Supporting information for

Synthesis of 3-chloro-6-((4-(di-tert-butyl[^{18}F]fluorosilyl)-benzyl)oxy)-1,2,4,5-tetrazine ([^{18}F]SiFA-OTz) for fast Tetrazine-Based ^{18}F-Radiolabeling

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Experimental

Commercial Solvents and Reagents Used. Guanidine hydrochloride, hydrazine monohydride, 2,4-pentanedione, sodium nitrite, trichloroisocyanuric, 2,4,6-collidine, Kryptofix 2.2.2, oxalic acid, anhydrous acetonitrile, anhydrous dichloromethane, and anhydrous dimethyl sulfoxide were all purchased from Aldrich and were used as received. Potassium carbonate (Caledon), chloroform-d6 (Cambridge Isotope Laboratories) were also used as received. Highly enriched [^{18}O]water (>97%) was purchased from Rotem. The SAX cartridges (Sep-Pak Light (46 mg) Accell Plus QMA Carbonate, Sep-Pak C18 Light Cartridge, Sep-Pak Dry Sodium Sulfite Light Cartridge, Sep-Pak Silica Light Cartridge) were purchased from Waters (USA). SiFA-OH(**2**) was synthesised as per reference[1]. Dichlorotetrazine was synthesized following a slightly modified procedure developed by Hiskey.[2,3,4] An important concern however has to be highlighted: caution must be exercised when handling large quantities of tetrazine, as tetrazine is reported to be explosive.[5] SiFA-OH was synthesized following the referenced procedure.[15] All solvents were purchased from Aldrich and were used as received.

Instrumentation. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded using a Mercury 400 (400MHz) and Inova (500) MHz in deuterated chloroform solution and are reported in parts per million (ppm), with the residual protonated solvent resonance used as a reference. HR-MS (ESI, APCI) analyses were recorded in the McGill University, Department of Chemistry Mass Spectrometry Facility. Radio TLCs were monitored by a Mini Gita (Raytest) TLC reader. Analytical HPLC were performed on an Agilent Technologies 1200 system equipped with a Gabi radioactivity detector (Raytest).

Synthesis of 3-chloro-6-((4-(di-tert-butylfluorosilyl)-benzyl)oxy)-1,2,4,5-tetrazine (SiFA-OTz). Dichloroteterazine (21.0 mg, 0.14 mmol), (4-(di-tert-butylfluorosilyl)phenyl)methanol (SiFA-OH, 34.0 mg, 0.13 mmol), and 16.8 μL of anhydrous 2,4,6-collidine (0.13 mmol) were dissolved in 10 mL of anhydrous CH_2Cl_2 under Ar. The solution turned from orange to pink in 5 min. The reaction continued for 35 min and the solvent was then removed by rotary evaporation. SiFA-O-Tz was then purified by flash chromatography and gave 45.4 mg of SiFA-O-Tz as a red crystal with a 95% separation yield. SiFA-O-Tz has been stored in solid form for over three months, or in solution for 2 weeks, and no dissociation was detected by NMR and TLC.^{1}H NMR ($CDCl_3$, 400MHz) δ(ppm): 7.68 (d, J = 8 Hz, 2H), 7.56 (d, J = 8 Hz, 2H), 5.70 (s, 2H), 1.06 (s, 18H). ^{13}C NMR (CDCl3, 60MHz) δ(ppm): 166.5, 164.5, 135.2 (d, J = 13.7 Hz), 134.8, 134.4 (2C, d, J = 4.3 Hz), 127.8(2C), 71.9, 27.3 (6C), 20.3 (2C). HR-MS (APCI), exact mass ($[M+H]^+$, $C_{17}H_{25}ClFN_4OSi$) calc. 383.1470; found: 383.1480.

Model reaction of SiFA-OTz with trans-cyclooctenol. 5mg (13.0 μmol) of SiFA-OTz was dissolved in 5 mL of dichloromethane. 1.65 mL of trans-cyclooctenol/DMSO solution (1mg/mL,

13.0 μmol) was added under stirring. The reactants color fades from pink to pale yellow instantly. The crude mixture was analyzed by HPLC-MS and HRMS. Unfortunately, different isomers cannot be isolated by liquid flash chromatography or HPLC due to their similar structure and polarity. HPLC-MS (ESI) showed one single peak at 11.6 min, m/z 481.36 $[M+H]^+$ at 254 nm. HRMS (APCI), exact mass ($[M+H]^+$, $C_{25}H_{39}ClFN_2O_2Si$) calc. 481.2453; found: 481.2449.

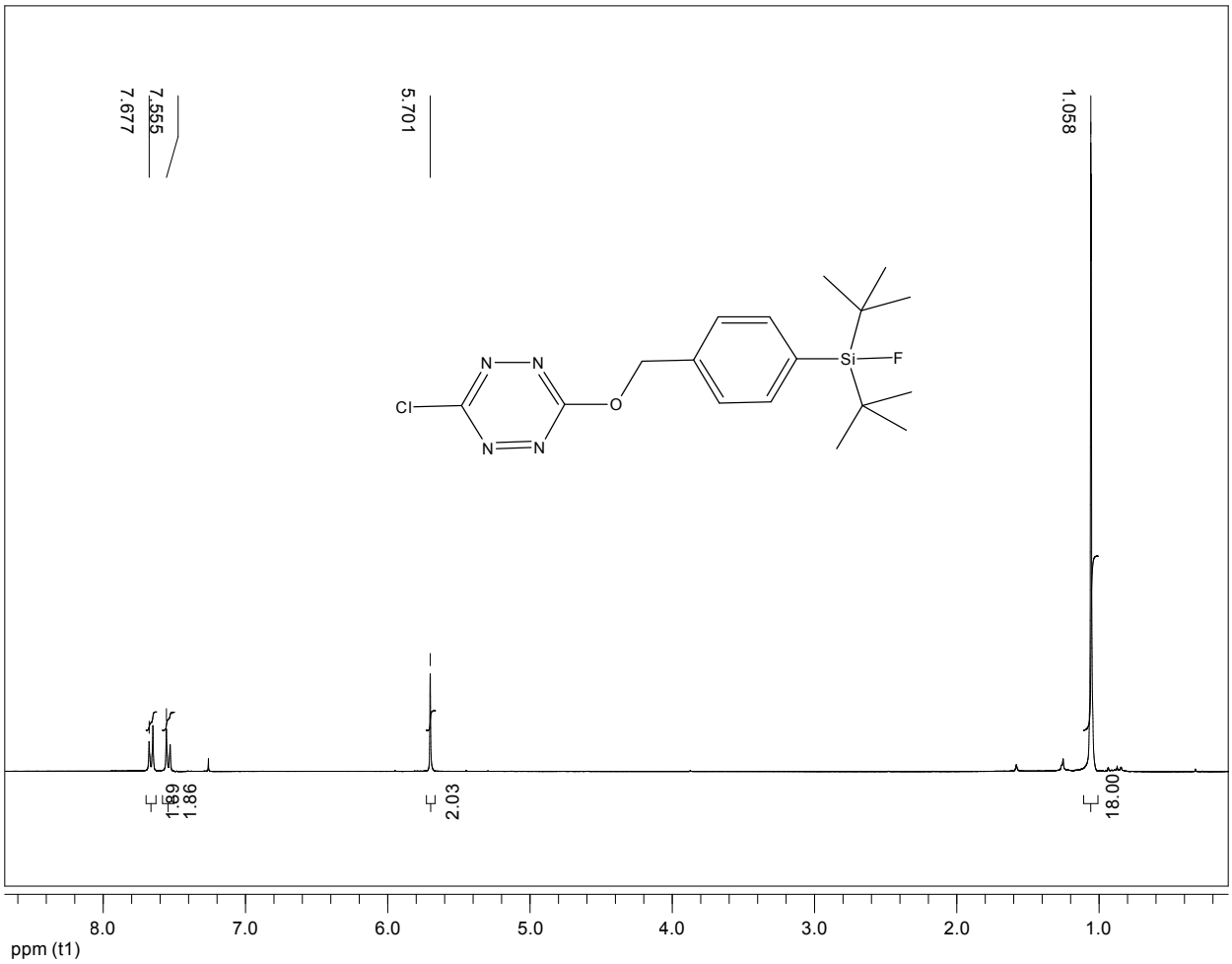

Figure S1. ^1H NMR of compounds of 3-chloro-6-((4-(di-tert-butylfluorosilyl)benzyl)oxy)-1,2,4,5-tetrazine (SiFA-O-Tz)

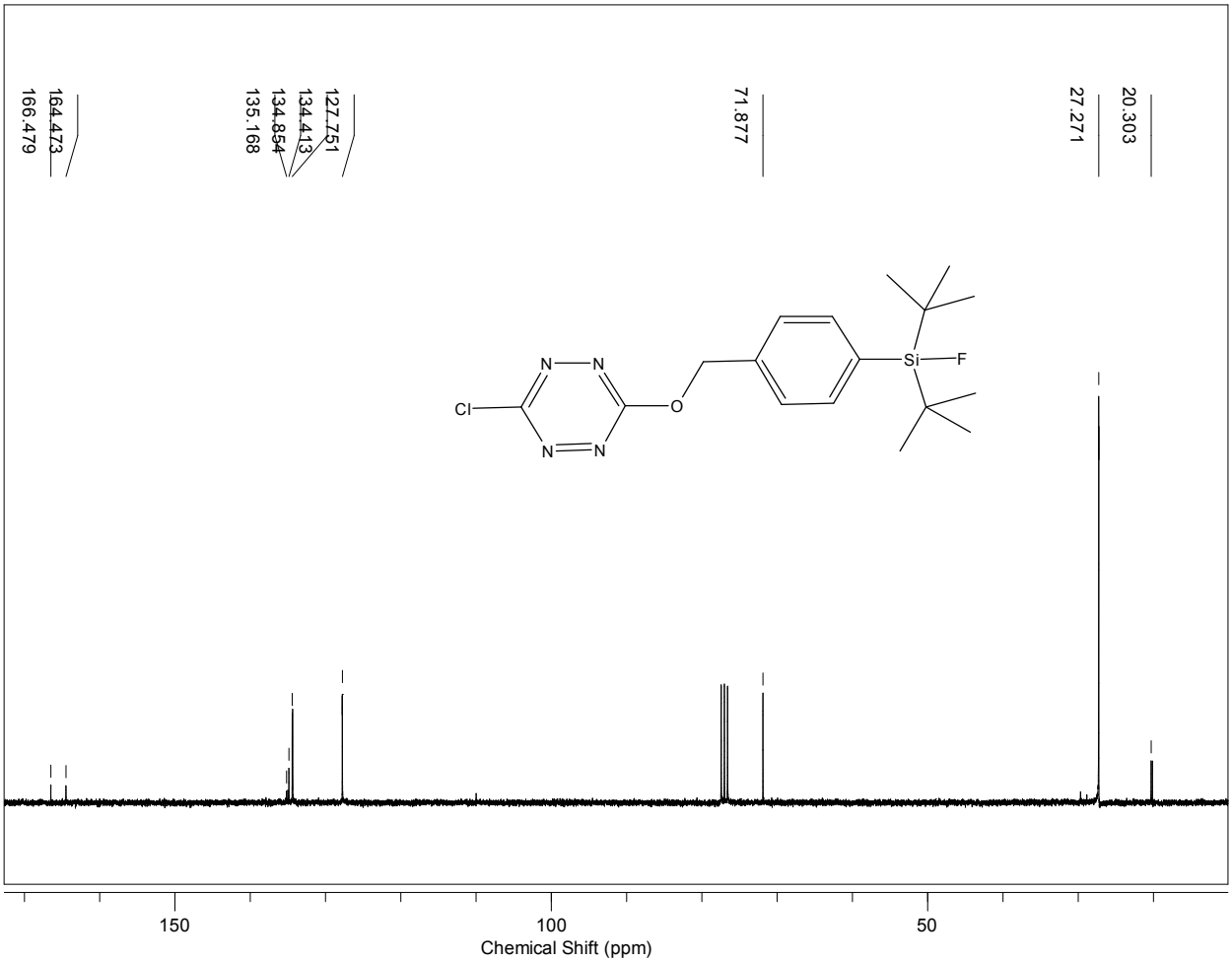

Figure S2. ^{13}C NMR of compounds of 3-chloro-6-((4-(di-tert-butylfluorosilyl)benzyl)oxy)-1,2,4,5-tetrazine (SiFA-O-Tz)

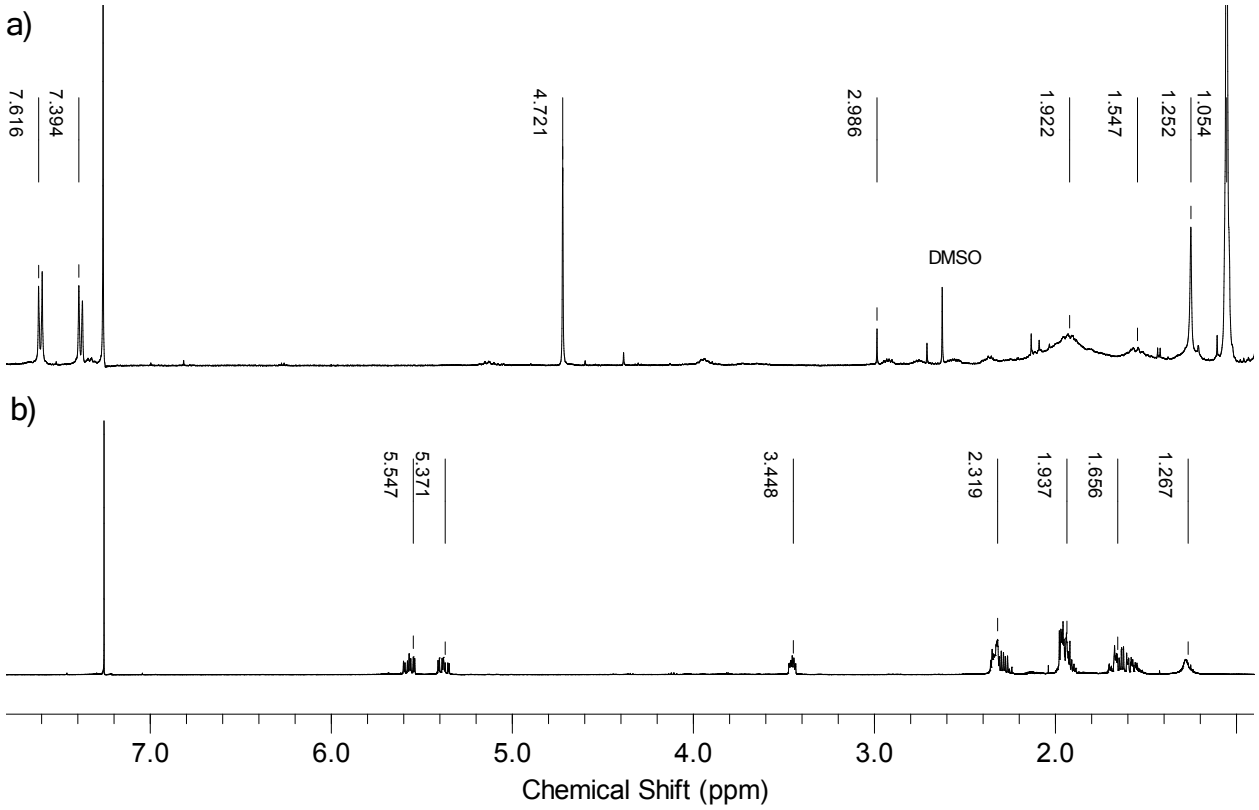

Figure S3. [1]H NMR spectrum of a) crude product of 3-chloro-6-((4-(di-tert-butylfluorosilyl)benzyl)oxy)-1,2,4,5-tetrazine (SiFA-O-Tz) with trans-cyclooctenol (**4**) and b) trans-cyclooctenol (**4**). Disappearance of peak signals from the double bond proton of trans-cyclooctenol suggests that the reaction is quantitative.

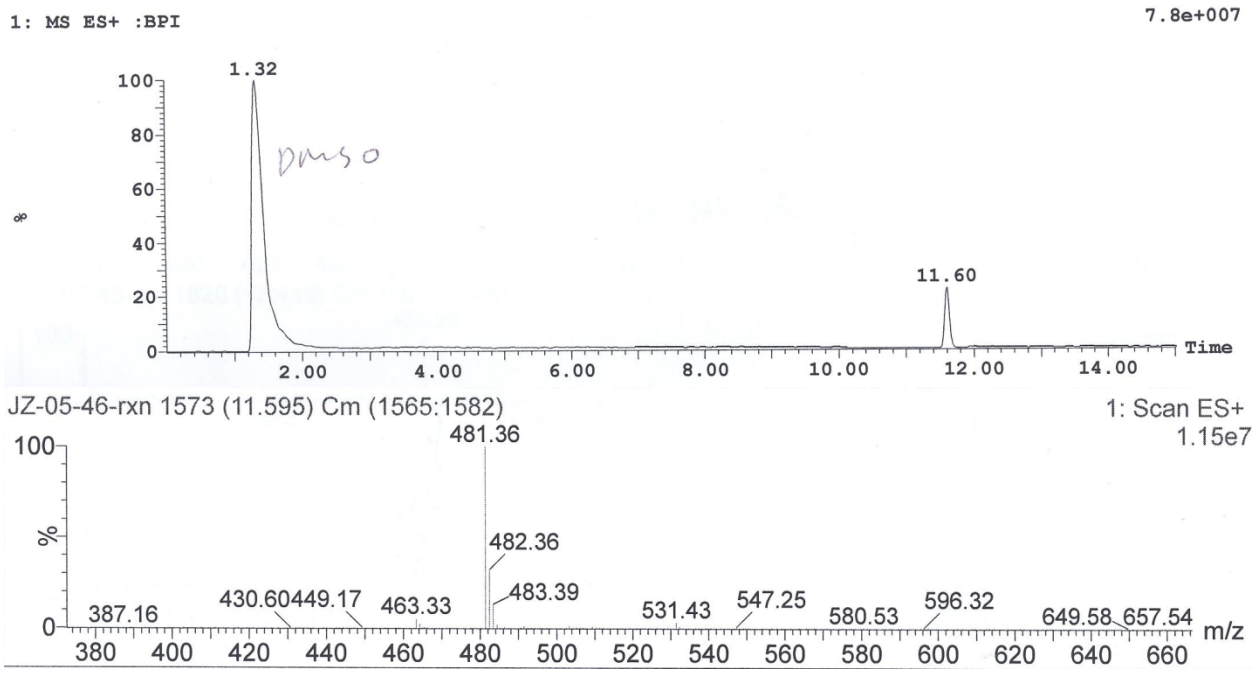

Figure S4. HPLC-MS (ESI) spectrum of crude product of 3-chloro-6-((4-(di-tert-butylfluorosilyl)benzyl)oxy)-1,2,4,5-tetrazine (SiFA-O-Tz) (**3**) with trans-cyclooctenol (**4**). HPLC-MS showed one single peak at 11.6 min at 254 nm with a m/z 481.36 ($[M+H]^+$) proved the formation of compound **5**.

Optimization conditions for the preparation of [^{18}F]-SiFA-OH.

[^{18}F]fluoride was dried following the Munich method.[13] Kryptofix2.2.2 (0.410 g, 1.10 mmol) was dissolved in 1 mL of 1M KOH (1.00 mmol) solution. The solution was split into 10 Eppendorf tubes and each portion was diluted with 0.9 mL of water. The resulting solutions were lyophilized to dryness for two days and stored at 4 ºC before use. Each portion contains 41 mg of Kryptofix2.2.2 and 100 µmol of KOH. The [^{18}F] eluent cocktail was prepared by adding 0.5 mL of anhydrous acetonitrile to the previously prepared Kryptofix2.2.2/KOH complex immediately before use. Diluted the $^{18}F^-$/[^{18}O]H_2O into 1mL of water then passed through a preconditioned SAX cartridge (Sep-Pak Accell Plus QMA Carbonate Light (46 mg), preconditioned with 10 mL of water). The cartridge was flushed with 20 mL of air, followed by rinsing with 10 mL of

anhydrous acetonitrile and 20 mL of air. $^{18}F^-$ was then recovered from the cartridge with the previous prepared cocktail followed by flushing with 5 mL of air.

For optimization of the labeling condition, the collected dry $^{18}F^-$ solution was split into 5 portions (each portion contains 20 μmol of KOH). 0, 10, 20, 30, 40 μL of 0.5 M of oxalic acid in anhydrous acetonitrile solution (corresponding to 0, 1:4, 2:4, 3:4, 4:4 mole ratio of oxalic acid:KOH) was added. Anhydrous acetonitrile was added (40, 30, 20, 10, 0 μL) to make up to constant volume, followed by 100 μL (0.745 μmol) of a SiFA-OH/anhydrous acetontrile solution (2 mg/mL). The isotope exchange reaction was performed for 5 min and the exchange ratio was determined by radio-TLC. The highest exchange rate can be achieved under neutral or weakly basic conditions. Strong basic (no oxalic acid added) or acidic conditions result in low or no isotope-exchange reaction. We thus used the oxalic acid:KOH = 1:4 in all subsequent studies.

In a parallel study, we also optimized the amount of SiFA-OH needed for the corresponding labeling. 100 μL of $^{18}F^-$ solution was mixed with 10 μL of oxalic acid. 100, 50, 25, 12.5, 5 μL of the 0.745 mM SiFA-OH/anhydrous acetonitrile solution was first diluted with 30, 80, 105, 117.5, and 125 μL of volume compensate acetonitrile then mixed with the labeling solution. After mixing for 5 min, radio-TLC was collected to determine the isotope exchange rate. The results showed that the best exchange rate (> 90%) can be achieved with > 12.5 μL of the SiFA-OH solution (93 nmol).

Purification of [^{18}F]-SiFA-OH. The labeling mixture was diluted into 10 X volume of the labeling mixing of water (in this case 2.3 mL) then passed through a pre-conditioned C18 cartridge (10 mL of ethanol, then 10 mL of water). The cartridge was washing with 10 mL of water then dried with 20 mL of air. The [^{18}F]-SiFA-OH was recovered by flushing the C18 cartridge-Na_2SO_4 cartridge with 1.5 mL of anhydrous dichloromethane. The eluent was collected

in 0.5 mL increments. Fractions containing radioactivity were combined and characterized by HPLC. ^{18}F-SiFA-OH showed a narrow band and collected in a 1 mL fraction.

During the course of the optimization, we observed that the isotope exchange reaction with the conditions used here is independent of time. Reactions performed for 2 to 5 min do not show a RCY difference as monitored by radio-TLC. We are thus able to minimize the labeling and separation time to 4 to 5 min.

Table 1S. Optimization radiolabeling condition for the preparation of ^{18}F-SiFA-OH.

Entry	SiFA-OH	KOH	Oxalic Acid	RCY
1	200 µg	20 µmol	0 µmol	0 %
2	200 µg	20 µmol	5 µmol	98 %
3	200 µg	20 µmol	10 µmol	97 %
4	200 µg	20 µmol	15 µmol	30 %
5	200 µg	20 µmol	20 µmol	4 %
6	100 µg	20 µmol	5 µmol	98 %
7	50 µg	20 µmol	5 µmol	97%
8 (>3)	25 µg	20 µmol	5 µmol	>97%
9 (= 3)	10 µg	20 µmol	5 µmol	69 ± 3%

Optimization conditions for the synthesis of ^{18}F labeled 3-chloro-6-((4-(di-tert-butylfluorosilyl)-benzyl)oxy)-1,2,4,5-tetrazine ([^{18}F]-SiFA-OTz).

For the purpose of optimization, [^{18}F]-SiFA-OH, 2,4,6-collidine and dichlorotetrazine were mixed in anhydrous dichloromethane. The reaction was monitored by HPLC; optimized reaction

conditions are summarized in Table S2. The highest RCY of 94% was obtained (n>3) when 25 µg of ^{18}F-SiFA-OH (93 nmol), 22 µg of 2,4,6-collidine (102 nmol) and dichlorotetrazine (931 nmol) were mixed for 15 min. The final product ^{18}F-SiFA-OTz was separated by loading on a silica cartridge, followed by flushing with 2 mL of anhydrous dichloromethane. The pink narrow band corresponding to ^{18}F-SiFA-OTz was collected in 0.5 - 0.7 mL of eluent. This solution is concentrated enough for the next step. The total synthesis and cartridge purification time is about 17-18 min. Dichloromethane solvent can be removed by fitting with a vacuum for 2 min, however, no difference was detected when using the dichloromethane solution directly or [^{18}F]-SiFA-OTz/DMSO solution for the subsequent inverse electron demand Diels-Alder reaction. [^{18}F]-SiFA-OTz shows excellent stability and no noticeable dissociation 3 was detected by radio-HPLC after 2h (one half life decay).

Table 2S.Optimization reaction condition for the preparation of [^{18}F]-SiFA-OTz

Entry	SiFA-OH	Tz	2,4,6-collidine	RCY
10	25 µg (1)	15 µg (1)	11 µg (1)	30 %
11	25 µg (1)	150 µg (10)	11 µg (1)	70 %
12 (n>5)	25 µg (1)	150 µg (10)	22 µg (2)	92 ± 2 %
13	25 µg (1)	300 µg (20)	22 µg (2)	60 %

General procedure for the synthesis of ([^{18}F]-SiFA-OTz and the Inverse electron demand Diels-Alder reaction of [^{18}F]-SiFA-OTz with trans-cyclooctenol.

Diluted 27.4 - 35.0 mCi of $^{18}F^-$/[^{18}O]H_2O into 1mL of water then passed through a preconditioned SAX cartridge (Sep-Pak Accell Plus QMA Carbonate Light (46 mg),

preconditioned with 10 mL of water). The cartridge was flushed with 20 mL of air, followed by rinsing with 10 mL of anhydrous acetonitrile and 20 mL of air. $^{18}F^-$ was then recovered from the cartridge with the [^{18}F] eluent cocktail (contains 41 mg of Kryptofix2.2.2 (110 μmol), 100 μmol of KOH, and 25 μmol of oxalic acid in 1.0 mL of anhydrous acetonitrile followed by flushing with 5 mL of air. Followed by mixing with 12.5 μL of 2mg/mL SiFA-OH/anhydrous acetonitrile solution (0.093 μmol). The isotope exchange reaction was performed for 2 min and the labeling mixture was diluted into 10 mL of water, passed through a pre-conditioned C18 cartridge (10 mL of ethanol, then 10 mL of water). The cartridge was washed with 10 mL of water then dried with 20 mL of air. [^{18}F]-SiFA-OH was recovered by flushing the C18 cartridge-Na_2SO_4 cartridge with 2.0 mL of anhydrous dichloromethane and collected in 0.5 mL portions. 1 mL of the eluent contains 24.8 - 30.1 mCi of [^{18}F]-SiFA-OH was collected. The [^{18}F]-SiFA-OH solution was then mixed with 22.6 μg of 2,4,6-collidine (0.186 μmol) and 148.0 μg of dichlorotetrazine (0.931 μmol) for 15 min. The final product [^{18}F]-SiFA-OTz was separated by loading on a silica cartridge, followed by flushing with 2 mL of anhydrous dichloromethane. 17.8 - 21.6 mCi of [^{18}F]-SiFA-OTz (pink narrow band) was collected in 0.5 – 0.7 mL of eluent. This solution is concentrated enough for the next step. The total synthesis and cartridge purification time is about 20-25 min. Subsequently, the [^{18}F]-SiFA-OTz (17.8 - 21.6 mCi) solution was mixed with 11.7 uL of 1 mg/mL trans-cyclooctenol (0.093 μmol) DMSO solution. The reactant mixture color immediately changed from pink to pale yellow within 1 min and the product [^{18}F]-**5** were generated quantitatively as confirmed by radio-HPLC. [^{18}F]-**5** was allowed to fully decay in lead containers for one week and characterized by MS to confirm the formation of [^{18}F]-**5**.

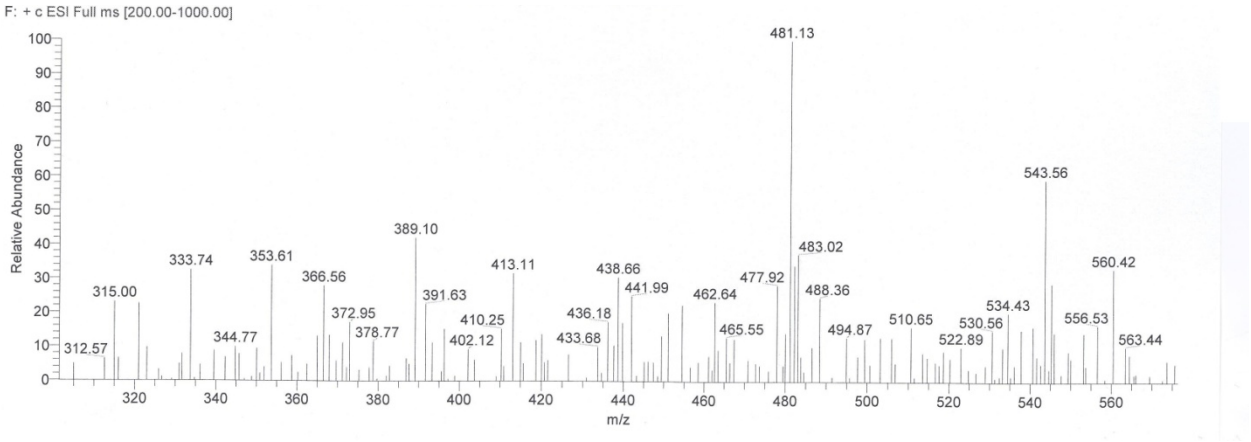

Figure S5. MS (ESI) spectrum of **5** after fully decay of [^{18}F]-**5** in a lead container for one week. m/z ([M+H]$^+$) of 481.13 indicate the formation of [^{18}F]-**5**.

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