Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 4994–5003
Bioorganic & Medicinal Chemistry

No carrier added synthesis of *O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine via a novel type of chiral enantiomerically pure precursor, Ni^{II} complex of a (*S*)-tyrosine Schiff base

Raisa N. Krasikova,[a,*] Olga F. Kuznetsova,[a] Olga S. Fedorova,[a] Victor I. Maleev,[b] Tatyana F. Saveleva[b] and Yuri N. Belokon[b]

[a]*Institute of Human Brain Russian Academy of Science, Laboratory of Radiochemistry, Acad. Pavlova Str. 9, St. Petersburg 197376, Russia*
[b]*A.N. Nesmeyanov Institute of Organoelement Compounds Russian Academy of Science, B-334, Vavilova Str. 28, Moscow 119991, Russia*

Received 3 October 2007; revised 5 March 2008; accepted 14 March 2008
Available online 17 March 2008

Abstract—*O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) has gained much attention as a promising amino acid radiotracer for tumor imaging with positron emission tomography (PET) due to favorable imaging characteristics and relatively long half-life of ^{18}F (110 min) allowing remote-site application. Here we present a novel type of chiral enantiomerically pure labeling precursor for [^{18}F]FET, based on NiII complex of a Schiff's base of (*S*)-[*N*-2-(*N*′-benzylprolyl)amino]benzophenone (BPB) with alkylated (*S*)-tyrosine, Ni-(*S*)-BPB-(*S*)-Tyr-OCH2CH2X (X = OTs (**3a**), OMs (**3b**) and OTf (**3c**)). A series of compounds **3a**–**c** was synthesized in three steps from commercially available reagents. Non-radioactive FET as a reference was prepared from **3a** in a form of (*S*)-isomer and (*R*,*S*) racemic mixture. Radiosynthesis comprised two steps: (1) n.c.a. nucleophilic fluorination of **3a**–**c** (4.5–5.0 mg) in the presence of either Kryptofix 2.2.2.or tetrabutylammonium carbonate (TBAC) in MeCN at 80 °C for 5 min, followed by (2) removal of protective groups by treating with 0.5 M HCl (120 °C, 5 min). The major advantages of this procedure are retention of enantiomeric purity during the ^{18}F-introduction step and easy simultaneous deprotection of amino and carboxy moieties in **3a**–**c**. Radiochemically pure [^{18}F]FET was isolated by semi-preparative HPLC (C18 μ-Bondapak, Waters) eluent aq 0.01 M CH_3COONH_4, pH 4/C_2H_5OH 90/10 (v/v). Overall synthesis time operated by Anatech RB 86 laboratory robot was 55 min. In a series of compounds **3a**–**c**, tosyl derivative **3a** provided highest radiochemical yield (40–45%, corrected for radioactive decay). Enantiomeric purity was 94–95% and 96–97%, correspondingly, for Kryptofix and TBAC assisted fluorinations. The suggested procedure involved minimal number of synthesis steps and suits perfectly for automation in the modern synthesis modules for PET radiopharmaceuticals. Preliminary biodistribution study in experimental model of turpentine-induced aseptic abscess and Glioma35 rat's tumor (homografts) in Wistar rats has demonstrated the enhanced uptake of radiotracer in the tumor area with minimal accumulation in the inflamed tissues.

1. Introduction

Positron emission tomography (PET), based on the imaging of pharmaceuticals labeled with short-lived positron-emitting radionuclides, is a rapidly growing modality for the diagnosis and management of cancer.[1] At present 2-[^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is the most widely used PET radiotracer for tumor imaging,[1,2] which exploits the abnormal glucose metabolism of malignant cells.[3] Although [^{18}F]FDG has been effective radiotracer for a variety of malignancies,[1,2] it has limited application in brain tumors imaging, because of high glucose uptake in a grey matter. [^{18}F]FDG accumulation in inflamed tissues and granulation cells makes it difficult to differentiate malignant tumors from benign lesions as the main source of false-positive PET findings.[4,5] Labeled amino acids constitute an alternate class of PET tracers for indicating tumor activity by measuring trans-membrane transport rate, which is accelerated in malignant cells.[6–8] They demonstrated a minimal uptake in the normal brain parenchyma and relatively low accumulation in inflamed tissues. Among amino acids

Keywords: Fluorine-18; *O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine; Labeling precursor; Asymmetric synthesis.
* Corresponding author. Tel.: +7 812 234 2359; fax: +7 812 234 3247; e-mail: raisa@ihb.spb.ru

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.03.040

radiotracers, L-[^{11}C-methyl]methionine (L-[^{11}C]MET) remains the most popular one, mainly due to the ease of its synthesis. However the short half-life of carbon-11 ($t_{1/2}$ = 20.4 min) has a potential disadvantage of being used only in PET centers with an expensive in-house cyclotron. Fluorinated compounds labeled with longer-lived fluorine-18 ($t_{1/2}$ = 109.8 min) have practical benefits, they can be transported to centers remote from production/cyclotron site.

Despite a variety of ^{18}F-labeled amino acids ([^{18}F]FAAs) have being suggested as tumor seeking agents for PET,[7,8] their clinical application has been hampered by difficult synthesis. Particularly difficult is nucleophilic synthesis of the [^{18}F]FAAs with label introduced into the aromatic moiety. Such procedures involve several synthetic steps and are difficult to adapt for routine productions.[9–11] Alternatively the introduction of ^{18}F-label into the alkyl chain can be achieved via aliphatic nucleophilic substitution and allows to produce radiotracers in clinically relevant amounts. Of several structurally similar *o*-fluoroalkyl substituted tyrosines, *O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET),[12] *O*-(3-[^{18}F]fluoropropyl)-L-tyrosine,[13] and *O*-(2′-[^{18}F]fluoromethyl)-L-tyrosine,[14] [^{18}F]FET has gained particular attention. An initial study demonstrated that an uptake of [^{18}F]FET in brain tumors is similar to that of L-[^{11}C]MET.[15] [^{18}F]FET is not metabolized and not incorporated into proteins, it is actively transported into tumor cells by specific amino acid transport system L and in part by Na^+-dependent system B^{0+}.[16,17] Further reports have shown high uptake of [^{18}F]FET in cerebral[18–21] and peripheral tumors.[22] In contrast to L-[^{11}C]MET and [^{18}F]FDG, [^{18}F]FET exhibits negligible uptake in inflammatory tissues in animals model.[23,24] In the recent studies in humans the usefulness of [^{18}F]FET as a molecular probe for the differentiation of tumor and inflammation has been confirmed.[25]

Growing interest to clinical application of [^{18}F]FET has stimulated developments of new synthesis strategies that could be suitable for routine use. In the first published synthesis[12] [^{18}F]FET was prepared via alkylation of the di-potassium salt of L-tyrosine with [^{18}F]fluoroethyltosylate. The process involved separation of the ^{18}F-alkylating intermediate from starting tosyloxyethane by semi-preparative HPLC. This laborious procedure with double preparative HPLC steps was not easy adaptable to automation. Later the purification of [^{18}F]fluoroethyltosylate on disposable SPE cartridges was reported,[26] however this method was not ideal for automated productions due to multiplicity of purification steps using various solvents.

The synthesis of [^{18}F]FET was substantially improved by the implementation of direct nucleophilic radiofluorination process on protected alkyl tyrosine derivative, *O*-(2-tosyloxyethyl)-*N*-trityl-L-tyrosine *tert.*-butylester (**1**, Fig. 1).[27] Deprotection of amino and carboxy functions was carried out in solution of trifluoroacetic acid in dichloromethane. Enantiomerically pure [^{18}F]FET was obtained in a radiochemical yield of 60% using remote-controlled synthesis module.[28] However, the necessity to remove the solvent and an aggressive TFA by SPE technique preceding the final HPLC purification step resulted in a relatively long and complex radiochemistry sequence.

In the further studies similar precursor structure with different protecting groups, *O*-(2-tosyloxyethyl)-*N*-*tert.*-butyloxycarbonyl-L-tyrosine benzyl ester (**2**, Fig. 1), was suggested.[29] It should be noted that the change-over of the protecting groups may lead to racemization of amino acid moiety within the course of ^{18}F-fluorination reaction, performing under basic conditions.[27] Unfortunately, the enantiomeric purity of [^{18}F]FET obtained via ^{18}F-nucleophilic fluorination of **2** was not reported.[29]

Based on our experience in asymmetric synthesis of amino acids,[30–33] we pursued a different approach to [^{18}F]FET by elaborating a new leading structure of labeling precursor.[34] The novel type of the precursor arose from Ni^{II} complex of a Schiff's base of (*S*)-[*N*-2-(*N*′-benzylprolyl)amino]benzophenone (BPB) with alkylated (*S*)-tyrosine, Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2X (**3**, Fig. 1); X = OTs (**3a**), OMs (**3b**), OTf (**3c**). The precursor has some advantages which include (a) simple preparation from commercial reagents; (b) stability under reaction basic conditions and retention of desired (*S*)-configura-

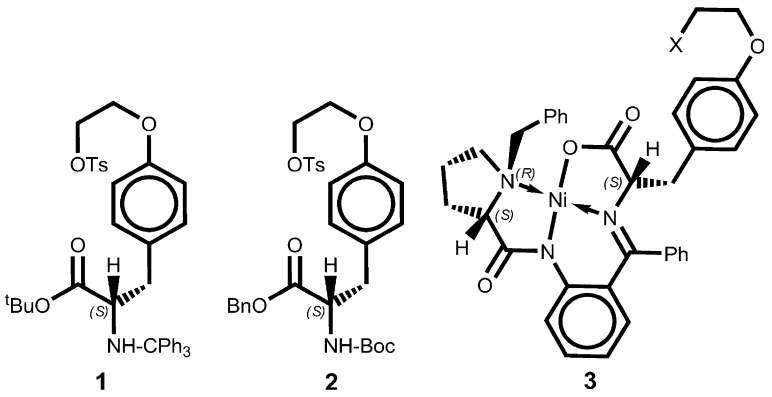


Figure 1. The structure of labeling precursors for [^{18}F]FET, including Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2X (**3**) of the present work; X = OTs (**3a**), OMs (**3b**), OTf (**3c**).

tion of amino acid;[30,31] (c) one stage removal of the chiral auxiliary and simultaneous deprotection of amino and carboxy functions under mild conditions.

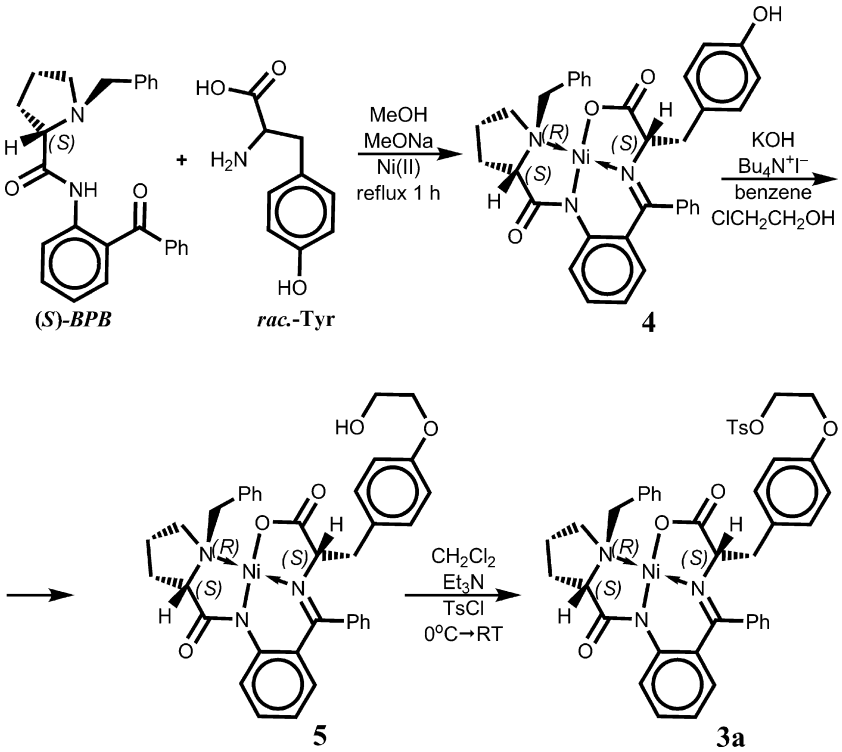
Herein, we describe the preparation of this novel type of precursor **3** and authentic FET. We also report one-pot two-steps radiosynthesis of [^{18}F]FET via direct nucleophilic fluorination of **3** with no carrier added (n.c.a.) [^{18}F]fluoride in the presence of two phase transfer catalysts, tetra butylammonium carbonate (TBAC) and Kryptofix 2.2.2. (K2.2.2.)/K_2CO_3 complex. Biological evaluation of [^{18}F]FET obtained via new synthesis approach has been performed in experimental rat's model of tumor and inflammation.

2. Results and discussion

2.1. Chemistry

The synthesis of a novel chiral diastereoisomerically pure precursor for [^{18}F]FET, a Ni^{II} complex of a Schiff's base of (*S*)-[*N*-2-(*N*′-benzylprolyl)amino]benzophenone (BPB) with (*S*)-tyrosine, in which the OH-group of the tyrosine moiety was converted into OCH_2CH_2OTs derivative Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2OTs (**3a**), is depicted in Scheme 1. At the first synthesis stage the Ni-(*S*)-BPB-(*S*)-Tyr complex (**4**) was easily prepared from a nickel nitrate salt, (*S*)-BPB and racemic tyrosine in the presence of base. Under the reaction basic conditions the epimerization of the unstable intermediate ((*R*,*S*)-diastereoisomer) leads to the formation of a stable (*S*,*S*)-diastereoisomer as the only red colored reaction product. The next two stages include O-alkylation and tosylation of the *O*-hydroxyethyl-group under basic conditions. Using this synthesis route a series of precursors carrying mesylate and triflate leaving groups in an alkyl moiety, **3b** and **3c**, respectively, were prepared (Fig. 1). All intermediate compounds and the final products were characterized by ^{1}H and ^{13}C NMR spectroscopy.

It should be emphasized that it is most beneficial to use in PET studies single-enantiomer tracers. The distribution and kinetics of (*S*)-isomers of amino acids are known to be different from those for (*R*)-isomers, and the (*S*)-configuration is generally preferred for brain tumors imaging.[17] Finally, for precursors **1** and **2** the reactions in the basic solutions may be a cause of possible amino acid moiety racemization either during ^{18}F-introduction or in the process of cold synthesis of the standard.[30] The reason for that is relatively high CH-acidity of their amino acid moieties. Fortunately, in the case of **3** it is no longer racemization but epimerization of the amino acid moiety that would take place under the reaction conditions, leading to an equilibrium ratio of (*R*,*S*)- and (*S*,*S*)-diastereoisomers, namely Ni-(*S*)-BPB-(*S*)-Tyr-[18(19)F]OCH_2CH_2F and Ni-(*S*)-BPB-(*R*)-Tyr-[18(19)F]OCH_2CH_2F. Due to the chiral structure of diastereoisomerically pure complex **3** and greater thermodynamic stability of its (*S*,*S*)-form, relative to (*S*,*R*)-isomer, ^{18}F-fluorination should proceed with the retention of desired (*S*)-configuration of the tyrosine moiety. In other words even if the α-proton of the (*S*)-tyrosine moiety leaves under the effect of a base, it should come back preferentially from the same side and thus the danger of side reaction of racemization becomes insignificant.



Scheme 1. Synthesis of labeling precursor, Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2OTs (**3a**).

The reference compound, (*S*)-FET, was obtained via reaction of **3a** with KF in the presence of tertabutylammoinim fluoride, followed by the decomposition of the resulting complex and purification of product by column chromatography. The compound was characterized by ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy. Racemic FET was prepared according to earlier described procedure.[35]

2.2. Radiochemistry

The two-step reaction sequence for the preparation of [^{18}F]FET using new labeling precursors **3a–c** is outlined in Scheme 2. The three compounds were carefully investigated for their applicability in routine production procedures, including radiofluorination effectiveness, chemical stability, and other related characteristics.

2.2.1. ^{18}F nucleophilic substitution reactions on labeling precursors 3a–c. One-pot radiosynthesis of [^{18}F]FET was accomplished in two steps: (1) n.c.a. nucleophilic fluorination of **3a–c** in the presence of phase transfer catalyst (PTC) followed by (2) removal of protective groups by treating with aqueous solution of HCl. The first stage was carried out under routine conditions for nucleophilic displacement reactions on the aliphatic substrates using n.c.a. [^{18}F]fluoride. Typically, 4.5–5.0 mg of **3a–c** was used, radiofluorinations were conducted in acetonitrile at 80 °C for 5 min. In previous reports[27,29] TBAC was chosen as a PTC for this type of reaction because it showed higher ^{18}F-incorporation rate comparing to the commonly employed Kryptofix 2.2.2/K_2CO_3 complex ([K/K2.2.2]$^+$[^{18}F]$^-$). Because the Kryptofix system is a generally preferred one for the most of n.c.a. fluorinations, we compared the performance of ^{18}F-nucleophilic displacement reactions on the **3a–c** in the presence of both complexing agents. Detailed procedure for the preparation of the activated [K/K2.2.2]$^+$ [^{18}F]$^-$ complex has been described elsewhere.[11] Briefly, [^{18}F]fluoride generated in water cyclotron target via ^{18}O(p,n)^{18}F nuclear reaction was collected on the preconditioned QMA cartridge. It was eluted from the QMA resin by passing 2 mL of stock solution of either TBAC (pH 8) or K2.2.2/K_2CO_3in MeCN/H_2O. The eluate was collected into 5 mL conic vial, the solvents were removed under stream of nitrogen gas and heated until dryness. Two portions of acetonitrile (0.5 mL) were added in succession and each evaporated in turn, to give the corresponding ^{18}F-reactive complex as a slightly brown residue.

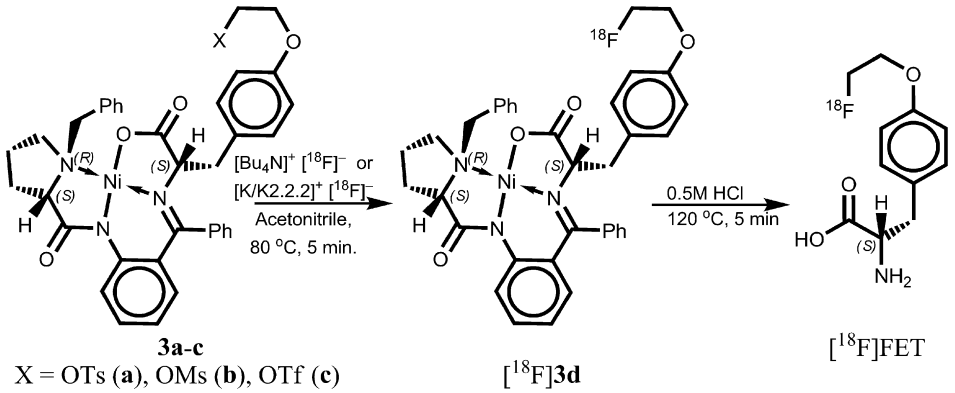
The course of radiofluorination reactions was monitored by radio TLC. Initially, the aliquot of the reaction mixture was quenched with water. A radio TLC chromatogram showed only two peaks of unbounded [^{18}F]fluoride and ^{18}F-fluorinated precursor **3d**. Radiochemical yields of ^{18}F-incorporation (incorporation rates) were evaluated from the TLC data and defined as the ratio of ^{18}F-labeled intermediate **3d** over total fluorine-18 radioactivity (Table 1).

Concerning the influence of the leaving group, precursors **3a** and **3b** gave similar and reasonably good radiofluorination yields of 50–60%, when using TBAC as a phase transfer catalyst (PTC). Although not systematically investigated, increasing the amount of precursors up to 9–10 mg did not further increase the radiochemical yields. Noteworthy, radiochemical yields for **3c** were much lower, and no further attempts were made to improve the yield. As for a choice of PTC, similar incorporation rates were obtained for precursor **3a** for both complexing agents. However, from the current data, the fluorination yields for **3b** were substantially lower in cryptate-assisted reactions (Table 1). Also, we observed reduction in fluorination efficiency of **3b** in TBAC mediated process from 50% to 60% to 15% within 6 months of storage period at 4 °C. Nevertheless, no chemical instability of **3a** was observed within 2.5 years.

2.2.2. Hydrolysis/deprotection of [^{18}F]-3d. In terms of automation of the synthesis procedure, the hydrolysis/deprotection step warrants particular attention. In the previous communication[27] the cleavage of protective groups in the labeled intermediate, *N*-trityl-*O*-(2-

Table 1. ^{18}F-incorporation rates into **3a–c**

Precursor	^{18}F-incorporation rate in %, TBAC	^{18}F-incorporation rate in %, Kryptofix
3a	56.7 ± 9.8 (n = 5)	57.3 ± 8.5 (n = 3)
3b	51.3 ± 9.6 (n = 5)	28.0 ± 14.0 (n = 4)
3c	<10	<5



3a-c
X = OTs (**a**), OMs (**b**), OTf (**c**)

[^{18}F]**3d**

[^{18}F]FET

Scheme 2. Radiosynthesis of [^{18}F]FET via direct nucleophilic fluorination of **3a–c**.

[^{18}F]fluoroethyl)-L-tyrosine *tert.*-butylester, was carried out in a non-aqueous solution consisting of trifluoroacetic acid (TFA) in dichloromethane. In order to remove the corrosive TFA and to separate the radiotracer from the water insoluble triphenylcarbinol, the crude [^{18}F]FET was preliminarily isolated on Silica Sep-Pak. In our view this represents a difficult procedure to perform if automated synthesis has to be accomplished. In our workup a removal of the chiral auxiliary and simultaneous deprotection of amino and carboxy functions of tyrosine was easily achieved under moderate aqueous conditions (0.5 M HCl, 5 min, 120 °C). It should be noted that aqueous acid solution has to be added into the reactive vial which contained acetonitrile used in the previous radiofluorination step. Our studies depicted that the presence of acetonitrile on that stage of the synthesis was a critical factor for an exhaustive cleavage of protective groups. The completeness of decomposition of labeling intermediate [^{18}F]-**3d** and removal of the protective groups was confirmed by radio-TLC analysis of the aliquot of the crude reaction mixture.

2.2.3. The HPLC purification and the formulation steps. Crude product obtained after hydrolysis/deprotection step was partly neutralized and directly injected into semi-preparative HPLC (C18 μ-Bondapak column, Waters) 300 × 7.8 mm, 10 μm; eluent aq 0.01 M CH_3COONH_4, pH 4/C_2H_5OH 90/10 (v/v). An intermediary cartridge purification suggested earlier[27] was eliminated and thus radiochemistry sequence was simplified. HPLC analysis of the product fraction revealed high radiochemical (>99%) and chemical purities of [^{18}F]FET. The enantiomeric purity was analyzed by chiral HPLC (Fig. 2) and (*R,S*)-FET was used as a reference. The enantiomeric purity was in range of 94–95% and 96–97%, correspondingly, for Kryptofix and TBAC assisted fluorinations.

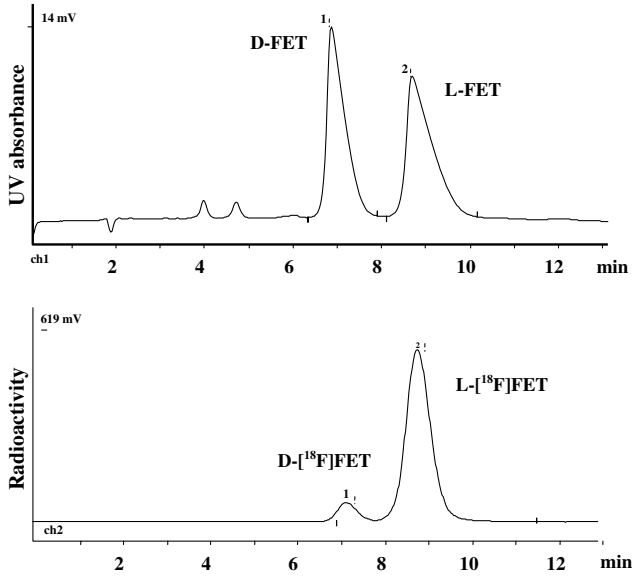


Figure 2. A typical chiral HPLC radiochromatogram of purified [^{18}F]FET obtained by n.c.a. [^{18}F]fluorination of **3a** (down) and UV chromatogram of racemic D,L-tyrosine (up); Crownpack CR (+), eluent $HClO_4$ pH 2/CH_3OH 90/10 (v/v), flow rate 0.8 mL/min; λ 254 nm.

To ensure that the final dose is devoid of any nickel impurities, the nickel content of a few batches of [^{18}F]FET was determined by the inductively coupled plasma mass spectroscopy. The nickel content was negligible, ranging between 0.2 and 0.5 parts per million. The radiochemical yield of [^{18}F]FET using **3a** as an optimal labeling precursor was 40–45% (corrected for radioactive decay). The specific radioactivity of the [^{18}F]FET was >14 GBq/μmol at the end of the synthesis. Synthesis time operated by laboratory robot Anatech RB 86 was 55 min including HPLC purification.

2.3. Preliminary in vivo evaluation in rats

The biodistribution of [^{18}F]FET was investigated in Wistar rats using experimental model of turpentine-induced aseptic abscess and Glioma35 rats tumor (homografts). To compare the radiotracer uptake in tumor and inflamed areas, the corresponding tumor-to-muscle and abscess-to-muscle ratios were calculated (Tables 2 and 3).

As expected from the literature data,[23,24] the uptake of [^{18}F]FET was not significantly different in the inflamed muscle than in the contralateral healthy tissues. The abscess-to-muscle ratios were 1.30, 1.32, 1.39, and 1.33 at 20, 40, 60, and 120 min of post injection, correspondingly. At the same time points the radiotracer uptake in Glioma35 rat's tumor was almost double: the tumor-to-muscle ratios reached the values of 3.17, 2.95, 2.54 and 2.93, correspondingly. The results obtained in our experimental models confirmed that [^{18}F]FET may serve as a suitable radiotracer for the differentiation of tumor and inflammatory tissues. This observation is in accordance with previously reported findings.[23]

3. Conclusions

In conclusion, the nucleophilic substitution of the leaving group in the novel labeling precursor, Ni^{II} complex of a Schiff's base of (*S*)-[*N*-2-(*N*′-benzylprolyl)amino]-benzophenone ((*S*)-BPB) with alkylated (*S*)-tyrosine, Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2X (**3a**–**c**), is a feasible way to a fully automated production of [^{18}F]FET, an important amino acid tracer for tumor diagnosis with PET. Based on the comparison within a series of labeling precursors **3a**–**c**, the tosylate derivative **3a** has appeared to be the best for routine preparation of [^{18}F]FET. According to the current results, TBAC seems to be more suitable phase transfer catalyst for the nucleophilic fluorination of **3a** with n.c.a. [^{18}F]fluoride providing high radiochemical and enantiomeric purity. The simple procedure with minimal intermediate purifications steps, the mild conditions for the removal of protective groups in [^{18}F]-**3d** offer a better opportunity to automate the overall synthesis process. In terms of large scale productions, using Curie amounts of cyclotron-produced [^{18}F]fluoride, the amenability of the syn-

Table 2. Tissue distribution of radioactivity in five Wistar rats bearing Glioma35 rat tumor at 40 min post-injection of [^{18}F]FET, in % ID/g

Blood	Kidney	Heart	Liver	Urinary bladder	Brain	Muscle	Tumor	Tumor-to-muscle ratio
1.24	0.73	0.89	1.03	2.51	0.54	0.75	2.53	3.36
1.05	0.73	0.77	0.85	1.59	0.38	0.62	2.35	3.77
1.20	0.86	0.88	0.82	1.42	0.45	0.84	2.44	2.89
1.16	0.70	0.98	0.71	7.02	0.41	0.74	2.00	2.69
1.12	0.66	0.73	0.56	1.12	0.46	0.61	1.25	2.06

thetic approach to automation becomes increasingly important. Taking into account a high price of both precursor **1** and authentic reference of FET from commercial vendor, the availability of a non-expensive alternative might be another important aspect slashing the costs of single clinical dose of [^{18}F]FET. Preliminary biodistribution study in rats has demonstrated the suitability of [^{18}F]FET as a radiotracer for the differentiation of tumor and inflammatory tissues.

4. Materials and methods

4.1. Chemistry

4.1.1. Materials and general procedures. All NMR spectra were recorded on a Bruker Avance 400 instrument (400.13 MHz for ^{1}H, 100.16 MHz for ^{13}C and 161 MHz for ^{19}F) using $CDCl_3$ as a solvent (unless indicated otherwise). Chemical shifts of peaks are presented in ppm (δ-scale with 0.00 ppm for TMS) with, using residual deuterated solvent signal as an internal standard. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in a thermostated cell at 25 °C. All solvents were distilled prior to use. Melting points were determined on Electrothermal Melting Point Apparatus and are uncorrected. Elemental analysis was carried out in the laboratory of Microanalysis of INEOS RAS using Carlo-Erba 1106. Enantiomeric analysis of FET was performed on a Bischoff HPLC instrument (detector: UV at 254 nm) using the 'Chirobiotic T' column (250× 4.0 mm), eluent MeOH/H_2O (1:1), flow rate 1 mL/min, temperature 25 °C. Retention time: 5.3 min for (*R*)-enantiomer and 5.8 min for (*S*)-enantiomer. For TLC analysis Alugram® SIL G/UV from Macherey-Nagel was used. Chiral auxiliary BPB was available from Acros. (*R*,*S*)-tyrosine, 2-chloroethanol and other reagents were available from Aldrich and were used without additional purification.

4.1.2. Ni-(*S*)-BPB-(*S*)-Tyr (4). Racemic tyrosine (1.8 g, 1×10^{-2} mol), (*S*)-BPB × HCl (0.84 g, 2×10^{-3} mol), $Ni(NO_3)_2 \times 6H_2O$ (1.16 g, 4×10^{-3} mol), and 10 mL of MeOH were placed in a three neck round-bottom flask equipped with a mechanical stirrer, thermometer, and reflux condenser. The mixture was heated up to 45 °C, the thermometer was replaced with a funnel and then 6.5 mL of 4 N MeONa (2.6×10^{-2} mol) was quickly added under stirring. The mixture was refluxed with stirring for 70 min. The reaction was monitored by TLC (SiO_2, $CHCl_3$/Me_2CO, 7:1), following the disappearance of the parent BPB spot on the chromatogram. Then the reaction mixture was neutralized with 1.5 mL of AcOH and diluted with 25 mL of water and the precipitate (the target product Ni-(*S*)-BPB-(*S*)-Tyr admixed with some residual initial tyrosine impurity) was filtered off. The precipitate was treated with $CHCl_3$ (the target product was dissolved) and the residual tyrosine was removed by filtration. The solution was evaporated to give 1.16 g (96%) of the target product Ni-(*S*)-BPB-(*S*)-Tyr. The final complex was used without additional purification. To get high-quality analytical data the portion of the product was additionally purified by gel-filtration chromatography (Sephadex LH-20, elution with C_6H_6/EtOH (3:1)). ^{1}H NMR: 1.65 (m, 1H, Pro), 1.94 (m, 1H, Pro), 2.34 (m, 3H, Pro), 2.72 and 2.92 (AB part of ABX, J_{AB} = 13.7 Hz, J_{AX} = 4.6 Hz, J_{BX} = 2.1 Hz, 2H, –CH_2-Tyr), 3.07 (m, 1H, δ-Pro), 3.33 (m, 1H, α-Tyr), 4.24 (m, 1H, α-Pro), 3.32 and 4.21 (AB, J_{AB} = 12 Hz, 2H, –CH_2-Bn), 6.64 (m, 2H, Ar), 6.83 (d, 1H, Ar), 6.91 and 7.00 (AB, J_{AB} = 7.7 Hz, 4H, Tyr), 7.13 (m, 2H, Ar), 7.27 (m, 3H, Ar), 7.41 (m, 1H, Ar), 7.52 (m, 2H, Ar), 8.05 (d, 2H, Ar), 8.14 (d, 1H, Ar), 8.98 (s, 1H, OH).

^{13}C NMR: 23.13 γ-C [Pro], 30.98 β-C [Pro], 38.54 β-C [Tyr], 57.85 δ-C [Pro], 63.81 CH_2 [Bn], 70.81 α-C [Tyr], 71.83 α-C [Pro], 116.01 *m*-Ar [Tyr], 121.13 3-Ar [2-ABPh], 123.64 5-Ar [2-ABPh], 126.21 *i*-Ar [Tyr], 126.49 1-Ar [2-ABPh], 127.24 4-Ph [Bn], 127.86 6-Ar [2-ABPh], 128.94 3,3′-Ph [Bn], 129.00 4-Ph [2-ABPh], 129.24 2-Ar [Tyr], 129.98 4-Ar [2-ABPh], 131.53 3.3′-Ph [2-ABPh], 131.67 2,2′-Ph [Bn], 132.42 2-Ph [2-ABPh], 133.55 *i*-Ph [Bn], 133.63 2′-Ph [2-ABPh], 134.19 *i*-Ph [2-ABPh], 142.28 2-Ar [2-ABPh], 157.14 4-Ar [Tyr], 171.24 –CONH–, 179.33 >C=N–, 180.79 –COO–.

Table 3. Tissue distribution of radioactivity in five Wistar rats with turpentine-induced aseptic abscess at 40 min post-injection of [^{18}F]FET, in % ID/g

Blood	Kidney	Heart	Liver	Urinary bladder	Brain	Muscle	Abscess	Abscess-to-muscle ratio
1.45	1.58	1.47	1.24	2.84	1.00	1.32	1.75	1.33
1.15	0.89	1.28	0.88	2.30	0.66	1.16	1.41	1.22
1.19	1.13	1.00	1.04	0.19	0.87	0.85	1.17	1.37
1.29	0.85	1.16	1.17	3.94	0.96	0.92	1.52	1.66
1.13	0.64	1.08	1.03	2.50	0.85	0.97	0.98	1.02

Found, %: C 68.18; H 5.24; N 6.71. Calculated for $C_{34}H_{31}N_3NiO_4$, %: C, 67.57; H, 5.17; N, 6.95. Mp 180–182 °C. $[\alpha]_{25}^{D}$ +2154 (*c* 0.031, MeOH).

4.1.3. Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OH (5). Ni-(*S*)-BPB-(*S*)-Tyr (**4**) (1 g, 1.65×10^{-3} mol) was placed in a round-bottom flask, 2-chloroethanol (4 g, 4.96×10^{-2} mol) was added, and the mixture was stirred with a magnetic stirrer until the substance was dissolved. Then benzene (15 mL) and $Bu_4N^+I^-$ (0.6 g, 1.65×10^{-4} mol) were added to the flask, followed by 12 mL of aqueous KOH (50%) solution in portions of 1 mL over a period of 3 h under vigorous stirring. The reaction mixture was additionally stirred for 14 h at room temperature, neutralized with 1 mL of AcOH, and treated with 3 mL of $CHCl_3$. The chloroform extract was separated, washed with water (3× 5 mL), dried over Na_2SO_4 and evaporated in vacuo. The residue was twice treated with 5 mL of acetone and each time evaporated to remove the remaining 2-chloroethanol, 10 mL of acetone was added to the solid residue, and the suspension filtered off and washed with acetone (2× 5 mL) to give 0.754 g (70.5%) of Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OH. The product was additionally purified by gel-filtration chromatography (Sephadex LH-20, mixture C_6H_6/EtOH (3:1)). ^{1}H NMR: 1.66 (m, 1H, Pro), 1.96 (m, 1H, Pro), 2.34 (m, 3H, Pro), 2.78 and 3.01 (AB part of ABX, J_{AB} = 14 Hz, J_{AX} = 5.7 Hz, J_{BX} = 4.3 Hz, 2H, –CH_2-Tyr), 3.09 (m, 1H, δ-Pro), 3.29 (m, 1H, α-Tyr), 3.41 (s, 1H, OH), 4.21 (m, 1H, α-Pro), 3.42 and 4.25 (AB, J_{AB} = 12.5 Hz, 2H, –CH_2-Bn), 3.94 (m, 2H, OCH_2-CH_2OH), 4.07 (m, 2H, OCH_2CH_2OH), 6.64 (d, 2H, Ar), 6.86 (d, 1H, Ar), 6.93 and 7.07 (AB, J_{AB} = 8.4 Hz, 4H, Tyr), 7.13 (m, 2H, Ar), 7.28 (m, 3H, Ar), 7.42 (m, 1H, Ar), 7.51 (m, 2H, Ar), 8.00 (d, 2H, Ar), 8.19 (d, 1H, Ar). ^{13}C NMR: 23.13 γ-C [Pro], 30.66 β-C [Pro], 38.88 β-C [Tyr], 57.35 δ-C [Pro], 61.35 CH_2 [Bn], 63.46 –OCH_2-CH_2OH, 69.33 α-C [Tyr], 70.41 α-C [Pro], 71.61 –OCH_2-CH_2OH, 114.82 *m*-Ar [Tyr], 120.66 3-Ar [2-ABPh], 123.38 5-Ar [2-ABPh], 126.17 1-Ar [2-ABPh], 127.19 4-Ph [Bn], 127.85 6-Ar [2-ABPh], 128.19 *i*-Ar [Tyr], 128.79 3,3′-Ph [Bn], 128.90 2-Ar [Tyr], 129.12 4-Ph [2-ABPh], 129.80 4-Ar [2-ABPh], 131.51 3.3′-Ph [2-ABPh], 131.64 2,2′-Ph [Bn], 132.36 2-Ph [2-ABPh], 133.31 *i*-Ph [Bn], 133.53 2′-Ph [2-ABPh], 134.20 *i*-Ph [2-ABPh], 142.82 2-Ar [2-ABPh], 154.39 4-Ar [Tyr], 171.07 –CONH–, 178.66 >C═N–, 180.33 –COO–.

Found, %: C, 65.37; H, 5.40; N, 5.91. Calculated for $C_{36}H_{35}N_3NiO_5 \times EtOH$, %: C, 65.72; H, 5.95; N, 6.05. Mp 250–252 °C. $[\alpha]_{25}^{D}$ +1206 (*c* 0.03, MeOH).

4.1.4. Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OTs (3a). To the stirred solution of (5) (0.1 g, 1.54×10^{-4} mol) in 2 mL of dry CH_2Cl_2 at 0 °C was added Et_3N (0.07 mL, 4.78×10^{-4} mol) followed by $CH_3C_6H_4SO_2Cl$ (0.09 g, 4.72×10^{-4} mol). After the addition was complete, the cooling was stopped; the reaction mixture was allowed to warm up to an ambient temperature and the mixture was additionally stirred for 20 h. The reaction was monitored by TLC (SiO_2, elution with $CHCl_3$/Me_2CO (3:1)) by the disappearance of the initial Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OH spot. After the reaction was complete, solvent was removed in vacuo and Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OTs was separated by preparative TLC (SiO_2, elution with $CHCl_3$/Me_2CO (7:1)). The complex was additionally purified by gel-filtration chromatography (Sephadex LH-20, elution with C_6H_6/EtOH (3:1)), dried on air. Yield 67% (0.08 g). ^{1}H NMR: 1.65 (m, 1H, Pro), 1.95 (m, 1H, Pro), 2.30 (m, 3H, Pro), 2.42 (s, 3H, $CH_3C_6H_4SO_3$–), 2.77 and 2.99 (AB part of ABX, J_{AB} = 13.9 Hz, J_{AX} = 5.7 Hz, J_{BX} = 4.3 Hz, 2H, –CH_2-Tyr), 3.07 (m, 1H, δ-Pro), 3.29 (m, 1H, α-Tyr), 4.20 (m, 1H, α-Pro), 3.42 and 4.24 (AB, J_{AB} = 12.5 Hz, 2H, –CH_2-Bn), 4.12 (m, 2H, OCH_2CH_2OTs), 4.34 (m, 2H, OCH_2CH_2OTs), 6.64 (d, 2H, Ar), 6.84 (d, 1H, Ar), 6.80 and 7.04 (AB, J_{AB} = 9 Hz, 4H, Tyr), 7.12 (m, 2H, Ar), 7.27 (m, 3H, Ar), 7.41 (m, 1H, Ar), 7.51 (m, 2H, Ar), 7.33 and 7.79 (AB, J_{AB} = 8.2 Hz, 4H, Ts), 8.00 (d, 2H, Ar), 8.19 (d, 1H, Ar).

^{13}C NMR: 21.70 CH_3-[Ts], 23.10 γ-C [Pro], 30.70 β-C [Pro], 38.92 β-C [Tyr], 57.35 δ-C [Pro], 63.42 CH_2 [Bn], 65.58 –OCH_2CH_2OTs, 68.15 α-C [Tyr], 70.35 α-C [Pro], 71.56 –OCH_2CH_2OH, 114.75 *m*-Ar [Tyr], 120.59 3-Ar [2-ABPh], 123.37 5-Ar [2-ABPh], 126.11 1-Ar [2-ABPh], 127.19 4-Ph [Bn], 127.82 6-Ar [2-ABPh], 128.01 2,2′-Ar [Ts], 128.58 *i*-Ar [Tyr], 128.78 3,3′-Ph [Bn], 2-Ar [Tyr], 128.90 4-Ph [ABPh], 129.12 4-Ph [2-ABPh], 129.80 4-Ar [2-ABPh], 129.79 4-Ar [Ts], 129.92 3,3′-Ar [Ts], 131.52 3.3′-Ph [2-ABPh], 131.61 2,2′-Ph [Bn], 132.34 2-Ph [2-ABPh], 133.33 *i*-Ph [Bn], 133.51 2′-Ph [2-ABPh], 134.20 *i*-Ph [2-ABPh], 142.85 2-Ar [2-ABPh], 145.03 *i*-Ar [Ts],157.72 4-Ar [Tyr], 171.05 –CONH–, 178.55 >C═N–, 180.34 –COO–.

Found, %: C, 62.54; H, 4.90; N, 4.90. Calculated for $C_{43}H_{41}N_3NiO_7S \times 1.25H_2O$, %: C, 62.6; H, 5.31; N, 5.09. Mp 220–223 °C. $[\alpha]_{25}^{D}$ +1922 (*c* 0.03, MeOH).

4.1.5. Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OMs (3b). To the stirred solution of (5) (0.087 g, 1.34×10^{-4} mol) in 2 mL of dry CH_2Cl_2 at 0 °C was added Et_3N (0.02 mL, 1.42×10^{-4} mol) followed by $MeSO_2Cl$ (0.01 mL, 1.34×10^{-4} mol). After the addition was complete, the cooling was stopped and the reaction mixture was stirred for another 18 h at ambient temperature. Then additionally Et_3N (0.02 mL, 1.42×10^{-4} mol) and $MeSO_2Cl$ (0.01 mL, 1.34×10^{-4} mol) were added sequentially, and the mixture was stirred extra 6 h at room temperature. The reaction was monitored by TLC (SiO_2, $CHCl_3$:Me_2-CO (3:1)) by the disappearance of the spot of the initial Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OH complex. After completion of the reaction, the solvent was removed in vacuo and Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OMs was separated by preparative TLC (SiO_2, elution with $CHCl_3$/Me_2CO (3:1)). The complex was additionally purified by gel-filtration chromatography (Sephadex LH-20, elution with C_6H_6/EtOH (3:1)), dried on air. Yield, 68% (0.066 g). ^{1}H NMR: 1.71 (m, 1H, Pro), 1.83 (m, 1H, Pro), 1.96 (m, 1H, Pro), 2.34 (m, 2H, Pro), 2.42 (m, 1H, Pro), 2.81 and 3.00 (AB part of ABX, J_{AB} = 14 Hz, J_{AX} = 6 Hz, J_{BX} = 4.2 Hz, 2H, –CH_2-Tyr), 3.07 (s, 3H, CH_3SO_3–), 3.11 (m, 1H, δ-Pro), 3.31 (m, 1H, α-Tyr), 4.20 (m, 1H, α-Pro), 3.44 and 4.26 (AB, J_{AB} = 12.5 Hz, 2H, –CH_2-

Bn), 4.22 (m, 2H, OCH_2CH_2OMs), 4.55 (m, 2H, OCH_2-CH_2OMs), 6.67 (d, 2H, Ar), 6.87 (d, 1H, Ar), 6.92 and 7.08 (AB, J_{AB} = 8.4 Hz, 4H, Tyr), 7.14 (m, 2H, Ar), 7.28 (m, 3H, Ar), 7.43 (m, 1H, Ar), 7.53 (m, 2H, Ar), 8.01 (d, 2H, Ar), 8.19 (d, 1H, Ar).

^{13}C NMR: 23.14 γ-C [Pro], 30.67 β-C [Pro], 37.78 $MeSO_2$–, 38.99 β-C [Tyr], 57.25 δ-C [Pro], 63.42 CH_2 [Bn], 65.92 –OCH_2CH_2OMs, 68.03 α-C [Tyr], 70.33 α-C [Pro], 71.54 –OCH_2CH_2OMs, 114.78 *m*-Ar [Tyr], 120.66 3-Ar [2-ABPh], 123.38 5-Ar [2-ABPh], 126.15 1-Ar [2-ABPh], 127.22 4-Ph [Bn], 127.82 6-Ar [2-ABPh], 128.34 *i*-Ar [Tyr], 128.82 2-Ar [Tyr], 3,3′-Ph [Bn], 129.16 4-Ph [2-ABPh], 129.84 4-Ar [2-ABPh], 131.52 3.3′-Ph [2-ABPh], 131.73 2,2′-Ph [Bn], 132.40 2-Ph [2-ABPh], 133.27 *i*-Ph [Bn], 133.53 2′-Ph [2-ABPh], 134.18 *i*-Ph [2-ABPh], 142.80 2-Ar [2-ABPh], 157.64 4-Ar [Tyr], 171.10 –CONH–, 178.56 > C═N–, 180.29 –COO–.

Found, %: C, 60.45; H, 4.970; N, 5.38. Calculated for $C_{37}H_{37}N_3NiO_7S \times 0.1CHCl_3$, %: C, 60.35; H, 5.06; N, 5.69. Mp 125–127 °C. $[\alpha]_{25}^{D}$ +1975 (*c* 0.032, MeOH).

4.1.6. Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OTf (3c). To the stirred solution of (**5**) (0.2 g, 3.08×10^{-4} mol) in 3.5 mL of dry CH_2Cl_2 at 0 °C was added Et_3N (0.14 mL, 9.59×10^{-4} mol) followed by CF_3SO_2Cl (0.15 mL, 1.41×10^{-3} mol). After the addition was complete, the cooling was stopped; the reaction mixture was allowed to warm up to an ambient temperature and the mixture was stirred for another 20 h. The reaction was monitored by TLC (SiO_2, eluent $CHCl_3$/Me_2CO (7:1)) following the disappearance of the initial Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OH. After the reaction was complete, the solvent was removed in vacuo and Ni-(*S*)-BPB-(*S*)-Tyr-CH_2CH_2OTf was separated by preparative TLC (SiO_2, elution with $CHCl_3$/Me_2CO (7:1)). The complex was additionally purified by gel-filtration chromatography (Sephadex LH-20, elution with C_6H_6/EtOH (3:1)), dried on air. Yield 33% (0.08 g). ^{1}H NMR: 1.67 (m, 1H, Pro), 1.96 (m, 1H, Pro), 2.33 (m, 3H, Pro), 2.77 and 3.00 (AB part of ABX, J_{AB} = 13.8 Hz, J_{AX} = 5.7 Hz, J_{BX} = 4.4 Hz, 2H, –CH_2-Tyr), 3.10 (m, 1H, δ-Pro), 3.29 (m, 1H, α-Tyr), 3.43 and 4.25 (AB, J_{AB} = 12.5 Hz, 2H, –CH_2-Bn), 3.80 (m, 2H, OCH_2CH_2OTf), 4.21 (m, 3H, OCH_2CH_2OTs and α-Pro), 6.64 (d, 2H, Ar), 6.87 (d, 1H, Ar), 6.93 and 7.09 (AB, J_{AB} = 8.6 Hz, 4H, Tyr), 7.13 (m, 2H, Ar), 7.28 (m, 3H, Ar), 7.42 (m, 1H, Ar), 7.51 (m, 2H, Ar), 8.00 (d, 2H, Ar), 8.20 (d, 1H, Ar).

^{13}C NMR: 23.12 γ-C [Pro], 30.647 β-C [Pro], 38.85 β-C [Tyr], 42.08 –OCH_2CH_2OTf, 57.32 δ-C [Pro], 63.39 CH_2 [Bn], 68.13 α-C [Tyr], 70.34 α-C [Pro], 71.57 –OCH_2-CH_2OMs, 72.52 CF_3SO_2-, 114.91 *m*-Ar [Tyr], 120.58 3-Ar [2-ABPh], 123.38 5-Ar [2-ABPh], 126.12 1-Ar [2-ABPh], 127.19 4-Ph [Bn], 127.84 6-Ar [2-ABPh], 128.57 *i*-Ar [Tyr], 128.78 2-Ar [Tyr], 3,3′-Ph [Bn], 129.11 4-Ph [2-ABPh], 129.79 4-Ar [2-ABPh], 131.52 3.3′-Ph [2-ABPh], 131.69 2,2′-Ph [Bn], 132.35 2-Ph [2-ABPh], 133.29 *i*-Ph [Bn], 133.50 2′-Ph [2-ABPh], 134.25 *i*-Ph [2-ABPh], 142.89 2-Ar [2-ABPh], 157.90 4-Ar [Tyr], 171.06 –CONH–, 178.54 >C═N–, 180.33 –COO–. ^{19}F NMR spectra: −72.92, s, CF_3SO_2–.

Found, %: C, 63.115; H, 4.98; N, 5.81. Calculated for $C_{37}H_{34}F_3N_3NiO_7S$, %: C, 66.94; H, 4.39; N, 5.38. Mp 193–195 °C with decomposition. $[\alpha]_{25}^{D}$ +2226 (*c* 0.03, MeOH).

4.1.7. *O*-(2′-[^{19}F]fluoroethyl)-(*S*)-tyrosine (FET): (*S*)-FET and (*R*,*S*)-FET. To the solution of Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2OTs, (1.29 g 1.57×10^{-3} mol) in 15 mL of dry MeCN were added dry KF, 2.33 g (4.02×10^{-2} mol) and 1.6 mL of Bu_4NF (1 N solution in THF). The reaction mixture was stirred for 10 h at 80 °C, then the mixture was cooled to ambient temperature. The inorganic salts formed were filtered and the filtrate was washed with dry MeCN. The combined filtrates were evaporated and the target product Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2F was separated by column chromatography on SiO_2 column (300×35 mm, $CHCl_3$/Me_2CO,7:1). The yield was 0.53 g (8.14×10^{-4} mol), 52%. ^{1}H NMR: 1.68 (m, 1H, Pro), 1.96 (m, 1H, Pro), 2.35 (m, 3H, Pro), 2.78 and 3.02 (AB part of ABX, J_{AB} = 14 Hz, J_{AX} = 5.6 Hz, J_{BX} = 4.2 Hz, 2H, –CH_2-Tyr), 3.11 (m, 1H, δ-Pro), 3.31 (m, 1H, α-Pro), 4.16 (dd, 1H, α-Tyr), 3.43 and 4.26 (AB, J_{AB} = 12.6 Hz, 2H, –CH_2-Bn), 4.19 and (m, 2H, OCH_2CH_2F, J_{H-F} = 20.1 Hz), 4.74 (m, 2H, OCH_2CH_2F, J_{H-F} = 47.1 Hz), 6.65 (d, 2H, Ar), 6.86 (d, 1H, Ar), 6.95 and 7.10 (AB, J_{AB} = 9 Hz, 4H, Tyr), 7.14 (m, 2H, Ar), 7.29 (m, 3H, Ar), 7.42 (m, 1H, Ar), 7.51 (m, 2H, Ar), 8.00 (d, 2H, Ar), 8.19 (d, 1H, Ar). ^{19}F NMR: −226, (–CH_2F). Mp 130–132 °C. $[\alpha]_{25}^{D}$ +2300 (*c* 0.038, MeOH). Found, %: C, 66.49; H, 5.31; N, 6.38. Calculated for $C_{36}H_{34}FN_3NiO_4$, %: C, 66.48; H, 5.27; N, 6.46.

Enantiomerically pure (*S*)-FET (ee > 99.8%) was prepared after the decomposition of the complex Ni-(*S*)-BPB-(*S*)-Tyr-OCH_2CH_2F under standard procedure conditions.[30] ^{1}H NMR: 3.15 and 3.26 (AB part of ABX, J_{AB} = 15 Hz, J_{AX} = 7.56 Hz, J_{BX} = 5.4 Hz, 2H, –CH_2Ar), 4.26 (m, 2H, –OCH_2CH_2F), 4.34 (m, 1H, α-H), 4.73 (m, 2H, –OCH_2CH_2F J_{H-F} = 47 Hz), 6.99 and 7.25 (AB, J_{AB} = 8.7 Hz, 4H, Ar).

^{13}C NMR: 34.75 (–CH_2Ar), 54.25 (α-C), 67.57 (–OCH_2CH_2F, J_{C-F} = 18 Hz), 82.71 (–OCH_2CH_2F, J_{C-F} = 164 Hz), 115.35 (C4, Ar), 126.99 (C1, Ar), 130.75 (C2, Ar), 157.37 (C4, Ar), 171.59 (COOH). ^{19}F NMR: −223 (–CH_2F). $[\alpha]_{25}^{D}$ −3.94 (*c* 0.5, 6 N HCl).

Found, %: C, 58.19; H, 6.19; N, 6.11. Calculated for $C_{11}H_{14}FNO_3$, %: C, 58.14; H, 6.21; N, 6.16.

To prepare the racemic FET, part of the (*S*)-FET was racemized, using earlier described procedure.[35]

4.2. Radiochemistry

4.2.1. Materials and reagents. The commercially available reagents and solvents were used without further purification unless stated otherwise. ^{18}O-enriched water (97%) was purchased from the Global Scientific Technologies, Sosnovy Bor, Russia; anhydrous potassium carbonate, 4,7,13,16,20,24-hexaoxa-1,10-diazabicyclo-[8.8.8]hexacosane (Kryptofix, K2.2.2) were obtained from Aldrich; anhydrous acetonitrile (DNA-quality) was bought from

Merck; Sep-Pak Light Waters Accell™ Plus QMA cartridges were purchased from Waters. *O*-(2-fluoroethyl)-L-tyrosine (trifluoroacetate salt) was delivered by ABX, Germany. Tetrabutylammonium hydrogen carbonate was obtained by bubbling carbon dioxide through the solution of commercially available 20% aqueous tetrabutylammonium hydroxide (Merck) to the pH 8; the stock solution obtained was kept in a sealed vial at 4 °C.

4.2.2. HPLC, TLC and other equipment and conditions. The HPLC purification system consisted of a Gilson Pump 305; an automatic sample injector (type VICI Valco) equipped with 2 mL loop; Gilson 116 UV absorbance detector in series with a Beckman 170 radiodetector to monitor the effluent radioactivity; semi-preparative C18 μ-Bondapak column, Waters, 300 × 7.8 mm; eluent aq 0.01 M CH_3COONH_4, pH 4/C_2H_5OH 90/10 (v/v); flow rate 4.0 mL/min; λ 254 nm. Analytical HPLC system included a Gilson Pump 305, a Rheodyne type injector fitted with 20 μL loop, Gilson 116 UV absorbance detector in series with a Beckman 170 radiodetector; Chrom & Spec data acquisition software, Ampersand, Russia; system A: column Nucleosil C18, Supelco, 250 × 4.6 mm, 5 μm; eluent aq 0.05 M CH_3COONH_4, pH 3/C_2H_5OH 90/10 (v/v), flow 1.0 mL/min, λ 254 nm; system B: column Crownpak CR (+), Daicel Chemical Industries, Ltd, 150 × 4 mm; eluent $HClO_4$ pH 2/CH_3OH 90/10 (v/v), flow rate 0.8 mL/min; λ 254 nm. TLC analyses were run on precoated plates of silica gel F_{254}, Sorbfil, Lenchrom, Russia; radioactivity spots were detected using an automatic radioTLC scanner, raytest GmBH, Germany. Authentic FET was visualized by spraying with 0.2% ninhydrin solution and heating. The nickel content of a few batches of the final [^{18}F]FET was determined by the inductively coupled plasma mass spectroscopy (ICP-MS PQ-3; Plasma Quad, VG, UK).

4.2.3. Radioisotope production. N.c.a. [^{18}F]fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by the proton bombardment (17 MeV, 15 μA) of [^{18}O]H_2O (97% isotopic enrichment) in a small volume low pressure target of the MC-17 cyclotron, Scanditronix, Sweden. Radioactivity was measured with a dose calibrator PTW Curiementor-2, Germany. The entire radiochemical synthesis was operated with the use of computer-assisted Anatech RB 86 laboratory robot (Anatech, Uppsala, Sweden) and associated ARC software. The system has been described elsewhere.[36]

4.2.4. Preparation of the $[Bu_4N]^+[^{18}F]^-$ and [K/K2.2.2]$^+[^{18}F]^-$ Fluorinating agents. [^{18}F]fluoride generated in water cyclotron target was collected on the Sep-Pak Light Waters Accell™ Plus QMA cartridge pre-conditioned by passing 10 mL of 0.5 M K_2CO_3 and 15 mL of water. Cartridge was flushed with helium for at least 5 min to maximally remove the last traces of [^{18}O]water. The [^{18}F]fluoride was than eluted from the QMA resin by passing 2 mL of stock solution A or B of the following composition: A: 2 mL MeCN, 0.075 mL of 20% aq solution of TBAC, pH 8; B: 9.5 ± 0.4 mg (0.025 mmol) of K2.2.2, 1.7 ± 0.2 mg (0.012 mmol) of K_2CO_3, 2 mL of MeCN/H_2O (96/4 v/v). The resulting solution was collected into 5 mL conic vial, the solvents were gently removed under a stream of nitrogen gas at 130 °C until dryness. Two portions of acetonitrile (0.5 mL) were added in succession and each was evaporated in turn, to give the corresponding ^{18}F-reactive complex as a slightly brown residue.

4.2.5. General ^{18}F-labeling procedure. The appropriate precursor **3a**–**c** (4.5–5.0 mg) was dissolved in 0.5 mL of anhydrous acetonitrile. This solution was added through the Teflon rubber septa into the vial containing the dry [^{18}F]fluorinating agent. Reaction mixture was heated at 80 °C for 5 min without stirring. The aliquot of the reaction mixture was taken by syringe, quenched with water, and then analyzed by radioTLC (SiO_2 plate, eluent: EtOAc/$CHCl_3$/CH_3COOH (4:1:1)); (R_f [^{18}F]fluoride: 0.1; R_f [^{18}F]4: 0.53). Immediately, 0.5 mL of aqueous 0.5 M HCl was added following heating at 120 °C for 5 min. An aliquot of the crude reaction mixture was analyzed by radioTLC (SiO_2 plate, eluent: *n*-BuOH/CH_3COOH/H_2O/EtOH (4:1:1.6:0.5)); (R_f [^{18}F]fluoride: 0.1; R_f [^{18}F]FET: 0.63).

4.2.6. Semi-preparative HPLC purification. Crude reaction mixture was cooled down, partly neutralized until pH 3, and diluted three times with HPLC eluent until total volume of 2 mL. The resultant solution was introduced onto HPLC loop with the help of a motor-driven 5 mL disposable plastic syringe. The radioactive fraction containing [^{18}F]FET, eluted between 8.0 and 9.5 min, was collected into the sterile 10 mL sealed vial. Finally, the solution was passed through a sterile filter (0.22 μm pore size, Millipore) and mixed with PBS buffer, to adjust pH, isotonic formula, and ethanol concentration in injectable solution (Table 3).

4.2.7. Quality control. The radiochemical and chemical purities of the final preparation were determined by reversed phase HPLC (system A, Section 4.2.2). The identity of [^{18}F]FET was confirmed under the same conditions by co-injection of the authentic sample available from commercial vendor (ABX, Germany) or synthesized within the present study. t_R FET: 4.54 min; t_R [^{18}F]FET: 4.99 min (radiodetector is situated after UV detector). Enantiomeric purity of [^{18}F]FET was determined by chiral HPLC (system B, Section 4.2.2) using optically pure (*S*)-FET and a racemic mixture of the (*R*,*S*)-isomers, as the reference standards. t_R (*R*)-[^{18}F]FET: 7.21 min; t_R (*S*)-[^{18}F]FET: 8.85 min. Specific radioactivity was determined by HPLC (system A), using reference (*S*)-FET of known concentration.

4.3. Preliminary in vivo evaluation in rats

The biodistribution study was carried out in Wistar rats (males, weighing 150–200 g) bearing Glioma35-derived rats tumors (homografts) and turpentine-induced inflammatory foci (aseptic abscess). For the induction of the tumor we used previously reported procedure.[11] Briefly, 10% suspension of Glioma35 tumor cells in 5% dimethylsulfoxide solution in saline, stored in liquid nitrogen, was thawed quickly at 37 °C and injected subcutaneously into the experimental animal (0.5 mL per

rat). When the tumor size reached the weight of about 50 g, the rats were killed by cervical dislocation, tumor tissues were taken, pulverized in saline (1:10 w/v) by passing through the sieve with 1 mm pore size and pumping through the injection needle with 1 mm inner diameter. This suspension (0.5 mL per rat) was injected subcutaneously or into the muscle of the right hind limb of rats. The tumors were allowed to grow for 14 days. To induce aseptic inflammation, 10 μL of turpentine oil was inoculated subcutaneously into the muscle of the right hind limb for 24 h before the experiment.

The rats were injected with 150–180 μCi (4.0–5.0 MBq) of [^{18}F]FET in 0.3 mL of the isotonic solution via jugular vein. The animals were sacrificed at different time points; organs and tissues of interest were dissected, isolated, wiped of excess blood, weighed, and counted in a gamma counter. The biodistribution of radioactivity was accessed in 20, 40, 60, and 120 min after injection (five rats for each time point) and the percentage of injected dose per gram (% ID/g) was calculated. The tumor-to-muscle or abscess-to-muscle ratios were then calculated using right hind limb as a contralateral region. The animal distributions experiments were performed in the laboratory of the Institute of Experimental Medicine RAMS, St. Petersburg, under Institutional Animal Care and National regulations governing the safe and humane use of laboratory animals in research.

Acknowledgments

The authors thank the cyclotron engineers Mr. Alexandr Demianov and Mr. Vladimir Obolencev for performing the irradiations of the fluorine-18 target. This work was supported by Grant 2780 from the ISTC and a Grant 06-0308089-ofi from Russian Foundation of Basic Researches.

References and notes

1. Chen, W. *J. Nucl. Med.* **2007**, *48*, 1468.
2. Reske, S. N.; Kotzerke, J. *Eur. J. Nucl. Med.* **2001**, *28*, 1707.
3. Gallagher, B. M.; Fowler, J. S.; Gutterson, N. I.; MacGregor, R. R.; Wan, C. N.; Wolf, A. P. *J. Nucl. Med.* **1978**, *19*, 1154.
4. Cook, G. J. R.; Wegner, E. A.; Foegelman, I. *Semin. Nucl. Med.* **2004**, *34*, 122.
5. Strauss, L. G. *Eur. J. Nucl. Med.* **1996**, *23*, 1409.
6. Jager, P. L.; Vaalburg, W.; Pruim, J.; de Vries, E. G.; Langen, K. J.; Piers, D. A. *J. Nucl. Med.* **2001**, *42*, 432.
7. Laverman, P.; Boerman, O. C.; Corstens, F. H. M.; Oyen, J. G. W. *Eur. J. Nucl. Med.* **2002**, *29*, 681.
8. Couturier, O.; Luxen, A.; Chatal, J-F.; Vuillez, J-P.; Rigo, P.; Hustinx, R. *Eur. J. Nucl. Mol. Imaging* **2004**, *31*, 1182.
9. Lemaire, C.; Damhaut, P.; Plenevaux, A.; Comar, D. *J. Nucl. Med.* **1994**, *35*, 1996.
10. Lemaire, C.; Gillet, S.; Guillouet, S.; Plenevaux, A.; Aerts, J.; Luxen, A. *Eur. J. Org. Chem.* **2004**, 2899.
11. Krasikova, R. N.; Zaitsev, V. V.; Ametamey, S. M.; Kuznetsova, O. F.; Fedorova, O. S.; Mosevich, I. K.; Belokon, Yu. N.; Vyskočil, Š.; Shatik, S. V.; Nader, M.; Schubiger, P. A. *Nucl. Med. Biol.* **2004**, *31*, 597.
12. Wester, H.; Herz, M.; Weber, W.; Heiss, P.; Senekovitsch-Schmidtke, R.; Schwaiger, M.; Stocklin, G. *J. Nucl. Med.* **1999**, *40*, 205.
13. Tang, G. H.; Wang, M. F.; Tang, X. L.; Luo, L.; Gan, M. Q. *Nucl. Med. Biol.* **2003**, *30*, 733.
14. Iwata, R.; Furumoto, S.; Pascali, C.; Bogni, A.; Ishiwata, K. *J. Label. Compd. Radiopharm.* **2003**, *46*, 555.
15. Weber, W. A.; Wester, H. J.; Grosu, A. L.; Herz, M.; Dzewas, B.; Feldmann, H. J.; Molls, M.; Stocklin, G.; Schwaiger, M. *Eur. J. Nucl. Med.* **2000**, *27*, 542.
16. Heiss, P.; Mayer, S.; Herz, M.; Wester, H. J.; Schwaiger, M.; Senekovitsch-Schmidtke, R. *J. Nucl. Med.* **1999**, *40*, 1367.
17. Langen, K. J.; Hamacher, K.; Weckesser, M.; Floeth, F.; Stoffels, G.; Bauer, D.; Coenen, H. H.; Pauleit, D. *Nucl. Med. Biol.* **2006**, *33*, 287.
18. Popperl, G.; Gotz, C.; Rachinger, W.; Gildehaus, F. J.; Tonn, J. C.; Tatsch, K. *Eur. J. Nucl. Med. Mol. Imaging* **2004**, *31*, 1464.
19. Weckesser, M.; Langen, K. J.; Rickert, C. H.; Kloska, S.; Straeter, R.; Hamacher, K.; Kurlemann, G.; Wassmann, H.; Coenen, H. H.; Schober, O. *Eur. J. Nucl. Med. Mol. Imaging* **2005**, *32*, 422.
20. Rachinger, W.; Goetz, C.; Popperl, G.; Gildehaus, F. J.; Kreth, F. W.; Holtmannspotter, M.; Herms, J.; Koch, W.; Tatsch, K.; Tonn, J. C. *Neurosurgery* **2005**, *57*, 505.
21. Floeth, F. W.; Pauleit, D.; Sabel, M.; Stoffels, G.; Reifenberger, G.; Riemenschneider, M. J.; Jansen, P.; Coenen, H. H.; Steiger, H. J.; Langen, K. J. *J. Nucl. Med.* **2007**, *48*, 519.
22. Pauleit, D.; Stoffels, G.; Schaden, W.; Hamacher, K.; Bauer, D.; Tellmann, L.; Herzog, H.; Broer, S.; Coenen, H. H.; Langen, K. J. *J. Nucl. Med.* **2005**, *46*, 411.
23. Rau, F. C.; Weber, W. A.; Wester, H. J.; Herz, M.; Becker, I.; Kruger, A.; Schwaiger, M.; Senekowitsch-Schmidtke, R. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 1039.
24. Kaim, A. H.; Weber, B.; Kurrer, M. O.; Westera, G.; Schweitzer, A.; Gottschalk, J.; von Schulthess, G. K.; Buck, A. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 648.
25. Pauleit, D.; Zimmermann, A.; Stoffels, G.; Bauer, D.; Risse, J.; Fluss, M. O.; Hamacher, K.; Coenen, H. H.; Langen, K. J. *J. Nucl. Med.* **2006**, *47*, 256.
26. Tsukada, H.; Sato, K.; Fukumoto, D.; Kakiuchi, T. *Eur. J. Nucl. Med. Mol. Imaging* **2006**, *33*, 1017.
27. Hamacher, K.; Coenen, H. H. *Appl. Radiat. Isot.* **2002**, *57*, 853.
28. Hamacher, K.; Blessing, G.; Nebeling, B. *App. Radiat. Isot.* **1990**, *41*, 49.
29. Wang, H.-E.; Wu, S.-Y.; Chang, C.-W.; Liu, R.-S.; Hwang, L.-C.; Lee, T.-W.; Chen, J.-C.; Hwang, J.-J. *Nucl. Med. Biol.* **2005**, *32*, 367.
30. Belokon, Yu. N. *Pure Appl. Chem.* **1992**, *64*, 1917.
31. Belokon, Yu. N.; Tararov, V. I.; Maleev, V. I.; Saveleva, T. F.; Ryzhov, M. G. *Tetrahedron: Asymm.* **1998**, 4249.
32. Krasikova, R. N.; Fedorova, O. S.; Mosevich, I. K.; Kuznetsova, O. F.; Korsakov, M. V.; Ametamey, S. M.; Schubiger, P. A. *J. Label. Compd. Radiopharm.* **1999**, *42*(1), S102.
33. Krasikova, R. N.; Kuznetsova, O. F.; Fedorova, O. S.; Mosevich, I. K.; Maleev, V. I.; Belokon, Yu. N.; Saveleva, T. F.; Sagyan, A. S.; Dadayan, S. A.; Petrosjan, A. A. *Radiochemistry* **2007**, *49*, 512.
34. Kuznetsova, O. F.; Krasikova, R. N.; Fedorova, O. S.; Maleev, V. I.; Saveleva, T. F.; Pripadchev, D. A.; Babayan, E. P.; Belokon, Yu. N. *Quart. J. Nucl. Med. Mol. Imag.*, **2006**, *50*, 42.
35. Yamada, S.; Hongo, C.; Yoshioka, R.; Chibata, I. *J. Org. Chem.* **1983**, *48*, 843.
36. Krasikova, R. N. *Radiochemistry* **1998**, *40*(4), 364.