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Research Article

Reaction of [^{18}F]4-fluorobenzenediazonium cations with cysteine or the cysteinyl group: preparation of ^{18}F-labeled S-aryl-cysteine and a radiolabeled peptide

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Summary

A reaction route for the preparation of no-carrier-added (n.c.a.) [^{18}F]S-4-fluorophenylcysteine **7** via the [^{18}F]-4-fluorobenzenediazonium ion **4** is described. The key step in this radiosynthesis is the reaction of **4** with cysteine forming [^{18}F]4-fluorophenyldiazocysteine **6**, which is subsequently converted into **7** by irradiation with 366 nm light. **4** was synthesized by reacting 1,4-dinitrobenzene **1** with [^{18}F]-fluoride in acetonitrile in a PEEK-capillary in a microwave oven. After dilution of the reaction mixture with methanol, the resulting [^{18}F]4-fluoro-1-nitrobenzene **2** was submitted to reduction by means of H_2 with Pd on C catalyst. The resulting [^{18}F]4-fluoroaniline **3** was purified by HPLC and diazotized to **4**. The preparation of **4** was optimized with regard to yield and purity. The radiochemical yield of **6** was $>90\%$ (based on **3**) while after UV irradiation and HPLC purification 45% of **7** (based on **3**) was obtained (yields corrected for decay). The suitability of this method for labeling peptides with fluorine-18 was demonstrated by application to the tripeptide, glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine) **8**.

Key Words: fluorine-18; diazonium ion; *S*-aryl-cysteine; cysteinyl; peptide

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Introduction

Metabolic imaging in oncology is an important application of positron emission tomography (PET). Some amino acids, labeled with positron-emitters, such as ^{11}C and ^{18}F ($t_{1/2} = 20.3$ and 109.7 min, respectively), are useful tracers in oncology for reporting protein synthesis and/or amino acid transport (for a review see Reference 1). Thus, the ^{18}F-labeling of *S*-aryl substituted cysteine[2] via ^{18}F-labeled diazonium ions, as described below, may result in new tracers for tumor imaging with PET and provide a new method for radiolabeling peptides.

Feliu described the radiosynthesis of [^{18}F]WIN 44577 via the production of [^{18}F]4-fluorophenylhydrazine and diazonium coupling.[3] In this procedure [^{18}F]4-fluorophenylalanine (**3**) was obtained and diazotized in high yield, as shown by monitor reactions. Although diazothio-compounds are note suitable for a direct introduction of ^{18}F into an aromatic ring, the high reactivity of the sulfhydryl-group of cysteine with diazonium ions to form diazothio-compounds[4,5] and arylthioethers by thiodediazonation[6–8] may provide a possible reaction pathway for the synthesis of radiopharmaceuticals containing the ^{18}F-aryl thioether moiety. In this paper, we describe an adaptation of the synthesis of the diazonium ion **4** and its application to the synthesis of ^{18}F-labeled **7** (Figure 2). Furthermore, we describe the application of this method to the radiolabeling of the model tripeptide, glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine), **8**.

Results and discussion

The method for the production of **4** described by Feliu[3] was modified in certain respects (Figure 1): acetonitrile was used instead of dimethyl

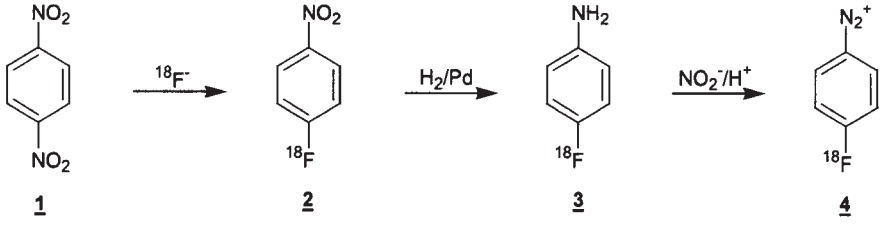

Figure 1. Modified procedure for the preparation of [^{18}F]4-fluorobenzenediazonium ions (Feliu)[3]

sulfoxide as solvent. Acetonitrile could be easily removed by evaporation and did not impair the reduction step. Thus the SepPak purification of **2**, which leads to losses in yield due to incomplete trapping of **2** on the stationary phase of the cartridge, could be avoided. For the catalytic reduction of **2**, hydrogen was used instead of sodium borohydride to avoid contaminating the product with boron compounds that may interfere with the chromatographic separation of product. **3** was purified by HPLC to improve the purity of the diazonium salt solution. A simplified one-pot procedure without HPLC purification of **3** was shown to give moderate radiochemical yields of **6** (60% based on **3**), but after irradiation only a rather low amount of the desired product **7** was obtained. The low UV intensity (UV hand lamp 3 W) and the high energy absorption by other substances in the reaction mixture may explain this observation.

The microwave procedure for NO_2 for ^{18}F exchange is superior compared to thermal heating procedures, because a considerably lower amount of precursor can be used with a shorter reaction time. In the polar aprotic solvent, acetonitrile, the main effects of microwave power may be superheating and creation of hot spots. Nevertheless, other effects like stabilization of the transition state of the reaction are possible (for a review see References[9–11]). Performing the reaction within PEEK-tubing in a domestic microwave oven is a low-cost alternative to dedicated radiochemistry microwave units and allows the usage of low boiling solvents like acetonitrile. Consecutive intervals of 30 s full microwave power (30 s, 750 W) followed by 30 s cooling time each, resulted in radiochemical yields $>70\%$ (2×30 s, 29%; 4×30 s, 49%; 6×30 s, 73%). For reduction of the nitro-compound, the crude reaction mixture was diluted with methanol. Complete reduction of **2** was achieved within 10 min by passing a stream of hydrogen through the reaction mixture in the presence of Pd/C catalyst. It was found, that acetonitrile does not impair the reduction. After filtration of the mixture, purification by normal phase HPLC, and evaporation of the solvent, **3** was obtained in $>60\%$ overall radiochemical yield. Figure 2 shows the reaction scheme of the following process. Diazotization of purified **3** and subsequent coupling to cysteine gave **6** in quantitative radiochemical yield. The retention time of this radioactive product agreed with that of the reference compound, as detected by MS ($m/z = 244.06$ molecular ion) (Figure 3). After irradiation with 366 nm UV light and HPLC purification the cysteine conjugate **7** was obtained in 45% radiochemical yield (based on **3**). The retention time of this

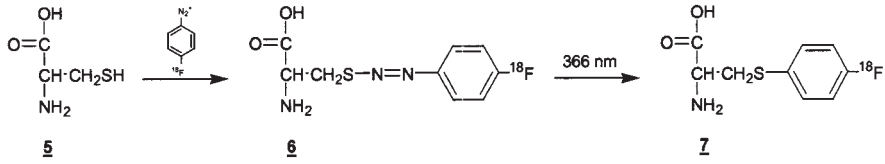

Figure 2. Preparation of [^{18}F]4-fluorophenyl-cysteine by reacting cysteine with [^{18}F]4-fluorobenzenediazonium ion and subsequent irradiation with 366 nm light

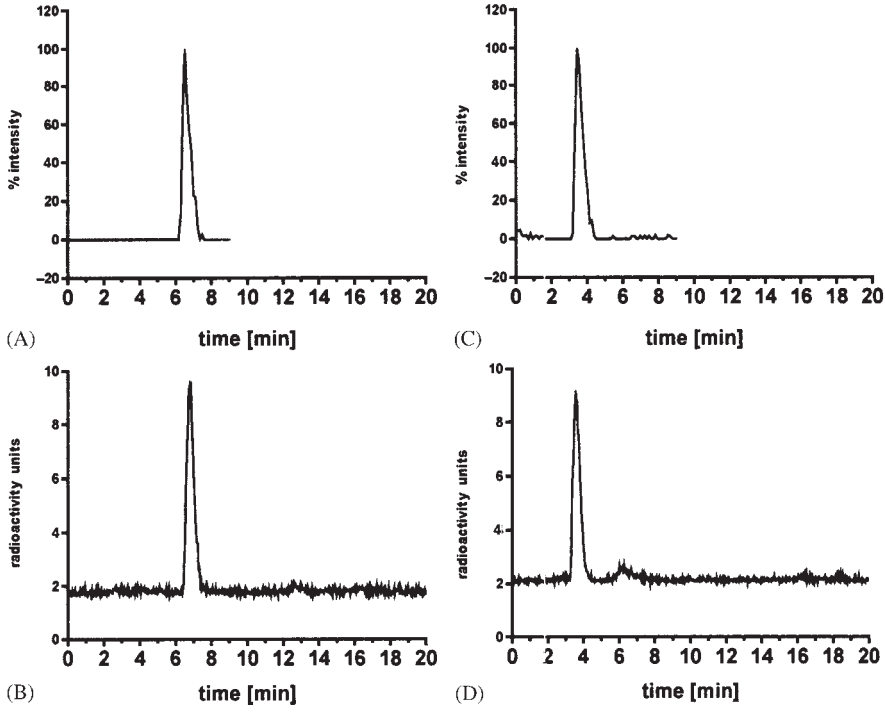

Figure 3. HPLC–MS chromatogram (A,C) and radio-chromatogram (B,D) of 6 and 7. (A) extracted ion chromatogram ($m/z = 244.1 \pm 0.2$) of 6, (B) radio chromatogram of 6, (C) extracted ion chromatogram ($m/z = 216.1 \pm 0.2$) of 7, (D) radio chromatogram of 7

radioactive product on HPLC agreed with that of the authentic reference compound, as detected by MS ($m/z = 216.05$) (Figure 3). The reference compound 7 was purified by HPLC and characterized by ^{1}H-NMR and ^{19}F-NMR spectroscopy. The melting point of the product was 179–181°C after HPLC purification and in agreement with a melting point of 185–186°C reported by Goodman *et al.*[2]

The reaction of arenediazonium ions with thiolate ions is a well-known procedure for the preparation of arylthioethers. The

mechanism of thiodediazonation is considered to consist of four reaction steps:[7,8]

1. the reaction of an electron donor with the aryldiazothio-compound to form a radical anion,
2. the cleavage of the C–N and N–S bonds in the radical anion to form aryl radical, nitrogen, and thiolate anion,
3. the recombination of aryl radical and thiolate anion and
4. the reoxidation of the radical anion to the arylthioether product.

Our results with n.c.a. **6** show that the propagation step (electron transfer from the radical anion of step 4 to the aryldiazothio-compound in step (1)) is not crucial for the reaction. The rapid recombination of the aryl radical with the thiolate anion has been thoroughly investigated by Dell'Erba *et al*..[7] To explain the minor influence of solvent changes or the presence of a competing nucleophile (CN^-) on the reaction, Dell'Erba *et al*. proposed a 'cage effect'. Because of the high reactivity and the mechanism, this reaction also appears useful for the radiolabeling of peptides and other biologically active compounds. To test the applicability of this method for the radiolabeling of peptides, **8** was reacted with **4**. A radiochemical yield of 75% (based on **3**) of [^{18}F] *S*-4-fluorophenyldiazo-GSH **9** was obtained for an amount of 0.5 µmol of **8** in the reaction mixture. Higher amounts of precursor peptide **8** resulted in quantitative conversion. The thiodediazonation step to [^{18}F]*S*-4-fluorophenyl-GSH **10** could be performed with 33% yield. This step is not optimized yet, and might be improved by use of a dedicated UV-irradiation apparatus. These results illustrate the suitability of this labeling procedure for peptides.

Experimental

General: Solvents for HPLC and LC–MS and chemicals were purchased in gradient grade quality or analytical quality by Merck, Germany unless otherwise stated. HPLC chromatograms were recorded on an Agilent Chemstations for LC systems equipped with an NaI/Tl detector connected to the HPLC system via a Dual Channel Interface HP35900E (Agilent). The low pressure gradient system used for semipreparative separations consisted of quaternary HPLC pump and vacuum degasser, autosampler and diode array detector. The high pressure gradient system consisted of vacuum degasser, binary HPLC pump,

autosampler, multi wavelength detector and was connected to the mass spectrometer. TLC were exposed to BAS-MS2325 imaging plate and read out with the BAS-1800II IP-Reader (Fujifilm). The image was evaluated with AIDA software (raytest isotopenmessgeräte, Straubenhardt, Germany). Mass spectra and LC-MS chromatograms were obtained using a Mariner Biospectrometry Workstation (Applied Biosystems) (MS-TOF, positive ion mode, nozzle potential 100 V, skimmer 1 potential 7.5 V). NMR spectra were recorded on a BRUKER DRX-400 (AVANCE), ^{19}F-NMR spectra were measured at room temperature in D_2O with trifluroacetic acid as internal reference ($\delta(CF_3COOH) = 0$ ppm). ^{1}H-NMR spectra were recorded in D_2O with the HDO peak as reference ($\delta(HDO) = 4.67$ ppm). For UV-irradiation of the sample a UV-lamp for TLC-detection (3 W, 366 nm) was used, which was positioned 4–5 cm above the sample surface. The sample was irradiated in a small glass beaker. Radiochemical yields were corrected for decay.

*Synthesis of 4-fluorophenylcysteine (**7**)*

A solution of 0.01 M 4-fluoroaniline (for synthesis) in 1 M HCl (1 ml) diazotized at 0°C with 0.1 M sodium nitrite solution (0.1 ml). The solution of the diazonium salt was added to a stirred solution of cold (0°C) 0.1 M L-cysteine (for biochemistry) in 1 M NaOH (1 ml). The pH was adjusted to a slightly alkaline value (8–9). The 4-fluorophenyldiazocysteine was not isolated but an aliquot of the mixture was filtered and analyzed by HPLC-MS (2 Zorbax XDB-C8, 4.6 mm × 15 cm columns in series, acetonitrile 15%, water (2.5% acetic acid) 85%, 5 min, gradient to acetonitrile 75% at 15 min, 1 ml/min, retention time for **6** 6.4 min). The remaining mixture was irradiated with UV light for 30 min. The mixture was diluted with eluent, filtered and the product purified by HPLC (Supelcosil LC-ABZ+, 250 × 7.6 mm, acetonitrile 15%, water containing 2.5% acetic acid 85%) (injection volume 500 µl, 5 ml/min, retention time for **7** 4.3 min, **6** 6.9 min). The separated product was analyzed by ^{1}H-NMR, ^{18}F-NMR mass spectrometry and melting point.

^{1}H-NMR (D_2O) δ ppm: 7.6 (2 H, m); 7.21 (2 H, m); 3.85 (1 H, dd; 3.9 Hz; 8.3 Hz); 3.59 (1 H,dd, 3.9 Hz, 14.9 Hz); 3.35 (1 H, dd, 8.3 Hz, 14.9 Hz).
^{19}F-NMR (D_2O): −39 ppm.
m/z: 216.05 $(M+H)^+$ (Turbo Ion Spray).
Melting point: 179–181°C (decomposition).

*Synthesis of n.c.a. [^{18}F]1-fluoro-4-nitrobenzene (**2**)*

Heating-block procedure. Aminopolyether 2.2.2 (Kryptofix 222) (10 mg, 26 μmol), K_2CO_3 (13 μmol) and n.c.a. [^{18}F]fluoride (100–1000 MBq, obtained via the $^{18}O(p,n)^{18}F$ nuclear reaction) in water and acetonitrile (1 ml) were mixed in a 5 ml reaction vial (Alltech, Germany). The water was evaporated under a stream of nitrogen (heating block 110–120°C). Acetonitrile (1 ml) was added and re-evaporated three times. A solution of **1** (for synthesis) in acetonitrile (5 mg in 500 μl) was added and the mixture was refluxed for 30 min. Radio-TLC-analysis of the reaction mixture showed 50–80% conversion to product (Polygram SIL-G/UV254 (Macherey-Nagel, Germany); petroleum ether–ethyl acetate (4/1, v/v), $R_f = 0.7$).

For purification of the product, the solvent was evaporated and the residue dissolved in ethanol (200 μl) and diluted with water (10 ml). The mixture was passed through an RP-18 cartridge (Sep Pak light, preconditioned with 5 ml ethanol and 5 ml water). An additional amount of water (5 ml) was passed through the cartridge, which was subsequently dried with a nitrogen gas stream. The product was eluted with methanol and used without further purification (radiochemical yield: 30–50%).

Microwave procedure. The reaction was performed in a microwave oven (for household use, 750 W). The reaction mixture was enclosed in a PEEK-capillary (0.5 mm inner diameter, completely filled with reaction mixture, manually closed with 2 PEEK fittings and a PEEK union) and placed on the glass tray in the microwave oven. Before performing a radioactive production, a bursting experiment was performed with inactive reaction mixture in a bended PEEK-tube. The 750 W on time was chosen well below the time recorded for capillary rupture in this experiment. The reaction mixture was prepared as described above. The amount of **1** in acetonitrile was reduced to (0.75 mg in 500 μl). The reaction mixture was treated with several intervals (30 s) of microwave energy followed by 30 s cooling time each.

*Production of [^{18}F]4-fluoroaniline (**3**) by catalytic reduction of **2***

The acetonitrile solution (microwave method) containing **2** was transferred with methanol (2 ml) from the PEEK loop into a reaction vessel containing Pd/C (10% Pd; 1–2 mg). A stream of hydrogen was passed through the mixture for 10 min. The progress of the reduction

was monitored by TLC (Polygram SIL-G/UV254, Macherey-Nagel, Germany), petroleum ether-ethyl acetate, 2/1 (v/v), R_f (**3**) = 0.45, R_f(**2**) = 0.9). The catalyst was filtered off, the filtrate washed with ethyl acetate and the solution evaporated. The residue was redissolved in eluent and injected on a semipreparative HPLC column (LiChrosorb Si 60, 250 × 10 mm, ethyl acetate/methanol, 80/20, v/v, 4 ml/min, retention time for **3** 5.0 min) (**1** 4.2 min, **2** 4.3 min, 4-nitroaniline 4.5 min, 1,4-phenylenediamine 19 min). The product peak was collected, 1 M HCl (100 µl) was added and the eluent evaporated off. The product was dissolved in 1 M HCl (1–2 ml).

*Synthesis of n.c.a. [^{18}F]S-4-fluorophenyldiazocysteine (**6**)*

3 in 1 M hydrochloric acid (100 µl) was treated with 1 M sodium nitrite solution (10 µl) at 0°C for 2 min. Ammonia solution (25%; 30 µl) was added followed by 0.1 M cysteine (**5**) solution in 1 M NaOH (100 µl). An aliquot of the reaction mixture was analyzed by HPLC (2 Zorbax XDB-C8, 150 × 4.6 mm columns in series, acetonitrile 15% water (2.5% acetic acid) 85% 5 min, gradient to acentonitrile 75% at 15 min, 1 ml/min, retention time for **6** 6.4 min) (Figure 3).

*Synthesis of n.c.a. [^{18}F]S-4-fluorophenylcysteine (**7**)*

The reaction mixture containing **6** was irradiated for 20 min with 366 nm light. The solution was filtered and **7** was separated by HPLC (Supelcosil LC-ABZ+, 250 × 7.6 mm, acetonitrile 15%, water containing 2.5% acetic acid 85%) (5 ml/min, retention time for **7** 4.3 min, **6** 6.9 min). The yield (46%, corrected for decay) was determined by collecting the appropriate product fraction and counting product fraction and aliquot sample. The radiochemical purity of the product was determined by radio-HPLC, 2 Zorbax XDB-C8, 150 × 4.6 mm columns in series, acentonitrile 15%, water (2.5% acetic acid) 85%, 5 min, gradient to acetonitrile 75% at 15 min, 1 ml/min (Figure 3).

*Synthesis of n.c.a. [^{18}F]S-4-fluorophenyl-GSH (**10**)*

3 in 1 M hydrochloric acid (100 µl) was treated with 1 M sodium nitrite solution (10 µl) at 0°C for 2 min. Hydroxylamine solution (1 M, 20 µl) was added (1 min). A solution (200 µl) containing ammonia (0.5 M), sodium acetate (0.5 M) and **8** (154 µg, 0.5 µmol) was added and the

mixture was neutralized by addition of 1 M ammonia (40 µl). An aliquot of the reaction mixture was analyzed by radio-HPLC, 2 Zorbax XDB-C8, 150 × 4.6 mm columns in series, acetonitrile 15% water (2.5% acetic acid) 85%, 5 min, gradient to acetonitrile 75% at 15 min, 1 ml/min, to determine the amount of **9** formed. The mixture was diluted with 400 µl water and irradiated for 10 min with 366 nm UV-light. The yield of **10** was determined by radio-HPLC. The synthesis procedure described above was repeated with 100 µl of a 0.002 M solution of **3** in 1 M hydrochloric acid which was reacted with a solution (200 µl) containing ammonia (0.5 M), sodium acetate (0.5 M) and **8** (307 µg, 1 µmol). An aliquot of the reaction mixture was analyzed before and after irradiation with UV-light by the HPLC method described above with the HPLC–MS–TOF connected to the system.

m/z: 430.11 D, retention time = 12.2 min, $(m + H)^+$ of **9**.
m/z: 402.11 D, retention time = 11.2 min, $(m + H)^+$ of **10**, after UV irradiation.

Conclusion

The reaction of ^{18}F-labeled diazonium ions with the amino acid, cysteine, is a versatile reaction route for the production of non-naturally occurring amino acids with biological activity in good radiochemical yields. Furthermore, the procedure described might be useful for labeling peptides containing a cysteinyl residue. Further improvement of this method should be possible by optimization of the UV-irradiation step.

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