Preparation of No-Carrier-Added 6-[18F]fluoro-L-tryptophan via Cu-Mediated Radiofluorination

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Abstract: 18F-Labeled aromatic amino acids exhibit great potential for diagnostic applications using positron emission tomography. However, the introduction of 18F into aromatic compounds remains challenging, and novel fluorination methods facilitating easy access to 18F-labeled arenes are highly sought after. In recent years, novel metal-mediated fluorination methods have been reported and transferred into radiochemistry. Based on Cu-mediated radiofluorination, a two-step synthesis of no-carrier-added (n.c.a.) 6-[18F]fluoro-L-tryptophan was developed. 6-[18F]fluoro-L-tryptophan was synthesized with an overall radiochemical yield of 16 ± 4% within 110 min and a specific activity of 280 GBq μmol⁻¹. The radiochemical purity was more than 99 %. The developed method allowed access to radiofluorinated tryptophan derivatives in high radiochemical yields and opens new ways to provide radiofluorinated amino acids. Furthermore, the reaction conditions were optimized to facilitate automation.

Introduction

Positron emission imaging tomography (PET) is one of the most important molecular imaging techniques in clinical practice. PET imaging exploits the decay of positron emitters. The emitted positron is annihilated when in contact with an electron and produces a pair of photons that can be detected by a dedicated PET scanner, revealing a 3D-image of the radionuclide distribution. For these purposes, different PET nuclides, such as 11C, 13N, 15O, and 18F are available.[1] Among them, 18F plays an outstanding role because of its favorable decay properties with low positron emission but highly intense energy and convenient half-life of 109.7 min, enabling multistep radiosyntheses to be performed.

The indole motif occurs in numerous natural products,[2] as well as in the essential α-amino acid tryptophan, which is involved in a range of physiological processes. In vivo, tryptophan is either converted enzymatically over two steps into serotonin, which is involved in various neurological functions and diseases,[3] or it is metabolized through the kynurenine pathway.[4] Kynurenine seems to be involved in many neurodegenerative disorders such as Alzheimer’s disease,[5] Huntington’s disease,[6] and multiple sclerosis.[7] Recently published results describe kynurenine as an important factor in “progressive” tumor growth and immune system suppression.[8] As described by Optiz et al.,[9] some tumors overexpress the liver enzyme tryptophan dioxygenase (TDO), resulting in an increase of tryptophan uptake into the tumor cells. Furthermore, tryptophan participates in the serotonin pathway and therefore a radiolabeled variant of tryptophan could enable to detect small alterations of tryptophan uptake in regions of serotoninergic neurons to be traced.

First attempts to radiolabel tryptophan with positron emitters by using nucleophilic aromatic substitution (SνAr) were reported by Atkins et al. in 1972.[10] In their work, the Balz–Schiemann reaction with subsequent hydrolysis was applied, providing carrier-added (c.a.) 5- or 6-[18F]fluoro-D/L-tryptophan as a racemic mixture in radiochemical yields (RCY) of 7–10 %. The biodistribution of 5- and 6-[18F]fluoro-D/L-tryptophan was studied in mice and rats. However, the results were of limited value because 6-fluorotryptophan is itself an inhibitor of tryptophan hydroxylase and lower molar concentrations are mandatory.[3c,11] Moreover, the lack of enantioselectivity and low specific activities precluded further investigations. The uptake of the tryptophan analogue 6-fluorotryptophan through the blood brain barrier (BBB) has been confirmed[12] and, furthermore, the interaction of 6-fluorotryptophan with tryptophan hydroxylase was examined.[3e]

Alternative approaches to 18F-labeled tryptophan derivatives include electrophilic 18F-fluorination of 5-hydroxytryptophan (5-HT) leading to a mixture of 4- and 6-[18F]fluoro-5-hydroxytryptophan.[13] Moreover, 18F-fluoroalkylation reactions at different positions of the indole motif were carried out.[14] Recently, a three-step synthesis using isotopic exchange through SνAr was reported, giving c.a. 4-[18F]fluoro-L-tryptophan in 13 % RCY and a specific activity of 70 MBq mmol⁻¹.[15]
In the last few years, several innovative $^{18}$F-fluorination methods via $^{18}$F to electron-rich or electron-neutral arylamines or thiophenes have been developed that allow direct introduction of $^{18}$F into a wide range of substrates with high yields and high specific activity.

In the case of 7-bromo-1-indole, the indole nitrogen could be protected with tosyl chloride, which generated the boron pinacol ester substituted indole derivatives in yields of 80 ± 2 %. The Suzuki–Miyaura coupling of the unprotected compounds revealed that protective groups were not mandatory.

Radiolabeling of Indolyl Pinacolyl Boronates

Radiofluorination of Indolyl Pinacolyl Boronates

Synthesis of Indole Precursors

In preliminary studies, the most reactive substitution position of indole using the copper-mediated $^{18}$F-fluorination had to be determined. Therefore, an appropriate precursor synthesis was developed. As depicted in Scheme 1, the precursor synthesis started from the corresponding bromo-indole derivatives.

The aim of this work was firstly to determine the most reactive position of the indole scaffold towards substitution using copper-mediated $^{18}$F-fluorination. Then, a method to prepare $^{18}$F-fluorotryptophan derivatives in high specific activity and enantiomeric excess was developed.

Results and Discussion

Radiofluorination of Indolyl Pinacolyl Boronates

Table 1. Dependence of RCC on the substitution positions of the indole ring. Reaction conditions: $[^{18}$F$]fluoride$ (ca. 30 MBq), precursor (25 μmol), Cu(OTf)$_2$(py)$_4$ (5.6 μmol), DMF/MeCN (10:1), 110 °C for 20 min. All reactions were carried out at least in triplicate.

<table>
<thead>
<tr>
<th>Indole derivative</th>
<th>RCC [%]</th>
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<tbody>
<tr>
<td>$[^{18}$F$]4b$</td>
<td>11 %</td>
</tr>
<tr>
<td>$[^{18}$F$]4c$</td>
<td>17 %</td>
</tr>
<tr>
<td>$[^{18}$F$]4d$</td>
<td>2 %</td>
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</table>

The presence of a pinacol ester at the C-4 position on the indole motif delivered only a low RCC of 7 %. This is in contrast to a previous study in which the authors observed the highest $^{18}$F-labeling RCCs in the C-4 position of the indole motif using isotope exchange of carbonyl activated compounds. Given that copper-mediated $^{18}$F-labeling showed the best results at the C-6 position of the indole motif, only the precursor synthesis of 6-pinacolester-substituted tryptophan was further investigated.
Synthesis of tert-Butyl 3-[[2R,5S]-3,6-Dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazin-2-ylmethyl]-6-tetramethyl-1,3,2-dioxaborolan-2-yl-1H-indole-1-carboxylate

An appropriate precursor for 6-[18F]fluoro-L-tryptophan was synthesized within a linear six-step synthesis starting from 6-bromoindole according to Konas et al.[26] (cf. Scheme 3). Finally the Suzuki–Miyaura coupling[24] was used as a last step for the introduction of the boronic ester group (cf. Scheme 4).

Radiosynthesis of 6-[18F]Fluoro-L-tryptophan

The two-step radiosynthesis of the desired radiolabeled tryptophan [18F]13 is shown in Scheme 5. In the first step, the corresponding precursor 11 was radiolabeled in the presence of a Cu source in sulfolane and acetonitrile, based on similar conditions reported by Tredwell et al.[22] Highest hydrolysis RCCs were achieved by using 50 % H2SO4.

Optimization of the Radiosynthesis

Different nucleophilicity enhancers of 18F– for the copper-mediated 18F-fluorination were examined. In the original publication,[22] [18F]fluoride was dried azeotropically in a separate vessel, redissolved in acetonitrile (1 mL), and only small aliquots (30 μL) were used for subsequent 18F-labeling reactions. Since small aliquots are inappropriate for large-scale productions, a one-pot approach using the whole [18F]fluoride target solution is indispensable. During our synthesis development (cf. Table 2), Zlatopolskiy et al. published optimized conditions for copper-mediated 18F-fluorination.[30] In this report, the authors discovered the base sensitivity of the Cu complex and developed a “low-base” protocol in which only small amounts of K2CO3 (3 mg vs. 60 μg) and Kryptofix® 2.2.2 were used.

Table 2. RCC dependency of different bases for 18F-labeling of 11 in the presence of [Cu(OTf)2(py)4]. Reaction conditions: base and [18F]fluoride were azeotropically dried,[a] then DMF (300 μL), MeCN (30 μL), precursor 11 (25 μmol), and Cu(OTf)2(py)4 were added and heated up to 110 °C for 20 min. The reaction was quenched by addition of water (200 μL) and the radiochemical conversions were determined by radio-TLC. Each experiment was performed at least in triplicate.

<table>
<thead>
<tr>
<th>Base</th>
<th>Amount [μmol]</th>
<th>RCC [%]</th>
</tr>
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<tbody>
<tr>
<td>K2CO3 + Bu4NHCO3</td>
<td>0.5 ± 0.2</td>
<td>7.3 ± 3</td>
</tr>
<tr>
<td>Bu4NHCO3</td>
<td>7.3 ± 3</td>
<td>32 ± 8</td>
</tr>
<tr>
<td>Et4NHCO3</td>
<td>1.0 ± 0.0</td>
<td>32 ± 8</td>
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</table>

[a] Aqueous [18F]fluoride was added to the reaction vial with the respective base and dried azeotropically tree times with MeCN (1 mL) at 80 °C and 500 mbar.

The utilization of tetraethylammonium hydrogen carbonate in an amount of 1 μmol resulted in the highest RCCs for [18F]12 (32 ± 8 %) from precursor 11 (cf. Table 1). Recently published procedures described a significant shortening of the drying process. These methods consist of the fixation of the aqueous
[18F]fluoride on a cartridge, followed by flushing with anhydrous solvents and elution of [18F]fluoride with an appropriate base dissolved in anhydrous organic solvents. Brichard et al.[31] used n-hexane for flushing and TEAHCO3 in acetonitrile for elution. In our study, according to Richarz et al.[32] the best results were obtained by using anhydrous methanol as flushing reagent and also methanolic TEAHCO3 (0.4–1.0 mg, low boiling point of methanol: 64.7 °C) for eluting the [18F]fluoride from the cartridge. Compared with the conventional drying approach, the time required for the drying step was reduced from 20 to less than 5 minutes. 

After drying the reaction vial containing TEA[18F]F, the vial was flushed with air, which seems to improve reaction yields.[22]

As a compromise, less acidic hydrobromic acid at 165 °C for 25 min was used to obtain [18F]fluoride from the reaction vial and syringes were taken into account. Each experiment was performed at least in triplicate.

Hydrolysis of [18F]12

In general, the Schöllkopf chiral auxiliary can be easily cleaved with 2 n hydrochloric acid (HCl) followed by 2 n sodium hydroxide (NaOH) within 2 h.[29] However, radiolabeling with short-lived radionuclides requires rapid reactions and harsher reaction conditions with regard to temperature and acidity.[34] A one-step deprotection with HCl at 150 °C for 30 min led to only incomplete hydrolysis of [18F]13 (20.1 ± 6 % RCC, n = 5). In contrast, significant decomposition of the radiofluorinated amino acid was observed by using the more acidic hydroiodic acid (130 °C, 15 min). As a compromise, less acidic hydrobromic acid at 165 °C for 25 min was used to obtain [18F]13 in 36.1 ± 5 % RCC (n = 5). Using shorter times for acidic hydrolysis with HCl followed by saponification in the presence of NaOH did not improve the overall hydrolysis yield (up to 21 % hydrolysis). Use of trifluoroacetic acid, followed by lithium hydroxide, also did not provide the desired compound. Unidentified byproducts were observed in the reaction mixture. Finally, using 50 % sulfuric acid at 130 °C for 15 min yielded [18F]13 in the highest RCCs (43.3 ± 6 %; n = 4). Table 4 shows all hydrolysis reagents and their corresponding RCCs.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>RCC</th>
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<tbody>
<tr>
<td>HCl</td>
<td>20.0 ± 6.1 (n = 5)</td>
</tr>
<tr>
<td>HBr</td>
<td>36.1 ± 5.0 (n = 6)</td>
</tr>
<tr>
<td>HI</td>
<td>– (n = 3)</td>
</tr>
<tr>
<td>HCl/NaOH</td>
<td>17.2 ± 4.5 (n = 3)</td>
</tr>
<tr>
<td>TFA/LiOH</td>
<td>– (n = 3)</td>
</tr>
<tr>
<td>50 % H2SO4</td>
<td>43.3 ± 5.7 (n = 4)</td>
</tr>
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</table>

The use of aprotic solvents with a very high dipole moment substantially improved radiochemical yields. Accordingly, the highest labeling yields were obtained in sulfolane followed by dimethyl sulfoxide,[33] and is therefore the solvent of choice for this kind of radiolabeling.

The final purification was performed by using semipreparative high-performance liquid chromatography (HPLC) with 10 % ethanol in water as mobile phase. The isolated product could be used directly for further biological evaluations. The total radiosynthesis time of 6-[18F]fluoro-L-tryptophan [18F]13 amounted to 110 min including HPLC purification with a total radiochemical yield of 15.8 ± 4 % (n = 4). 6-[18F]Fluoro-L-tryptophan was obtained with a radiochemical purity of more than 99 % and an enantiomeric excess of 89 % (94.4 % l-enantiomer) and a specific activity of 280 GBq μmol⁻¹.

The relatively high amount of the o-enantiomer is inherent in the Schöllkopf auxiliary system.[29] Moreover, taking into account the fair hydrolysis yield, the synthesis of a precursor that can be deprotected under milder conditions is underway. However, the presented radiosynthesis provides a practical route to otherwise difficult-to-access 18F-labeled tryptophan.

Conclusions

First, the influence of the substitution position for 18F-fluorination in the indole structure was studied. Nucleophilic aromatic substitution was highest at the 6-position with 17 ± 1 % RCC (n = 4). The appropriately elaborated precursor synthesis for 6-[18F]fluoro-L-tryptophan [18F]10 consisted of a linear six-step synthesis with an overall yield of 37 %. The copper-mediated 18F-labeling step (53 ± 10 % RCC, n = 5) and the following hydrolysis (43 ± 6 % RCC, n = 6) were optimized. 6-[18F]Fluoro-L-tryptophan was obtained in an overall RCY of 16 ± 4 % (n = 4). The enantiomeric excess amounted to 89 % and the specific activity was 280 GBq μmol⁻¹ within a total synthesis time of 110 min.

The reported labeling results of n.c.a. 6-[18F]fluoro-L-tryptophan open up new opportunities for PET diagnostics. Furthermore, the simplified procedure is amenable to remote-controlled synthesis.[35] Moreover, it should enable new insights into the serotonergic and kynurenine pathways. Further biological evaluations of 6-[18F]fluorotryptophan will demonstrate the scope and limitations of the presented probe.
Experimental Section

General: Chemicals were purchased from Chempur (Karlsruhe, Germany), Merck (Darmstadt, Germany) or Sigma–Aldrich (Taufkirchen, Germany).

Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 M (Merck, Darmstadt, Germany) and detection was carried out at 254 nm. Detection of radioactive products on the TLC was performed with a Raytest minigita device (Raytest, Straubenhardt, Germany).

Separations via high-performance liquid chromatography (HPLC) were performed with a Knauer pump, a Knauer K-2500 UV/Vis detector (Knauer, Berlin, Germany), and a Rhodyne manual injector (20 μl or 5 ml loop), and for radioactivity detection a NaI(Tl) well-type scintillation detector model 276 Photomultiplier Base with an ACE mate Amplifier and BIAS supply (EG&G Ortec, Metemek, Meersbusch, Germany). The measured data were analyzed by using Gina software (Version 2.18, Raytest).

Table 5. The k-values of labeled compounds analysed by radio-HPLC.

<table>
<thead>
<tr>
<th>HPLC System</th>
<th>k</th>
</tr>
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<tbody>
<tr>
<td>18F</td>
<td>13</td>
</tr>
<tr>
<td>18F</td>
<td>13</td>
</tr>
<tr>
<td>18F</td>
<td>13</td>
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4-Bromo-1-(4-methylphenylsulfonyl)-1H-indole (2a): NaH (60 % in mineral oil, 200 mg, 5.0 mmol) was added to a solution of 4-bromo-1H-indole (650 mg, 3.31 mmol) in THF (10 mL) at 0 °C. The resulting solution was stirred at 0 °C for 30 min before 4-toluenesulfonyl chloride (978 mg, 5.0 mmol) was added. The resulting mixture was allowed to reach room temperature (room temp.) and stirred overnight. After quenching the reaction with water, the mixture was repeatedly extracted with Et3O, and the combined organic layers were dried with Na2SO4. After evaporation of the solvent, purification was performed by flash chromatography to give the desired compound (980 mg, 2.68 mmol, 80 %) as a white solid. Rf = 0.25 (PE/EA, 95:5); m.p. 117 °C. 1H NMR (400 MHz, [D6]DMSO): δ = 7.99–7.93 (m, 2 H), 7.90 (d, J = 8.4 Hz, 2 H), 7.49 (d, J = 7.8 Hz, 1 H), 7.40 (d, J = 8.1 Hz, 2 H), 7.29 (t, J = 8.1 Hz, 1 H), 6.78 (dd, J = 3.7, 0.5 Hz, 1 H), 2.32 (s, 3 H) ppm. 13C NMR (101 MHz, [D6]DMSO): δ = 145.87, 134.30, 133.83, 130.57, 130.34 (2 C), 128.07, 126.81 (2 C), 126.21, 126.08, 114.12, 112.55, 108.35, 21.01 ppm. HRMS: m/z calc. for C15H12BrNO2S [M]+ 348.9767; found 348.9766.

6-Bromo-1-(4-methylphenylsulfonyl)-1H-indole (2b): Prepared as described for 4-bromo-1-tosyl-1H-indole but starting from 5-bromo-1H-indole giving the desired compound as colorless solid in 82 % yield. Rf = 0.17 (PE/EA, 95:5); m.p. 134 °C. 1H NMR (400 MHz, [D6]DMSO): δ = 7.93–7.79 (m, 5 H), 7.48 (dd, J = 8.8, 1.9 Hz, 1 H), 7.38 (d, J = 8.3 Hz, 2 H), 6.81 (d, J = 3.7 Hz, 1 H), 2.31 (s, 3 H) ppm. 13C NMR (101 MHz, [D6]DMSO): δ = 145.72, 133.89, 132.92, 132.42, 130.29 (2 C), 128.42, 127.20, 126.70 (2 C), 124.04, 116.09, 114.97, 108.77, 21.00 ppm. HRMS: m/z calc. for C15H12BrNO2S [M]+ 349.9845; found 349.9845.

1-(4-Methylphenylsulfonyl)-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole (3a): The borylation was carried out as described by Ishiyama et al. [4] in a dry round-bottomed flask, 4-bromo-1-(4-methylphenylsulfonyl)-1H-indole (732 mg, 2.0 mmol), potassium acetate (567 mg, 6.0 mmol), bis(pinacolato)diboron (1.0 mg, 4.0 mmol) and PdCl2[R-bis(diphenylphosphanylferrrocene) (120 mg, 0.2 mmol) were dried in fine vacuum and flushed repeatedly with dry argon. Dry DMF (18 mL) was added and the stirring reaction mixture was heated to 100 °C for 3.5 h under argon. After cooling to room temp., the reaction was quenched with EtOH/EtO (1:1), filtered through silica gel, washed with water, and extracted repeatedly with the quenching solvent. The combined organic phases were washed with water and dried with Na2SO4. The solvent was removed in vacuo and the crude product was purified via flash chromatography (PE/EA, 95:5) and reversed-phase flash chromatography (gradient 50 % MeCN in water up to 70 % MeCN in water), to give the desired compound (520 mg, 1.25 mmol, 62 %) as a white solid. Rf = 0.35 (PE/EA, 95:5); 0.20 (RP-TLC, MeCN/H2O, 70:30); m.p. 208 °C (decomp.). 1H NMR (400 MHz, [D6]DMSO): δ = 8.07 (d, J = 8.3 Hz, 1 H), 7.86–7.83 (m, 3 H), 7.59 (dd, J = 7.2, 0.8 Hz, 1 H), 7.41–7.33 (m, 3 H), 7.08 (d, J = 3.7 Hz, 1 H), 2.32 (s, 3 H), 1.32 (s, 12 H) ppm. 13C NMR (101 MHz, [D6]DMSO): δ = 145.46, 135.13, 134.08, 133.61, 130.63, 130.20 (2 C), 127.55, 126.63 (2 C), 124.07, 121.52 (broad), 116.07, 110.68, 83.63 (2 C), 24.68 (4 C), 20.98 ppm. HRMS: m/z calc. for C31H31BO6NS [M + H]+ 539.1952; found 539.1951.

5-Bromo-1-(4-methylphenylsulfonyl)-1H-indole (2b): Prepared as described for 4-bromo-1-tosyl-1H-indole but starting from 5-bromo-1H-indole giving the desired compound as colorless solid in 78 % yield. Rf = 0.17 (PE/EA, 95:5); m.p. 132 °C. 1H NMR (600 MHz, CDCl3): δ = 8.17 (s, 1 H), 7.76 (d, J = 8.4 Hz, 2 H), 7.53 (d, J = 3.7 Hz, 1 H), 7.38 (d, J = 8.3 Hz, 1 H), 7.33 (dd, J = 8.3, 1.7 Hz, 1 H), 7.24 (d, J = 8.1 Hz, 2 H), 6.61 (dd, J = 3.7, 0.6 Hz, 1 H), 2.35 (s, 3 H) ppm. 13C NMR (151 MHz, CDCl3): δ = 145.43, 135.59, 135.17, 130.17 (2 C), 129.67, 126.94 (2 C), 127.68, 122.59, 118.35, 116.71, 108.89, 21.72 ppm. HRMS: m/z calc. for C15H12BrNO2S [M + H]+ 349.9848; found 349.9845.

C15H12BrNO2S: calc. C 63.49, H 6.09, N 3.53; found C 62.61, H 6.03, N 3.49.
1-(4-Methylphenylsulfonyl)-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole (3b): The reaction conditions were similar to those reported for the preparation of 1-(4-methylphenylsulfonyl)-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole, but using a reaction time of 16 h. For purification, the reaction was quenched with EA/TEtO (1:1), filtered through silica, washed repeatedly with brine and the combined organic layers were dried with Na2SO4. After evaporation of the solvent, the crude product was dissolved in MeCN (5 mL) and 2 N NaOH (5 mL) were added. The mixture was then stirred for 30 min at room temperature. After quenching with satd. aq NH4Cl solution, the mixture was extracted repeatedly with EA/TEtO. The organic layers were combined, washed with brine, dried with Na2SO4 and the solvent was evaporated. The crude product was dissolved in a small amount of dichloromethane (CH2Cl2)/PE and given onto a flash chromatography column (gradient PE/CH2Cl2, 80:20 to 50:50) to give the desired product (360 mg, 0.9 mmol, 83.77 ppm). HRMS: [M + H]+ 397.1513; found 397.1514. C21H24BNO4S (397.29): calcd. C 63.49, H 6.09, N 3.53; found C 63.27, H 6.24, N 3.53.

3-(Tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole (3d): In a dry round-bottom flask, 7-bromo-1H-indole (480 mg, 2.5 mmol), potassium acetate (710 mg, 7.5 mmol), bis(pinacolato)diboron (950 mg, 3.75 mmol) and PdCl2[R-bis(diphenylphosphino)ferrocene] (160 mg, 0.25 mmol) were dried by repeated application of fine vacuum and flushed with dry argon. Anhydrous DMF (20 mL) was added and the stirring reaction mixture was heated to 100 °C for 4 h under argon. After cooling to room temp., the reaction was quenched by adding Et3O and water. The mixture was filtered through silica gel, extracted repeatedly with the Et3O, and the organic phases were combined. After washing the organic layer three times with brine and drying over Na2SO4, the solvent was removed in vacuo. The crude product was purified by flash chromatography (PE/EA, 98:2 to 90:10) to give the desired compound (270 mg, 1.11 mmol, 44%) as a white solid. Rf = 0.57 (PE/EA, 9:1); m.p. 88 °C. 1H NMR (600 MHz, [D6]DMSO): δ = 10.16 (s, 1 H), 7.67 (d, J = 7.8 Hz, 1 H), 7.43 (dd, J = 7.0, 0.9 Hz, 1 H), 7.32–7.31 (m, 1 H), 7.06–6.95 (m, 1 H), 6.43 (dd, J = 3.0, 2.0 Hz, 1 H), 1.33 (s, 12 H) ppm. 13C NMR (151 MHz, [D6]DMSO): δ = 139.30, 138.39, 123.07, 125.87, 123.78, 118.50, 110.24 (br), 100.92, 83.49 (2 C), 24.67 (4 C) ppm. HRMS: m/z calcd. for C14H16BrNO3 [81BrM + Na]+ 326.0293; found 326.0295. C14H14BrNO3 (324.17): calcd. C 51.87, H 4.92, N 4.32; found C 51.87, H 4.37, N 4.40.

tert-Butyl 6-Bromo-3-formylindole-1-carboxylate (8): The reduction was performed by adding NaBH4 (626 mg, 16.6 mmol) to a stirred solution of 7 (3.6 g, 11.1 mmol) in THF/EtOH (2:1) at 0 °C. The mixture was warmed to room temp. and stirred for 30 min. NH4Cl was added and the mixture was extracted repeatedly with Et3O. After drying the combined organic phases over Na2SO4 and removal of the solvent in vacuo the desired compound (3.6 g, 11.1 mmol, quantitative) was obtained as a white solid. Rf = 0.11 (PE/EA, 9:1); m.p. 90 °C. 1H NMR (600 MHz, [D6]DMSO): δ = 8.20 (s, 1 H), 7.60 (d, J = 8.4 Hz, 1 H), 7.54 (s, 1 H), 7.39 (dd, J = 8.4, 1.8 Hz, 1 H), 5.12 (t, J = 5.5 Hz, 1 H), 4.61 (dd, J = 5.5, 0.9 Hz, 2 H), 1.61 (s, 9 H) ppm. 13C NMR (151 MHz, [D6]DMSO): δ = 148.75, 135.76, 132.85, 128.37, 125.92, 120.26, 114.59, 112.83, 86.25, 27.45 (3 C) ppm. HRMS: m/z calcd. for C9H9BrNO3 [81BrM + H]+ 326.0293; found 326.0295. C9H9BrNO3 (324.17): calcd. C 51.87, H 4.35, N 4.32; found C 51.87, H 4.37, N 4.40.
a silica column and eluted with a mixture of PE/EE (6:1). Removal of solvents gave the desired product (68 %, 3.65 g, 94 mmol) as an off-white solid. Because of the fast decomposition, the next step was performed immediately after drying. Rf = 0.68 (PE/EE, 4:1); m.p. 83 °C (decomp.). 1H NMR (600 MHz, D2O/DMSO): δ = 8.20 (s, 1 H), 7.88 (s, 1 H), 7.64 (d, J = 8.4 Hz, 1 H), 7.47 (dd, J = 8.4, 1.3 Hz, 1 H), 4.90 (s, 2 H), 1.60 (s, 9 H) ppm. 13C NMR (151 MHz, D2O/DMSO): δ = 133.62, 118.28, 118.27, 117.34, 84.75, 74.00, 73.50 ppm. HRMS: m/z calc. for C23H26BrN3O4 [M+H]+ 492.41; found C 56.10, H 6.14, N 8.53.

tert-Butyl 6-Bromo-3-[(2R,5S)-3,6-dimethoxy-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl](methyl)-6-tetramethyl-1,3,2-dioxaborolan-2-yl-indole-1-carboxylate (11): nbLu (3.4 mL, 8.5 mmol in n-hexane) was added slowly to a stirred solution of (3S,3R)-2,5-dimethoxy-3-hydroxy-(5-azido-2H-indole-1-carboxylate (10): nbLi (3.4 mL, 8.5 mmol in n-hexane) was added slowly to a stirred solution of (3S)-3-tert-butyl,2,5-dimethoxy-3-hydroxy-(5-azido-2H-indole-1-carboxylate (1.54 g, 8.5 mmol) in THF (10 mL) at –78 °C. After stirring for further 30 min at ~78 °C, tert-butyl 6-bromo-3-(bromomethyl)-1H-indole-1-carboxylate (3.0 g, 7.71 mmol) in THF (12 mL) was added slowly and the resulting solution was stirred for 1 h at ~78 °C. After quenching with saturated NH4Cl, the aqueous phase was extracted with Et2O and the combined organic phases were washed with water, filtered through silica gel, extracted repeatedly with Et2O and the combined organic phases were washed with water (3 mL). The RCCs were determined by radiosyntheses were carried out as described by Tredwell et al.[22]

**Radiochemistry**

**Preparation of Tetraethylammonium [18F]Fluoride:** No-carrier-added [18F]fluoride was obtained through an [18F]fluoride nuclear reaction by the bombardment of isotopically enriched [18O]water as target with 17 MeV protons at the Jsy cyclotron BC 1710 (INM-5, Forschungszentrum Jülich).[1c] An aliquot of aqueous [18F]fluoride was diluted with methanol (500 μL) and loaded onto a 46 mg Sep-Pak® QMA light cartridge (Waters, Eschborn, Germany). Then, anhydrous methanol (1 mL) followed by air (20 mL) were flushed through the cartridge. Elution of the [18F]fluoride was conducted by washing tetraethylammonium hydrogen carbonate (0.8 mg) in anhydrous methanol (800 μL) slowly through the cartridge into the reaction vial. Afterwards, the cartridge was washed with anhydrous methanol (500 μL). The solvent was evaporated at 80 °C and 500 mbar followed by an evaporation for 3 min at <10 mbar.

**General Procedure for Radiosynthesis of [18F]Fluorindoles:** The radiosyntheses were carried out as described by Tredwell et al.[22] 4a-c (10 mg) or 4d (15 mg), the corresponding pinacol boronate indole derivative (25 μmol) and Cu(OTf)2(py)4 (3.6 mg, 5.6 μmol) were dissolved in DMF/MeCN (10:1, 330 μL) and added on the dried residue of TEA[18F]F. The reaction mixture was heated to 110 °C for 20 min and quenched with water (3 mL). The RCCs were determined by radioTLC (Table 6).

<table>
<thead>
<tr>
<th>Indication</th>
<th>Indole derivative</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18F]4a</td>
<td>4-F-1-tosyl-1H-indole</td>
<td>0.80</td>
</tr>
<tr>
<td>[18F]4b</td>
<td>5-F-1-tosyl-1H-indole</td>
<td>0.54</td>
</tr>
<tr>
<td>[18F]4c</td>
<td>6-F-1-tosyl-1H-indole</td>
<td>0.63</td>
</tr>
<tr>
<td>[18F]4d</td>
<td>7-F-1H-indole</td>
<td>0.76</td>
</tr>
</tbody>
</table>

**General Procedure for Radiosynthesis of 6-[18F]Fluoro-l-tryptophan:** A solution of the precursor (10 mg, 19 μmol) and Cu(OTf)2(py)4 (3.8 mg, 5.6 μmol) in sulfolane (300 μL) with acetonitrile (30 μL) was added to the dried residue of TEA[18F]F. The reaction mixture was heated to 115 °C for 20 min. Then, after a short cooling time, the mixture was diluted with CH2Cl2 (2 mL) and passed through a 2 g silica cartridge into a dry reaction vial. The cartridge was washed with CH2Cl2 (3 mL). After removal of solvent at 500 mbar and 80 °C, 1 mL of 50 % sulfuric acid was added and the reaction mixture was heated to 130 °C for 15 min. The resulting mixture was diluted with water (1 mL), filtered through a 0.2 μm Millex® PTFE membrane filter (Merck, Darmstadt, Germany) and purified by semipreparative HPLC (System C).

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**Keywords:** Radiopharmaceuticals · Radiochemistry · Isotopic labeling · Positron emission tomography · Imaging agents · Amino acids


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