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Prostate cancer PET bioprobes: Synthesis of [^{18}F]-radiolabeled hydroxyflutamide derivatives

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Abstract—Approximately 80–90% of prostate cancers are androgen dependent at initial diagnosis. The androgen receptor (AR) is present in most advanced prostate cancer specimens and is believed to have a critical role in its development. Today, treatment of prostate cancer is done by inhibition of AR using antiandrogens such as flutamide (pro-drug of hydroxyflutamide), nilutamide, and bicalutamide. However, there is currently no noninvasive imaging modalities to detect, guide, and monitor specific treatment of AR-positive prostate cancer. (*R*)-3-Bromo-*N*-(4-fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methyl-propanamide [^{18}**F**]-**1** and *N*-(4-fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide [^{18}**F**]-**2**, derivatives of hydroxyflutamide, were synthesized as a fluorine-containing imaging agent candidates. A three-step fluorine-18 radiosynthesis route was developed, and the compounds were successfully labeled with a $10 \pm 3\%$ decay corrected radiochemical yield, 95% radiochemical purity, and a specific activity of 1500 ± 200 Ci/mmol end of bombardment ($n = 10$). These labeled biprobes not only may enable for the future quantitative molecular imaging of AR-positive prostate cancer using positron emission tomography but may also allow for image-guided treatment of prostate cancer.

1. Introduction

The androgen receptor (AR) is a member of the steroid/thyroid hormone receptor superfamily and plays a critical role in the development and maintenance of male secondary sexual phenotypes such as muscle, hair and bone mass, prostate growth, and spermatogenesis.[1–4] The AR is a cellular regulatory protein that upon androgen binding migrates into the nucleus, binds to specific DNA sequences called androgen response elements, and modulates the transcription of target genes.[1]

The AR is also believed to be involved in prostate carcinogenesis, and amplification of AR is present in most advanced prostate cancer specimens.[5,6] Males who are castrated at a young age do not develop prostate cancer, implying that androgens are risk factors for prostate cancer development.[6] In addition, prostate-specific expression of an androgen receptor transgene in a transgenic mouse induces prostate intraepithelial neoplasia.[6]

Prostate cancer is estimated to represent 30% of new cancer cases in US men.[6] Approximately 80–90% of prostate cancers are androgen dependent upon initial diagnosis and endocrine therapy of prostate cancer is directed toward the reduction of serum androgens and inhibition of AR.[7] On the other hand, some very aggressive forms of prostate cancer were shown to have lost the expression of AR and are insensitive to inhibition of the AR.[6,7] Testosterone and 5α-dihydrotestosterone (5α-DHT) are natural steroids that serve as the natural ligands for the AR and can be used as replacement therapy for androgen

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deficiency.[8,9] Different ligands of the AR were reported and can be divided into two main chemical structures—steroidal and nonsteroidal—with two different functionalities—androgenic and antiandrogenic classes.[1] Antiandrogens are used to counteract the undesirable actions of excessive androgens. Nonsteroidal antiandrogens, such as flutamide, nilutamide, and bicalutamide (Fig. 1), were shown to bind exclusively to the AR and, therefore, have few side effects.[1,4] These agents are advantageous over steroidal antiandrogens (e.g., megestrol acetate and cyproterone acetate) in terms of specificity, selectivity, and pharmacokinetic properties and are successfully used in the clinic for the treatment of AR-dependent prostate cancer.[1,10] Since AR is a specific target for prostate cancer treatment and loss of expression of AR has been observed in several aggressive tumors, it is critical to determine its role in individual patients to guide and monitor treatment. To date, imaging tools, including positron emission tomography (PET) for diagnosing local recurrence and metastatic sites of prostate cancer, are suboptimal.[11–13] PET is a nuclear medicine imaging modality, which allows for the three-dimensional, quantitative determination of the distribution of radioactivity within the human body.[14] PET allows accurate measurement of radioactivity concentration in small volume elements in vivo as well as the ability to follow tracer kinetics. PET requires the administration to the subject of a molecule labeled with a positron-emitting nuclide such as ^{15}O, ^{13}N, ^{11}C, and ^{18}F, which have half-lives of 2.037, 9.965, 20.39, and 109.8 min, respectively. The major effort to visualize AR in prostate cancer using PET has been focused on the labeling of steroids, such as testosterone and dihydrotestosterone, or synthetic steroid, mibolerone and metribolone (R1881) derivatives.[15] The most advanced PET agent for AR is a derivative of dihydrotestosterone ($K_i = 0.28$ nM)[4]—[^{18}F]fluoro-dihydrotestosterone.[15i,j,k] Although the metabolic rate of this compound was rapid, fast tumor uptake and prolonged retention of radioactivity were observed in human studies.[15i,j,k] While androgenic steroid radiopharmaceuticals have high affinity to the AR, they often have inadequate AR selectivity and like other steroids, tend to bind with other steroid receptors.[16] Anilide analogs such as flutamide, nilutamide, and bicalutamide (Fig. 1) were the first group of nonsteroidal androgen antagonists to be used as drugs, and their chemical structures are natural candidates for PET imaging agent development.

We report our efforts on the design, synthesis and radiosynthesis of novel prostate cancer PET imaging agent candidates labeled with fluorine-18. We will begin by first discussing our unsuccessful approach to aliphatically incorporate the radiolabeled isotopes, and finally report our successful radiosynthesis of (*R*)-3-bromo-*N*-(4-fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide [^{18}**F**]-**1** and *N*-(4-fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide [^{18}**F**]-**2** hydroxyflutamide derivatives (Fig. 2). These novel radiopharmaceuticals may not only enable future noninvasive-specific molecular imaging of AR-dependent prostate cancer using PET but also for image-guided treatment of prostate cancer.

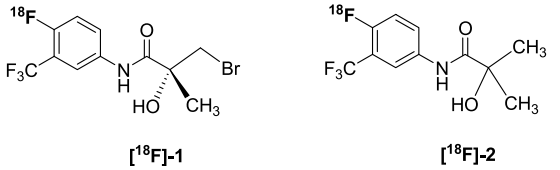

Figure 2. ^{18}F-radiolabeled nonsteroidal antiandrogen derivatives.

2. Results and discussion

Hydroxyflutamide is a symmetric nonchiral molecule ($K_i = 175$ nM);[17] however, substitution of a single hydrogen atom by halogen on one of the methyl groups of the hydroxyflutamide molecule, introduces an asymmetry, which results in the formation of a chiral center. Several reports have indicated the large differences in biological activity (AR-binding constants) of different stereoisomers of hydroxyflutamide derivatives such as bromine, fluorine, and iodine relative to flutamide and bicalutamide. It was found that the *R*-enantiomers of these derivatives would typically have much higher AR-binding constants than their *S* counterparts [(*R*)-3-bromo hydroxyflutamide $K_i = 0.3$ nM; (*S*)-3-bromo hydroxyflutamide, $K_i = 16.5$ nM] flutamide and bicalutamide (11 nM).[2] According to the present working hypothesis, it was assumed that radiolabeled ligands with higher AR-binding constants would produce better images, due to higher target affinity. On the basis of this assumption, a radiochemical transformation was designed to synthesize the (*R*)-enantiomer of 3-[^{18}F]fluoro-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)-phenyl)propanamide [^{18}**F**]-**6** in a one-step radiosynthesis (Fig. 4). Since the nonlabeled fluorine derivative only served as the chromatographic reference standard for the identification of the labeled analog by HPLC, it was prepared as a racemic mixture by a three-step synthesis (Fig. 3), starting with biphasic reaction of fluoroacetone solution in diethyl ether with aqueous NaCN to form the corresponding cyanohydrin **3**[18] with a 50% yield (Fig. 3). Refluxing of **3** in concentrated HCl produced (±)-3-fluoro-2-hydroxy-2-methylpropanoic acid

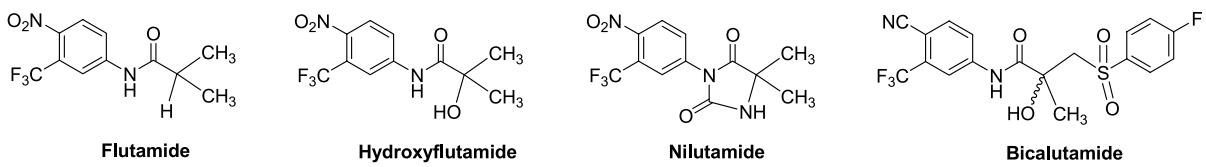

Figure 1. Nonsteroidal antiandrogens.

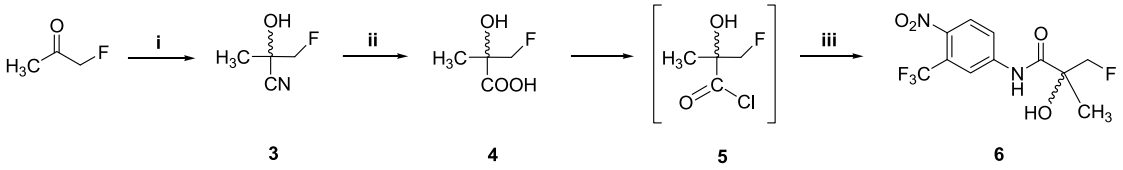

Figure 3. Synthesis of compound **6**. Reagents and conditions: (i) $NH_4Cl_{(q)}$, $NaCN_{(aq)}$, diethyl ether, 10 °C; (ii) concd $HCl_{(aq)}$, reflux, 3 h; (iii) thionyl chloride, dimethylacetamide; 4-nitro-3-(trifluoro-methyl)benzenamine, −12 °C.

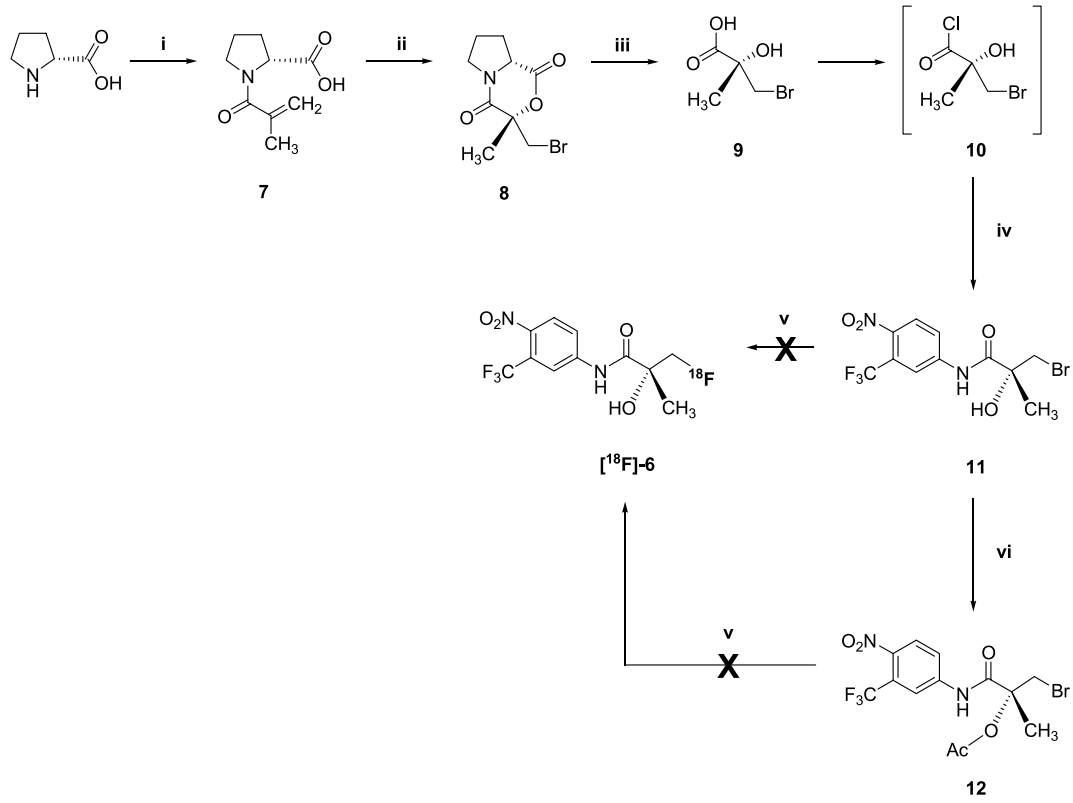

Figure 4. First attempt to synthesize [^{18}F]-radiolabeled hydroxyflutamide derivative [^{18}F]-6. Reagents and conditions: (i) methacryloyl chloride, $NaOH_{(aq)}$, acetone, 0 °C; (ii) *N*-bromosuccinimide, CCl_4, DMF, 0 °C; (iii) 24% $HBr_{(aq)}$, 105 °C; (iv) thionyl chloride, dimethylacetamide; 4-nitro-3-(trifluoro-methyl)benzenamine, −12 °C; (v) K[^{18}F]F·Kryptofix complex, DMSO, 110 °C; (vi) acetic anhydride, dimethylaminopyridine, pyridine, 35 °C.

4[18] with a 32% yield similar to the reported procedure. Attempts to improve the yield of this latter step by using different acids such as H_2SO_4 or H_3PO_4, or under other temperature conditions, resulted in even lower yields. In the third step, the coupling process was performed via in situ generation of acyl chloride **5** that was obtained by the reaction of hydroxy acid **4** with thionyl chloride in dry dimethylacetamide at −12 °C. Formation of compound **6** was found to be sensitive to the reaction temperature. Optimization of this parameter showed that the highest yield of the product of 45% was obtained when the reaction temperature was kept within −12 ± 5 °C range.

Compound **11**[19] and its derivatives served as precursors in attempts to radiolabel the (*R*)-enantiomer of 3-[^{18}F]fluoro-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoro-methyl)phenyl)propanamide [**^{18}F**]-**6** (Fig. 4). On the basis of the published procedures, pure (*R*)-enantiomer of compound **11** was prepared in four steps (Fig. 4). The required chirality was defined by utilizing D-Proline as a chiral auxiliary.[19] The synthesis was started by coupling of methacryloyl chloride with D-Proline in 2.0 M NaOH aqueous solution in acetone at 0 °C.[20] It was found that the use of freshly distilled methacryloyl chloride and careful control of pH conditions within a 10.3 ± 0.3 range are prerequisites for this reaction and the desired (*R*)-1-(2-methylacryloyl)pyrrolidine-2-carboxylic acid **7**[19] was isolated with a 81% yield. In the following cyclization step, substantial improvement in the yield of (3*R*,8α*R*)-3-(bromomethyl)-3-methyl-tetrahydro-3*H*-pyrrolo[2,1-*c*][1,4]oxazine-1,4-dione **8**[19] (from 64% to 96%) was achieved by using a light-protected reaction vessel and a mixture of dry CCl_4 and DMF (4:5) as the solvent. Compound **8** was then hydrolyzed in aqueous HBr at 105 °C to the corresponding hydroxy acid **9**[19] with a 84% yield. Conversion of **9** into (*R*)-3-bromo-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoro-methyl)phenyl)-propanamide **11**[19] was achieved by reacting in situ generated acyl chloride **10** with 4-nitro-3-(trifluoromethyl)-benzenamine in dry

dimethylacetamide at −12 °C. Compound **11** was obtained with a 45% yield after silica gel chromatography followed by selective precipitation from a diethyl ether/hexanes solution.

Attempts to substitute the bromine of compound **11** with [^{18}F]fluoride ion were unsuccessful and only H[^{18}F]F gas was detected. It was proposed that under fluorination conditions, the hydroxyl group of compound **11** would undergo deprotonation. This would interfere with the labeling reaction since it would capture most of the fluoride ions. Thus, the acetyl protecting group was introduced by reacting **11** with acetic anhydride and dimethylaminopyridine in dry pyridine. Similarly, the protected precursor, compound **12**, did not produce any fluorination products and therefore, it was concluded that the bromide atom should be replaced by a much better leaving group. Several good leaving groups, based on sulfonyl moiety, are frequently used in ^{18}F-radiopharmaceutical precursors, among them being the nosyl, tosyl, and triflate groups. Compound **12** was subjected to a series of reactions where silver salts of triflate, nosylate, and tosylate in dry acetonitrile or THF were used, under light-protecting conditions (Fig. 5). Due to the expected reactivity of sulfonyl derivatives, a preliminary analysis of crude reaction mixtures was performed by ^{1}H, ^{13}C NMR, and MS, immediately after silver bromide filtration and evaporation of the reaction solvent. It was found that reaction of **12** with silver triflate furnished the epoxide **13**;[20] reaction with silver nosylate in dry THF at 60 °C furnished the (*S*)-3-hydroxy-2-methyl-1-(4-nitro-3-(trifluoro-methyl)phenylamino)-1-oxopropan-2-yl acetate **14**. The only desired derivative that could be obtained with a 39% yield, following RP-C_{18} column chromatography, was the acetyl-protected tosylate derivative **15**, by the reaction of **12** and silver tosylate (Fig. 5).

Attempts to convert precursor **15** to the target compound [**^{18}F**]-**6** included a two-step reaction, in which **15** was reacted with K[^{18}F]F·Kryptofix complex in dry DMSO at 110 °C, followed by treatment with 1.0 M aqueous HCl at 105 °C (Fig. 6). The radio-HPLC analysis of the products at the end of the reaction with [^{18}F]fluoride indicated formation of low quantities of compound [**^{18}F**]-**16** probably because of (a) the tosyl group is not a sufficient leaving group (b) The amide N—H is also acidic because the electronegativity associated with the aromatic ring also undergoes deprotonation that interferes with the labeling reaction by capturing some of the fluoride ions. However, acid hydrolysis did not produce the desired product [**^{18}F**]-**6**. Increasing the temperature of the hydrolysis step above 110 °C resulted in the formation of side products, most probably due to the hydrolysis of the amide bond. It was then suggested that a better leaving and labile protecting groups may produce better results.

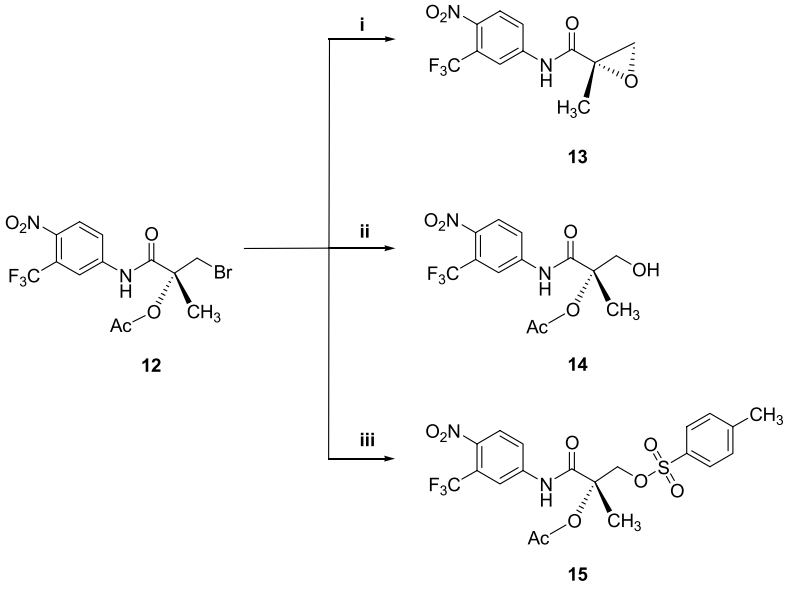

Figure 5. Synthesis of compound **15**. Reagents and conditions: (i) silver triflate, acetonitrile, 60 °C; (ii) silver nosylate, THF, 60 °C; (iii) silver tosylate, acetonitrile, 60 °C.

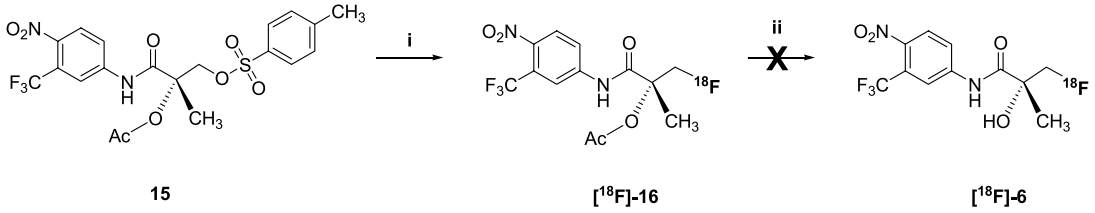

Figure 6. Attempt to convert potential precursor **15** to compound [**^{18}F**]-**6**. Reagents and conditions: (i) K[^{18}F]F·Kryptofix complex, DMSO, 110 °C; (ii) 1.0 M $HCl_{(aq)}$, 105 °C.

Following a search for a better protecting group that could be easily removed, the O-BOC derivative **18** was selected.[21] Compound **11** was treated with $(BOC)_2O$ under various conditions. Figure 7 and Table 1 present the results obtained during the development of suitable conditions for the synthesis of the target BOC-derived intermediate **18**.

Reaction of **11** with $(BOC)_2O$, dimethylaminopyridine, and pyridine in acetonitrile at 40 °C produced exclusively epoxide **13**.[3] Replacing the media to a less polar solvent such as toluene resulted in the formation of lactam **17**, as a major product. The changes in products obtained under the different conditions could be described with two different reaction mechanisms (Fig. 8). With acetonitrile, the formation of epoxide **13** was derived from a base-assisted intramolecular attack of the hydroxyl group on the methylenebromide moiety. On the other hand, with toluene, due to intramolecular hydrogen bonds,[19,22] the preferred conformation of **11** was placing the anilide nitrogen toward the methylene group and enabling intramolecular ring closure to form lactam **17**. These suggested mechanisms are supported by the work of Morris and co-workers[23] who performed an infrared spectroscopic study of hydroxyflutamide derivatives and related compounds. By examining the intramolecular hydrogen bonding in these types of molecules, they concluded that a single dominant conformation could have an important influence on the ability of the OH group to act as a hydrogen bond donor. Moreover, these proposed mechanisms are also supported by ^1H NMR measurements of compound **11** in CD_3CN and C_6D_6. Substantial changes in chemical shifts, from 4.69 ppm (in CD_3CN) to 2.30 ppm (in C_6D_6) of the OH proton in compound **11,** are indicative that with acetonitrile, intramolecular hydrogen bonds are disrupted by the solvent, making the proton of the OH group to be more acidic (Table 2).

Performing the reaction in the absence of dimethylaminopyridine produced a mixture of products from which the desired BOC derivative **18** was isolated with a 25% yield (Fig. 7). Upon increasing the temperature of the latter process to 60 °C, a substantial reduction in the yield of the desired product was observed. Attempts to obtain the nosylate derivative of **18** by reaction with silver nosylate in dry acetonitrile at room temperature and up to 40 °C resulted in the recovery of the starting material, while increased temperatures led to the decomposition of **18**. It was suggested that since the BOC group was too bulky, it may have blocked the S_N2 attack on the methylenebromide moiety.

At that point, the strategy was reevaluated and another position for incorporating the fluorine atom was selected. Specifically, the labeling was performed on

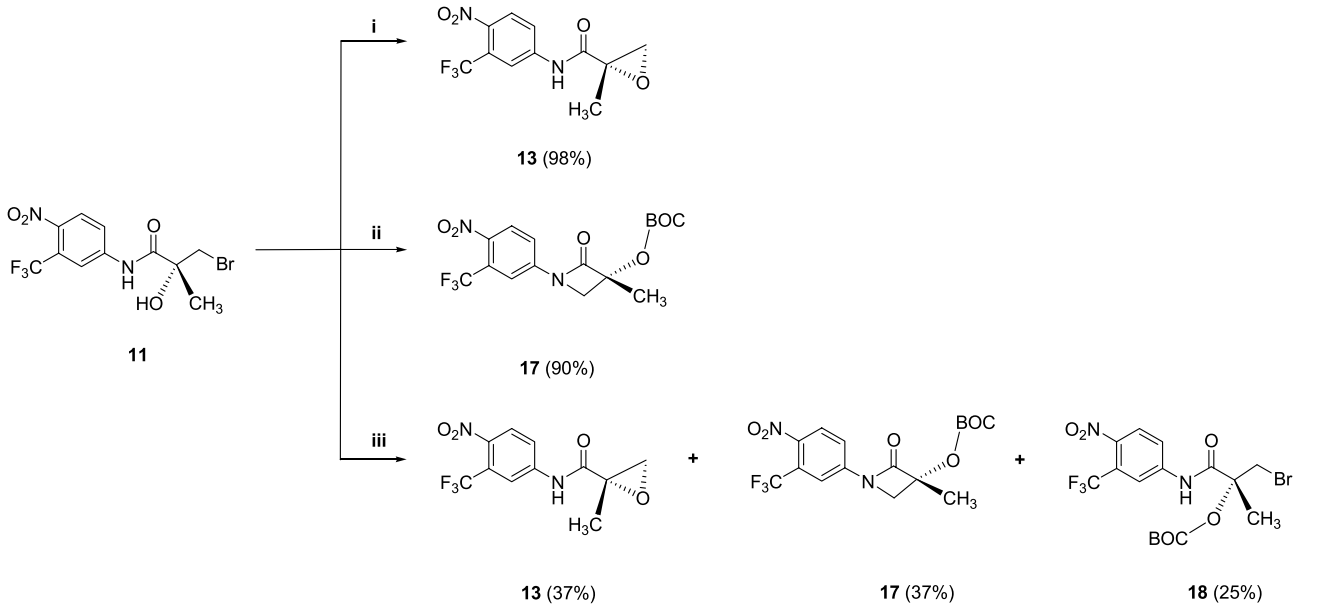

Figure 7. Reaction of compound **11** with $(BOC)_2O$ under various conditions. Reagents, specific conditions and obtained product yields are presented in Table 1.

Table 1. Changes in product distribution due to variations in reaction conditions of **11** with $(BOC)_2O$. Indicated yields of products correspond to the actual isolated yields of the products

	Solvent	Amount of base	Amount of $(BOC)_2O$ (equiv)	Temperature (°C)	Yield of **13** (%)	Yield of **17** (%)	Yield of **18** (%)
i.	Acetonitrile	0.25 equiv DMAP + 1.50 equiv pyridine	2.0	40	98	0	0
ii.	Toluene	0.25 equiv DMAP + 1.50 equiv pyridine	5.0	40	<2	90	<2
iii.	Toluene	1.50 equiv pyridine	5.0	40	37	37	25

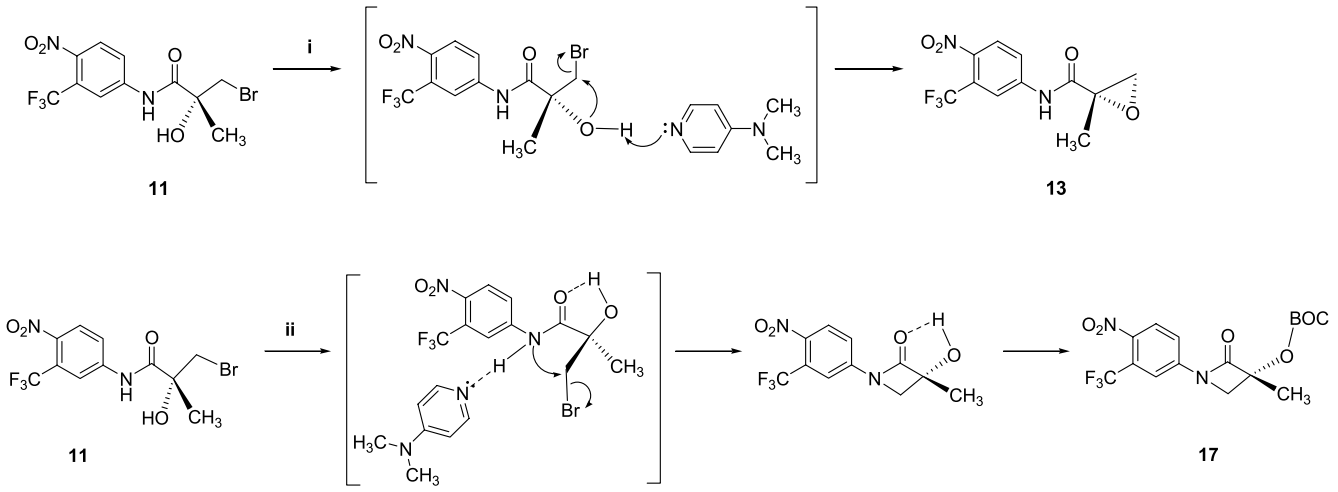

Figure 8. Proposed reaction mechanisms of the conversion of **11** to: (i) **13** in acetonitrile and (ii) **17** in toluene.

Table 2. Chemical shifts in ^{1}H NMR spectra of NH and OH protons of compound **11** and of flutamide in CD_3CN and C_6D_6

Solvent	O*H* (**11**) δ	N*H* (**11**) δ	N*H* (flutamide) δ
CD_3CN	4.69	9.87	10.16
C_6D_6	2.30	8.36	10.12

the aromatic ring replacing the nitro group to form new labeled derivatives of hydroxyflutamide [**^{18}F**]-**1** and [**^{18}F**]-**2** (Fig. 2). On the basis of the published studies, these derivatives should also have high affinity to the AR.[1] Nonlabeled chromatographic standards **1** and **2** were synthesized. Compound **9** and 2-hydroxy-2-methylpropanoic acids were used as starting materials for the syntheses of compounds **1** and **2**, under similar conditions described above, and were obtained with 56% (**1**) and 61% (**2**) isolated yields.

Compound **19** served as the key starting material for the radiosynthesis of compounds [**^{18}F**]-**1** and [**^{18}F**]-**2** (Fig. 9). It could be obtained by oxidation of the commercial 4-nitro-3-(trifluoromethyl)-aniline, using various oxidation methods, such as 3-chloro-peroxybenzoic acid[24] or combination of hydrogen peroxide and trifluoroacetic anhydride.[25] Both of these methods resulted with very low conversions (20%). The final method used for oxidation was with the HOF·CH_3CN complex in aqueous acetonitrile[26] which furnished **19** with a 95% yield and a synthesis time of 5 min. Compound **19** was reacted with the K[^{18}F]F·Kryptofix complex in DMSO, using a conventional microwave oven heating. [^{18}F]fluoride S_N2 nucleophilic attack took place on the most reactive elecrophilic aromatic carbon attached to the nitro group and α to the trifluoromethyl group[27] as analyzed by cold standards coinjection to the HPLC. The corresponding fluorine-18-labeled derivative [**^{18}F**]-**20** was obtained exclusively with a 60% radiochemical yield. The second step of the radiosynthesis was the reduction of the nitro residue to the corresponding amino [**^{18}F**]-**21**, with a 50% radiochemical yield, using Raney nickel and hydrazine hydrate at 55 °C.[27] The third and final step was coupling of compound **9** and 2-hydroxy-2-methylpropanoic acid chlorides[28] with the labeled amino moiety (30% radiochemical yield) to give the labeled hydroxyflutamide derivatives [**^{18}F**]-**1** and [**^{18}F**]-**2** with an overall decay corrected yield of 10 ± 3% (end of synthesis), a total synthesis time of 4 h and SA of 1500 ± 200 Ci/mmol EOB (determined by HPLC and calibration curve with nonlabeled standard; $n = 10$).

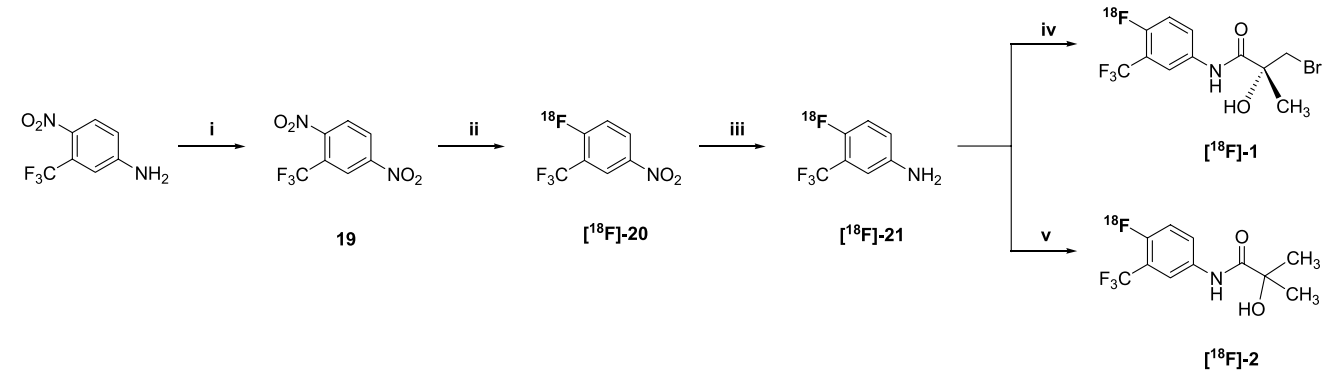

Figure 9. Radiosynthesis of [^{18}F]-radiolabeleld hydroxyflutamide derivatives [**^{18}F**]-**1** and [**^{18}F**]-**2**. Reagents and conditions: (i) HOF·$CH_3CN_{(aq)}$, acetonitrile, dichloromethane, 0 °C; (ii) K[^{18}F]F·Kryptofix complex, DMSO, microwave; (iii) Raney nickel, hydrazine hydrate; (iv) compound **9**, oxalyl chloride, dimethylformamide, 0 °C; (v) 2-hydroxy-2-methylpropanoic acid, oxalyl chloride, dimethylformamide, 0 °C.

3. Conclusions

The objective of the described work was to develop radiosynthetic methodologies for the preparation of novel ^{18}F-containing hydroxyflutamide derivatives, with potential affinity for the androgen receptor. These compounds may present a better alternative to radiolabeled steroid-based androgen ligands for noninvasive molecular imaging of AR-dependent prostate cancer using PET. This developed methodology for nonsteroidal anilide-type ^{18}F-labelled radiopharmaceuticals can serve as a platform for the preparation of a wide spectrum of specific prostate cancer PET imaging agents and may lead to image-guided treatment of prostate cancer.

4. Experimental

4.1. General methods

All operations with air- and moisture-sensitive compounds were performed by the Schlenk techniques under argon atmosphere. All solvents were of analytical grade or better. Toluene and THF were distilled over sodium/benzophenone; other solvents were purchased as anhydrous. ^{1}H and ^{13}C NMR spectra were recorded on 200 or 400 MHz spectrometers in $CDCl_3$ or DMSO-d_6. ^{1}H and ^{13}C NMR signals are reported in ppm. ^{1}H NMR signals are referenced to the residual proton (7.26 ppm for $CDCl_3$ or 2.50 ppm for DMSO-d_6) of a deuterated solvent and for ^{13}C NMR spectra, the signal of $CDCl_3$ (77.16 ppm) or DMSO-d_6 (39.52 ppm) was used as a reference. ^{13}C NMR spectra interpretations were supported by DEPT experiments. Mass spectra were obtained on a spectrometer equipped with CI, EI, and FAB probes and on a spectrometer equipped with ESI probe. HRMS results were obtained on MALDI-TOF and ESI mass spectrometers. IR spectra were recorded on FTIR spectrometer. Optical activity of the chiral molecules was measured in a polarimeter equipped with optical rotation 1 dc cell in CH_2Cl_2 or DMSO solutions. The progress of reactions was monitored by TLC (SiO_2) and visualized by UV light or developed with vanillin spray (1.0 g in 95.0 mL ethanol, 5.0 mL water, and 1.0 mL concentrated H_2SO_4) or with iodine chamber. Flash chromatography was carried out on SiO_2 (0.04–0.063 mm). Microwave heating was performed in a conventional oven operating at 500 W (full power). HPLC was performed on system with variable wavelength detector operating at 254 nm, unless otherwise noted, and with a radioactivity detector with a NaI crystal. Two systems were used: a reverse phase system employing C-18 column (5 μm, 250× 10 mm) and 35% CH_3CN/65% H_2O as eluent, at flow rate of 3.5 mL/min. Fractions of 4.0 mL were collected. Analysis of formulated radiotracer was performed on a reversed phase system using a C-18 column (10 μm, 300× 3.9 mm) and 40% CH_3CN/60% H_2O as solvents at a flow rate of 1 mL/min. The selected eluent fractions were diluted with water, loaded onto an activated (EtOH) and equilibrated (water) C-18 Sep-Pak (classic, short body). The cartridge was washed with 10% EtOH and 90% saline.

4.1.1. (±)-3-Fluoro-2-hydroxy-2-methylpropanenitrile (3). To a stirred solution of NH_4Cl (16.0 g, 299 mmol) in water (45 mL), a solution of fluoroacetone (17 mL, 236 mmol) in diethyl ether (50 mL) at 10 °C was added. Then, to the resulting emulsion, a solution of NaCN (13.0 g, 265 mmol) in water (30 mL) was added and the reaction mixture was stirred at room temperature for 10 h. After that time, organic and aqueous phases were separated, the aquoeus phase was extracted with diethyl ether (3× 200 mL), and ethereal extracts were combined with organic phase and dried over $MgSO_4$. Evaporation of organic solution gave **3** as a yellow oil (12.13 g, 50%) that was used further without purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.47 (d, 3H, J = 2 Hz), 4.35, 4.46 (ABq, 2H, J = 2.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.22 (d, J = 9 Hz), 66.75 (d, J = 19 Hz), 86.44 (d, J = 178 Hz), 121.06 (d, J = 3.5 Hz). ^{19}F NMR (376 MHz, $CDCl_3$): δ −129.53 (dd, J_{H-F} = 54 .41 Hz). IR (CH_2Cl_2): 3562, 2980, 1601, 1045, 885/cm.

4.1.2. (±)-3-Fluoro-2-hydroxy-2-methylpropanoic acid (4). A stirred suspension of **3** (12.0 g, 116 mmol) in concentrated aqueous HCl (30 mL) was refluxed for 3 h. After cooling to room temperature, a white precipitate was filtered out and washed with cold ether to give **4** as a white solid (4.59 g, 32%). Mp 97.0–98.6 °C (lit.[18] 99–100 °C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23 (d, 3H, J = 2 Hz), 4.38 (ddd, 2H, J = 57, 48, 9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.51 (d, J = 18 Hz), 73.20 (d, J = 18 Hz), 87.72 (d, J = 173 Hz), 174.02 (d, J = 4.5 Hz). ^{19}F NMR (376 MHz, DMSO-d_6): δ −131.98 (ddd, J = 722, 278, 55 Hz). HR-MS (CI): m/z 123.0 (MH^+, 100); m/z calcd for $C_4H_8FO_3$ (MH^+): 123.0457. Found: 123.0452. IR (KBr): 3109, 2933, 1753, 1157, 815/cm.

4.1.3. (±)-3-Fluoro-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)phenyl)propanamide (6). To a solution of **4** (4.59 g, 37.62 mmol) in dry dimethylacetamide (44 mL), thionyl chloride (3.45 mL, 48 mmol) was added dropwise at −12 °C under Ar. At the same temperature, the resulting mixture was stirred for 3 h and then a solution of 4-nitro-3-(trifluoromethyl)benzenamine (7.75 g, 38 mmol) in dry dimethylacetamide (51 mL) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for additional 6 h. After dimethylacetamide evaporation, the resulting orange oil was purified by flash chromatography (SiO_2; CH_2Cl_2; R_f = 0.3) to give crude **6** as a yellow solid that was further purified by selective precipitation from diethyl ether/hexanes to yield pure **6** as a light-yellow solid (5.25 g, 45%). Mp 99.8–101.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71 (d, 3H, J = 2 Hz), 4.70 (dd, 1H, J = 48, 10.5 Hz), 4.81 (dd, 1H, J = 48, 10.5 Hz), 8.03 (dd, 1H, J = 8.5, 12 Hz), 7.07 (d, 1H, J = 8.5 Hz), 8.14 (d, 1H, J = 2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.21 (d, J = 4.5 Hz), 83.12 (d, J = 180 Hz), 85.18 (d, J = 18.5 Hz), 121.44 (q, J = 272 Hz), 124.32, 126.41, 1344.77, 1447.05, 151.63, 170.71 (d, J = 3.5 Hz). HRMS (MALDI-TOF): m/z 133.03 (MNa^+, 100); m/z calcd for $C_{11}H_{11}N_2O_4F_4$ (MH^+): 311.0649. Found: 311.0650.

IR (KBr): 3447, 1735, 1032, 519/cm. UV–vis (MeCN): λ_{max} 201, 253 nm.

4.1.4. (*R*)-1-(2-Methylacryloyl)pyrrolidine-2-carboxylic acid (7). To a solution of D-proline (50.0 g, 434 mmol) in 2.0 M aqueous NaOH (240 mL) and acetone (240 mL), a solution of methacryloyl chloride (32 mL, 443 mmol) in acetone (240 mL) was added dropwise at 0 °C. During the addition, the reaction pH was monitored and kept within 10.3 ± 0.3 range via simultaneous dropwise addition of 2.0 M aqueous NaOH. After the end of addition, the reaction mixture was allowed to warm up to room temperature and stirred for additional 4 h. Following the evaporation of acetone, the mixture was acidified to pH 2 with concentrated HCl and NaCl was added till saturation. The resulting solution was extracted with ethyl acetate (3× 250 mL) and combined organic extracts were dried over $MgSO_4$. After solvent evaporation, the crude product was purified by crystallization (ethyl acetate/hexanes) to yield **7** as a white solid (64.05 g, 81%). Mp 103.5–104.5 °C (lit.[19] 102.5–103.5 °C). ^{1}H NMR (200 MHz, DMSO-d_6) [Major rotamer]: δ 1.94 (s, 3H), 1.90–2.10 (m, 2H), 2.25–2.40 (m, 2H), 3.35–3.55 (m, 2H), 4.32–4.35 (m, 1H), 5.20, 5.33 (ABq, 2H, J = 12.0 Hz); [Minor rotamer]: δ 1.88 (s, 3H), 1.90–2.10 (m, 2H), 2.25–2.40 (m, 2H), 3.60–3.65 (m, 2H), 4.52–4.55 (m, 1H), 5.20–5.33 (ABq, 2H, J = 24 Hz). ^{13}C NMR (50 MHz, DMSO-d_6) [Major rotamer]: δ 19.53, 24.72, 28.91, 48.77, 58.25, 116.44, 140.83, 169.13, 173.27; [Minor rotamer]: δ 19.66, 22.35, 30.97, 40.67, 60.25, 115.28, 141.60, 170.01, 174.04. MS (CI): m/z 184.1 (MH^+, 100). IR (KBr): 3509, 2960, 1734, 1587, 1458, 1174/cm.

4.1.5. (3*R*,8α*R*)-3-(Bromomethyl)-3-methyl-tetrahydro-3*H*-pyrrolo[2,1-*c*][1,4]oxazine-1,4-dione (8). To a solution of **7** (112.59 g, 615 mmol) in dry CCl_4 (320 mL) and dry DMF (400 mL), a solution of NBS (142.20 g, 800 mmol) in dry DMF (500 mL) was added dropwise in light-protected environment at 0 °C. After the end of addition, the reaction mixture was stirred at 0 °C for 2 h, allowed to warm up to room temperature and stirred for additional 48 h. Following the evaporation of CCl_4, saturated NaCl aqueous solution (700 mL) was added and the mixture was extracted with ethyl acetate (3× 400 mL). The combined organic extracts were washed with water (3× 200 mL) and dried over $MgSO_4$. After ethyl acetate evaporation, the crude product was purified by crystallization (CH_2Cl_2/hexanes) to yield **8** as a white solid (154.64 g, 96%). Mp 158.1–159.9 °C (lit.[19] 152–154 °C). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.67 (s, 3H), 1.85–2.10 (m, 2H), 2.31–2.43 (m, 2H), 3.48–3.69 (m, 2H), 3.96, 4.13 (ABq, 2H, J = 12.0 Hz), 4.81 (dd, 1H, J = 7.5, 9.5 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 21.63, 22.95, 29.01, 37.86, 45.47, 57.265, 83.92, 163.11, 167.33. MS (CI): m/z 262.0 (MH^+, 100). IR (KBr): 3861, 1744, 1686, 1449, 1061, 649/cm.

4.1.6. (*R*)-3-Bromo-2-hydroxy-2-methylpropanoic acid (9). A solution of **8** (10.0 g, 38.15 mmol) in 24% aqueous HBr (170 mL) was heated to 105 °C for 90 min. After cooling to room temperature, solid NaCl was added till saturation and the mixture was extracted with ethyl acetate (4× 200 mL). The combined ethyl acetate fractions were extracted with saturated $NaHCO_3$ aqueous solution (4× 200 mL), the resulting aqueous solution was acidified to pH 1, and then was extracted with ethyl acetate (4× 200 mL). The combined ethyl acetate extracts were dried over $MgSO_4$ and evaporated to yield **9** as a white solid (5.86 g, 84%). Mp 111.4–113.1 °C (lit.[19] 106.5–109.0 °C). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.47 (s, 3H), 3.62, 3.75 (ABq, 2H, J = 10.0 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 24.55, 41.02, 73.19, 174.60. MS (CI): m/z 182.9 (M^+, 25), 103.0 (M^+−Br, 100).

4.1.7. (*R*)-3-Bromo-2-hydroxy-2-methylpropanoyl chloride (10). A round bottom flask with a nitrogen flow connector was charged with compound **9** (40 mg, 0.22 mmol) in 2.0 mL of dry dichloromethane. To this solution, oxalyl chloride (38.5 μL, 0.44 mmol) in dry DMF (100 μL) was added under nitrogen at −5 °C to −10 °C. The resulting mixture was stirred for 3 h under the same conditions. The excess of oxalyl chloride and the dichloromethane were evaporated under vacuum. Compound **10** was used for the radiosynthesis without further purification.

4.1.8. (*R*)-3-Bromo-2-hydroxy-2-methyl-*N*-(4-nitro-3-(trifluoromethyl)phenyl)propanamide (11). To a solution of **9** (5.0 g, 27.32 mmol) in dry dimethylacetamide (32 mL), thionyl chloride (2.5 mL, 31.90 mmol) was added dropwise at −12 °C under Ar. At the same temperature, the resulted mixture was stirred for 3 h and then a solution of 4-nitro-3-(trifluoromethyl)benzenamine (5.63 g, 27.32 mmol) in dry dimethylacetamide (37 mL) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for additional 6 h. After dimethylacetamide evaporation, the crude orange oil was purified by flash chromatography (SiO_2; CH_2Cl_2; R_f = 0.3) to give a yellow wax that was further purified by selective precipitation from diethyl ether/hexanes solution to give **11** as a light-yellow solid (4.56 g, 45%). Mp 103.0–103.8 °C (lit.[19] 100.0–101.5 °C). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.50 (s, 3H), 3.60, 3.85 (ABq, 2H, J = 10.5 Hz), 6.43 (s, 1H), 8.21 (d, 1H, J = 9.0 Hz), 8.37 (dd, 1H, J = 2.0, 9.0 Hz), 8.57 (d, 1H, J = 2.0 Hz), 10.60 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.79, 41.28, 74.35, 118.21 (q, J = 6 Hz), 122.37, 122.98 (q, J = 33 Hz), 123.02, 127.15, 141.60, 142.89, 173.55. MS (CI): m/z 373.0 (MH^+, 7), 291.1 (MH^+−HBr, 100).

4.1.9. (*R*)-3-Bromo-2-methyl-1-(4-nitro-3-(trifluoromethyl)phenylamino)-1-oxopropan-2-yl acetate (12). To a solution of **11** (400 mg, 1.078 mmol) and DMAP (13 mg, 0.106 mmol) in dry pyridine (5.0 mL) acetic anhydride (310 μL, 3.279 mmol) was added by syringe. The mixture was heated at 35 °C under Ar for 10 h. After solvent evaporation, the crude yellow oil was purified by flash chromatography (SiO_2; ethyl acetate/hexanes, 2:3) to give **15** as a light-yellow oil (270 mg, 61%). $[\alpha]_D^{20}$ −17 (c 1.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.90 (s, 3H), 2.29 (s, 3H), 3.99, 4.31 (ABq, 2H, J = 11 Hz), 7.94 (d, 1H, J = 10 Hz), 8.02–8.03 (m, 2H), 8.43 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ

21.84, 21.97, 34.84, 82.85, 119.01 (q, $J = 6$ Hz), 120.40, 123.17, 125.19 (q, $J = 34$ Hz), 127.06, 141.18, 143.51, 168.62, 169.44. HR-MS (CI): *m/z* 413.0 (MH^+, 100); *m/z* calcd for $C_{13}H_{12}BrF_3N_2O_5$ (MH^+): 412.99241. Found: 412.99296. IR (CH_2Cl_2): 3411, 1753, 1532, 1170, 912/cm. UV–vis (MeCN): λ_{max} 224, 286 nm.

4.1.10. (*S*)-*N*-(3-(Trifluoromethyl)-4-nitrophenyl)-2-methyloxirane-2-carboxamide (13). To a solution of **11** (300 mg, 0.808 mmol) and DMAP (25 mg, 0.205 mmol) and dry pyridine (100 μL, 1.236 mmol) in dry acetonitrile (32 mL) heated to 40 °C under Ar, di-*tert*-butyl dicarbonate (372 μL) was added by syringe in one portion. The resulting yellow solution was stirred at 40 °C for 10 h. After solvent evaporation, the crude product was purified by chromatography (SiO_2; CH_2Cl_2/ethyl acetate/ hexanes, 18%:18%:64%; $R_f = 0.38$) to give **13** as a yellow oil (230 mg, 98%). $[\alpha]_D^{20}$ −36 (*c* 1.6, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85 (s, 3H), 3.73, 3.85 (ABq, 2H, $J = 12$ Hz), 8.02 (dd, 1H, $J = 9$, 2 Hz), 8.07 (d, 1H, $J = 9$ Hz), 8.12 (d, 1H, $J = 2$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.21, 33.60, 84.31, 123.96, 125.24 (q, $J = 34$ Hz), 126.46, 129.13, 134.64, 147.15, 151.50, 171.20. MS (ESI): *m/z* 289.20 ($M-H^+$, 100), 306.90 ($M+H_2O$, 100). IR (CH_2Cl_2): 3023, 2932, 1758, 1540, 1250, 418/cm. UV–vis (MeCN): λ_{max} 253 nm. Compound **13** was also obtained by reaction of **12** with silver triflate in acetonitrile at 60 °C.

4.1.11. (*S*)-3-Hydroxy-2-methyl-1-(4-nitro-3-(trifluoromethyl)phenylamino)-1-oxopropan-2-yl acetate (14). To a solution of **12** (77 mg, 0.187 mmol) in dry THF (15 mL), solid silver *p*-nitrobenzenesulfonate (64 mg, 0.208 mmol) was added in a light-protected environment at room temperature under Ar, the vessel was sealed and stirred at room temperature for 10 h. After filtration of silver bromide and solvent evaporation, the crude brown wax was purified by flash chromatography (SiO_2; ethyl acetate/hexanes, 2:3) to give **14** as a yellow solid (62 mg, 95%). Mp 116.5–117.1 °C. $[\alpha]_D^{20}$ −15 (*c* 2.3, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (s, 3H), 2.16 (s, 3H), 4.43, 4.51 (ABq, 2H, $J = 12$ Hz), 8.03 (d, 1H, $J = 9$ Hz), 8.07 (dd, 1H, $J = 9$, 2 Hz), 8.13 (d, 1H, $J = 2$ Hz), 9.22 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.96, 23.55, 29.88, 69.86, 118.38 (q, $J = 6$ Hz), 122.18, 127.26, 141.53, 172.20, 172.93. MS (CI): *m/z* 351.1 (MH^+, 100). HR-MS (CI): *m/z* calcd for $C_{13}H_{14}F_3N_2O_6$ (MH^+): 351.080396. Found: 351.080649. IR (KBr): 3487, 3316, 2928, 1526, 1142, 807/cm. UV–vis (MeCN): λ_{max} 198, 227, 294 nm.

4.1.12. (*S*)-2-Methyl-1-(4-nitro-3-(trifluoromethyl)phenylamino)-1-oxo-3-(tosyloxy)propan-2-yl acetate (15). To a solution of **12** (77 mg, 0.187 mmol) in dry THF (15 mL) solid silver *p*-toluenesulfonate (78 mg, 0.280 mmol) was added in a light-protected environment at room temperature under Ar, the vessel was sealed and heated at 65 °C for 10 h. After filtration of silver bromide and solvent evaporation, the crude brown wax was purified by flash chromatography (RP-C_{18}; CH_3CN) to give **15** as a yellow solid (37 mg, 39%). Mp 144–146 °C. $[\alpha]_D^{20}$ −4 (*c* 0.6, MeOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.73 (s, 3H), 2.23 (s, 3H), 2.44 (s, 3H), 4.31, 4.83 (ABq, 2H, $J = 11$ Hz), 7.31 (d, 2H, $J = 8$ Hz), 7.71 (d, 2H, $J = 8$ Hz), 7.94–7.99 (m, 3H), 8.42 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.77, 19.98, 22.44, 70.67, 82.41, 119.45, 123.39, 127.51, 128.45, 130.62, 132.75, 141.51, 146.16, 169.03, 169.41. MS (CI): *m/z* 505.10 (MH^+, 15). UV–vis (MeCN): λ_{max} 290 nm.

4.1.13. *tert*-Butyl-(*S*)-1-(3-(trifluoromethyl)-4-nitrophenyl)-3-methyl-2-oxoazetidin-3-yl carbonate (17). To a solution of **11** (300 mg, 0.808 mmol) and DMAP (25 mg, 0.205 mmol) and dry pyridine (100 μL, 1.236 mmol) in dry toluene (32 mL) heated to 40 °C under Ar, di-*tert*-butyl dicarbonate (930 μL) was added by syringe in one portion. The resulting yellow solution was stirred at 40 °C for 10 h. After solvent evaporation, the crude product was purified by chromatography (SiO_2; CH_2Cl_2/ethyl acetate/hexanes, 18%:18%:64%; $R_f = 0.54$) to give **17** as a yellow solid (284 mg, 90%). Mp 142–145 °C. $[\alpha]_D^{20}$ +24 (*c* 2.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51 (s, 9H), 1.79 (s, 3H), 3.84, 4.20 (ABq, 2H, $J = 3$ Hz), 7.69 (dd, 1H, $J = 1$, 4.5 Hz), 7.75 (d, 1H, $J = 1$ Hz), 8.00 (d, 1H, $J = 4.5$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.70, 27.68, 52.97, 84.46, 115.59 (q, $J = 5.5$ Hz), 119.69, 125.81 (q, $J = 34$ Hz), 127.50, 141.49, 143.05, 151.448, 165.27. HRMS (MALDI-TOF): *m/z* 413.08 (MNa^+, 80); *m/z* calcd for $C_{16}H_{17}N_2O_6F_3Na$ (MNa^+): 413.0936. Found: 413.0812. IR (KBr): 2971, 1766, 1576, 1136, 826/cm. UV–vis (MeCN): λ_{max} 225, 307 nm.

4.1.14. (*R*)-2-(3-(Trifluoromethyl)-4-nitrophenylcarbamoyl)-1-bromopropan-2-yl-*tert*-butyl carbonate (18). To a solution of **11** (300 mg, 0.808 mmol) in dry pyridine (100 μl, 1.236 mmol) and dry toluene (32 mL) heated to 42 °C under Ar, di-*tert*-butyl dicarbonate (930 μL) was added by syringe in one portion. The resulting yellow solution was stirred at 42 °C for 10 h. After solvent evaporation, the crude product was purified by chromatography (SiO_2; CH_2Cl_2/ethyl acetate/hexanes, 18%:18%:64%; $R_f = 0.58$. Under these chromatographic conditions R_f of **11** = 0.17, R_f of **13** = 0.38 and R_f of **17** = 0.54) to give **18** as a yellow oil (95 mg, 25%). $[\alpha]_D^{20}$ −4 (*c* 2.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 9H), 1.87 (s, 3H), 3.99, 4.33 (ABq, 2H, $J = 11$ Hz), 7.99 (d, 1H, $J = 9$ Hz), 8.04 (dd, 1H, $J = 9$, 2 Hz), 8.07 (d, 1H, $J = 2$ Hz), 8.61 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.80, 27.26, 57.99, 79.98, 84.65, 118.73, 122.76, 124.10, 126.48, 129.15, 140.60, 143.31, 14.74, 149.49, 150.57, 168.71. HRMS (MALDI-TOF): *m/z* 493.02 (MNa^+, 100); *m/z* calcd for $C_{16}H_{18}N_2O_6F_3$-NaBr (MNa^+): 493.0192. Found: 493.0151. IR (CH_2Cl_2): 3401, 2929, 1754, 1533, 1153, 811/cm. UV–vis (MeCN): λ_{max} 290 nm.

4.1.15. (*R*)-3-Bromo-*N*-(4-fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (1). To a solution of **9** (1.0 g, 5.464 mmol) in dry dimethylacetamide (10 mL), thionyl chloride (0.5 mL, 6.892 mmol) was added dropwise at −12 °C under Ar. At the same temperature, the resulted mixture was stirred for 3 h and then 4-fluoro-3-(trifluoromethyl)benzenamine (0.8 mL, 6.222 mmol) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for

additional 16 h. After dimethylacetamide evaporation, the crude orange oil was purified by flash chromatography (SiO_2; CH_2Cl_2; $R_f = 0.2$). Compound **1** was obtained as a white solid (1.04 g, 56%). Mp 103.0–104.5 °C. $[\alpha]_D^{20}$ −28 (*c* 0.5, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ 1.66 (s, 3H), 3.39 (s, 1H), 3.63, 4.04 (ABq, 2H, $J = 10.5$ Hz), 7.21 (t, 1H, $J = 9.0$ Hz), 7.77 (dt, 1H, $J = 9.0$, 4.0 Hz), 7.92 (dd, 1H, $J = 6.0$, 4.0 Hz), 8.85 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.91, 41.41, 75.44, 117.49, 117.65 (d, $J = 22$ Hz), 118.84 (q, $J = 5.0$ Hz), 122.35 (q, $J = 122.0$ Hz), 125.27 (d, $J = 8.0$ Hz), 133.28, 156.40 (d, $J = 253.0$ Hz), 171.48. ^{19}F NMR (376 MHz, $CDCl_3$): δ −119.34, −61.93. MS (CI): *m*/*z* 344.1 (MH^+, 100), 343.1 (M^+, 20). HRMS (MALDI-TOF): *m*/*z* calcd for $C_{11}H_{10}NO_2F_4NaBr$ (MNa^+): 365.9723. Found: 365.9692. IR (KBr): 3333, 2928, 1674, 1513, 1133, 609/cm. UV–vis (MeCN): λ_{max} 283, 241 nm.

4.1.16. *N*-(4-Fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide (2). To a solution of 2-hydroxy-2-methylpropanoic acid (1.5 g, 14.408 mmol) in dry dimethylacetamide (35 mL), thionyl chloride (1.3 mL, 17.920 mmol) was added dropwise at −12 °C under Ar. At the same temperature, the resulted mixture was stirred for 3 h and then 4-fluoro-3-(trifluoromethyl)benzenamine (1.90 mL, 14.76 mmol) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for additional 16 h. After dimethylacetamide evaporation, the crude orange oil was purified by flash chromatography (SiO_2; CH_2Cl_2; $R_f = 0.1$). Compound **2** was obtained as a light yellow solid (2.327 g, 61%). Mp 93.3–94.9 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 1.58 (s, 6H), 2.79 (s, 1H), 7.19 (t, 1H, $J = 9.5$ Hz), 7.75 (dt, 1H, $J = 4.0$, 9.0 Hz), 7.93 (dd, 1H, $J = 3.0$, 6.0 Hz), 8.91 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.89, 75.40, 118.41 (d, $J = 22$ Hz), 119.44 (q, $J = 6$ Hz), 123.33 (q, $J = 271$ Hz), 125.87 (d, $J = 8$ Hz), 134.73 (d, $J = 3.5$ Hz), 157.10 (d, $J = 250$ Hz), 175.77. ^{19}F NMR (376 MHz, $CDCl_3$): δ −120.08, −61.94. MS (CI): *m*/*z* 266.2 (MH^+, 100). HRMS (CI): *m*/*z* calcd for $C_{11}H_{12}NO_2F_4$ (MH^+): 266.080417. Found: 266.080291. IR (KBr): 3291, 2926, 1668, 1134, 671/cm. UV–vis (MeCN): λ_{max} 284, 241 nm.

4.1.17. 1,4-Dinitro-2-(trifluoromethyl)benzene (19). A solution of 3-(trifluoromethyl)-4-nitrobenzenamine (1.0 g, 4.854 mmol) in CH_2Cl_2 (50 ml) was added to a solution of HOF[26] in aqueous CH_3CN (0.4 M, 24 mL) at 0 °C. The reaction mixture was stirred for 5 min. Evaporation gave **19** as a yellow solid (1.145 g, 95%). Mp 35.0–36.0 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 8.46 (d, 1H, $J = 9$ Hz), 8.69 (d, 1H, $J = 2.5$ Hz), 8.77 (dd, 1H, $J = 9$, 2.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 119.80, 122.37–122.71 (m), 123.80 (q, $J = 5$ Hz), 127.36, 129.95, 149.18, 150.17. ^{19}F NMR (376 MHz, DMSO-d_6): δ −59.99. MS (CI): *m*/*z* 237.1 (MH^+, 25). IR (KBr): 3102, 1550, 1294, 1172, 839/cm. UV–vis (MeCN): λ_{max} 248 nm.

4.1.18. K[^{18}F]F·Kryptofix 2.2.2 complex. [^{18}F]Fluoride ion was produced by the $^{18}O(p,n)$ ^{18}F nuclear reaction on 2 mL enriched [^{18}O]water (95% isotopic purity) as a target at the Hadassah-Hebrew University IBA 18/9 cyclotron and then transferred into a fluorination module by a flow of argon. After trapping, it was loaded on an anion exchange column, dried, eluted with 1 mL of K_2CO_3 solution (2.76 mg/mL), and transferred to the reactor and finally to the collecting vial. Reactive organic [^{18}F]fluoride ion was prepared by adding 50–100 μL of the K[^{18}F]F solution to Kryptofix 2.2.2 (1.0 mg, 2.7 μmol) in acetonitrile. Azeotropic removal of water and acetonitrile was achieved by heating under a stream of nitrogen. The dried K[^{18}F]F·Kryptofix 2.2.2 complex was then dissolved in 300 μL anhydrous DMSO for use in the radiolabeling.

4.1.19. (*R*)-3-Bromo-*N*-(4-[^{18}F]fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide ([^{18}F]-1). The K[^{18}F]F·Kryptofix 2.2.2 complex in DMSO (300 μL) was added to **19** (2–3 mg, 8–13 μmol) in a screw-cap test tube (8 mL). The tube was capped, shaken and heated in the microwave for 3.5 min. After cooling to ambient temperature in a water bath, the vial content was diluted with 10 mL of water and loaded onto an activated (EtOH) and equilibrated (water) C_{18} Sep-Pak cartridge (classic, short body). The cartridge was washed with water (10 mL) and [^{18}**F**]-**20** was eluted with EtOH (2 mL) into a small glass test tube. [^{18}**F**]-**20** was analyzed using HPLC and compared to cold standards (Sigma) by coinjection; this analysis indicated that only the nitro group at the ortho to the trifluoromethyl group was substituted exclusively. The reduction vessel was prepared by adding to a V-vial (5 mL), sequentially, a few borosilicate glass beads, EtOH–water (100 μL, 4:1), Raney nickel (50% slurry in water, 250 μL), and hydrazine monohydrate (60 μL, 1.2 μmol). After capping with a septum-equipped screw cap (vented with a large diameter needle), the vial was shaken and placed in a 55 °C heating block for 5 min. The solution of [^{18}**F**]-**20** in ethanol was diluted with water (0.5 mL) and was added slowly to the reduction vessel. After 5 min, an additional portion of hydrazine hydrate (40 μL, 0.8 μmol) was added to the reaction vessel. The reaction was stirred for additional 10 min and then the vessel was cooled to ambient temperature in a water bath and the vial content was filtered through a 0.45 mm filter (polypropylene) into another glass test tube. [^{18}**F**]-**21** was analyzed using HPLC and compared to cold standard (Sigma) by coinjection. To the filtered solution of [^{18}**F**]-**21** water (10 mL) was added and loaded onto an activated (EtOH) and equilibrated (water) C_{18} Sep-Pak cartridge (classic, short body). The cartridge was washed with dry dimethylacetamide (0.7 mL) into a 10 mL flask, which contained **10** under nitrogen at 0 °C. The reaction solution was stirred for 0.5 h in an ice bath and additional 1 h at room temperature to give 30% conversion to the product [^{18}**F**]-**1**. The solution was filtered through a 0.2 mm filter (nylon) and injected onto the reverse phase HPLC (semipreparative column). [^{18}**F**]-**1** was eluted with a retention time of 28 min with a decay corrected radiochemical yield of 10% (SOS). The product was then analyzed by analytical reversed phase HPLC [retention time = 15.2 min; chemical purity 90%; radiochemical purity 95%, SA of 1500 Ci/mmol (EOB)] and compared with nonlabeled standard by coinjection.

4.1.20. *N*-(4-[^{18}F]Fluoro-3-(trifluoromethyl)phenyl)-2-hydroxy-2-methylpropanamide ([^{18}F]-2). Compound [^{18}F]-**2** was prepared in the same manner as described above and obtained with a decay corrected radiochemical yield of 10% (SOS). [^{18}F]-**2** was analyzed by analytical reversed phase HPLC with a retention time of 10 min, chemical purity of 95%; radiochemical purity of 95%, SA of 1500 Ci/mmol (EOB) and compared with nonlabeled standard by coinjection.

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