Labeling with Radioactive Fluorine

DOI: 10.1002/anie.200705854

Silicon-Based Building Blocks for One-Step ^{18}F-Radiolabeling of Peptides for PET Imaging

Linjing Mu, Aileen Höhne, P. August Schubiger, Simon M. Ametamey, Keith Graham, John E. Cyr, Ludger Dinkelborg, Timo Stellfeld, Ananth Srinivasan, Ulrike Voigtmann, and Ulrich Klar**

Positron emission tomography (PET) is an important diagnostic tool in modern medicine due to its ability to locate and assess abnormalities in neurology,[1a–e] oncology,[1f–j] and cardiology.[1k,l] The application of ^{18}F-labeled small bioactive peptides for diagnostic imaging has emerged as an important and interesting field in nuclear medicine.[2] However, currently established ^{18}F-labeling procedures require scrupulously dry, strongly basic reaction conditions at high temperature, which are not suitable for biomolecules such as peptides and proteins. Therefore, the labeling of peptides and proteins is usually achieved by using suitable prosthetic groups labeled with ^{18}F. This approach, however, requires a multistep reaction sequence and is time-consuming.[3] Owing to the short half-life (110 min) of ^{18}F and the chemical properties of biomolecules, a more efficient, one-step method for site-specific labeling under mild conditions is required.

Based on the high silicon–fluorine bond energy (135 kcal mol^{-1} vs. 116 kcal mol^{-1} for C–F) and the experimental results of Whitmore et al.,[4] the concept of exploiting the fluoride substitution at silicon for the ^{18}F-labeling of biomolecules has been discussed and tested by different research groups.[5] Up to now, site-specific ^{18}F-radiolabeling of organosilanes under mild conditions has been achieved, however, most methods still require at least a two-step procedure. Recently, Choudhry et al. evaluated the hydrolytic stability of four model trialkylfluorosilanes and proposed to use the most stable compound as a building block for the direct ^{18}F-labeling of biomolecules.[6] Schirrmacher et al. also reported on the direct radiolabeling of an organosilicon-modified peptide by an isotope exchange reaction,[7a] but the product contains predominantly the corresponding non-radioactive ^{19}F compound, which leads to relatively low specific radioactivity. Very recently, the same group used the highly effective labeling reagent *p*-(di-*tert*-butylfluorosilyl)benzaldehyde for coupling to N-terminal aminooxy (N-AO) derivatized peptides to achieve high specific activities with a two-step procedure.[7b] Ting et al. published the carrier-added ^{18}F-labeling of trialkoxysilanes with multiple fluorine atoms attached to silicon,[8] and the alkyltetrafluorosilicate was moderately stable in aqueous media.

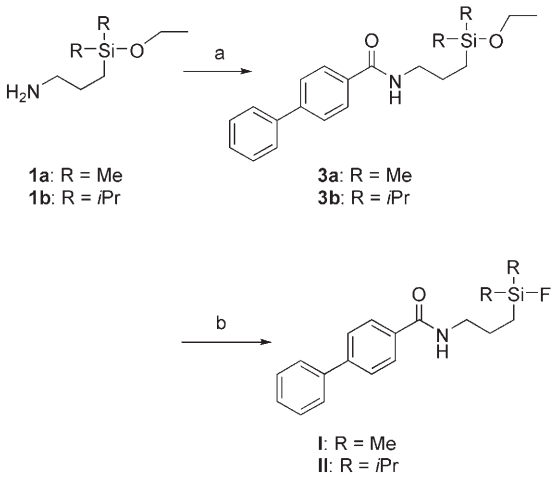
A one-step no-carrier-added nucleophilic ^{18}F-fluorination of biomolecules such as peptides using silicon–fluorine chemistry is the main goal of our study. For the silicon-based ^{18}F imaging agent to be effective as a PET probe, the Si–F bond needs to be sufficiently stable under physiological conditions. It is known that the hydrolytic stability of the silicon–halogen bond is determined by the nature of the substituents on the silicon atom. Therefore, a series of bifunctional silicon building blocks were designed and synthesized, which contained different substituents and leaving groups suitable for fluorination and linkers suitable for subsequent coupling to a biomolecule. Model fluorosilanes using non-radioactive fluoride (^{19}F$^-$) were also prepared. These compounds were used for stability studies and as standard reference compounds.

The amides **3a** and **3b** were synthesized from commercially available dimethyl- and diisopropylsilylamines **1a** and **1b**, respectively. Fluorination of **3a** and **3b** with $BF_3{\cdot}OEt_2$ afforded compounds **I** and **II** as standard references (Scheme 1).

Scheme 2 depicts the synthetic pathway towards silane derivatives with an aryl linker. Compound **5a** was synthesized by nucleophilic substitution of diisopropylchlorosilane with an ate complex generated from **4**, isopropylmagnesium bromide, and *n*BuLi. Compound **5b** was synthesized by nucleophilic substitution of di-*tert*-butylchlorosilane with {4-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]phenyl}lithium, which was generated in situ by metal–halogen exchange of bromide **4** with *n*BuLi. Basic hydrolysis of **5b** with KOH in EtOH yielded the silanol **6**. Treatment of compound **5a**, **5b**, and **6** with toluenesulfonic acid in ethanol gave the alcohols **7, 8a**, and **8b**, respectively. Compound **7** was oxidized by means of a Jones oxidation to give the carboxylic acid **9a**. Compound **9b** was obtained by oxidizing **9a** with Pd/C in a H_2O–CCl_4 mixture. The di-*tert*-butyl-substituted compounds **10a** and **10b** were obtained in good yields by Jones oxidation of the crude **8a** and **8b** directly. Coupling of **10a** or **10b** with

[*] Dr. L. Mu,[+] A. Höhne,[+] Prof. Dr. P. A. Schubiger, Prof. S. M. Ametamey
Animal Imaging Center-PET
Center for Radiopharmaceutical Science of ETH, PSI and USZ
ETH-Hönggerberg, D-CHAB IPW HCI H427
Wolfgang-Pauli-Strasse 10, 8093 Zurich (Switzerland)
Fax: (+41) 446-331-367
E-mail: simon.ametamey@pharma.ethz.ch
Dr. K. Graham, Dr. J. E. Cyr, Dr. L. Dinkelborg, Dr. T. Stellfeld, Dr. A. Srinivasan, Dr. U. Voigtmann, Dr. U. Klar
Bayer Schering Pharma AG
Global Drug Discovery, 13342 Berlin (Germany)
Fax: (+49) 304-689-2635
E-mail: ulrich.klar@bayerhealthcare.com
[+] These authors contributed equally to the work.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Synthesis of silane derivatives with alkyl linkers. a) Biphenyl-4-carbonyl chloride (**2**), NEt_3, dioxane, RT, 1 h for **3a**, 61%; 1.5 h for **3b**, 55%; b) $BF_3{\cdot}OEt_2$, Et_2O, reflux, 15 min for compound **I**, 63%; 30 min for compound **II**, 97%.

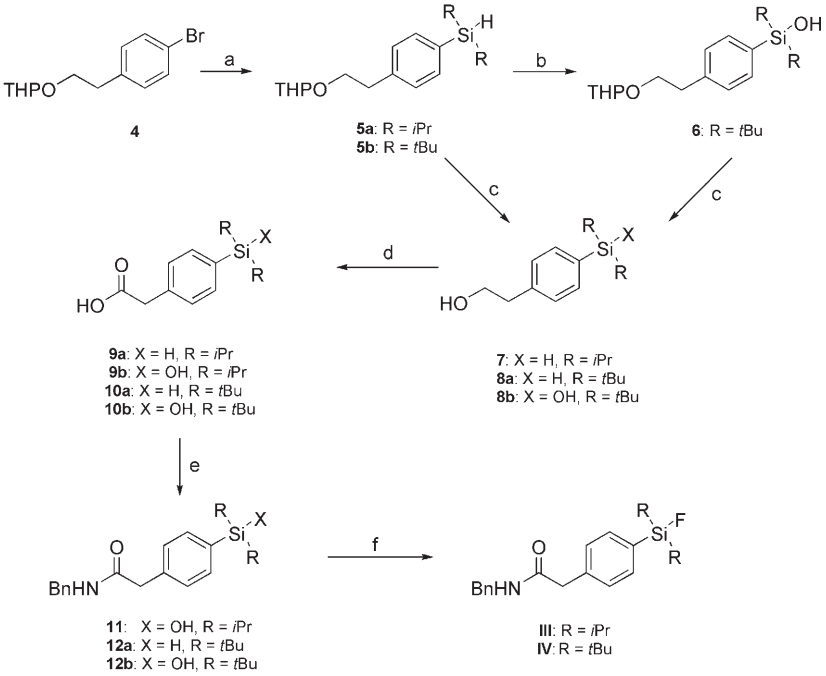
benzylamine using 1-ethyl-3-(3-di-methyl-aminopropyl)carbodiimide hydrochloride (EDC·HCl) as the coupling reagent provided amides **12a** and **12b**, respectively. Compound **11** was obtained from compound **9a** in three steps: activation of the acid with *N*-hydroxysuccinimide, hydrolysis with Pd/C in H_2O–CCl_4, and amidation with benzylamine. Fluorination of **11** and **12a** with potassium fluoride in the presence of cryptand-222 and acetic acid yielded compounds **III** and **IV**.

Scheme 3 depicts the radiolabeling conditions. Good to high yields of ^{18}F-incorporation were achieved under mild labeling conditions with acetic acid as an additive (Table 1). In

Scheme 3. ^{18}F-radiolabeling of silicon-based building blocks.

most cases, the addition of acetic acid enhances the radiolabeling yield significantly. For example, with acetic acid as an additive, the ^{18}F-incorporation for the diisopropylsilanol at 65 °C was 90%, whereas without acetic acid it was only 3% (Table 1, entries 14, 15). The enhanced yields are probably due to the protonation of hydroxy and alkoxy groups, which makes them better leaving groups. It is not surprising that with hydrogen as the leaving group, the addition of acetic acid did not have any dramatic effect on the radiochemical yield (Table 1, entries 22 and 23). Under the same reaction conditions, the di-*tert*-butylsilyl model compounds gave a much higher radiolabeling yield with the hydrogen leaving group than with the hydroxy leaving group (Table 1, entries 16–23). Therefore, we decided to use the silane building block for the subsequent coupling reactions instead of the silanol building block. As shown in entry 10 in Table 1, decreasing the amount of the precursor resulted in a fourfold lower yield of ^{18}F-incorporation at 30 °C. However, ^{18}F-incorporation with lower amounts of precursor could be improved from 19% to 88% by increasing the reaction temperature from 30 °C to 90 °C (Table 1, entries 10, 11). With the diisopropyl derivatives, a temperature of 30 °C was sufficient to achieve high ^{18}F-incorporation, whereas with the di-*tert*-butyl derivatives, a relatively higher reaction temperature was required.

The time-dependent hydrolysis of fluorosilane model compounds **I** to **IV** was determined at pH 7.0 and their hydrolytic half-lives ($T_{1/2}$) were calculated (**I**: <5 min, **II**: 12 h, **III**: 8 h, **IV**: $\geqslant 170$ h).[9] The model fluorosilane compound **I** (R = Me) is stable in anhydrous organic solvents such as acetonitrile and diethyl ether, but is readily hydrolyzed in the presence of water. Introducing bulkier isopropyl and *tert*-butyl groups into the fluorosilanes (compounds **II**, **III**, and **IV**) significantly increased their stability towards hydrolytic cleavage. Based

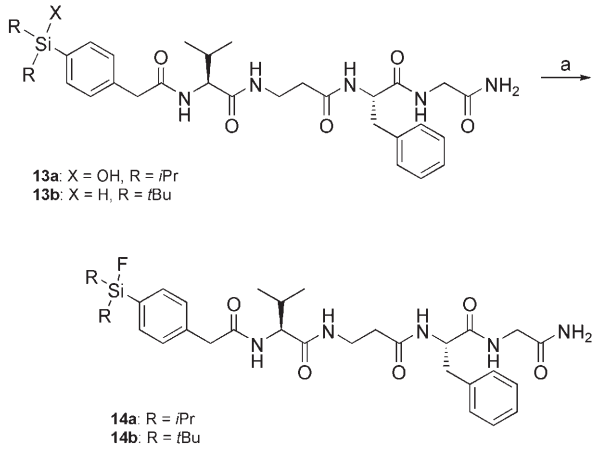


Scheme 2. Synthesis of silane derivatives with aryl linkers. a) **5a**: *n*BuLi, *i*PrMgBr, THF, iPr_2SiClH, 97%; **5b**: *n*BuLi, THF, tBu_2SiClH, 74%; b) **6**: KOH, EtOH, 71%; c) **7**: *p*TsOH, EtOH, 93%; **8a** and **8b**: TsOH, EtOH; d) **9a**: Jones reagent, acetone, 69%; **9b**: Pd/C, H_2O, CCl_4 from **9a** 69%; **10a**: from **5b**, 1. *p*TsOH, EtOH, 2. Jones reagent, acetone, overall yield 80%; **10b**: from **6**, 1. TsOH, EtOH, 2. Jones reagent, acetone, overall yield 77%; e) **11**: starting with **9a**, 1. NHS, EDC·HCl, CH_2Cl_2, 83%; 2. Pd/C, H_2O, CCl_4, 78%; 3. $BnNH_2$, CH_2Cl_2, 76%; **12a** and **12b**: EDC·HCl, $BnNH_2$, CH_2Cl_2, 66% for **12a** and 76% for **12b**; f) KF/crypt-222, AcOH, THF, 81% for compound **III**, 98% for compound **IV** from **12a**. THP = tetrahydropyranyl, Bn = benzyl, Ts = toluenesulfonyl.

Table 1: [18]F radiolabeling of various silicon model compounds.[a]

Entry	Precursor (mg)	*T* [°C]	Acetic acid [μL]	Conversion [%][b]
1	**3a** (5)	30	3	84
2	**3a** (5)	30	0	18
3	**3a** (5)	65	3	92
4	**3a** (5)	65	0	2
5	**3b** (5)	30	3	93
6	**3b** (5)	30	0	24
7	**3b** (5)	65	3	96
8	**3b** (5)	65	0	13
9	**3b** (3)	30	3	79
10	**3b** (1)	30	3	19
11	**3b** (1)	90	3	88
12	**11** (5)	30	3	53
13	**11** (5)	30	0	9
14	**11** (5)	65	3	90
15	**11** (5)	65	0	3
16	**12b** (5)	30	3	15
17	**12b** (5)	30	0	0
18	**12b** (5)	65	3	23
19	**12b** (5)	65	0	4
20	**12a** (5)	30	3	59
21	**12a** (5)	30	0	24
22	**12a** (5)	65	3	69
23	**12a** (5)	65	0	69

[a] [18]F-labeling experiments were carried out in 300 μL DMSO for 15 min. [b] Determined from the radio-HPLC chromatogram: ratio of the radioactivity area of product to the total radioactivity area.



Scheme 4. [18]F-radiolabeling of silicon tetrapeptides. a) K[[18]F]F, 5 mg crypt-222, 1 mg K_2CO_3, 3 μL AcOH, 1 mg **13a** in 0.3 mL DMSO, 15 min, 90 °C, 53 % conversion for **14a**; K[[18]F]F, 5 mg crypt-222, 1 mg K_2CO_3, 2 mg **13b** in 0.3 mL DMSO, 15 min, 65 °C, 45 % conversion for **14b**.

on these current results, steric hindrance at the R position seems to play a key role in the stabilization of the silicon–fluorine bond. Interestingly, the linker appeared to have less steric influence in our model compounds; fluorosilanes with aryl linkers were not substantially better with regard to hydrolytic stability than those with alkyl linkers. Of all the compounds tested, the fluorosilane **IV** with *tert*-butyl substituents and an aryl linker exhibited the best hydrolytic stability.

To test the practical utility of these new silicon building blocks, the acids **9b** and **10a** were coupled to a tetrapeptide by using standard solid-phase peptide synthesis protocols.[10] The [18]F-labeling of these peptides was performed under similar reaction conditions to those reported for the silicon model compounds (Scheme 4). Around 50 % [18]F-incorporation was achieved after a reaction time of 15 min for compounds **13a** and **13b**. The preliminary stability test for the silicon-based tetrapeptide **13b** was very promising. After two hours of incubation in human plasma, no degradation products were observed.

A series of bifunctional silicon building blocks with different linkers, leaving groups, and substituents were synthesized and labeled with [18]F. The hydrolytic stability of the fluorosilane center appears to depend on steric hindrance at the silicon center. The most stable building block was successfully used for the synthesis of a radiolabeled tetrapeptide, which showed a hydrolytic stability in the range required for PET studies. In vivo PET imaging and biodistribution studies with bioactive peptides labeled with [18]F using this new labeling methodology are currently ongoing. In conclusion, we have developed a unique silcon-based method for the facile [18]F-labeling of biomolecules under mild conditions and using a one-step approach.

Received: December 20, 2007
Revised: February 20, 2008
Published online: May 21, 2008

Keywords: fluorine · peptides · PET imaging · radiolabeling · silicon

[1] a) F. Roesch, G. Gruender, M. Schreckenberger, G. Dannhardt, *Pharm. Unserer Zeit* **2005**, *34*, 474–482; b) A. R. Troiano, A. J. Stoessl, *Bioimaging Neurodegener.* **2005**, 25–35; c) R. Sanchez-Pernaute, A.-L. Brownell, B. G. Jenkins, O. Isacson, *Toxicol. Appl. Pharmacol.* **2005**, *207*, S251–S256; d) L. Cai, R. B. Innis, V. W. Pike, *Curr. Med. Chem.* **2007**, *14*, 19–52; e) R. M. Cohen, *Mol. Imaging Biol.* **2007**, *9*, 204–216; f) J. R. Mercer, *J. Pharm. Pharm. Sci.* **2007**, *10*, 180–202; g) N. Oriuchi, T. Higuchi, T. Ishikita, M. Miyakubo, H. Hanaoka, Y. Iida, K. Endo, *Cancer Sci.* **2006**, *97*, 1291–1297; h) D. Le Bars, *J. Fluorine Chem.* **2006**, *127*, 1488–1493; i) H. Fukuda, S. Furumoto, R. Iwata, K. Kubota, *Int. Congr. Ser.* **2004**, *1264* (PET and Molecular Imaging), 158–165; j) A. M. Scott, *Positron Emiss. Tomogr.* **2005**, 311–325; k) M. N. Maisey, *Nucl. Med. Commun.* **2000**, *21*, 234–236; l) S. Furst, *MTA* **1999**, *14*, 506–508, 510.

[2] a) A. J. Fischman, J. W. Babich, H. W. Strauss, *J. Nucl. Med.* **1993**, *34*, 2253–2263; b) D. Blok, R. I. J. Feitsma, P. Vermeij, E. K. J. Pauwels, *Eur. J. Nucl. Med.* **1999**, *26*, 1511–1519; c) J. C. McAfee, R. D. Neumann, *Nucl. Med. Biol.* **1996**, *23*, 673–676; d) S. M. Okarvi, *Eur. J. Nucl. Med.* **2001**, *28*, 929–938; e) H. J. Wester, K. Hamacher, G. Stöcklin, *Nucl. Med. Biol.* **1996**, *23*, 365–372.

[3] a) M. R. Kilbourn, C. S. Dence, M. J. Welch, C. J. Mathias, *J. Nucl. Med.* **1987**, *28*, 462–470; b) T. Toyokuni, J. C. Walsh, A. Dominguez, M. E. Phelps, J. R. Barrio, S. S. Gambhir, N. Satyamurthy, *Bioconjugate Chem.* **2003**, *14*, 1253–1259; c) G. Vaidyanathan, M. R. Zalutsky, *Bioconjugate Chem.* **1994**, *5*, 352–356; d) S. Guhlke, H. H. Coenen, G. Stöcklin, *Appl. Radiat. Isot.* **1994**, *45*, 715–727; e) Y. Shai, K. L. Kirk, M. A. Channing, B. B. Dunn, M. A. Lesniak, R. C. Eastman, R. D. Finn, J. Roth,

K. A. Jacobson, *Biochemistry* **1989**, *28*, 4801 – 4806; f) L. Lang, W. C. Eckelman, *Appl. Radiat. Isot.* **1994**, *45*, 1155 – 1163; g) K. Bruus-Jensen, T. Poethko, M. Schottelius, A. Hauser, M. Schwaiger, H.-J. Wester, *Nucl. Med. Biol.* **2006**, *33*, 173 – 183.

[4] N. S. Marans, L. H. Sommer, F. C. Whitmore, *J. Am. Chem. Soc.* **1951**, *73*, 5127 – 5130.

[5] a) J. C. Walsh, K. M. Akhoon, N. Satyamurthy, J. R. Barrio, M. E. Phelps, S. S. Gambhir, T. Toyokuni, *J. Labelled Compd. Radiopharm.* **1999**, *42*, S1 – S3; b) L. G. Hutchins, A. L. Bosch, M. S. Rosenthal, R. J. Nickles, S. J. Gatley, *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 375 – 378.

[6] U. Choudhry, R. Paul, S. C. Biagini, P. J. Blower, *Eur. J. Nucl. Med. Mol. Imaging* **2007**, *34*, S332.

[7] a) R. Schirrmacher, G. Bradtmoeller, E. Schirrmacher, O. Thews, J. Tillmanns, T. Siessmeier, H. G. Bucholz, P. Bartenstein, B. Wängler, C. M. Niemeyer, K. Jurkschat, *Angew. Chem.* **2006**, *118*, 6193 – 6197; *Angew. Chem. Int. Ed.* **2006**, *45*, 6047 – 6050; b) E. Schirrmacher, B. Wängler, M. Cypryk, G. Bradtmoeller, M. Schäfer, M. Eisenhut, K. Jurkschat, R. Schirrmacher, *Bioconjugate Chem.* **2007**, *18*, 2085 – 2089.

[8] R. Ting, M. J. Adam, T. J. Ruth, D. M. Perrin, *J. Am. Chem. Soc.* **2005**, *127*, 13094 – 13095.

[9] For stability studies, the fluorosilane model compound (2 mg) was dissolved in acetonitrile/aqueous buffer (2:1, 1.5 mL, pH 7.0) at room temperature (23 °C) and hydrolysis was monitored by reversed-phase HPLC after different intervals.

[10] W. C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis—A Practical Approach*, Oxford University Press, Oxford, **2003**.

