An approach to the asymmetric synthesis of ^{18}F-labeled analog of L-*threo*-3,4-dihydroxyphenylserine (6-L-*threo*-[^{18}F]FDOPS) — a new radiotracer for visualization of norepinephrine transporters by positron emission tomography*

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An asymmetric synthesis method has been suggested as a feasible approach for the preparation of fluorine-18-labeled ($T_{1/2}$ 110 min) analog of L-*threo*-3,4-dihydroxyphenylserine, *i.e.*, 6-L-*threo*-[^{18}F]FDOPS ((2*S*,3*R*)-2-amino-3-(2-[^{18}F]fluoro-4,5-dihydroxyphenyl)-3-hydroxypropionic acid), a new radiotracer for the evluation of norepinephrine transporters by positron emission tomography (PET). The approach is based on the condensation reaction of 2-[^{18}F]fluoro-4,5-bis(methoxymethoxy)benzaldehyde with a chiral nickel(II) complex and glycine (Ni-(*R*)-*BPB*-Gly) with subsequent removal of protection from hydroxy groups by acid hydrolysis. The radiochemical synthesis includes three steps and can be easily implemented into modern automated modules for the synthesis of radiopharmaceutical agents for PET.

Key words: fluorine-18, radiopharmaceutical agents, positron emission tomography, asymmetric synthesis, (2*S*,3*R*)-2-amino-3-(2-[^{18}F]fluoro-4,5-dihydroxyphenyl)-3-hydroxypropionic acid, 6-L-*threo*-[^{18}F]FDOPS, norepinephrine transporters.

Positron emission tomography (PET) is a rapidly developing nuclear imiging technique, which makes it possible to obtain *in vivo* information on the alteration of physiological and biochemical processes on molecular level. This method is based on the use of radiotracers (radiopharmaceuticals, RPs), *i.e.*, biologically active compounds labeled with short-lived positron emitting radionuclides. Among four cyclotron-produced PET radionuclides (^{15}O, ^{13}N, ^{11}C, ^{18}F), fluorine-18 has gained a prominent interest; its half-life (110 min) allows one to carry out complicated radiochemical syntheses and deliver RPs to the centers without their own cyclotron. During the years of development of PET, various RPs were synthesized and introduced into clinical diagnostics, among which glycolysis radiotracer 2-[^{18}F]-2-deoxy-D-glucose[1] is the most important.

The most widely PET is used for evaluation of various processes in tumor: amino acid transport, proliferation, hypoxia, apoptosis, and angiogenesis,[2,3] as well as in the receptor studies.[4] At the same time, the possibilities of PET in cardiodiagnostics are far from being completely implemented. Sympathetic innervation of the myocardium is more often evaluated by single photon emission computed tomography (SPECT), which is considerable inferior to PET in sensitivity and resolution. The most important radiopharmaceutical agent for SPECT is 1-(3-iodobenzyl)guanidine (methaiodobenzyl guanidine, [^{123}I]MIBG),[5] the iodine-123-labeled analog of norepinephrine neurotransmitter (noradrenaline, 4-[(1*R*)-2-amino-1-hydroxy]-1,2-dihydroxybenzene, NE). The agent

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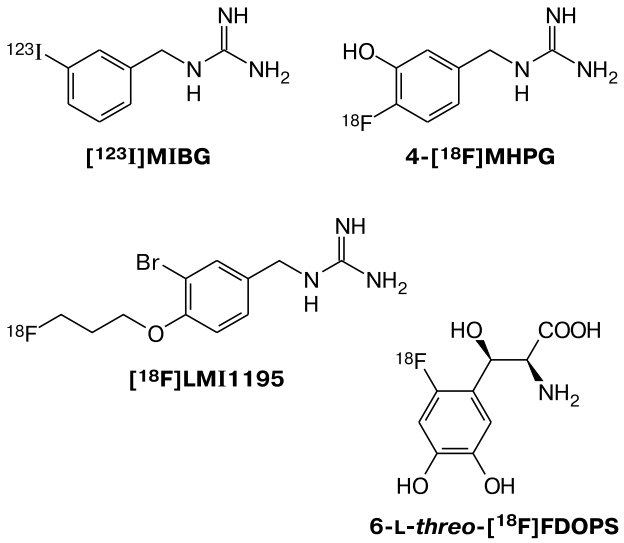
[^{123}I]MIBG is also used in the SPECT diagnostics of tumors with increased expression of norepinephrine transporters (NET) (pheochromocytoma, neuroblastoma).[6] It should be noted that analogs of NE labeled with carbon-11 (see Ref. 7) are not widely used in PET because of the short half-life of ^{11}C (20.4 min), whereas analogs of MIBG labeled with fluorine-18 were not introduced into practice because of the multi-step method for their synthesis.[8] In the recent years, the interest has been renewed to PET radiotracers based on the structural analogs of guanidine. Thus, hydroxyphenyl guanidine labeled with fluorine-18 (4-[^{18}F]MHPG)[9] was obtained in 1—2% radiochemical yield in a four-step synthesis, but this process requires optimization. In several reports,[10—12] *N*-[3-bromo-4-(3-[^{18}F]fluoropropoxy)benzyl]guanidine ([^{18}F]LMI1195) was suggested as a substrate of NET. At a first glance, the synthetic procedure does not seem difficult, but synthetic details were not reported. The agent [^{18}F]LMI1195 is currently under preclinical trials as an agent for visualization of the sympathetic nervous system of the heart[10,11] and diagnostics of neuroendocrine tumors.[12]



The fluorine-18-labeled analogs of L-*threo*-3,4-dihydroxyphenylserine (L-DOPS), a synthetic amino acid, which is transformed to NE by the reaction with L-amino acid aromatic decarboxylase (LAAAD), can serve as radiotracers for visualization of processes involving NET.[13]

The purpose of the present work is development of an approach to the synthesis of fluorinated analog of L-DOPS, *viz.*, (2*S*,3*R*)-2-amino-3-(2-[^{18}F]fluoro-4,5-dihydroxyphenyl)-3-hydroxypropionic acid (6-L-*threo*-[^{18}F]FDOPS). Similarly to L-DOPS, it can be expected that metabolism of 6-L-*threo*-[^{18}F]FDOPS would result in the formation of 6-[^{18}F]FNE (4-[(1*R*)-2-amino-5-[^{18}F]fluoro-1-hydroxy]-1,2-dihydroxybenzene), the accumulation of which in tumors with increased expression of NET can be detected by PET. Besides, 6-L-*threo*-[^{18}F]FDOPS can also be used in the studies of the heart sympathetic system, in which NE is a major neuromediator. By now, no information on the application of labeled analogs of L-DOPS is available, though, a possibility of their use in the diagnostics by PET has been considered as early as in 1990s.[14] Recently, attempted synthesis of the fluorine-18-labeled L-DOPS derivatives by direct electrophilic radiofluorination using [^{18}F]F_2 led to the formation of a mixture of isomers of 2-amino-3-(3-[^{18}F]fluoro-4,5-dihydroxyphenyl)-3-hydroxypropionic and 2-amino-3-(2-[^{18}F]fluoro-3,4-dihydroxyphenyl)-3-hydroxypropionic acids (in the cited work: [^{18}F]5- and [^{18}F]2-FDOPS, respectively), which were not characterized.[15] At the same time, comparative studies of two fluorinated analogs of L-DOPS (see Ref. 16), *viz.*, (2*S*,3*R*)-2-amino-3-hydroxy-3-(2-fluoro-3,4-dihydroxyphenyl)propionic and (2*S*,3*R*)-2-amino-3-hydroxy-3-(2-fluoro-4,5-dihydroxyphenyl)propionic acids (in the cited work: 2-F-L-*threo*-DOPS and 6-F-L-*threo*-DOPS) showed that only isomer 6-F-L-*threo*-DOPS was involved in the methabolytic process with the formation of 6-FNE. Taking into account these results, we have chosen the corresponding labeled analog, *viz.*, 6-L-*threo*-[^{18}F]FDOPS, as the object of our studies.

For the preparation of 6-L-*threo*-[^{18}F]FDOPS, we suggested a new approach to the asymmetric synthesis based on the condensation reaction of fluorine-18-labeled substituted benzaldehyde with chiral nickel(II) complex with glycine, Ni-(*R*)-*BPB*-Gly. In this case, the introduction of the fluorine-18 label in the molecule of substituted benzaldehyde is possible by the reaction of nucleophilic substitution based on [^{18}F]fluoride widely used in radiochemistry. Asymmetric methods were successfully used in the synthesis of ^{18}F-fluorinated amino acids ([^{18}F]FAA) with fluorine-18 label in the aromatic ring, including 6-[^{18}F]fluoro-3,4-dihydroxy-L-phenylalanine (6-[^{18}F]-L-FDOPA),[17—20] 2-[^{18}F]fluoro-L-tyrosine,[18] and other well established PET radiotracers. Preliminary results on the synthesis of 6-L-*threo*-[^{18}F]FDOPS were reported at a conference.[21]

Results and Discussion

Specific features of methods for the synthesis of [^{18}F]FAA. The most commonly used method for the preparation of compounds labeled with fluorine-18 is a nucleophilic substitution reaction, in which a [^{18}F]fluoride serves as the nucleophile.[22] Modern medical cyclotrons provide generation of carrier-free [^{18}F]fluoride with high (to 25 Ci) radioactivity level *via* ^{18}O(p,n)^{18}F nuclear reaction and irradiation of water-^{18}O (95—97% enrichment) with 16.5 MeV protons. To be involved in the nucleophilic fluorination reaction, [^{18}F]fluoride is recovered from the irradiated water-^{18}O by absorption on an anion-exchange resin and activated by addition of phase-transfer catalysts (PTC): tetrabutylammonium salts, crown-ethers, or cryptands. A necessity to carry out synthesis within a strictly

limited period of time when working with the short-lived isotope fluorine-18 requires the number of synthetic steps and intermediate purifications to be the minimal. For the synthesis of most RPs used in clinical practice of PET,[22] methods were developed which are based on the reaction of direct nucleophilic substitution of a leaving group in the substrate molecule, in which functional groups with labile hydrogen atoms are protected to prevent attack by the nucleophile at the unwanted position. The subsequent step of the synthesis consists in the removal of protecting groups (hydrolysis), then, the final product is isolated from the reaction mixture by semipreparative HPLC or solid-phase extraction. Direct nucleophilic radiofluorination method is fully automated[23] and applied for the synthesis of most clinically relevant RPs.[2,3] This technique suits well for the labeling aliphatic amino acids[24] or alkyl derivatives of aromatic amino acids,[25,26] however, it cannot be used for the direct introduction of the label in the aromatic ring of [^{18}F]FAA, since position of the nucleophilic attack is not activated. For the aromatic substrates to be fluorinated, the presence of strong electron-withdrawing substituents (NO_2, CHO, COR, CN, *etc.*) at *ortho*- or *para*-position to the leaving group is necessary,[22] that is the case in substituted nitrobenzaldehydes. That is why these compounds are used in the first step (the ^{18}F-fluorination reaction) of asymmetric synthesis of [^{18}F]FAA containing the label in the benzene ring. Asymmetric methods of synthesis stipulated by a thorough selection of chiral agent and reaction conditions allow one to obtain [^{18}F]FAA with high (more than 95%) enantiomeric purity,[17—20] that meets the PET requirements.

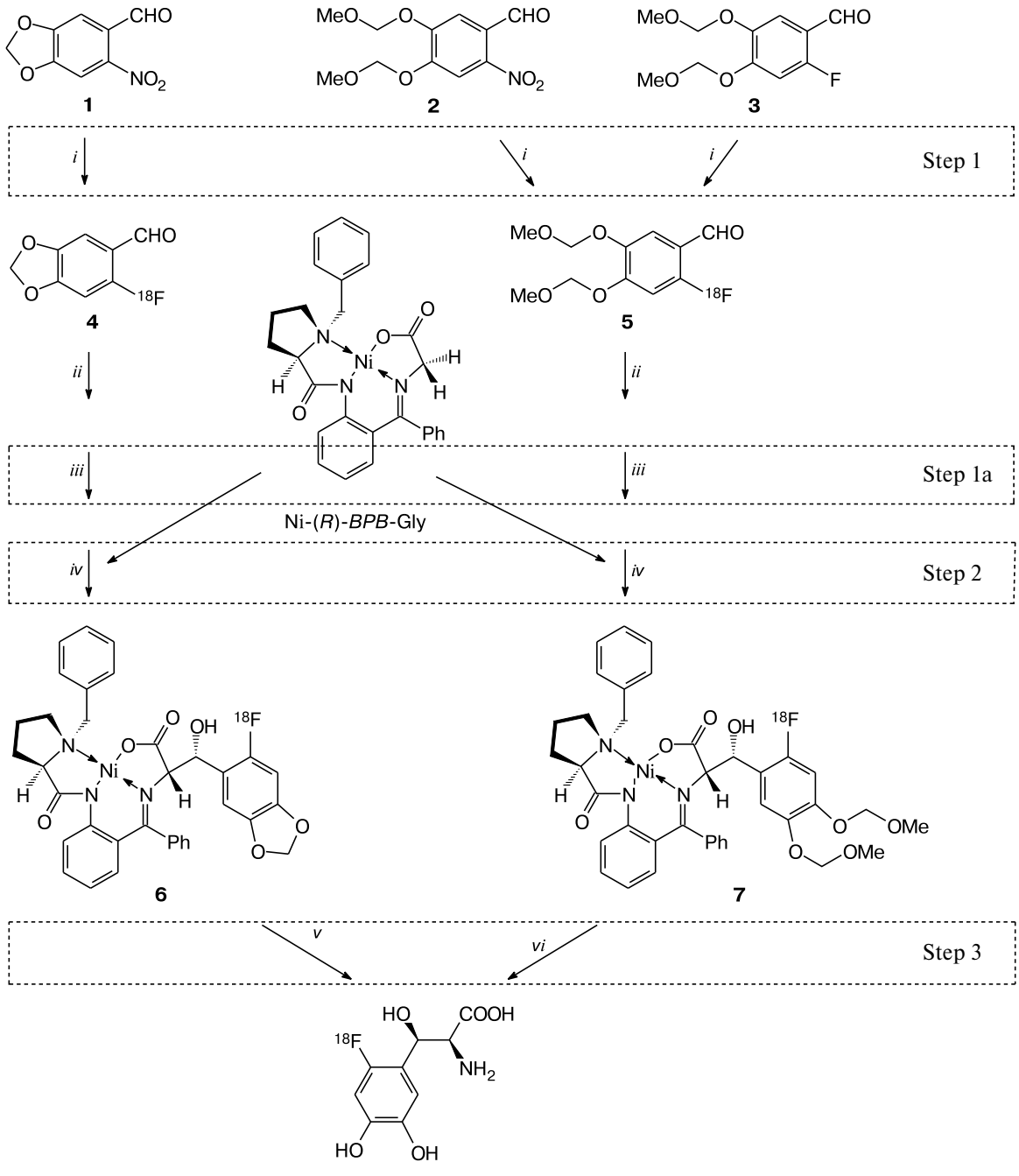
Synthesis of 6-L-*threo*-[^{18}F]FDOPS. We suggested an original method of asymmetric synthesis for the preparation of 6-L-*threo*-[^{18}F]FDOPS, which is based on the condensation reaction of substituted benzaldehyde labeled with fluorine-18 and a Ni^{II} complex of glycine Schiff base with the chiral agent (*R*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboximide (Ni-(*R*)-*BPB*-Gly). The complex Ni-(*R*)-*BPB*-Gly undergoes deprotonation in the presence of a base with its subsequent addition to benzaldehyde at the carbonyl group with the formation of the C—C bond. In nonradioactive experiments, this reaction reaches completion within 30 min (methanol, in the presence of MeONa, 25 °C) with very high (>96%) diastereo- and enantiostereoselectivity. Liberation of free amino acid and removal of protection from hydroxy groups were achieved by acid hydrolysis without isolation of the intermediate complexes. The principal steps of radiochemical version of the synthesis are shown in Scheme 1.

Introduction of fluorine-18 in the aromatic ring of 6-L-*threo*-[^{18}F]FDOPS. Nucleophilic substitution of leaving groups (NO_2, Hal, and others) in the molecule of substituted benzaldehydes with [^{18}F]fluoride in the presence of PTC is a standard method for the introduction of fluorine-18 in aromatic substrates.[22] Thus, the first step in the synthesis of 6-[^{18}F]-L-FDOPA[17,18] (whose molecule contains the same aromatic fragment as that of 6-L-*threo*-[^{18}F]FDOPS) is the fluorination reaction of 4,5-methylenedioxy-2-nitrobenzaldehyde (nitropiperonal (**1**)), proceeding with high yield.[18] Removal of protecting groups in the synthesis of 6-[^{18}F]-L-FDOPA requires rather drastic conditions (57% HI, 180—200 °C, 20 min), under which, as it was found in the present work, the molecule of 6-L-*threo*-[^{18}F]FDOPS is unstable. The use of protecting methoxymethoxy groups (MOM) gives an advantage, since they can be removed just in dilute aqueous HCl. In the present work, we synthesized 4,5-bis(methoxymethoxy)-2-nitrobenzaldehyde (**2**) and 2-fluoro-4,5-bis(methoxymethoxy)benzaldehyde (**3**) (Scheme 2), which were not previously studied in the radiofluorination reaction. Plenty of studies were devoted to the choice of leaving and protecting groups and their mutual arrangement in the molecule of substituted benzaldehydes, especially in the early period of development of PET.[19,27—29] Thus, it was shown[22] that the presence of leaving NO_2, ^{19}F, and other groups at *ortho*- and *para*-positions of the molecule of substituted benzaldehyde secure high efficiency of radiofluorination. As of now, there are no reports on the use of MOM protecting groups in the synthesis of fluorine-18-labeled benzaldehydes.

Comparison of the results of radiofluorination of compounds under study showed that under similar conditions (PTC: kryptofix 2.2.2, K_2CO_3, DMF, 140 °C, 10 min), the yield of fluorination largely varied and was 90, 50—65, and 15% for compounds **1**, **2**, and **3**, respectively. Radiofluorination in the system 18-crown-6/$KHCO_3$ in DMSO made it possible to increase the yield of fluorination of compound **3** to 40%, but appeared to be not that efficient for compound **2** (30%). Varying conditions of the synthesis (temperature and reaction time within 110—180 °C and 5—40 min, respectively) and amount of the starting benzaldehydes **1**, **2**, or **3** (5—10 mg) did not lead to the increase in the efficiency of radiofluorination.

For the condensation reaction to be successful (see Scheme 1, step 2), the efficiency of purification of labeled 2-[^{18}F]fluoro-4,5-bis(methoxymethoxy)benzaldehyde (**5**) is of great importance (see Scheme 1, step 1a). Unlike for compound **1**, fluorination of benzaldehydes **2** and, especially, **3** is accompanied by the formation of labeled side products (Fig. 1, *a*, *b*, *c*). Compound **5** was purified by a standard method of the solid-phase extraction on the reversed-phase sorbents (disposable cartridges C18 Plus, Waters; RP-18e, Merck; Oasis HLB, 3 cc, Waters). After the reaction mixture (10-fold diluted with water) was passed through the cartridge and additionally washed with water until unreacted [^{18}F]fluoride and solvent were completely removed, the product **5** was eluted with methanol (1.2 mL); this solvent was used for the condensation reaction. As it is seen from Fig. 1, radiochemical impurities are efficiently removed during purification of the reaction

Scheme 1

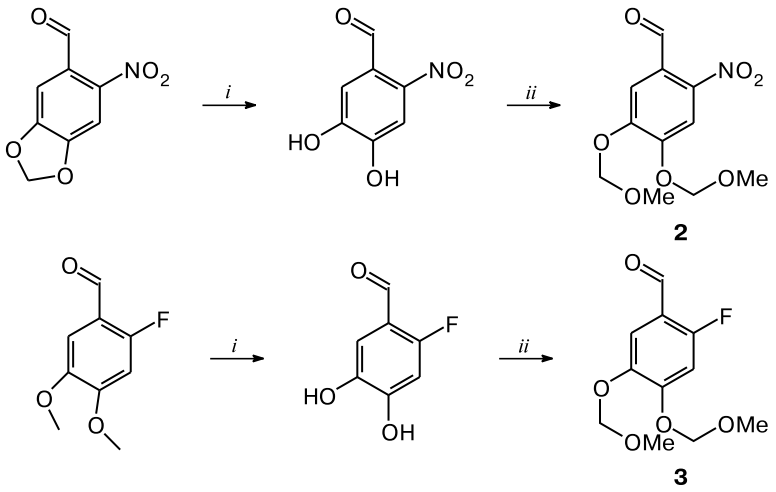


Reagents and conditions: *i*. [K/K2.2.2]$^{+}$^{18}F^{-}, DMF, 140 °C, 10 min; *ii*. dilution with water; *iii*. purification and change of solvent (SepPak C18 Plus or OASIS HLB cartridge); *iv*. MeOH, Bu^{t}OK, 10 min, 50 °C; *v*. 6 *M* HCl, 120 °C, 5 min (hydrolysis was not complete); *vi*. 1 *M* HCl, 50 °C, 5 min.

mixture obtained by fluorination of nitro derivative of compound **2**, but not that of **3**. Thus, of two benzaldehydes with MOM protecting groups, the higher yield of radiofluorination and a high degree of purification were reached in the case of compound **2.**

Condensation reaction of Ni-(*R*)-*BPB*-Gly and compound 5. The condensation reaction of compound **5** with the chiral complex Ni-(*R*)-*BPB*-Gly is a key step of the synthesis. In the presence of a base, the complex Ni-(*R*)-*BPB*-Gly undergoes deprotonation and adds at the carb-

Scheme 2



i. BBr_3, CH_2Cl_2, Ar, −78 °C, RT; *ii.* MOMCl, Bu^{t}OK, MeCN.

onyl group of benzaldehyde with the formation of the C—C bond (see Scheme 1). Earlier, it was unambiguously shown that under strongly basic conditions the reaction of the complex Ni-(*R*)-*BPB*-Gly with aldehydes led to the complex containing amino acid exclusively in the (*S*)-*threo*-configuration.[30] The optimal conditions for this step of radioactive synthesis were selected after a series of experiments. The maximal extent of incorporation of compound **5** into the structure of the complex (80%) was reached under the following conditions: MeOH, Bu^{t}OK, 50 °C, 10 min. The efficiency of the reaction was calculated based on the ratio of the peaks of compound **5** and the intermediate fluorine-18-labeled complex ((Ni-(*R*)-*BPB*-(*S*)-*threo*-6-[^{18}F]-FDOPS(OMOM)$_2$) (**7**) according to the data obtained by radio-TLC in the system 2 (Fig. 2). For the intermediate labeled condensation product **7** to be identified, we synthesized and characterized the corresponding nonradioactive complex Ni-(*R*)-*BPB*-(*S*)-*threo*-6-FDOPS(OMOM)$_2$ (**9**) (Scheme 3).

Hydrolysis of the intermediate complex 7 and removal of protection. The last step of the synthesis consists in the decomposition of the condensation product of complex **7** with simultaneous removal of protecting groups. Initially, we used nitrobenzaldehyde **1** with high efficiency of radiofluorination as the starting compound in the radiofluorination reaction (see Scheme 1, step 1). To remove protection, the intermediate product Ni-(*R*)-*BPB-(S)-threo*-6-[^{18}F]FDOPS(OCH$_2$O) (**6**) (see Scheme 1, step 3)

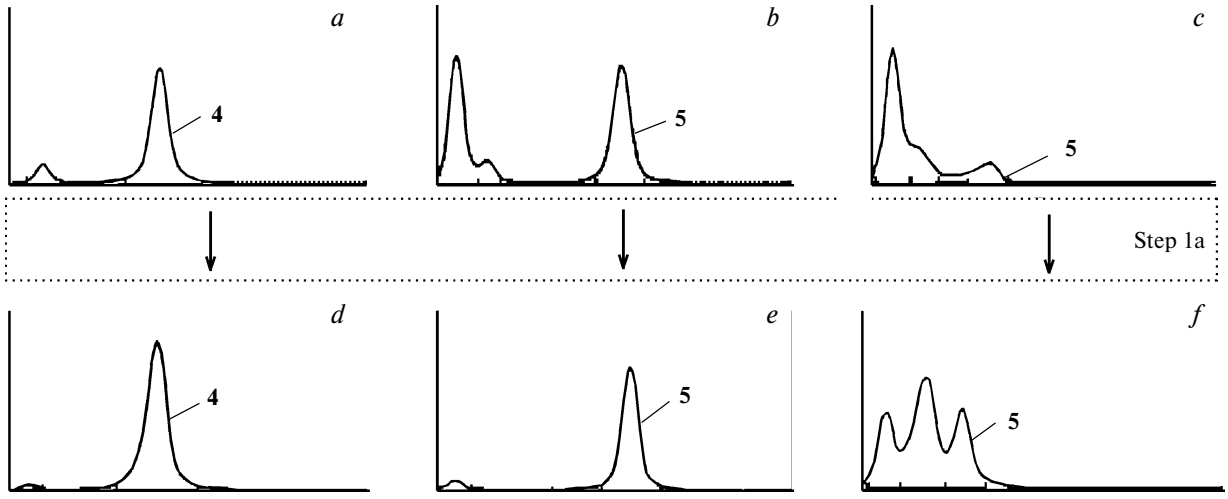


Fig. 1. Analysis by radio-TLC of the products (system 1) in the reaction mixture obtained by radiofluorination of compounds **1** (*a*, *d*), **2** (*b*, *e*), and **3** (s, *f*) in the presence of K2.2.2. (DMF, 140 °C, 10 min): before purification (*a*, *b*, *c*) and after purification (*d*, *e*, *f*) on a C18 Plus cartridge (Waters).

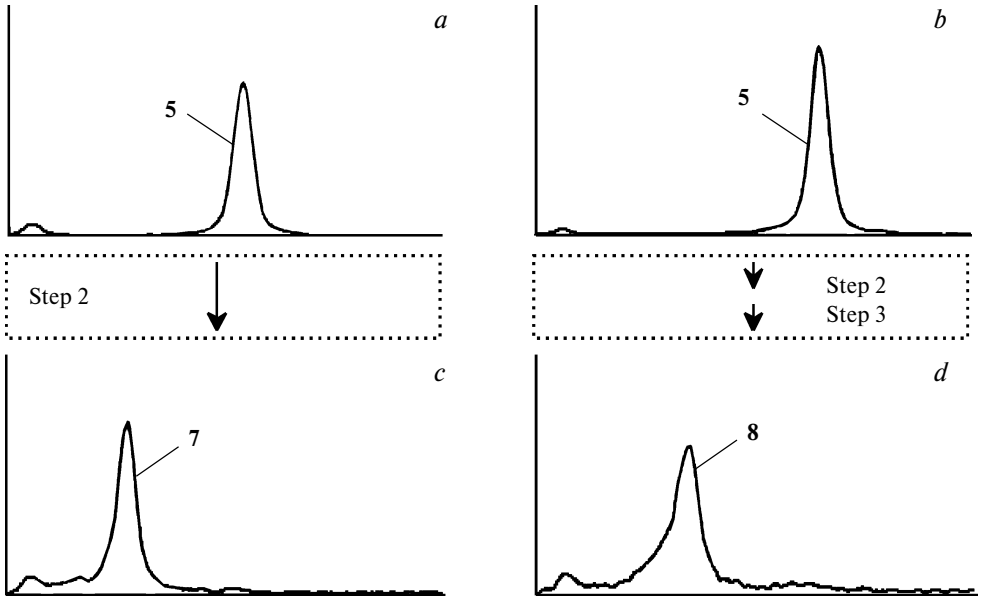
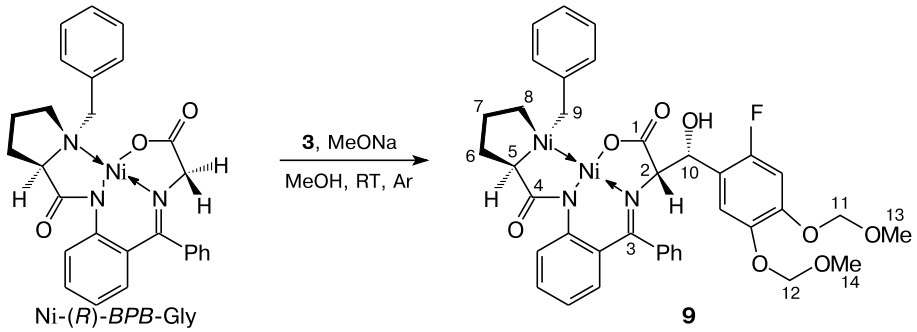


Fig. 2. Analysis by radio-TLC of the condensation products (*a*) in step 2 (system 2, *c*) and the hydrolysis products (*b*) in step 3 (system 3, *d*); the synthesis was carried out for compound **2**.

was treated with 6*N* HCl for 5 min at 120 °C. However, the use of even that high concentration of hydrochloric acid either did not cleave protecting group or removed it only partially. When compound **2** was used as the starting compound, decomposition of complex **7** under the same conditions was efficient enough, as well as with the lower concentration of the acid (1*N* HCl, 50 °C, 5 min).

We paid special attention to the identification of the reaction products in each step of the synthesis, since the compounds obtained differ in polarity, solubility, and other properties. Thus, the intermediate nickel(II) complexes (**6** and **7**, see Scheme 1) and the corresponding nonradioactive standards **10** and **9** are virtually insoluble in water, therefore, a nonpolar eluent was used in TLC (system 2) for the monitoring of step 2. At the same time, the final product is an amino acid, and it is better to use a polar eluent composed of water and acid (system 3) for the monitoring of the hydrolysis step and the formation of 6-L-*threo*-[^{18}F]FDOPS (**8**). Since the condensation reaction (see Scheme 1, step 2) is reversible, it was important to show that the intermediate complex **7** obtained in this step is not converted to compound **5**. This was confirmed by the absence of the peak of compound **5** on the TLC chromatogram of the reaction mixture after hydrolysis (see Fig. 2, *d*). A poor solubility of fluorinated amino acid 6-L-*threo*-[^{19}F]FDOPS in water and any other polar solvents made it difficult to analyze it by reversed-phases HPLC. In this case, the standard for the identification of the product by HPLC was obtained *in situ* by he decomposition of nonradioactive complex **9** under the same conditions, which were used for the hydrolysis in step 3 of the radioactive synthesis. The agreement of the retention time of the peak of the standard 6-L-*threo*-[^{19}F]FDOPS obtained *in situ* and the radioactive 6-L-*threo*-[^{18}F]FDOPS (including the time for the eluent to pass through two serially connected flow detectors: based on the absorption in the UV region of the spectrum and radioactivity detector) confirms the formation of 6-L-*threo*-[^{19}F]FDOPS as

Scheme 3



the major reaction product. The mild conditions of hydrolysis (5 min as compared to 2.5 h in the work[14]), as well as the data of preceding studies[30] indicate a possibility of retention of *threo*-configuration of the labeled amino acid in this step. To confirm this suggestion, we plan additional experiments with high initial radioactivity of fluorine-18, providing a possibility of isolation of the product from the reaction mixture by semipreparative radio-HPLC.

In conclusion, we for the first time demonstrated a principal possibility of the preparation of analog of L-*threo*-3,4-dihydroxyphenylserine labeled with fluorine-18, *viz.*, 6-L-*threo*-[^{18}F]FDOPS (**8**), a promising radiotracer for visualization of the processes involving NET by PET. The method of the asymmetric synthesis suggested in this work is based on the condensation reaction of substituted benzaldehyde labeled with fluorine-18, 4,5-bis(methoxymethoxy)-2-[^{18}F]fluorobenzaldehyde (**5**), with chiral nickel(II) complex with glycine, Ni-(*R*)-*BPB*-Gly. The radiochemical synthesis includes three steps and can be easily implemented into modern automated synthesis modules for PET RPs, which is necessary for the work with highly radioactive agents. At the present time, we continue our studies on optimization of each step of the synthesis in order to increase radiochemical yield of 6-L-*threo*-[^{18}F]FDOPS and confirm *threo*-configuration of the amino acid. A complete automation of the process, including purification by semipreparative HPLC, will make it possible to use this method for the preparation of 6-L-*threo*-[^{18}F]FDOPS in the dozes sufficient for pre-clinical trials.

Experimental

Commercially available 4,5-methylenedioxy-2-nitrobenzaldehyde (nitropiperonal) and 2-fluoro-4,5-bis(methoxy)benzaldehyde (fluoroveratrol) (Aldrich) were used in the work. The complex [({[2-(1S_N,2*R*)-1-benzylpyrrolidine-2-carboxamido]-phenyl}-phenylmethylene)aminoacetato-*N*,*N*′,*N*″,O]nickel(II) (Ni-(*R*)-BPB-Gly) was obtained according to the known procedure.[31] The methoxy- and methylenedioxy protecting groups were removed according to the modified procedure.[32] NMR spectra were recorded on a Bruker Avance 400 spectrometer (400.13 MHz for ^{1}H NMR, 100.16 MHz for ^{13}C NMR, and 161 MHz for ^{19}F NMR). Chemical shifts (δ) were measured relative to the residual signals of nondeuterated solvent ($CDCl_3$ in the case of ^{1}H and ^{13}C spectra) or Freon CHF_2Cl (in the case of ^{19}F spectra). Optical rotation was measured on a Perkin-Elmer 341 polarimeter in a 0.5-dm cell at 25 °C. Column chromatography was carried out on Kieselgel 60 silica gel (Merck), GPC on Sephadex LH-20 (Supelco). All the reactions were performed under inert atmosphere of dry argon in anhydrous solvents prepared immediately before use according to the standard procedures.[33]

4,5-Bis(methoxymethoxy)-2-nitrobenzaldehyde (2). A 1 *M* solution of BBr_3 in CH_2Cl_2 (6 mL) was added dropwise to a solution of compound **1** (0.5 g, $2.56\cdot10^{-3}$ mol) in anhydrous CH_2Cl_2 (6 mL) cooled to −78 °C, then the reaction mixture was warmed-up to 20 °C and stirred under these conditions for 18 h. Then, MeOH (5 mL) was added dropwise to the reaction mixture, a resulting solution was concentrated, the residue was diluted with MeOH (5 mL) and concentrated once more. This procedure was repeated two times. An oil obtained was extracted with a hot 1 : 1 mixture of hexane with ethyl acetate (6×10 mL). The solutions were concentrated to obtain a dry residue (0.5 g), which was dissolved in anhydrous MeCN (20 mL). Chloromethoxymethane (MOMCl) (0.21 mL, 0.22 g, $2.73\cdot10^{-3}$ mol) and (Bu^{t}OK 0.31 g, $2.73\cdot10^{-3}$ mol) were sequentially added to the solution. The reaction mixture was stirred for 1.5 h at ~20 °C, followed by addition of MOMCl (0.11 mL, 0.11 g, $1.37\cdot10^{-3}$ mol) and Bu^{t}OK (0.15 g, $1.36\cdot10^{-3}$ mol) and stirring for another 1 h. The reaction progress was monitored by TLC on silica gel in the system hexane—ethyl acetate, 3 : 1, observing the disappearance of the spot of the starting compound with R_f 0.15 and the appearance of the spot of the product with R_f 0.65. The intermediate monomethoxymethoxy product has an R_f value of 0.25. After the reaction reached completion, the mixture was filtered from the inorganic salts, the salts were washed with MeCN (2×6 mL). The combined solutions were concentrated to obtain a glassy residue. The product was purified by recrystallization from a mixture of benzene—hexane (2 : 1, 15 mL), washing a precipitate formed with hexane, which was dried over P_2O_5 and paraffin to obtain 4,5-bis(methoxymethoxy)-2-nitrobenzaldehyde (**2**) (0.486 g, $1.79\cdot10^{-3}$ mol, 70%). M.p. 91—92 °C (*cf.* Ref. 34: m.p. 100—101 °C). Found (%): C, 48.71; H, 4.96; N, 5.06. $C_{11}H_{13}NO_7$. Calculated (%): C, 48.71; H, 4.83; N, 5.16. ^{1}H NMR ($CDCl_3$), δ: 10.42 (s, 1 H, —CHO); 7.95 (s, 1 H, Ar); 7.72 (s, 1 H, Ar); 5.40 (s, 2 H, —OCH_2O—); 5.397 (s, 2 H, —OCH_2O—); 3.568 (s, 3 H, —OMe); 3.549 (s, 3 H, —OMe).

2-Fluoro-4,5-bis(methoxymethoxy)benzaldehyde (3). A 1 *M* solution of BBr_3 in CH_2Cl_2 (45 mL) was added dropwise to a solution of 2-fluoro-4,5-bis(methoxy)benzaldehyde (3 g, $1.6\cdot10^{-2}$ mol) in anhydrous CH_2Cl_2 (20 mL) cooled to −78 °C, then the reaction mixture was warmed-up to 20 °C and stirred under these conditions for 18 h. Then, MeOH (20 mL) was added dropwise to the reaction mixture, a resulting solution was concentrated, the residue was diluted with MeOH (25 mL) and concentrated once more. This procedure was repeated two times. The product was purified by column chromatography on silica gel (11×3 cm) in the system CH_2Cl_2—AcOEt, 5 : 1 to obtain 2-fluoro-4,5-dihydroxybenzaldehyde (2.4 g, 96%) as a light yellow oil very easy oxidizable in air. To avoid losses, it was immediately used in the subsequent step without additional purification and crystallization, thus alternating procedure described in the work.[35] The oil obtained was dissolved in anhydrous acetonitrile (120 mL), followed by a sequential addition of MOMCl (1.2 mL, 1.28 g, $1.6\cdot10^{-2}$ mol) and Bu^{t}OK (1.8 g, $1.6\cdot10^{-2}$ mol). The reaction mixture was stirred for 2 h at ~20 °C, then, MOMCl (1.2 mL, 1.28 g, $1.6\cdot10^{-2}$ mol) and Bu^{t}OK (1.8 g, $1.6\cdot10^{-2}$ mol) were add and the stirring was continued for 16 h. The reaction progress was monitored by TLC on silica gel in the the system hexane—ethyl acetate, 1 : 1, observing the disappearance of the spot of the starting compound with R_f 0.13 and the appearance of the spot of the product with R_f 0.54. The intermediate monomethoxymethoxy product has R_f 0.20. The product (3.5 g, 89%) was obtained as a colorless oil. Found (%): C, 53.94; H, 6.26. $C_{11}H_{13}FO_5\cdot1/3$AcOEt. Calculated (%): C, 54.14; H, 5.77. ^{1}H NMR ($CDCl_3$), δ: 10.27 (s, 1 H, CHO); 7.64 (d, 1 H, Ar, J = 6.8 Hz); 7.04 (d, 1 H, Ar, J = 11.8 Hz); 5.36 (s, 2 H, OCH_2O); 5.28 (s, 2 H, OCH_2O); 3.57 (s, 3 H, OMe); 3.57 (s, 3 H, OCH_3O). ^{19}F NMR ($CDCl_3$), δ: −48.8.

Addition of Ni-(*R*)-*BPB*-Gly to 2-fluoro-4,5-bis(methoxymethoxy)benzaldehyde. A solution of 2-fluoro-4,5-bis(meth-

oxymethoxy)benzaldehyde (0.6 g, $2.5 \cdot 10^{-3}$ mol) in MeOH (3 mL) and a 3.76 *N* solution of MeONa in MeOH (2 mL) were sequentially added to a solution of Ni-(*R*)-*BPB*-Gly (1.25 g, $2.5 \cdot 10^{-3}$ mol) in MeOH (10 mL) with stirring. The reaction mixture was stirred for 30 min, neutralized with AcOH (1 mL), and concentrated. The product was purified by column chromatography in the system THF—benzene (1 : 1) with additional purification by gel permeation chromatography in the system EtOH—benzene to obtain complex Ni-(*R*)-*BPB*-(*S*)-*threo*-6-FDOPS(OMOM)$_2$ (1.5 g, $2.02 \cdot 10^{-3}$ mol, 81%). M.p. 93—95 °C. $[\alpha]_D^{25}$ +1010 (*c* 0.08, MeOH). Found (%): C, 61.28; H, 5.18; F, 2.40; N, 5.20. $C_{38}H_{38}FN_3NiO_8 \cdot 2/3EtOH$. Calculated (%): C, 61.11; H, 5.48; F, 2.44; N, 5.44. ^{1}H NMR ($CDCl_3$), δ: 8.43 (d, 1 H, Ar, J = 8.8 Hz); 7.55 (m, 3 H, Ar); 7.33 (m, 4 H, Ar); 7.27 (m, 5 H, Ar); 7.15 (d, 1 H, Ar, J = 11.4 Hz); 6.82 (dd, 1 H, Ar, J = 8.3 Hz, J = 1.9 Hz); 6.73 (dd, 1 H, Ar, J = 8.3 Hz, J = 6.8 Hz); 5.19 (dd, 2 H, OCH_2O, J = 26.6 Hz, J = 6.8 Hz); 5.23, 5.16 (AB-system, 2 H, OCH_2O, J_{AB} = 6.8 Hz); 5.06, 5.04 (AB-system, 2 H, OCH_2O, J_{AB} = 6.5 Hz); 5.01 (m, 2 H); 4.38 (m, 1 H); 4.07, 3.63 (AB-system, 2 H, CH_2Ph, J_{AB} = 13.4 Hz); 3.70 (m, 1 H); 3.43 (s, 3 H, OMe); 3.34 (m, 1 H); 3.31 (s, 3 H, OCH_3); 2.39 (m, 1 H); 2.27 (m, 1 H); 1.97 (m, 1 H); 1.44 (m, 2 H). ^{13}C NMR ($CDCl_3$), δ: 181.83 (C(1)), 178.67 (C(3)), 174.45 (C(4)), 157.10 (C_{Ar}), 154.70 (C_{Ar}), 148.86 (C_{Ar}), 148.76 (C_{Ar}), 144.39 (C_{Ar}), 143.32 (C_{Ar}), 134.13 (C_{Ar}), 133.98 (C_{Ar}), 133.03 (C_{Ar}), 132.19 (C_{Ar}), 131.57 (C_{Ar}), 130.35 (C_{Ar}), 129.37 (C_{Ar}), 129.13 (C_{Ar}), 128.90 (C_{Ar}), 128.77 (C_{Ar}), 126.91 (C_{Ar}), 126.29 (C_{Ar}), 123.85 (C_{Ar}), 123.77 (C_{Ar}), 120.94 (C_{Ar}), 120.65 (C_{Ar}), 120.49 (C_{Ar}), 117.37 (C_{Ar}), 104.79 (C_{Ar}), 96.33 (C(12)), 95.79 (C(11)), 68.64 (C(5)), 66.97 (C(9)), 60.43 (C(8)), 56.60 (C(13)), 56.5 (C(14)), 55.26 (C(10)), 31.36 (C(6)), 23.76 (C(7)). ^{19}F NMR ($CDCl_3$), δ: −59.11.

The complex Ni-(*R*)-*BPB*-(*S*)-*threo*-6-FDOPS(OCH_2O) (**10**) was synthesized similarly using 2-fluoro-4,5-methylenedioxybenzaldehyde.

Radiochemical synthesis. The following equipment was used in the radiochemical synthesis: MC17 cyclotrons, Scanditronix (Sweden) and GE PETTrace 4 (Sweden) (energy of protons 17 and 16.5 MeV, respectively); a Von Gahlen hot cell (Netherlands) for the work with radioactivity; a PTW Curiementor-2 isotope calibrator (Germany); a Gilson analytical liquid chromatograph (France) (a model 305 pump, a model 316 UV detector, a Rheodyne model 7125 injector valve, Beckman 170 flow radiation detectors (USA)); a Minigita scanner for thin-layer radiochromatography (Raytest, Germany).

A remote-controlled semiautomatic module for nucleophilic radiofluorination developed in the N. P. Bechtereva Institute of Human Brain of the Russian Academy of Sciences and set up in a hot cell was used in the radioactive synthesis; valves, gas flows, temperatures were remotely operated from the external button panel. This system does not allow to manipulate with high levels of radioactivity, therefore, we used in the work 185—1100 MBq of fluorine-18, that was enough for the development of synthetic strategy and analysis of all the products.

Reagents and materials. A 97% enriched water-^{18}O (Global Scientific Technology, Sosnovyi Bor, Russia); commercially available anhydrous acid-free MeCN (DNA grade), potassium carbonate (anhydrous), potassium hydrogen carbonate, MeOH, Me_2CO (Merck); anhydrous DMF, DMSO, Bu^{t}OK, 4,7,13,16,20,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (kryptofix or K2.2.2.), 1,4,7,10,13,16-hexaoxocyclooctadecane (18-crown-6), 4,5-methylenedioxy-2-nitrobenzaldehyde (Aldrich) were used without additional purification. Reversed-phase cartridge for the solid-phase extraction C18 Plus, Oasis HLB 3 cc (Waters), and RP-18E (Merck) were conditioned before use by passing ethanol (10 mL) and water (15 mL). A Sep-Pak Light Waters Accell^{TM} Plus QMA (Waters) anion-exchange cartridge was activated by passing 0.5 *M* solution of potassium carbonate (10 mL) and water (15 mL). Radio-TLC was performed on the Sorbfil-type silica gel plates (Krasnodar) with plastic support.

Preparation of fluorine-18 and its complex with PTC. Radionuclide fluorine-18 ($T_{1/2}$ = 110 min) in the form of [^{18}F]fluoride was obtained by the ^{18}O(p,n)^{18}F nuclear reaction effected upon irradiation of [^{18}O]H_2O with protons in the cyclotron aqueous niobium target; the target sizes were 3.5 mL (MC17) and 2.3 mL (GE PETTrace 4). An aqueous solution of [^{18}F]fluoride obtained in the target was transferred with a flow of helium on a Sep-Pak Light Waters Accell^{TM} Plus QMA anion-exchange cartridge, the cartridge was purged with helium for 5—10 min to remove traces of water. Radionuclide ^{18}F was eluted with solutions of complex composition (2 mL) into a 5-mL conical reaction vessel placed into a heating block. Depending on PTC, eluents of the following composition were used: K2.2.2 (9.8 mg), K_2CO_3 (2.1 mg), H_2O (0.09 mL), MeCN (2 mL) or 18-crown-6 (14.9 mg), $KHCO_3$ (2.57 mg), H_2O (0.09 mL), MeCN (2 mL). The eluate containing radioactive [^{18}F]fluoride was heated in the flow of nitrogen during 4—6 min at 130 °C to remove the solvents, then MeCN (1 mL) was added and traces of water were additionally distilled off as an azeotrope. An activated complex obtained, for example [K/2.2.2.]$^+$^{18}F$^-$ in the case of kryptofix, was used in the following step.

Synthesis of 4,5-methylenedioxy-2-[^{18}F]fluorobenzaldehyde (4) and 4,5-bis(methoxymethoxy)-2-[^{18}F]fluorobenzaldehyde (5) (general procedure). Substituted benzaldehyde **1**, **2**, or **3** (5—8 mg) in DMSO or DMF (0.6 mL) was added to an activated complex containing fluorine-18, the mixture was stirred with a flow of nitrogen and heated for 10 min in a capped flask at 140 °C (see Scheme 1, step 1). Compounds **4** and **5** were isolated from the reaction mixture by solid-phase extraction on reversed-phase sorbents. Before passing, the reaction mixture was diluted 10-fold with water, the labeled product **4** or **5** adsorbed on the sorbent surface was eluted with methanol (1.2 mL) (see Scheme 1, step 1a). The same solvent was used in the following condensation step. Radio-TLC (system 1) was used to determine efficiency of radiofluorination.

Synthesis of 6-L-*threo*-[^{18}F]FDOPS (8). A solution of **4** or **5** in MeOH (1.2 mL) was added into a reaction vessel with a conical bottom (V = 5 mL), which was contained a chiral complex Ni-(*R*)-*BPB*-Gly (20.5 mg) and Bu^{t}OK (20 mg). The mixture was stirred with a flow of nitrogen and heated for 10 min at 50 °C in a capped flask (see Scheme 1, step 2). Decomposition of diastereomeric complex **6** or **7** and removal of protection from hydroxy groups were performed by heating the reaction mixture: 6 *N* HCl (0.3 mL), 5 min at 120 °C (in the case of compound **6**) and 1 *N* HCl (1 mL), 5 min at 50 °C (in the case of **7**) (see Scheme 1, step 3). Efficiency of steps 2 and 3 was evaluated by radio-TLC (systems 2 and 3).

Analysis by TLC. Some separate steps of the synthesis were monitored by TLC on the Sorbfil-type silica gel plates (10×100 mm) using the following eluents: dichloromethane (system 1); chloroform—acetone, 7 : 1 (system 2); Bu^{n}OH—AcOH—EtOH—H_2O, 4 : 1 : 0.5 : 1.6 (system 3). Positions of spots of nonradioactive compounds were determined under UV light (254 nm). Distribution of radioactivity was detected by radiometric method using a scanner for radioTLC. Retention factors (R_f)

for TLC in system 1: [^{18}F]fluoride 0.02; 4,5-methylenedioxy-2-nitrobenzaldehyde (**1**) 0.45; 4,5-methylenedioxy-2-[^{18}F]fluorobenzaldehyde (**4**) 0.45; 2-fluoro-4,5-bis(methoxymethoxy)benzaldehyde (**3**) 0.45; 2-[^{18}F]fluoro-4,5-bis(methoxymethoxy)benzaldehyde (**5**) 0.45. Retention factors (R_f) for TLC in system 2: [^{18}F]fluoride 0.02; 4,5-methylenedioxy-2-nitrobenzaldehyde (**1**) 0.65; 4,5-bis(methoxymethoxy)-2-nitrobenzaldehyde (**2**) 0.60; 2-fluoro-4,5-bis(methoxymethoxy)benzaldehyde (**3**) 0.65; 2-[^{18}F]fluoro-4,5-methylenedioxybenzaldehyde (**4**) 0.65; 2-[^{18}F]fluoro-4,5-bis(methoxymethoxy)benzaldehyde (**5**) 0.65; Ni-(*R*)-*BPB-(S)-threo*-6-FDOPS(OCH_2O) (**10**) 0.25; Ni-(*R*)-*BPB-(S)-threo*-6-[^{18}F]FDOPS(OCH_2O) (**6**) 0.25; Ni-(*R*)-*BPB*-(*S*)-*threo*-6-FDOPS$(OMOM)_2$ (**9**) 0.25; Ni-(*R*)-*BPB*-(*S*)-*threo*-6-[^{18}F]FDOPS$(OMOM)_2$ (**7**) 0.25. Retention factors (R_f) for TLC in system 3: [^{18}F]fluoride 0.02; 4,5-methylenedioxy-2-nitrobenzaldehyde (**1**) 0.65; 2-[^{18}F]fluoro-4,5-methylenedioxybenzaldehyde (**4**) 0.65; 6-L-*threo*-[^{18}F]FDOPS (**8**) 0.40.

Analysis by HPLC. The target product was analyzed by radio-HPLC on a column Lichrospher C18 (5 μm, 250×4.6 mm) under the following conditions: eluent 0.1% AcOH, the flow rate 1.0 mL min^{-1}; detection of nonradioactive standard 6-L-*threo*-[^{19}F]FDOPS (obtained *in situ* by decomposition of complex **9**): 254 nm, retention time 7.1 min. Retention time of complex **8** (7.6 min) was determined using a flow detector of radioactivity set up in the line after UV detector.

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