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Titania-Catalyzed Radiofluorination of Tosylated Precursors in Highly Aqueous Medium

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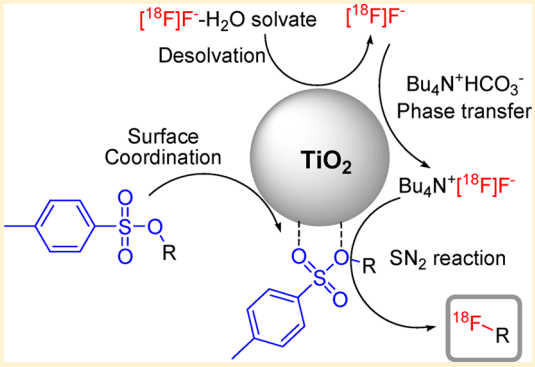
Supporting Information

ABSTRACT: Nucleophilic radiofluorination is an efficient synthetic route to many positron-emission tomography (PET) probes, but removal of water to activate the cyclotron-produced [^{18}F]fluoride has to be performed prior to reaction, which significantly increases overall radiolabeling time and causes radioactivity loss. In this report, we demonstrate the possibility of ^{18}F-radiofluorination in highly aqueous medium. The method utilizes titania nanoparticles, 1:1 (v/v) acetonitrile−thexyl alcohol solvent mixture, and tetra-*n*-butylammonium bicarbonate as a phase-transfer agent. Efficient radiolabeling is directly performed with aqueous [^{18}F]fluoride without the need for a drying/azeotroping step to significantly reduce radiosynthesis time. High radiochemical purity of the target compound is also achieved. The substrate scope of the synthetic strategy is demonstrated with a range of aromatic, aliphatic, and cycloaliphatic tosylated precursors.



1. INTRODUCTION

Positron emission tomography (PET) is an extremely effective imaging tool in clinical care, preclinical and clinical research, and drug discovery. PET enables visualization of physiological states or changes in the living body, investigation of the mechanism of disease, and quantification of biological processes such as receptor occupancy, cell proliferation, metabolic activity, apoptosis, and gene expression. Since PET allows for highly sensitive measurement of phenotypic changes associated with a malignant condition, the onset of a particular disease such as cancer can be detected, diagnosed, and treated at an early stage prior to the development of metastasis.[1] Typically, ^{18}F-labeled PET tracers are synthesized by nucleophilic fluorination of activated precursors with [^{18}F]fluoride/[^{18}O]H_2O obtained from a cyclotron.[2−5] Due to high ^{18}F ion solvation energy in water, aqueous fluoride is relatively unreactive unless it is released from its aqueous surroundings. This is usually achieved by mixing aqueous fluoride with a phase-transfer agent [e.g., K_2CO_3/Kryptofix-222 (K_{222}), tetra-*n*-butylammonium bicarbonate (TBAB), or Cs_2CO_3] and azeotropically drying the resulting solution with acetonitrile. The dried active complex (e.g., [^{18}F]KF/K_{222}) is then used for radiolabeling in an anhydrous organic solvent. Depending on the technical method of fluoride activation (e.g., cartridge-based solvent exchange followed by evaporation), this procedure may take up to 20−30 min, and some loss of initial radioactivity is observed.[6−8]

To reduce the need for a lengthy drying process, there has been recent interest in the development of radiofluorination methods that allow direct use of aqueous [^{18}F]fluoride without a separate activation step. Several novel approaches were reported, such as enzymatic[9−12] and transition-metal mediated/catalyzed[13−18] reactions, and the development of specific substrates and fluorinating reagents.[19,20] The majority of these methods directly use cyclotron-delivered [^{18}F]fluoride/[^{18}O]H_2O, and reactions are conducted in organic−aqueous media with water content of 0.5−20%. However, most of these methods are suited only for a narrow compound class (e.g., aromatic, benzylic nucleophilic fluorination), and the reported fluorination efficiency was typically low to moderate (4−48% of the desired fluorinated product). For enzymatic catalysis, the substrate scope is limited to only a few specific precursors.

We report herein a conceptually new method based on titania (titanium dioxide, TiO_2) nanoparticles (crystalline composition 45% rutile, 55% anatase; <200 nm size) as a catalyst. The precursor solution is first incubated with the particles, and then a mixture of phase-transfer agent and [^{18}F]fluoride/[^{18}O]H_2O (taken directly from cyclotron-delivered vial or trapped and eluted from QMA cartridge) is added and reacted, followed by removal of the catalyst particles by filtration and then purification and reformulation steps. The use of TiO_2 obviates the laborious synthesis and purification of a metal−precursor complex prior to radiolabeling, and the method is compatible with many commercially available or easily synthesized precursors. We have demonstrated that this route for radiofluorination is suitable for aromatic, aliphatic, and cycloaliphatic precursors in organic−aqueous media with a tolerated water content up to 25 vol %.

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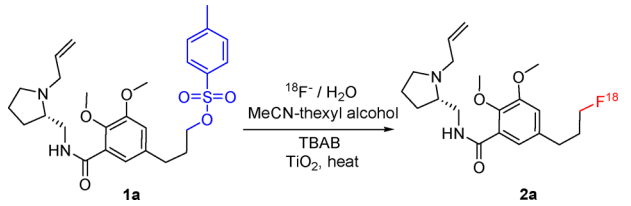
Titania is widely used in a variety of chemical processes, including electro- and photochemistry.[21,22] Along with broad catalytic activity, it possesses a strong ability to adsorb water from surrounding medium.[23−25] Numerous reports have demonstrated the mechanism of water dissociation at titania surface,[26,27] and we hypothesize that this feature of TiO_2 might be used for in situ desolvation of [^{18}F]fluoride from water to catalyze nucleophilic radiofluorination reactions in organic−aqueous solutions without preliminary drying of the [^{18}F]fluoride. We present an investigation of the influence of various reaction parameters on the radiofluorination of a model PET probe precursor and also discuss studies performed to further elucidate the mechanism of TiO_2-catalyzed radiofluorination.

2. RESULTS AND DISCUSSION

2.1. Preliminary Observations. As a model system for our primary studies, we have chosen the radiofluorination of tosyl-Fallypride **1a** to [^{18}F]Fallypride **2a** (Scheme 1), a highly specific dopamine D_2/D_3 receptor radioligand used in PET imaging of the brain to study receptor occupancy and density and which has potential clinical application in relation to neuropsychiatric conditions and aging.[28,29]

Scheme 1. Catalytic Formation of [^{18}F]Fallypride



The radiofluorination reaction was performed in a 1:1 (v/v) mixture of acetonitrile (MeCN) and 2,3-dimethyl-2-butanol (thexyl alcohol). Thexyl alcohol was included due to a report by Lee and co-workers,[30] showing that addition of alcohol to the reaction medium facilitates the radiofluorination S_N2 process, and a report by Javed et al.,[31] showing a substantial increase in fluorination efficiency of [^{18}F]Fallypride when this solvent mixture was used compared to pure MeCN. Reactions were performed in a sealed vial, heated to 110 °C for 7 min by use of a Peltier heater. The reaction mixture was mixed by refluxing solvent; magnetic stirring was not used. After completion of the reaction, methanol was added to the reaction mixture, and solid catalyst particles were removed by filtration through a 20 nm filter (Whatman Anotop 10 Plus). In general, we found that some [^{18}F]fluoride trapped onto the catalyst and could not be extracted. By use of a dose calibrator, radioactivity measurement of the filtered extract was compared to the initial radioactivity to determine the radioactivity extraction efficiency (REE): REE = [decay-corrected radioactivity recovered in the organic extract]/[initial radioactivity]. The fraction of initial radioactivity trapped onto the catalyst is calculated as 1 − REE. Analysis with radio-TLC (thin-layer chromatography) and radio-HPLC (high-performance liquid chromatography) were used to determine fluorination efficiency. This in turn enabled calculation of the radiochemical conversion (RCC): RCC = REE × fluorination efficiency. All results are averaged over at least $n = 3$ experiments. Identity of the radiofluorinated product was confirmed by coinjection with standard [^{19}F]Fallypride (ABX GmbH, Germany).

We performed a series of experiments (Table 1) to confirm the importance of each species in the reaction. Entries 1 and 2 show the conventional synthesis conditions where [^{18}F]fluoride is predried and reaction takes place in anhydrous organic medium. The improvement in RCC due to addition of thexyl alcohol is apparent. Entry 5 shows 68% RCC resulting from catalytic synthesis conditions with all species included as described above. Comparative runs without the phase-transfer catalyst resulted in only 18% RCC. Thus, the phase-transfer agent appears to be important as well, possibly due to better solubilization of in situ generated tetra-*n*-butylammonium fluoride ([^{18}F]TBAF), compared to [^{18}F]fluoride, in organic−aqueous medium. As expected, experiments in the absence of catalyst did not lead to formation of desired product **2a** in organic−aqueous medium (entry 3), indicating that TiO_2 is essential when there is water in the reaction mixture. If the [^{18}F]fluoride was dried (as in conventional synthesis) prior to catalytic reaction, no conversion was observed (entry 6). In fact, the filtrate contained neither fluorinated product nor parent [^{18}F]fluoride; all the radioactivity was found bound to the catalyst. This means that, in nonaqueous medium, TiO_2 addition results in total [^{18}F]fluoride trapping (REE ∼0%). On the other hand, REE was found to be remarkably constant (∼80%) for all of the TiO_2-catalyzed conditions containing water. Even if the reaction was performed without the precursor, the REE was unchanged (80% ± 2%).

Table 1. Influence of Reaction Components on Formation of 2a[a]

entry	catalyst	phase-transfer agent[b]	MeCN (μL)	thexyl alcohol (μL)	water[c] (μL)	REE (%)	RCC (%)
1	none	TBAB	40	0	0	100[d]	31 ± 2
2	none	TBAB	20	20	0	100[d]	64 ± 4
3	none	none	15	15	10	100[d]	0
4	TiO_2	none	15	15	10	79 ± 4	18 ± 3
5	TiO_2	TBAB	15	15	10	80 ± 3	68 ± 2
6	TiO_2	TBAB	20	20	0	0	0
7	TiO_2	K_2CO_3/K_{222}	15	15	10	78 ± 3	39 ± 6
8	none	K_2CO_3/K_{222}	20	20	0	100[d]	31 ± 4
9	$MgSO_4$	TBAB	15	15	10	99 ± 1	0
10	$CaCl_2$	TBAB	15	15	10	99 ± 1	0

[a]Reactions were performed with 2.3 μmol of **1a** and 140 μmol of TiO_2 in 40 μL reaction volume at 110 °C for 7 min without magnetic stirring. [b]Amounts of phase-transfer agent used, when applicable: 0.36 μmol of TBAB; 0.36 μmol of K_2CO_3, and 0.36 μmol of K_{222}. [c]For cases where water is 0 μL, [^{18}F]fluoride was added as dry complex with phase-transfer agent (1.5−4 mCi) reconstituted in 10 μL of MeCN−thexyl alcohol (1:1 v/v); for other cases, radioactivity was introduced as a solution of aqueous [^{18}F]fluoride (1.5−4 mCi) containing phase-transfer agent; [d]In the case of catalyst absence, no extraction was performed.

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The influence of the type of phase-transfer agent was evaluated by carrying out the reaction with K_2CO_3/K_{222} mixture (entry 7). This resulted in a substantial decrease in RCC, suggesting that TBAB is a superior phase-transfer agent for these reactions.

Finally, to assess whether the role of TiO_2 was simply to sequester water to facilitate fluorination in mixed aqueous–organic medium, comparative runs with particles of other common non-oxide drying agents, $MgSO_4$ and $CaCl_2$ (140 μmol each), were also performed (entries 9 and 10). These showed zero RCC, suggesting an effect of TiO_2 beyond simple water adsorption but most probably its ability of water splitting.

2.3. Hypothesized Mechanism of TiO_2-Catalyzed Radiofluorination. Previous reports show that oxo- and oxy-containing species readily coordinate on a TiO_2 surface through hydrogen bonding.[32−35] Thus, we hypothesize that in addition to an interaction with water, the TiO_2 catalyst could also serve to coordinate tosyl-Fallypride **1a** via oxygen atoms of the sulfonyl group, facilitating reaction with desolvated [^{18}F]fluoride in close proximity. Based on these ideas, the mechanism of catalyzed fluorination shown in Figure 1 is suggested.

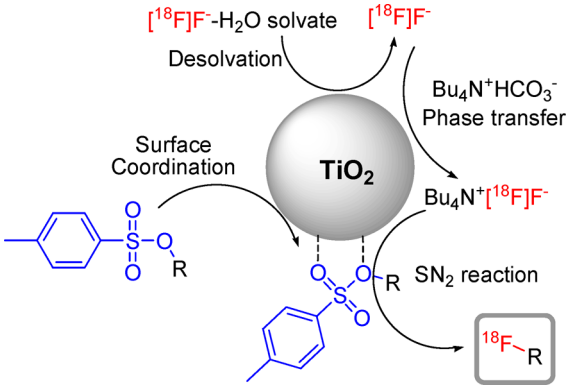

Figure 1. Proposed mechanism for TiO_2-catalyzed radiofluorination. S_N2 substitution is improved with alcohol as a cosolvent, perhaps by inclusion in intermediate complex formation as determined by Oh et al.[30] (not shown here).

Hydrogen bonding occurs between oxygen atoms of the sulfonyl moiety and TiO_2, which coordinates tosylated precursor to the surface of the catalyst; when aqueous [^{18}F]fluoride/TBAB solution is added, solvated [^{18}F]fluoride is adsorbed at active sites of TiO_2 where the aqueous shell is split, resulting in [^{18}F]fluoride release (i.e., activation); TBAB phase-transfer catalyst then serves as a [^{18}F]fluoride-trapping agent and intercepts activated [^{18}F]fluoride, subsequently conducting the phase transfer of [^{18}F]fluoride to the surface-coordinated precursor to faciliate the S_N2-type reaction. Because the coordination with the precursor is at the leaving group, the resulting radiofluorinated product is released upon formation from TiO_2.

2.4. Evidence for Coordination. To explore the role of coordination in the reaction mechanism, we performed radiolabeling after first incubating the tosylated substrate with catalyst. If coordination indeed occurs, prolonged exposure of the precursor to the catalyst should increase the amount of precursor bound to the surface of the catalyst and thus promote increased S_N2-reactions (i.e., radiolabeling efficiency). Thus, a solution of tosyl-Fallypride **1a** in 1:1 (v/v) MeCN–thexyl alcohol was added to the catalyst and incubated at room temperature for various durations. After incubation, aqueous [^{18}F]fluoride/TBAB mixture was added, and radiolabeling was performed by heating at 110 °C for 12 min in an oil bath. The reaction mixture was mixed by refluxing solvent; no magnetic stirring was used. We observed a modest enhancement in RCC starting after 20–30 min of incubation and reaching a maximum improvement after ∼1 h (Figure 2).

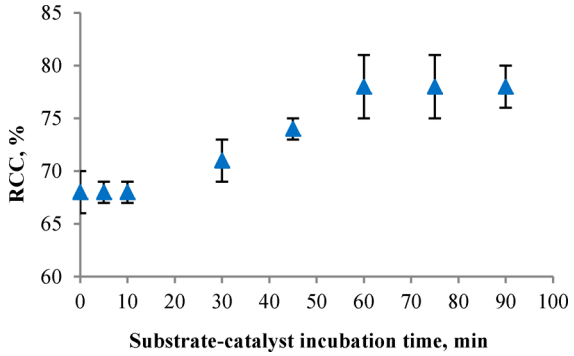

Figure 2. Effect of substrate–catalyst preincubation time on radiofluorination efficiency of **1a**.

Analytical samples of organic solution after incubation with catalyst contained significant amount of precursor **1a**; thus, it is likely that the observed saturation is due to occupation of all accessible active sites of TiO_2 capable of binding the oxygen atoms of the leaving group. Attempts to perform preincubation at elevated temperatures (30, 45, and 60 °C) showed similar results; that is, the time scale was not shortened. It should be noted that the preincubation step was performed before the introduction of [^{18}F]fluoride; therefore, incorporation of preincubation into the synthesis protocol does not introduce any delays that would affect the yield due to decay.

Studies were performed by running the radiofluorination reaction in the presence of various amounts of a nonreactive SO-containing compound (dimethyl sulfoxide, DMSO) to assess whether the presence of another cocoordinating species could block the binding of the precursor and lower the RCC (Figure 3).

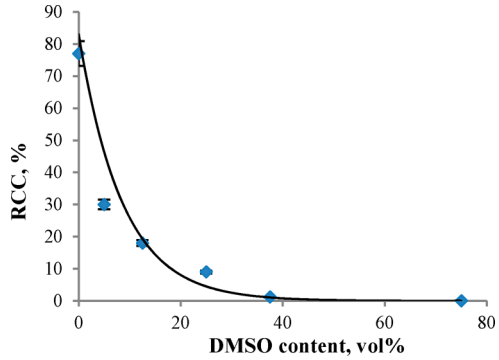

Figure 3. Influence of DMSO on TiO_2-catalyzed radiochemical conversion of **1a**.

It was revealed that even slight addition of DMSO dramatically affected the RCC; a 2-fold decrease in RCC was registered at 5 vol % DMSO, with nearly complete inhibition at 37.5 vol % DMSO, suggesting that DMSO molecules may indeed bind to active sites of TiO_2, thus reducing sites available for precursor coordination. Surprisingly, the REE was also significantly affected, and the amount of [^{18}F]fluoride trapped onto the catalyst increased with increasing DMSO content. Almost total

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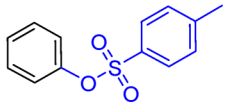
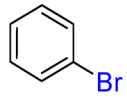
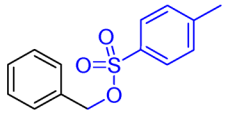
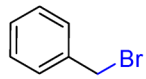
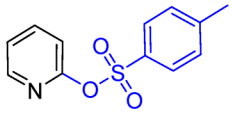
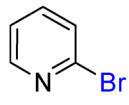
[^{18}F]fluoride trapping (90%) was registered at 75 vol % DMSO. This suggests that instead of merely affecting precursor coordination, DMSO may interact with the catalyst in additional ways leading to trapping of fluoride, such as DMSO−water cluster formation,[36−39] intercalation of DMSO−water clathrate inside the oxide structure,[40] or DMSO-induced creation of positively charged sites.[41]

To investigate further the need for leaving-group coordination at the TiO_2 surface, experiments were performed comparing tosylated compounds **1b**, **1c**, and **1s** with their brominated versions **3**−**5** (not suspected to coordinate with surface) in TiO_2-catalyzed [^{18}F]fluorination (Table 2). No product was observed

Table 2. TiO_2-Catalyzed Radiofluorination of Tosylated versus Brominated Substrates[a]

Entry	Structure	
1	 **1b** (78 ± 3% RCC)	 **3** (0 % RCC)
2	 **1c** (80 ± 3% RCC)	 **4** (0 % RCC)
3	 **1s** (70 ± 7% RCC)	 **5** (0 % RCC)

[a]Preincubation time 1 h, 2.3 μmol of precursor, 140 μmol of TiO_2, 130 °C for 5 min, 40 μL total reaction volume, no magnetic stirring; radioactivity was introduced as 10 μL solution of aqueous [^{18}F]fluoride (1.5−4 mCi) containing 0.36 μmol of TBAB. Radioactivity extraction efficiency (REE) ~80% was observed for every entry.

in the case of bromo derivatives, while high RCCs of ~80% were determined for tosylated precursors, which agrees with the necessity for the proposed oxygen-coordinating mechanism at the leaving group. The REE in the case of bromo-substituted substrates remained close to 80%, that is., similar to that for tosylated reactants.

2.5. Optimization of Catalyst Loading. It was found that the amount of catalyst added had a substantial effect on RCC. Studies with different catalyst amounts (see Supporting Information) revealed that the optimal loading of TiO_2 is 140 μmol for 40 μL reaction volume with 25 vol % water content for the catalyst particle size used (<200 nm). This translates to ~60:1 ratio of catalyst to precursor **1a** and enables production of target compound **2a** with the highest RCC (78%). The RCC drops for both increasing and decreasing amounts of catalyst.

Looking at fluorination efficiency, we observed catalyst amounts lower than the optimum to result in decreasing fluorination efficiency, perhaps due to reduced desolvation of fluoride. Surprisingly, increasing the amount of catalyst also decreased the fluorination efficiency. Perhaps as the catalyst amount is increased, one of the reaction components becomes depleted, reducing the interactions necessary for fluorination.

On the other hand, looking at extraction efficiency, we observed that REE decreases (i.e., trapping increases) with increasing amount of catalyst. Due to this linear relationship, we suspect there are specific trapping sites for fluoride on the TiO_2, the number of which depends on the amount of catalyst present. Perhaps trapping occurs via exchange of fluoride with terminal hydroxyl groups.[42−44] The consistent ~20% trapping for a fixed amount of catalyst may represent the equilibrium exchange between the surface and the reaction solution containing both fluoride and hydroxide ions. The case of 100% trapping under dry conditions (Table 1, entry 6), where the fluoride concentration dominates, could represent a shift in this equilibrium.[45]

To determine if the optimal amount of catalyst is universal or should be adjusted for every precursor, similar experiments were performed with substrates **1i**, **1q**, and **1t**. The loading of 140 μmol of TiO_2 remained optimal for these substrates as well (Figure 4).

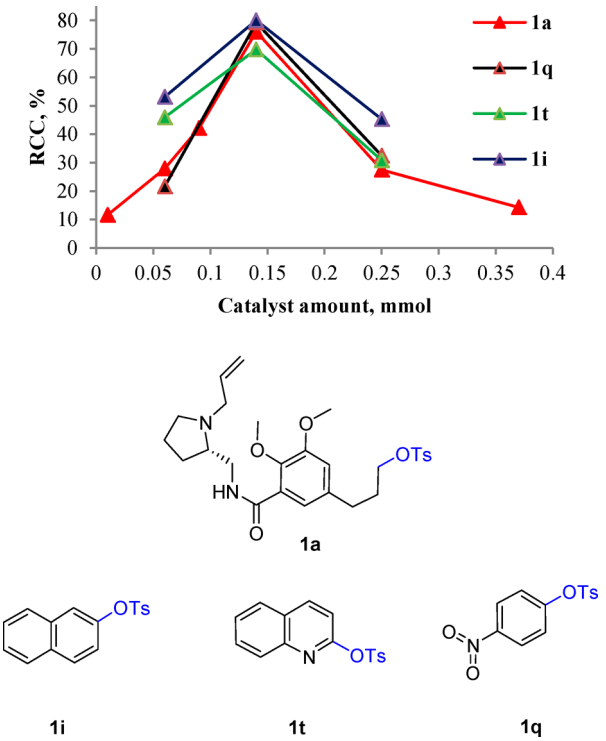


Figure 4. Determination of optimal catalyst amount for radiolabeling of **1a**, **1i**, **1q**, and **1t**.

2.6. Evaluation of Optimal Reaction Conditions. To maximize fluorination efficiency of the precursor **1a**, further evaluation of reaction conditions has been performed to determine optimal reaction parameters.

The range of water content that provided maximum RCC was found to be up to 25 vol % (see Supporting Information). In this aqueous range, the RCC and fluoride trapping remained remarkably constant. With higher water content, the trapping remained constant, but a strong decrease in [^{18}F]fluorination was observed. We hypothesize that higher water content exceeds the capacity of the catalyst to adsorb and split water, and thus the [^{18}F]fluoride is not as effectively desolvated, reducing the fluorination efficiency. If true, this suggests that increased catalyst

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Table 3. Titania-Catalyzed Production of [^{18}F]Fallypride[a]

parameter	run 1	run 2	run 3	average
initial activity (mCi)	5.20	5.19	5.23	5.20 ± 0.02
REE (%)	79.1	81.2	81.9	80.7 ± 1.5
RCC (%) (nonisolated)	79.1	81.2	81.5	80.6 ± 1.3
activity remaining after synthesis and extraction[b] (%)	79.1	81.2	81.9	80.7 ± 1.5
activity remaining after HPLC purification[b] (%)	75.7	76.5	77.6	76.6 ± 1.0
activity remaining after reformulation[b] (%)	71.2	68.6	73.2	71.0 ± 2.3
total loss (%)	28.8	31.4	26.8	28.9 ± 2.3
isolated RCY[c] (%)	71.2	68.6	73.2	71.0 ± 2.3

[a]Optimized reaction conditions: 1 h preincubation time, 2.3 μmol of precursor, 140 μmol of TiO_2, 40 μL total reaction volume, no magnetic stirring; radioactivity introduced as 10 μL solution of aqueous [^{18}F]fluoride (~2.6 mCi) containing 0.36 μmol of TBAB; heated to 130° for 5 min in an oil bath. For each run, two vials are pooled after extraction to improve accuracy of measurements. [b]Fractions of remaining radioactivity determined by measuring the radioactivity after the relevant step, correcting for decay, and dividing by the initial radioactivity. [c]RCY = radiochemical yield.

Table 4. Comparison of Catalytic Production of 2a to Known Procedures

ref	reactor type	radiolabeling conditions	mean RCY[a] (%)	total time (min)
Moon et al.[48]	macroscale automated. TracerLab FX	100 °C, 30 min	68	74
Pike et al.[46]	microscale automated, Nanotek Advion	150−190 °C, 4−23 min	16−88	50−218
Javed et al.[7]	microscale automated, EWOD chip	105 °C, 7 min	83	70
Lazari et al.[49]	macroscale automated, ELIXYS	105 °C, 7 min	66	56[b]
this report	small-volume vial, manual	130 °C, 5 min	71	50

[a]RCY = radiochemical yield. [b]Total time reported without reformulation step.

Table 5. Quality Control Tests of Injectable [^{18}F]Fallypride Solution

clinical QC test	clinical acceptance criteria	results of this study
optical clarity	clear and particle-free	clear and particle-free
pH	5.5−8.0	6.5
radiochemical purity (%)	>95	>99
radiochemical identity	matches retention time of standard	matches retention time of standard
^{18}F-radionuclide half-life (min)	105−115	111
endotoxin level (EU/mL)	<5	<1
filter integrity (psig)	>50	>100
MeCN content (ppm)	<410	<2
thexyl alcohol content (ppm)	<5000	<1
sterility	no growth in 14 days	no growth in 14 days
titanium content (ng)	none specified	36 ± 4

amount may enable improved water tolerance if desired, but in light of results in the previous section, it would also be necessary to increase the precursor amount in the same proportion as the catalyst.

We also studied RCC as a function of reaction time and temperature (see Supporting Information) to determine the optimal reaction time (5 min) and temperature (130 °C). The influence of the specific type of alcohol cosolvent was evaluated as well. Several alcohols were tested in place of thexyl alcohol, but relatively little difference in RCC was observed (see Supporting Information). Thexyl alcohol showed the highest RCC of alcohols tested.

2.7. Production and Quality Control of Clinically Relevant PET Probe. To demonstrate the overall radiochemical yield of isolated [^{18}F]Fallypride **2a**, we performed full production runs (radiofluorination, HPLC purification, and formulation). The final formulated product was obtained as a sterile, injectable solution. During production, radioactivity measurements were recorded at key steps to assess efficiency of each process and identify potential areas for optimization (Table 3). The biggest loss is during extraction from the catalyst (20% trapped), and an additional ~10% is lost during our purification and formulation processes. Total production times, isolated yields, and specific activities are compared to those previously reported in literature (Table 4). Generally, the isolated yield of titania-catalyzed reaction tends to be higher than the yield reported for macroscale automated production, while it is comparable to the yields for microfluidic procedures, such as syntheses on a digital microfluidic chip[7] or use of the Advion Nanotek capillary reactor.[46] By avoiding the need for fluoride drying/azeotroping, the synthesis process is simplified.

It is noteworthy to mention the high radiochemical purity of target compound **2a** that formed during reaction. Only two radioactive peaks were detected by analytical HPLC, which consisted of unreacted [^{18}F]fluoride and the desired [^{18}F]-Fallypride (see Supporting Information). Regarding HPLC analysis of the nonradioactive side products, the hydroxylated compound, resulting from hydrolysis, is clearly observed while no byproduct from β-elimination is apparent. With HPLC purification, the [^{18}F]fluoride and nonradioactive side products were effectively removed, and the reformulated solution was then examined by standard quality control (QC) tests[47] to evaluation its compliance with U.S. Food and Drug Administration requirements for injectable PET tracers (Table 5).

With the introduction of TiO_2 catalyst into the production procedure, an additional QC test to assess the titanium content

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in the reformulated solution may be needed. While the 20 nm filtration process and subsequent HPLC purification are expected to eliminate all particles, there is still the possibility of titanium ions in the final formulated solution. By use of inductively coupled plasma mass spectrometry (ICP-MS), the titanium content in representative samples was found to be 36 ± 4 ng (n = 9) of titanium per batch. Titanium is considered very inert and there do not appear to be established limits for titanium in injectable solutions. According to medicinal reports,[50−52] however, normal levels of titanium in human body range from 0 to ∼20 μg/L blood (∼0−100 μg per whole body of an adult) in patients without titanium implants, while reaching 100−150 μg/L blood (500−750 μg per whole body) in patients with artificial titanium joints. Thus, an injection of formulated [^{18}F]Fallypride produced by our method would have a very miniscule impact because it contains orders of magnitude less titanium than normally present in the blood. This could suggest that routine titanium testing may not be needed for every batch of injectable PET probe.[53,54]

We have, therefore, demonstrated the practical feasibility of the newly developed synthetic approach for synthesis of a clinically relevant PET probe, including compliance with FDA requirements for injectable solutions in clinical applications. By varying the precursor, other [^{18}F]fluorine-labeled PET probes could be produced by this procedure and similar QC results would be expected.

2.8. Determination of Specific Activity. High specific activity (SA) of PET tracers is essential to minimize the injected quantity of the nonradioactive form of the tracer, which can saturate rare biological targets, such as neurological receptors, and subsequently lower the image quality and possibly cause pharmacologic effects.[55] It has been confirmed that SA plays an important role in PET image quality,[29,56,57] especially in small animals.[58,59] By use of standard methods,[7] the SA for TiO_2-catalyzed synthesis of [^{18}F]Fallypride **2a** was found to be 5 ± 2 Ci/μmol (n = 5). This is higher than typically obtained from macroscale synthesis (0.4−3 Ci/μmol).[49,60] This evidence suggests TiO_2 catalysis could be successfully used for routine production of PET imaging probes that require high SA.

2.9. Attempts to Scale up Synthesis. Previous experiments utilized operating volumes of 40 μL, 10 μL of which was [^{18}F]fluoride solution. While we have been able to demonstrate the production of several millicuries of the isolated product starting from ∼5 mCi of [^{18}F]fluoride, it may be desirable in some cases to produce larger amounts of product (e.g., for clinical production), which requires a larger volume of the initial [^{18}F]fluoride solution.

One simple way to scale up the quantity of tracer produced is to run several reactions in parallel and pool the results; however, this would require multiple reaction vessels and may not be convenient to handle or automate. Another approach is to scale up the reaction volume, enabling a larger volume of [^{18}F]fluoride to be used while still keeping water content within the 25 vol % range, and thus enabling a larger amount of starting radioactivity. To test this possibility, we explored proportionally increasing the amounts of all of the reaction components (i.e., precursor, TiO_2, solvent, and aqueous [^{18}F]fluoride/TBAB solution). As an example, the catalytic fluorination of **1a** was scaled up by a factor of 3, such that the final volume of the reaction mixture comprised 120 μL. During these experiments, we found that 5 min reaction time was insufficient to efficiently fluorinate the precursor (fluorination efficiency = 50%). With increased heating time, it was possible to increase fluorination efficiency to the values seen prior to scaling (Figure 5). We suspect that longer time is needed for sufficient diffusive mixing in the larger volume. We also

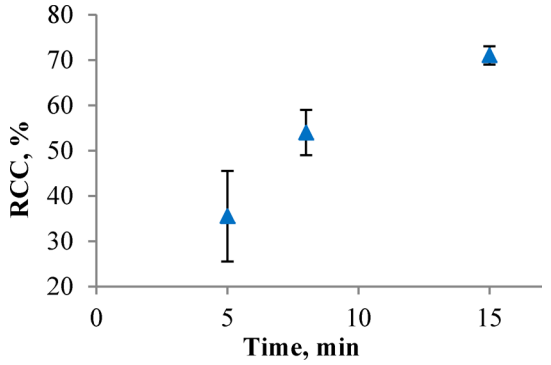

Figure 5. Correlation between reaction time and fluorination efficiency in scaled-up catalytic radiolabeling of **1a**. Values are averaged from n = 9 experiments for 5 min runs and n = 6 for each of 8 and 15 min experiments.

encountered some initial difficulties during the filtration step to remove the nanoparticles after the reaction was done. We observed clogging of the 20 nm filter due to the increased amount of TiO_2. This issue was resolved by incorporating an initial prefiltration step with a 0.22 μm filter prior to 20 nm fine filtering. Unfortunately, this had the effect of slightly reducing the extraction efficiency, from 80% ± 2% to 71% ± 13%.

The tested scale-up factor of 3 is sufficient for production of a human dose of [^{18}F]Fallypride, and further increases in scale are presumably possible, if desired; with higher volumes, stirring during the preincubation and reaction steps may become important, requiring additional optimization of reaction time and extraction procedures. Another reason to perform volume scale-up is to potentially enable automation in commercially available radiosynthesizers, which typically require at least several hundred microliters of solution in the reaction. Though there are advantages in performing reactions in extremely small volumes,[7,8] it will be some time before automated and commercialized versions of such technologies are widely available.

As an alternative way to increase the amount of radioactivity in the reaction without impacting reaction volume, solid-phase extraction procedures using microscale QMA cartridges could be used to concentrate the [^{18}F]fluoride to obtain higher starting radioactivity in volumes of the [^{18}F]fluoride/TBAB solution described here (i.e., 10 μL water content). Several reports have shown that an entire cyclotron target volume can be trapped and efficiently eluted in only 5−45 μL of eluent solution.[61−63] This would likely be the preferred approach to scale up the amount of radioactivity since no increase in reaction volume would be necessary.

2.10. Substrate Scope. In order to explore the utility of the TiO_2 catalytic approach for synthesis of other PET tracers, we investigated the scope of applicable substrates. We first considered the use of other leaving groups with sulfonyl moieties (i.e., triflate and nosylate). Surprisingly, radiofluorination of commercially available substrates **6** and **7**, precursors for 2′-deoxy-2′-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) and 3′-deoxy-3′-[^{18}F]fluoro-L-thymidine ([^{18}F]FLT) (Figure 6), resulted in RCC = 0%.

In the presence of catalyst, triflate and nosylate groups seemed to become overreactive, and immediate explosive hydrolysis was

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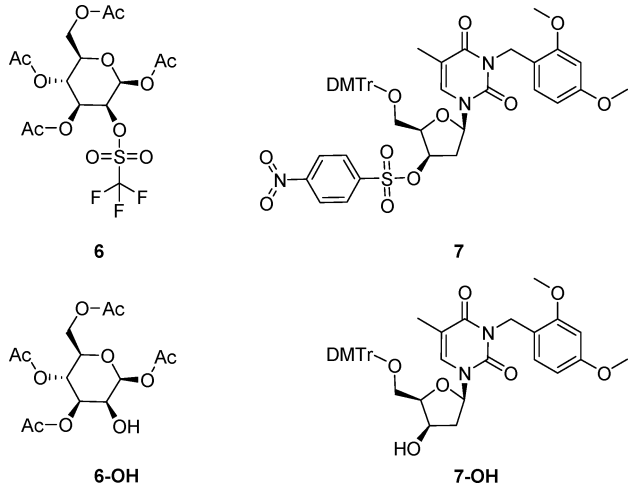


Figure 6. Additional substrates with sulfonyl-containing leaving groups and their corresponding hydrolysates.

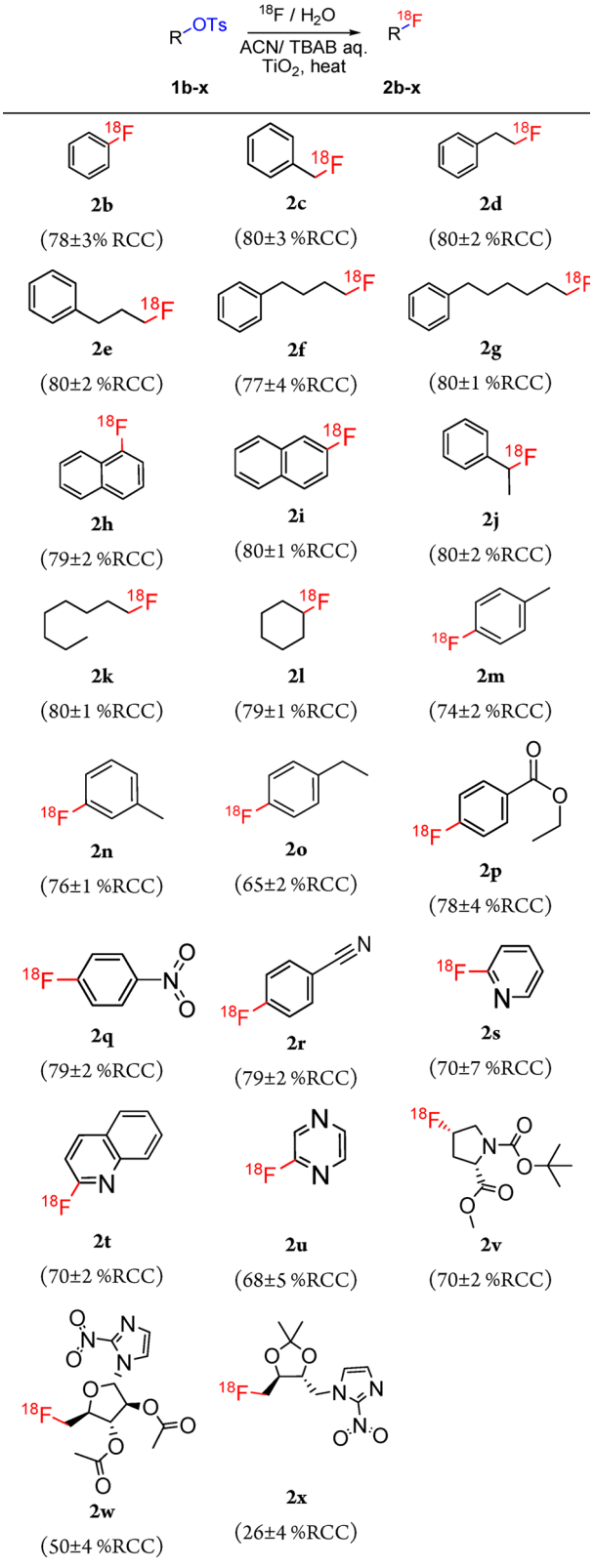
observed upon aqueous [^{18}F]fluoride addition. When analyzed, only unreacted [^{18}F]fluoride and hydroxylated compounds **6-OH** and **7-OH** were detected. This phenomenon suggests that reactivity of oxygen-containing leaving groups are increased when incubated with TiO_2. Due to this additional activation, the ideal leaving group should initially possess lower reactivity (tosyloxy preferred over triflyloxy or nosyloxy), otherwise concurrent side reaction of hydrolysis prevails over [^{18}F]-fluorination.

We next investigated the generality of TiO_2-catalyzed radiofluorination of tosylated precursors. A library of aromatic, aliphatic, and cycloaliphatic tosylates was tested, along with the commercially available tosylated PET probe precursors for [^{18}F]-4-fluoroproline ([^{18}F]-4-FP), [^{18}F]fluoroazomycin arabinoside ([^{18}F]FAZA), and [^{18}F]fluoroerythronitroimidazole ([^{18}F]-FETNIM) (Table 6). The methodology was highly efficient for low-molecular-weight precursors **1b−v** (65−80% RCC) but resulted in low to moderate yields with bulky and sterically hindered substrates **1w−x** (i.e., from the commercially available precursors for [^{18}F]FAZA and [^{18}F]FETNIM, respectively). These particular precursors also contain additional oxo moieties, which potentially lower yields by coordinating the precursor at the catalyst surface instead of at the O═S═O moiety of the tosylate leaving group. Such coordination could significantly reduce [^{18}F]fluoride interaction with the tosylate reactive center by placing the reaction center further from the catalyst surface where fluoride desolvation occurs. We are currently looking into methods to further understand the reaction mechanism to predict the effectiveness of different substrates and perhaps enable improved substrate design.

3. CONCLUSIONS

In conclusion, we have developed a novel method of TiO_2-catalyzed radiofluorination of tosylated presursors and demonstrated its use for the preparation of ^{18}F-labeled PET probes. The method avoids the need for drying of [^{18}F]fluoride/[^{18}O]H_2O from the cyclotron before fluorination. The wet [^{18}F]fluoride is mixed with a phase-transfer agent and added to a solution of precursor solution preincubated with TiO_2 nanoparticles and reacted for a short time. In this fashion, nucleophilic ^{18}F-fluorination is shown to proceed rapidly and efficiently in aqueous medium with up to 25 vol % water content, which to the best of our knowledge is the highest reported other than

Table 6. Substrate Scope for TiO_2-Catalyzed Radiofluorination of Tosylated Substrates[a]



[a]Optimized reaction conditions: 1 h preincubation time, 2.3 μmol of precursor, 140 μmol of TiO_2, 130 °C, 5 min, 40 μL total reaction volume, no magnetic stirring; radioactivity introduced as 10 μL solution of aqueous [^{18}F]fluoride (1.5−4 mCi) containing 0.36 μmol TBAB. For all entries, REE was observed to be ~80%.

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enzymatic methods. We have also demostrated the production of clinically relevant amounts of [^{18}F]Fallypride with this approach, as well as shown compliance of the final formulated PET tracer with QC requirements for clinical use. The product was found to have high specific activity even with low amounts of starting radioactivity. The applicability of the reported protocol to a range of tosylated substrates was also demonstrated for organic molecules containing aromatic, aliphatic, and cycloaliphatic moieties. Although extensive additional investigations are required to explore the substrate scope and further understand the mechanism, we anticipate that the facile procedure and high radiofluorination efficiency of this new method may provide a versatile tool for practitioners in the field of PET radiochemistry. On the basis of our hypothesized mechanism of reaction, further studies regarding the importance of the structure of the precursor and other effective catalysts are currently in progress in our group.

■ ASSOCIATED CONTENT

Supporting Information

Additional text, 11 figures, and nine tables with experimental details, as well as selected HPLC and NMR data and spectra (PDF); files for compounds **1a**−**1x** and **2a**−**2x** (CDX). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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