Radioactive Peptides

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^{18}F-Labeling of Peptides by means of an Organosilicon-Based Fluoride Acceptor**

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Fluorine-18 is among the most commonly used radionuclides for positron emission tomography (PET).[1] This non-invasive imaging technique is capable of providing in vivo information about the distribution of radiolabeled biomolecules by 180° coincidence detection of two simultaneously emitted photons from positron–electron annihilation. Although a number of different radiotracers have been successfully employed in PET, only a few, such as 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG)[2] and [^{18}F]fluorodopa,[3] have gained widespread application in nuclear medicine. The reason for this is that the regioselective introduction of ^{18}F into tracer molecules is often non-specific and radiochemical yields (RCY) of the ^{18}F-labelled product are low. The introduction of $^{18}F^-$ into tracer molecules requires high temperatures and often leads to undesired by-products.[1] The research over the last decade clearly indicates that the success of PET in nuclear medicine justifies the intense search for versatile labeling formulations for the syntheses of ^{18}F-radiopharmaceuticals. Especially the development of rational ^{18}F-labelling strategies for peptides, until now characterized by multistep procedures, is considered to be one of the most important tasks.[4] As an alternative to conventional ^{18}F-labelling chemistry, the use of [^{18}F]fluorosilanes as labeling synthons was first proposed by Rosenthal et al. who treated chlorotrimethylsilane with n.c.a. (no carrier added) $^{18}F^-$ in aqueous acetonitrile isolating the corresponding [^{18}F]fluorosilane in 65 % yield.[5] A preliminary in vivo evaluation revealed fast hydrolysis of the compound accompanied by high radioactivity (^{18}F) uptake by the bone making it unsuitable as a labeling synthon.

Another approach is based on the work by Pilcher et al.[6] who fluorinated organosilanoles with nonradioactive HF in high yields. An analogous labeling strategy was proposed in a symposium abstract.[7] However, so far no labeling experiments using aqueous $^{18}F^-$/[^{18}F]HF solutions have been reported. Most recently Ting et al. used organotriethoxysilanes as labeling precursors for the synthesis of [^{18}F]fluorosilanes but no practical application for the synthesis of potential radiopharmaceuticals has been demonstrated.[8]

Herein we report the syntheses of substituted [^{18}F]organofluorosilanes using organochlorosilanes as labeling precursors and their in vitro and in vivo stability. As an alternative labeling approach we also describe the ^{18}F–^{19}F isotopic exchange using [^{19}F]di-*tert*-butylphenyl fluorosilane as a highly efficient silicon-based fluoride acceptor (SiFA compound). As proof of applicability we transferred the SiFA approach to the development of a simple and practical formulation for the synthesis of a ^{18}F-labelled SiFA derivatized Tyr^3-octreotate, a peptide used in oncology for the visualization of neuro-endocrine tumors.[9]

We synthesized three [^{18}F]organofluorosilanes, namely [^{18}F]fluorotriphenylsilane (**1**), [^{18}F]fluoro-*tert*-butyldiphenylsilane (**2**), and [^{18}F]fluorodi-*tert*-butylphenylsilane (**3**), and evaluated their in vitro stability in human serum as well as their in vivo stability in rats, studied by animal-PET. These data are essential for finding the most suitable compound and for evaluating the labeling concept.

The reaction in acetonitrile of the triorganochlorosilanes (5–11.8 μmol mL^{-1}) Ph_3SiCl, *t*$BuPh_2SiCl$, and *t*$Bu_2PhSiCl$, with the azeotropically dried complex $^{18}F^-$/Kryptofix2.2.2./K^+ at room temperature provided almost quantitatively the corresponding [^{18}F]triorganofluorosilanes **1**–**3** (Figure 1), as demonstrated by means of radio-HPLC. Their identities were confirmed by coelution of the radioactive probes spiked with the related nonradioactive ^{19}F-analogues. The specific activity of **1**, **2**, and **3** was determined using UV-calibration curves and was in the range 1500–1700 GBq μmol^{-1}.

To investigate the applicability of the ^{18}F-labelled compounds for the development of Si–^{18}F containing radiopharmaceuticals, their in vitro stability in human serum was investigated (Figure 1). In agreement with previously published data,[10,11] the [^{18}F]triphenylfluorosilane **1** was found to be stable for 4 h in neutral water (data not shown) but unstable at pH 7.4–7.6 in human serum. In contrast, the *tert*-

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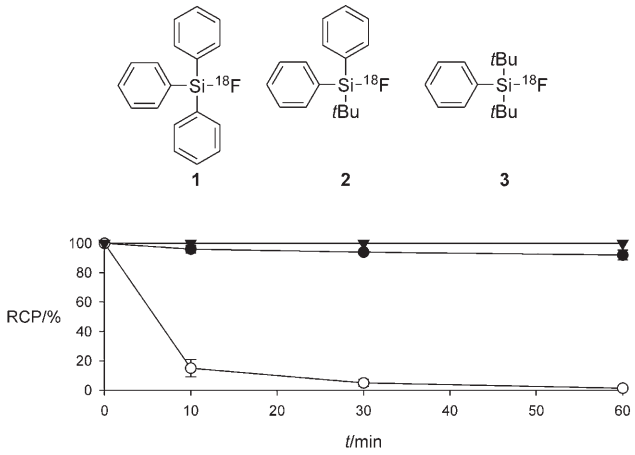

Figure 1. Top: ^{18}F-fluorosilanes **1**–**3** for in vitro evaluation in human serum and in vivo evaluation in rats by animal PET. Bottom: Only compounds **2** and **3** are stable in human serum (37.4 °C, pH 7.4–7.6; ○ = **1**, ● = **2**, ▼ = **3**). RCP = radiochemical purity.

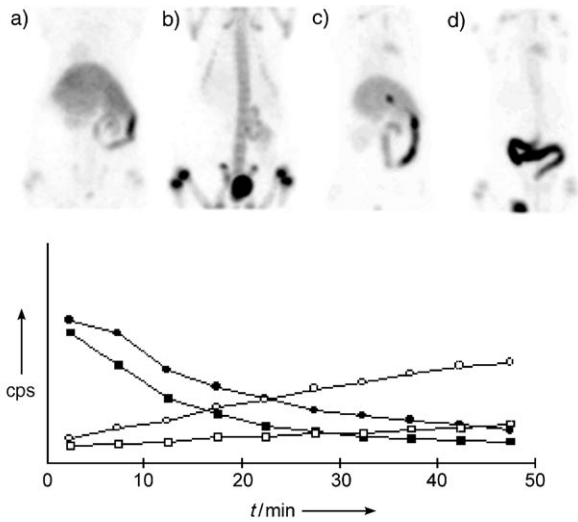

Figure 2. Top: Coronal maximum intensity projections (MIP) of the second frame (5–10 min after injection, a and c) and the last frame (45–50 min after injection, b and d). The data reveal a much higher bone uptake for compound **2** (a, b) than for **3** (c, d). Bottom: Plot of time activity in regions of interest in bone and liver for compound **2** (○ = lumbar vertebra, ● = liver) and **3** (□ = lumbar vertebra, ■ = liver). cps = normalized counts per second.

butyl-substituted derivatives **2** and **3** displayed a high stability in this biological matrix.

Encouraged by these results, we performed the first in vivo animal PET scans with compounds **2** and **3** in healthy Sprague-Dawley rats to investigate the in vivo stability of the Si–^{18}F bond. To this end, 20–40 MBq of HPLC-purified **2** and **3**, dissolved in physiological saline/10% ethanol, were injected into the rats and the thoraco-abdominal area was scanned using an animal PET scanner (Philips, Mosaic animal imaging) for 50 min with time frames of 5 min. Because unbound $^{18}F^-$ readily accumulates in bone (osteotropic), the stability of the Si–^{18}F bond was determined by measuring the time curve of radioactivity uptake in bone (lumbar vertebra) as a dynamic measurement of Si–^{18}F hydrolysis. In contrast to their rather similar in vitro stability (Figure 1), the in vivo stability of **2** and **3** was found to be markedly different. As shown on the PET scans, for the mono-*tert*-butyl-substituted [^{18}F]triorganofluorosilane **2**, fast bone uptake of radioactivity was detected, whereas in case of di-*tert*-butyl-substituted compound **3**, only a little uptake in late time frames was observed (Figure 2).

Moreover, PET studies indicated that, probably owing to their high lipophilicity, both compounds are metabolized mainly by the liver. Because **2** is hydrolyzed in vivo to give unbound $^{18}F^-$, the main excretion of radioactivity occurred through the bladder, in contrast to compound **3**, which displayed intestinal excretion (see Supporting Information). To use organosilanes for ^{18}F-labelling of radiopharmaceuticals, such as peptides for tumor imaging,[12] a suitable labeling strategy must include fast coupling steps to cope with the half-life of 110 min of ^{18}F. Ideally, as in ^{99m}Tc-labelling chemistry, no final HPLC purification step should be required for the formulation of the final radiopharmaceutical. Unfortunately, when applying triorganochlorosilanes as precursors for ^{18}F-labelling reactions, the resulting [^{18}F]triorganofluorosilane has to be separated from the triorganochlorosilane by means of HPLC. This step is time consuming, leads to a decreased RCY, and requires a trained radiochemist. Furthermore, organochlorosilanes as well as organoalkoxysilanes are sensitive to hydrolysis, which limits complex syntheses.

Another major problem in conventional ^{18}F-radiochemistry is that ^{19}F-compounds to be labeled with ^{18}F by isotopic exchange reactions are used in high concentrations (usually 10–100 μM) resulting in overall products of low specific activity. Therefore, the application of the concept of isotopic exchange to [^{18}F]triorganofluorosilanes would only be meaningful if 1) the concentration of the organofluorosilane is low enough to guarantee a high specific activity, 2) the ^{18}F–^{19}F exchange rate is sufficiently high, and 3) the isotopic exchange reaction is not negatively influenced by various functional groups (such as, NH_2, COOH) usually present in biological molecules, such as peptides. To circumvent these difficulties and to reduce the labeling to only one reaction step, preferably excluding HPLC purification, we investigated the reaction of [^{19}F]-*t*Bu$_2$PhSiF (**4**) with n.c.a. $^{18}F^-$/Kryptofix 2.2.2./K^+ complex in acetonitrile (Figure 3).

The reaction in acetonitrile of compound **4** (1 μg, 4.1 nM) with "dried" n.c.a. $^{18}F^-$/Kryptofix 2.2.2./K^+ complex (1 GBq) at room temperature in 100 μL acetonitrile without stirring gave [^{18}F]triorganofluorosilane **3** in yields between 80–95% after only 15 min. The specific activity of **3** was in the range of 194–230 GBq μmol^{-1}. This exchange reaction is remarkably fast and exceeds the RCY of 50–70% usually obtained by nucleophilic ^{18}F–^{19}F substitution of activated aromatic compounds, which normally requires high reaction temperatures and HPLC purification.[13,14] The specific activity obtained is competitive with clinically used radiotracers, which show specific activities between 100–1000 GBq μmol^{-1}. As a result of the very mild reaction conditions, a noteworthy advantage of the SiFA method is the exclusive formation of product **3** (Figure 3). Moreover, the specific activity, which is of crucial importance when imaging saturable neuroreceptors,[15] might

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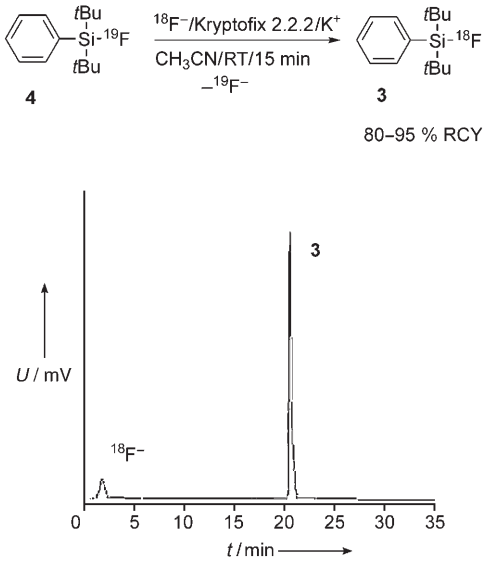
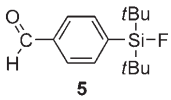


Figure 3. Top: synthesis of **3** using **4** and $^{18}F^-$/Kryptofix2.2.2/K^+; Bottom: radio-HPLC chromatogram of the crude reaction mix of **4** (1 μg (4.1 nM)) with $^{18}F^-$/Kryptofix2.2.2/K^+ complex (1 GBq) in CH_3CN (100 μL) after 15 min reaction time at room temperature.

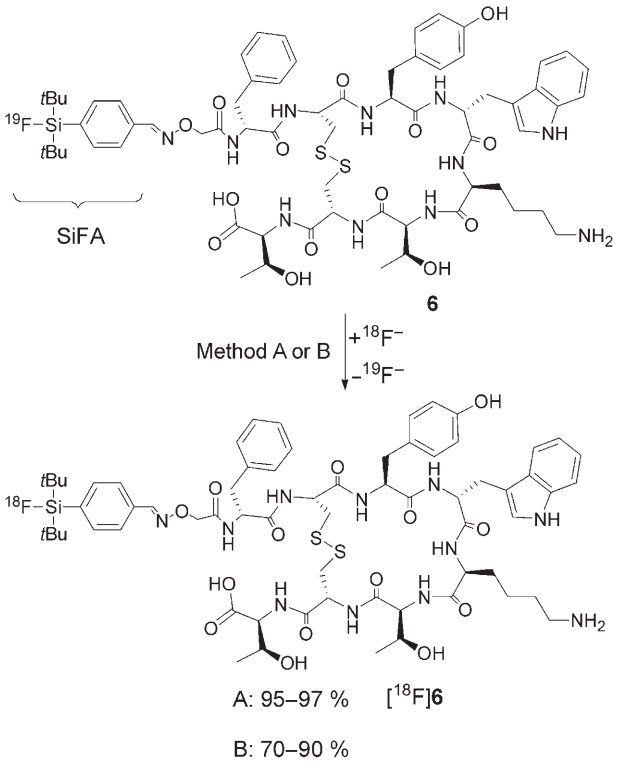
be further enhanced by starting with higher amounts of ^{18}F radioactivity.

Current conventional methods for the syntheses of ^{18}F-labelled peptides are based on multistep procedures involving prosthetic groups suitable for bioconjugation, for example, acylation, amidation, alkylation, photochemical conjugation,[4,12] and the recently introduced method of chemoselective oxime formation.[16,17] All these methods are complex, which is a major drawback owing to the short half-life of ^{18}F. Hence, in contrast to the radiolabeling of peptides with radiometals,[4,12] no suitable robust practical formulation has been described for the routine ^{18}F-labeling of peptides. Ideally the radiolabeling should proceed directly in the commercially available $^{18}F^-/[^{18}O]H_2O$ solution or in dried $^{18}F^-$/Kryptofix2.2.2/K^+ complex in dipolar aprotic solvents. To date, nucleophilic ^{18}F-fluorination from aqueous solution has been considered impossible because the fluoride ion is highly solvated and thus has low nucleophilicity. To circumvent these problems and to utilize our labeling chemistry described on potential radiopharmaceuticals, we synthesized *p*-(di-*tert*-butylfluorosilyl) benzaldehyde, *p*-(tBu$_2$FSi)C$_6$H$_4$C(O)H (**5**, see Supporting Information) which is amenable for chemoselective oxime ligation to aminooxy-functionalized peptides.[16]



5

The organosilicon-functionalized aldehyde **5** was coupled to an aminooxy-derivatized Tyr^3-octreotate,[18] a peptide which is used in nuclear medicine,[19] which was synthesized by Fmoc solid-phase-peptide-synthesis (see Supporting Information; Fmoc = (9H-fluoren-9-ylmethoxy)carbonyl) and coupled to compound **5** by oxime formation in acetonitrile/water (90:10) at pH 4, yielding peptide **6** in > 98 % purity after HPLC purification. In a first isotopic exchange experiment we treated **6** (100 μg, 74 nmol) of with $^{18}F^-$/Kryptofix2.2.2./K^+ complex (280–360 MBq) in 800 μL acetonitrile for 10–15 min at room temperature yielding [^{18}F]**6** in yields between 95–97 % (Scheme 1). This reaction is the fastest,



Scheme 1. Synthesis of [^{18}F]**6** by two different procedures. Method A: **6** (100 μg, 74 nmol), $^{18}F^-$/Krypto fix2.2.2/K^+ (280–360 MBq), CH_3CN (800 μL), room temperature, 10–15 min. Method B: **6** (100 μg, 74 nmol) in CH_3CN (40 μL), $^{18}F^-/[^{18}O]H_2O$ (200–300 μL, 175–250 MBq), 95 °C, 30 min.

most selective and mildest ^{18}F-labelling chemistry documented to date. To remove the toxic Kryptofix2.2.2, potassium ions and residual $^{18}F^-$, the acetonitrile was diluted with sodium dihydrogenphospate (0.25 N, pH 4.5, 10 mL) and passed through a C-18-solid-phase-extraction cartridge. The radiolabeled peptide [^{18}F]**6** was retained on the cartridge (trapping efficiency ≈ 80 %) and washed with water to reduce the amount of residual $^{18}F^-$ to < 1 %. To obtain [^{18}F]**6** as an injectable solution, the cartridge was eluted with ethanol (1 mL, elution efficiency ≈ 80 %) and diluted with isotonic saline (9 mL). Notably, the entire experimental procedure took only 25 min and the overall radiochemical yield was between 55–65 % giving [^{18}F]**6** in a purity of > 98 %.

To evaluate whether an isotopic exchange also occurs in commercially available $^{18}F^-/[^{18}O]H_2O$, we treated a solution of **6** (100 μg, 74 μmol) in acetonitrile (40 μL) with $^{18}F^-/[^{18}O]H_2O$ (200 μL, 175–250 MBq) at room temperature for 15 min. Only a 5 % yield of [^{18}F]**6** was detected by radio-HPLC. However, when conducting this reaction at 95 °C, the desired compound was obtained in yields between 70–90 % (HPLC) within 30 min (Scheme 1). No hydrolysis of the Si–^{18}F bond and no disintegration of peptide **6** was observed

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during HPLC quality control. Purification was performed in a similar manner as described above by solid-phase extraction using water (5 mL) for dilution. The calculated specific activity of [^{18}F]**6** for both methods was 3–5 GBq μmol^{-1} which is sufficient for in vivo PET studies. We anticipate, however, that even higher specific activities should be obtainable by using larger amounts of $^{18}F^-$ for the isotopic exchange.

In conclusion, we have described a rapid and versatile approach for the synthesis of ^{18}F-labeled peptides as illustrated by the example of a SiFA-derivatized Tyr^3-octreotate. This compound was labeled with $^{18}F^-$/Kryptofix2.2.2./K^+ complex in acetonitrile at room temperature as well as in aqueous $^{18}F^-$/[^{18}O]H_2O solution at 95 °C. We anticipate that the very mild reaction conditions and the fast and efficient labeling make the SiFA strategy a valuable tool for the development of ^{18}F-radiopharmaceuticals. The in vivo evaluation of compound [^{18}F]**6** as a tumor imaging agent is currently under investigation.

Experimental Section

Syntheses of the organosilanes *t*Bu_2PhSiX (X = H,[20] F,[21] Cl,[20] I[21]) and *t*$BuPh_2SiF$[22] have been described elsewhere, however, different procedures were applied herein and all details are given in the Supporting Information, together with the synthetic procedures for *p*-(di-*tert*-butylfluorosilyl) benzaldehyde (**5**) and peptide **6**. Noteworthy, in contrast to earlier reports *t*Bu_2PhSiF (**4**) was obtained as crystalline material and its molecular structure was determined by single-crystal X-ray diffraction.[23]

[^{18}F]**6**: Method A: Compound **6** (100 μg, 74 nmol) was dissolved in acetonitrile and $^{18}F^-$/Kryptofix2.2.2./K^+ complex (280–360 MBq) in acetonitrile was added to obtain a total volume of 800 μL. The solution was kept at room temperature without stirring for 10–15 min, diluted with sodium dihydrogenphospate (0.25 N, pH 4.5, 10 mL) and passed through a C-18-Sepak cartridge (Merck). The cartridge was washed with water (2 mL) and eluted with ethanol (1 mL) to obtain [^{18}F]**6** (160–240 MBq) as an injectable solution.

[^{18}F]**6**: Method B: Compound **6** (100 μg, 74 nmol) dissolved in acetonitrile (40 μL) was added to $^{18}F^-$/[^{18}O]H_2O (200–300 μL; 175–250 MBq; purchased from PetNet Erlangen) and heated at 95 °C for 30 min in a sealed reaction vial. Water (5 mL) was added and the solution was passed through a C-18-SepPac cartridge (Merck). The cartridge was washed with water (2 mL) and eluted with ethanol (1 mL) to obtain [^{18}F]**6** (95–150 MBq) as an injectable solution.

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[1] "Chemistry of Fluorine-18 Radiopharmaceuticals": S. E. Snyder, M. Kilbourn in *Handbook of Radiopharmaceuticals, Radiochemistry and Applications* (Eds.: M. Welch, C. S. Redvanly), Wiley, Chichester, **2003**.
[2] T. Ido, C.-N. Wan, J. S. Fowler, A. P. Wolf, *J. Org. Chem.* **1977**, *42*, 2341.
[3] G. Firnau, C. Nahmias, E. S. Garnett, *Int. J. Appl. Radiat. Isot.* **1973**, *24*, 182.
[4] P. Blower, *Dalton Trans.* **2006**, 1705.
[5] M. S. Rosenthal, A. L. Bosch, R. J. Nickles, S. J. Gatley, *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 318.
[6] A. S. Pilcher, H. L. Ammon, P. DeSong, *J. Am. Chem. Soc.* **1995**, *117*, 5166.
[7] 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.
[8] R. Ting, M. J. Adam, T. J. Ruth, D. M. Perrin, *J. Am. Chem. Soc.* **2005**, *127*, 13094.
[9] M. de Jong, W. A. P. Breeman, W. H. Bakker, P. P. M. Kooij, B. F. Bernard, L. J. Hofland, T. J. Visser, A. Srinivasan, M. A. Schmidt, J. L. Erion, J. E. Bugaj, H. R. Mäcke, E. P. Krenning, *Cancer Res.* **1998**, *58*, 437.
[10] C. G. Swain, R. M. Esteve, R. H. Jones, *J. Am. Chem. Soc.* **1949**, *71*, 965.
[11] H. Szmant, G. A. Brost, *J. Am. Chem. Soc.* **1950**, *72*, 5763.
[12] "Radiolabeled Peptides for Tumor Imaging": L. C. Knight in *Handbook of Radiopharmaceuticals, Radiochemistry and Applications* (Eds.: M. Welch, C. S. Redvanly), Wiley, Chichester, **2003**.
[13] R. Bolton, *J. Labelled Compd. Radiopharm.* **2002**, *45*, 485.
[14] M. S. Haka, M. R. Kilbourn, G. L. Watkins, S. A. Toorongian, *J. Labelled Compd. Radiopharm.* **1989**, *27*, 823.
[15] "Neurological Applications": N. I. Bohnen in *Principles and Practise of Positron Emission Tomography* (Eds.: R. L. Wahl, J. W. Buchanan), Lippincott Williams & Wilkins, Philadelphia, **2002**, and references therein.
[16] G. Thumshirn, U. Hersel, S. L. Goodman, H. Kessler, *Chem. Eur. J.* **2003**, *9*, 2717.
[17] T. Poethko, M. Schottelius, G. Thumshirn, U. Hersel, M. Herz, G. Henriksen, H. Kessler, M. Schwaiger, H. J. Wester, *J. Nucl. Med.* **2004**, *45*, 892.
[18] Tyr^3-octreotate was synthesized by Fmoc solid-phase peptide synthesis according to a published procedure (E. Schirrmacher, R. Schirrmacher, C. Beck, W. Mier, N. Trautman, F. Rösch, *Tetrahedron Lett.* **2003**, *44*, 9143).
[19] C. R. Reubi, M. Gugger, B. Waser, *Eur. J. Nucl. Med.* **2002**, *29*, 855.
[20] T. Kusukawa, W. Ando, *J. Organomet. Chem.* **1998**, *559*, 11.
[21] H.-W. Lerner, S. Scholz, M. Bolte, *Z. Anorg. Allg. Chem.* **2001**, *627*, 1638.
[22] R. Damrauer, R. A. Simon, B. Kanner, *Organometallics* **1988**, *7*, 1161.
[23] G. Bradtmöller, K. Jurkschat, M. Schürmann, *Acta Crystallogr. Sect. E* **2006**, *62*, 1303.

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