilled solvents. All reactions were monitored by thin-layer chromatography with Merck silica gel 60 F254 pre-coated aluminum plates (0.25 mm). Flash chromatography was performed with indicated solvents using silica gel (particle size 30–63 μm) purchased from Merck. The SAX cartridges (Sep-Pak Light (46 mg) Accell Plus QMA Carbonate) were purchased from Waters. 1H, 13C and 19F NMR (CDCl3) δ 173.2; 133.5; 130.8; 128.9; 128.6; 126.1; 125.6; 125.3; 77.4; 59.6; 45.4; 27.9; 12.8. IR (ATR, cm−1) 3053; 2928; 2863; 1501; 1075; 985; 795; 775. HR-MS (ESI+) calcd for C25H24NO4Si [M+H]+: 396.1940, found: 396.1941.

5.2.1. tert-Butyl 2-(2-nitro-1H-imidazol-1-yl)acetate 4. tert-Butyl 2-bromooacetate (0.18 g, 0.97 mmol) was added to a solution of 2-nitroimidazole (0.1 g, 0.88 mmol) and potassium carbonate (0.12 g, 0.88 mmol) in acetonitrile (2 mL) and reaction mixture was refluxed for 3 h. The mixture was filtered and solvent removed under reduced pressure to give the title compound 4 as a white solid (0.19 g, 98%). 1H NMR (CDCl3) δ 7.91 (1H, d, J = 5 Hz), 7.06 (1H, d, J = 1 Hz); 5.00 (2H, s), 1.47 (9H, s). 13C NMR (CDCl3) δ 161.3; 128.5; 126.6; 84.4; 51.8; 28.1. IR (ATR, cm−1) 3133; 2983; 1741; 1491; 1356; 1145; 830; 775; 750; 650. HR-MS (ESI+) calcd for C16H12N3O4 [M+H]+: 228.0984, found: 228.0983.

5.2.1.2. 3-(Ethoxydi(naphthalen-1-yl)silyl)propan-1-amine 3. tert-Butyl-(2-nitro-1H-imidazol-1-yl)acetate (0.19 g, 0.86 mmol) 4 was dissolved in a 30% solution of trifluoroacetic acid in dichloromethane and stirred for 1 h at room temperature. The solvent was removed in vacuo to give compound 5 as a white solid (0.13 g, 90%) which can be used without further purification: 1H NMR (MeOD) δ 7.65 (1H, s); 7.22 (1H, s); 5.23 (2H, s). 13C NMR (MeOD) δ 169.9; 129.0; 128.4; 51.8. IR (ATR, cm−1) 3148; 2987; 2517; 1746; 1496; 1366; 1145; 775; 705. HRMS (ESI−) calcd for C16H12N3O4 [M−H]−: 170.0202, found: 170.0195.

5.2.1.3. N-(3-(Ethoxy(naphthalen-1-yl)silyl)propyl)-2-(2-nitro-1H-imidazol-1-yl)acetamide 6. Compound 3 (0.09 g, 0.23 mmol) was added to a solution of 2-(2-nitro-1H-imidazol-1-yl)acetamide 5 (0.07 g, 0.25 mmol) in dry dichloromethane with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (0.04 g, 0.25 mmol). The reaction mixture was stirred at room temperature overnight. Then, the organic layer was washed with 10% HCl and saturated Na2CO3 solution. The organic layer was dried over Na2SO4 and concentrated in vacuo to give a pale yellow solid. The crude product was purified by flash chromatography using ethyl acetate as eluant. Yield was purified by preparative RP-HPLC to remove silanol side product than can be formed during the synthesis. Compound 6 was obtained as a white solid (0.08 g, 63%). 1H NMR (CDCl3) δ 8.20 (2H, 2d, J = 8 Hz); 7.94 (2H, d, J = 7 Hz); 7.50 (2H, d, J = 7 Hz); 7.49 (2H, d, J = 7 Hz); 7.41 (2H, td, J = 7 Hz, J = 1 Hz); 7.32 (2H, td, J = 8 Hz, J = 1 Hz); 3.75 (2H, q, J = 5 Hz); 3.23 (2H, t, J = 5 Hz); 1.71–1.62 (2H, m), 1.59–1.51 (2H, m); 1.25 (3H, d, J = 13 Hz). 13C NMR (CDCl3) δ 173.1; 135.3; 133.6; 133.5; 131.0; 128.9; 128.4; 126.2; 125.7; 125.3; 59.7; 54.3; 23.5; 15.6. IR (ATR, cm−1) 3053; 2923; 2902; 1261; 1270; 805; 770. HR-MS (ESI+) calcd for C40H32N6O12Si4 [M+Na]+: 573.1675, found: 573.1672.

5.2.1.4. N-(3-(Fluorodi(naphthalen-1-yl)silyl)propyl)-2-(2-nitro-1H-imidazol-1-yl)acetamide 7. Compound 6 (0.010 g, 0.02 mmol) was dissolved in dry THF. Hydrogen fluoride-pyridine complex was added (1.1 mg, 0.04 mmol) and the mixture was stirred for 20 min at room temperature. Then, the solvent was removed under vacuum to give pale a yellow solid in quantitative yield (0.10 g). 1H NMR (CDCl3) δ 8.07 (2H, 2d, J = 8 Hz); 7.61 (2H, d, J = 8 Hz); 7.59 (2H, d, J = 8 Hz); 7.49 (2H, d, J = 8 Hz); 7.23 (2H, d, J = 8 Hz); 3.23 (2H, t, J = 8 Hz); 3.75 (2H, q, J = 5 Hz); 2.64 (2H, t, J = 5 Hz); 1.48–1.41 (4H, m); 1.22 (3H, t, J = 5 Hz). 13C NMR (CDCl3) δ 137.2; 130.5; 134.0; 133.5; 130.8; 128.9; 128.6; 126.1; 125.6; 125.3; 77.4; 59.6; 45.4; 27.9; 12.8. IR (ATR, cm−1) 3053; 2928; 2863; 1501; 1075; 985; 795; 775. HR-MS (ESI+) calcd for C42H28N6O12Si4Cl4M+ [M+Cl]+: 573.1675, found: 573.1672.

5.2.1.5. N-(3-(Fluoro(naphthalen-1-yl)silyl)propyl)-2-(2-nitro-1H-imidazol-1-yl)acetamide 7. Compound 6 (0.010 g, 0.02 mmol) was dissolved in dry THF. Hydrogen fluoride-pyridine complex was added (1.1 mg, 0.04 mmol) and the mixture was stirred for 20 min at room temperature. Then, the solvent was removed under vacuum to give pale a yellow solid in quantitative yield (0.10 g). 1H NMR (CDCl3) δ 8.07 (2H, 2d, J = 8 Hz); 7.61 (2H, d, J = 8 Hz); 7.59 (2H, d, J = 8 Hz); 7.49 (2H, d, J = 8 Hz); 7.23 (2H, d, J = 8 Hz); 3.23 (2H, t, J = 8 Hz); 3.75 (2H, q, J = 5 Hz); 2.64 (2H, t, J = 5 Hz); 1.48–1.41 (4H, m); 1.22 (3H, t, J = 5 Hz). 13C NMR (CDCl3) δ 137.2; 130.5; 134.0; 133.5; 130.8; 128.9; 128.6; 126.1; 125.6; 125.3; 77.4; 59.6; 45.4; 27.9; 12.8. IR (ATR, cm−1) 3053; 2928; 2863; 1501; 1075; 985; 795; 775. HR-MS (ESI+) calcd for C42H28N6O12Si4Cl4M+ [M+Cl]+: 573.1675, found: 573.1672.
3295; 2933; 1663; 1491; 1367; 910; 779; 720; 447. HRMS (ESI) calc'd for C29H28N4O3Si [M+H]+: 513.1758, found: 573.1752.

5.2.2. Synthesis of analogue 11

5.2.2.1. 3-(2-Chloroethyl)ethoxy)prop-1-yn-8. Sodium hydride (0.16 g, 16.06 mmol) was suspended in THF (25 mL) and stirred at −20 °C. A solution of 2-(2-chloroethyl)ethanol (0.85 mL, 8.03 mmol) was added. The reaction mixture was stirred at −78 °C for 15 min then, a solution of propargyl bromide (0.83 mL, 9.64 mmol) in THF (5 mL) was added. The mixture was refluxed for 3 h.

5.2.2.2. 2-Nitroimidazole 9. Sodium iodide (0.12 g, 0.80 mmol) and potassium carbonate (0.12 g, 0.90 mmol) were added to a solution of 2-nitroimidazole (0.10 g, 0.88 mmol) and compound 8 (0.14 g, 0.90 mmol) in DMF (10 mL). The reaction mixture was stirred for one night at 110 °C then the solution was removed under reduced pressure and residue purified on silica gel (AcOEt/DCM: 1:1). Compound 9 was isolated in good yield (0.11 g, 60%).

5.2.2.3. 2-Nitroimidazol-1-yl)ethoxy)ethyl)methyl)-1H-imidazole 10. CuSO4·5H2O (0.02 g, 0.07 mmol) and sodium ascorbate (0.01 g, 0.07 mmol) were added to a solution of compound 9 (0.10 g, 0.42 mmol) and azide 2 (0.14 g, 0.35 mmol) in dioxane (20 mL). The reaction mixture was stirred at room temperature overnight and solvent was removed under reduced pressure. The residue was purified by flash chromatography (AcOEt/DCM/MeOH: 1:1:0.05) (0.10 g, 44%).

5.2.2.4. 1-(3-Ethoxy(4-naphthalen-1-yl) propyl)-4-(2-(2-Nitroimidazol-1-yl)ethoxy)ethoxy)methyl)-1H-1,2,3-triazole 11. Compound 10 (0.01 g, 0.02 mmol) was dissolved in dry THF. Hydrogen fluoride-pyridine complex was added (0.04 mmol) and the mixture was stirred for 20 min at room temperature. Then, solvent was removed under vacuum to give a pale yellow solid in quantitative yield (0.12 g). HRMS (ESI) calc'd for C39H28N4O3Si [M+H]+: 561.2751, found: 561.2772.

5.2.3. 3-(Di-tert-butylsilyl)benzyl alcohol 13a. A solution of tert-butyl lithium in pentanes (1.7 M, 1.13 mL) was added dropwise to a solution of (3-bromobenzyl)oxy)dimethylsilane (0.26 g, 0.86 mmol) in anhydrous THF (2 mL) over a period of 15 min at −4 °C. The reaction mixture was allowed to warm to room temperature overnight. The reaction was quenched by addition of saturated aqueous sodium chloride solution, and the product was extracted with Et2O (3 × 5 mL). The combined organic phase was dried over Na2SO4, filtered, and concentrated in vacuo to afford 12a as a pale-yellow liquid (0.29 g, 88%) that was carried forward without further purification.

5.2.3.3. 3-(Di-tert-butylsilyl)benzyl alcohol 13a. A concentrated aqueous hydrochloric acid solution (37 wt%, 25 μL) was added dropwise to a solution of crude 12a (0.28 g, 0.73 mmol) in MeOH (2.5 mL) at 25 °C, and the mixture was stirred at room temperature overnight. After all volatiles had been removed under reduced pressure, the residue was re-dissolved in ether (2 mL), and the solution was washed with saturated aqueous sodium carbonate solution (2 mL). The aqueous phase was extracted with ether (3 × 3 mL), and the combined organic phase was washed with H2O (3 mL), dried over Na2SO4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (9:1 to 7:1 hexanes/EtOAc) to afford 13a as a white solid (0.13 g, 65%).

5.2.3.4. 3-(Di-tert-butylsilyl)benzyl alcohol 13b. Following the previous procedure, tert-3-(Di-tert-butylsilyl)phenyl)methanol 13b was obtained as a colorless liquid without further purification (75%).

5.2.4. 3-(Di-tert-butylsilyl)benzoic acid 14. 3-(Di-tert-butylsilyl)phenyl)methanol 13b (0.12 g, 0.48 mmol) was solubilised in acetone (2.5 mL) and cooled to 0 °C. Jones Reagent (8 M,
0.26 mL, 2 mmol) was added dropwise. The reaction was stirred for 30 min at 0°C. Then the reaction was quenched with water (5 mL) and extracted with ethyl acetate (3 × 5 mL). The organic layers were combined, extracted with water, brine, dried over Na2SO4 and solvent removed under reduced pressure. Crude product was purified by flash chromatography on silica gel (Petroleum ether/AcOEt/AcOH: 90:9:1) to give 14 as a white solid (0.09 g, 71%).

1H NMR (CDCl3) δ 8.33 (1H, s); 8.11 (1H, dt, J = 5 Hz, J = 2 Hz); 7.81 (1H, dt, J = 5 Hz, J = 2 Hz); 7.45 (1H, t, J = 5 Hz); 3.93 (1H, s); 1.06 (18H, s). 13C NMR (CDCl3) δ 170.9; 141.2; 137.4; 130.8; 127.8; 29.0; 19.1. IR (ATR, cm⁻¹) 2928; 2855; 2102; 1677; 1586; 1467; 1428; 1288; 1142; 941; 774. HRMS (ESI) calcd for C15H23F2O3Si [M-H]: 263.1467, found: 263.1467.

5.2.3.6. 3-(Di-tert-butylfluorosilyl)benzaldehyde 15. A solution of 13a (0.08 g, 0.28 mmol) in anhydrous CHCl3 (50 mL) was added dropwise to a solution of pyridinium chlorochromate (0.15 g, 0.70 mmol) in anhydrous CH2Cl2 (15 mL) at 0°C. After drying, a solution of the precursor in DMSO or CH3CN using a stream of helium at 105°C. The product was confirmed by comparison with the HPLC retention times (RT) were 4.30 min and 4.85 min for 18F. The reaction mixture was added to water (2 mL) and brine (2 mL). After drying under Na2SO4, solvent was removed under reduced pressure and the crude product purified on silica gel (AcOEt/Petroleum ether: 82:0.5, 0.6%). 1H NMR (CDCl3) δ 7.94 (1H, s); 7.14 (1H, t, J = 5 Hz); 7.06 (1H, s); 6.91 (1H, s); 4.73 (2H, t, J = 5 Hz); 3.91 (2H, q, J = 5 Hz); 3.88 (1H, s); 1.02 (18H, s). 13C NMR (CDCl3) δ 169.2; 139.2; 136.8; 134.5; 132.7; 128.8; 127.9; 49.3; 40.4; 29.0; 19.1. IR (ATR, cm⁻¹) 2933; 2839; 1645; 1536; 1450; 1365; 1160; 830; 702. HRMS (ESI) calcd for C25H28F2O3Si [M+Na]: 402.1507, found: 402.1510.

5.3. Radiochemical synthesis

5.3.1. Production of [18F]-F[18F] was produced after a (p, n) reaction from the IBA Cyclone 18/9 cyclotron (Université Catholique de Louvain—UCL, Belgium) by irradiating [18O]-H2O with 16.5 MeV protons. The [18F]F⁻ was separated from the [18O]-H2O by trapping on an ion exchange resin (QMA, from Waters). QMA resin was previously conditioned with sodium bicarbonate (10 mL, 1 M) and water (20 mL). The [18F]F⁻ was eluted by a solution of Kryptofix 2.2.2 (15 mg, 40 mmol) and K2CO3 (3.5 mg, 25 mmol) in CH3CN/H2O (9:1, 1 mL). The residual water was removed by coevaporation to dryness with CH3CN using a stream of helium at 105°C. This step was repeated twice more with 0.5 mL CH3CN.

5.3.2. [18F] Radiolabeling of ethoxydi(naphthalen-1-yl)silyl)propyl derivatives 6 and 10

After drying, a solution of the precursor in DMSO or CH3CN (300 μL) with AcOH (5 μL) was added. The reaction mixture was stirred at 75°C for 15 min to effect labeling. The crude reaction mixture was analyzed by analytical reversed phase-HPLC (CH3CN/Water: 70:30, 1 mL min⁻¹, column: Nucleosil 100-5 C18, 150 × 4.6 mm, 5 μm) and by radio-TLC. The peak of the [18F]F⁻ labeled product was confirmed by comparison with the HPLC retention time of its non-radioactive reference molecule. The retention times (RT) were 4.30 min and 4.85 min for [18F]F⁻ ([N-(3-fluorodi(naphthalen-1-yl)silyl)propyl]-2-(2-nitro-1H-imidazol-1-yl)benzamide) and [18F]F⁻ ([N-(1-(3-fluorodi(naphthalen-1-yl)silyl)propyl)-4-(2(2-nitro-1H-imidazol-1-yl)ethoxy)ethoxy)ethyl)-(1H-1,2,3-triazole). Subsequently, the reaction mixture was added to water (800 mL) and loaded on a Waters SepPak silica cartridge. The latter had been preconditioned by subsequent rinsing with ethanol (5 mL) and water (10 mL). The trapped [18F]F⁻[ethoxydi(naphthalen-1-yl)silyl]propyl derivatives 6 or 10 were washed with water (5 mL) eluted from the cartridge with ethanol (3 × 2 mL). The second ethanol fraction was diluted with physiological saline solution (0.9%) to give an injection solution usable for animal experiments.

5.3.3. [18F] Radiolabeling SiFA compound 17a and 17b

To a dry [Kryptofix 2.2.2.][18F] complex (7–9 GBq), 17a (0.1 mg) or 17b (1.0 mg) and glacial acetic acid (5 μL) in anhydrous DMSO or CH3CN (300 μL) were added. After heating at 110°C for 15 min, the reaction mixture was diluted with HPLC eluent...
(2 mL, CH$_3$CN/H$_2$O 3:2). This solution was injected into a semi-preparative HPLC (isocratic, 3.0 ml/min, hypersil gold 150 × 10 mm, 5 μ), the product peak was identified by comparison with non-radioactive reference molecule and collected. The retention time (RT) of $^{[18F]}$F18 was 8.35 min. The decay corrected radiochemical yield of the-isolated product from Si–H precursor 17b was 70% with >98% radiochemical purity. The decay corrected radiochemical yield of the isolated product via isotopic exchange from precursor 17a was 64% with >98% radiochemical purity.

Alternative labeling method of $^{[18F]}$F18 from 17a: The $^{[18F]}$F- (40 mCi) was eluted from the QMA cartridge by a mixture of Kryptofix 2.2.2 (15 mg, 40 μl) and K$_2$CO$_3$ (3.5 mg, 25 μmol) in CH$_3$CN/H$_2$O (9:1; 1 mL). Glacial acetic acid (50 μL) was added to this mixture before the addition of 17a (0.1 mg). After heating at 100 °C for 15 min in a sealed vial, the reaction mixture was diluted with HPLC eluent (2 mL, CH$_3$CN/H$_2$O 3:2). This solution was injected into a semi-preparative HPLC (isocratic, 3.0 ml/min, hypersil gold 150 × 10 mm, 5 μ), the product peak was identified by comparison with non-radioactive reference molecule and collected. The retention time (RT) of $^{[18F]}$F18 was 8.35 min. The experimental conversion yield was between 65% and 75% under these conditions.

5.4. Biological evaluation

The wistar rats (300–350 g) were injected with 10 MBq of $^{[18F]}$F]-silafuorinated compound ($^{[18F]}$F7, $^{[18F]}$F11 or $^{[18F]}$F18). Dynamic acquisitions under Mosaic PET camera (Philips Medical Systems, Cleveland, OH, USA)$^{30}$ were performed with 5-min frames during 60 min after $^{[18F]}$F]-silafuorinated compound injection. Then, a static imaging was performed 90 min after injection. ROI’s were drawn on bones, liver, intestine, heart, bladder and muscle. Standard uptake values (SUVs) were calculated. All images contained at least 10$^3$ true events. All images were reconstructed with a fully 3D iterative algorithm (3D-RLMA). Before reconstruction, raw data were corrected for random and scattered coincidences and for system dead-time. Each reconstructed matrix was composed of 120 transverse 128 × 128 images with cubic voxels of 1 mm. Animals were maintained in a facility approved by the Belgian ministry of agriculture in accordance with current regulations and standards. ‘Principles of laboratory animal care’ (NIH publication No. 86–23, revised 1985) were strictly followed.

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References and notes