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Capturing aqueous [^{18}F]-fluoride with an arylboronic ester for PET: Synthesis and aqueous stability of a fluorescent [^{18}F]-labeled aryltrifluoroborate

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Abstract

The aqueous stability of aryltrifluoroborates is of importance to their use in transition metal mediated coupling reactions as well as their potential use in [^{18}F]-labeled aryltrifluoroborate PET imaging agents. Nevertheless, few studies have fully characterized the solvolysis of fluoride from an aryltrifluoroborate in water. Using [^{19}F] NMR, fluorescence and [^{18}F]-labeling techniques, we disclose the composition of an aryltrifluoroborate of exceptional kinetic stability with respect to solvolytic defluoridation. This work not only highlights the potential of using [^{18}F]-labeled aryltrifluoroborates for PET tracers, but provides a chemical platform and a general approach for evaluating the stability of other aryltrifluoroborates.

Keywords: Aryltrifluoroborate; PET; Synthesis; Kinetics; [^{18}F]-labeling

1. Introduction

For over a decade, aryltrifluoroborates have received considerable attention as boronic acid/ester equivalents in Suzuki-Miyaura cross-coupling reactions as they are exceptionally stable, inert to oxidation, easily isolated and incapable of boroxine formation [1–4]. Although aryltrifluoroborate stability has been attributed to the B–F bond strength, solvolytic defluoridation of the aryltrifluoroborate has been invoked as a necessary first step in transition metal mediated cross-coupling. That said, the stability of aryltrifluoroborates has been only briefly studied with sparse evidence as to rate of solvolytic defluoridation, which must ultimately regenerate the arylboronic acid via partially fluorinated species thereof [5–9]. In light of the extensive use of boronic esters and aryltrifluoroborates in synthetic chemistry, arylboronic acids represent potential captors of aqueous [^{18}F]-fluoride that would conveniently provide [^{18}F]-labeled PET-imaging agents composed of a pendant aryl-[^{18}F]-trifluoroborate [10]. Nevertheless, if aryltrifluoroborates are to be contemplated for *in vivo* PET-imaging, their resistance to solvolytic defluoridation would be of paramount importance.

In order to begin to address the aqueous stability of radiolabeled aryltrifluoroborates, herein we report the synthesis of an arylboronic acid with five non-exchangeable, spectator ^{19}F-nuclei manifesting four independent chemical shifts, which enable a quantitative ^{19}F NMR analysis of the arylboronic ester and its conversion to the aryltrifluoroborate. In addition, an ^{19}F-containing fluorescent BODIPY permits a fluorescent corroboration of the autoradiographic TLC analyses that address both the yield of radiolabeled aryl-[^{18}F]-trifluoroborate as well as its aqueous stability to solvolytic fluoride exchange (*vide infra*). Hence, we describe herein the preparation and evaluation of a very stable aryltrifluoroborate that may find use in the production of novel [^{18}F]-PET-imaging agents. Moreover, this report highlights a comprehensive approach for evaluating the stability of other aryltrifluoroborates.

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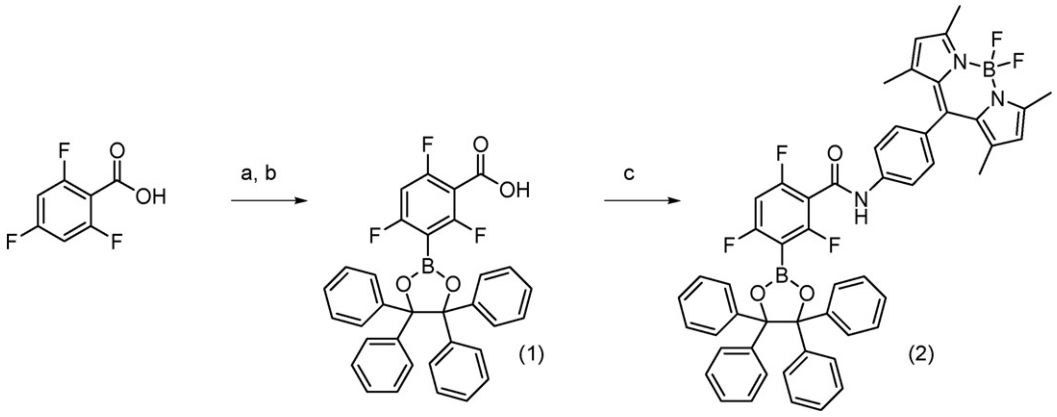


Scheme 1. (a) Two equivalent BuLi, $(MeO)_3B$, then HCl/dioxane (anhydrous); (b) tetraphenylpinacol in HCl (reflux); (c) HOBt/EDC, then BODIPY-NH_2.

2. Results and discussion

The synthesis of an [^{19}F]-labeled fluorescent boronic acid (**2**), as shown in Scheme 1, was accomplished in essentially three steps starting with 2,4,6-trifluorobenzoic acid which was cleanly lithiated, boronated and converted to its tetraphenylpincolate ester (**1**) upon acid work up in approximately 50% yield. Compound (**1**) was then coupled using standard protocols to amino-BODIPY (aminophenyl-di(dimethylpyrro)methene boron difluoride) [11] and the resulting amide was purified by standard flash chromatography to afford compound (**2**).

The rationale, in part, behind the choice of the compound (**2**) is as follows. The trifluorobenzoic acid was chosen to provide ultimately three discrete carbon-attached [^{19}F]-fluorine atoms whereby the ^{19}F NMR resonances on the target would be expected to serve as sensitive chemical-shift nuclei in non-deuterated solvents that would: (a) report on the kinetics of aryltrifluoroborate synthesis (fluoridation) and (b) would be sensitive enough to allow for ^{19}F NMR characterization of a radiolabeled aryltrifluoroborate that would be isolated at the relatively low concentrations and quantities that one might employ in a radiochemical synthesis. The BODIPY fluorophore was chosen largely for its fluorescence, which would allow for visualization on a TLC plate of sub-nanomole quantities of radiolabeled aryltrifluoroborate, which could also be independently revealed by autoradiography. It was also appreciated that the exchange-inert fluorine atoms on the BODIPY would afford additional internal ^{19}F NMR chemical shift references. The tetraphenylpinacol was chosen for the esterification of boron in (**1**) and (**2**) as it imparted high chromatographic mobility and enhanced target stability against protiodeboronation, an event that we often observed when working with the free boronic acid (data not shown).

With limiting amounts of arylboronate ester (**2**) at 2 mM and a large excess of free fluoride at 400 mM in the form of KHF_2 in the presence of 0.1 M HOAc/NaOAc pH 4.5 in 90:10 methanol:water, the kinetics of the conversion of the boronate ester (**2**) to the aryltrifluoroborate were readily followed by ^{19}F NMR, as shown in Fig. 1.

For this report, we deliberately used a large excess of fluoride to favor the formation of the aryltrifluoroborate and simplify the kinetics that might otherwise be very complicated given the various ionizable arylboronates, as well as mono- and di-fluorinated intermediates that might accumulate, particularly if fluoride were limiting. In Fig. 1, the chemical shifts for the aromatic carbon-attached fluorine atoms on the boronate ester (**2**) are found at approximately −18, −25 and −31 ppm, whereas the two boron-attached fluorine atoms on the BODIPY portion are found as a single peak at approximately −70 ppm. Free fluoride is observed in the range of −78 to −82 ppm while the internal reference, 2,2,2-trifluoroethanol, was locked at −0.6 ppm (off scale—not shown). After 72 h, full conversion to the aryltrifluoroborate is manifested by a new peak appearing at −60 ppm (actually a quartet due to coupling to the boron quadrupole) with the expected integration of 1:1:1:3:2 in the ^{19}F NMR. Accompanying this appearance is an anticipated large change in the chemicals shifts of the three carbon-attached fluorine atoms that now appear at −22.5, −30 and −40.5 ppm. Of note, and not unexpectedly, the chemical shift of the two boron-attached fluorine atoms on the BODIPY portion is largely unaffected. Despite a somewhat lengthy time for complete conversion, an appreciable amount of aryltrifluoroborate appears in a little as 96 min and the reaction is >75% complete after 8 h.

Somewhat to our surprise, an unidentified intermediate accumulates in the first 30 min and remains present under steady state throughout the early course of the reaction but disappears at completion (72 h). This intermediate is characterized by a new peak at −66 ppm and a concomitant loss of the peak at −31 ppm. Although, we have no proof as to nature of this intermediate, we hypothesize that it is the monofluoridated tetraphenylpinacolylfluoroborate because the chemical shift of the new peak at −66 ppm is more consistent with a boron-attached fluorine atom than that of a carbon-attached one. Nevertheless, if such is the case, we are still unable to account for the apparent loss of the resonance at −31 ppm that is attributed to one of the carbon-attached fluorine atoms. Also of note: we found that the ^{19}F-chemical shifts were very solvent dependent and that MeOH afforded the cleanest ^{19}F NMR analysis compared to other solvents (e.g. DMF, DMSO), which are equally effective in promoting fluoridation (data not shown). In addition, we were unable to use 100% water because

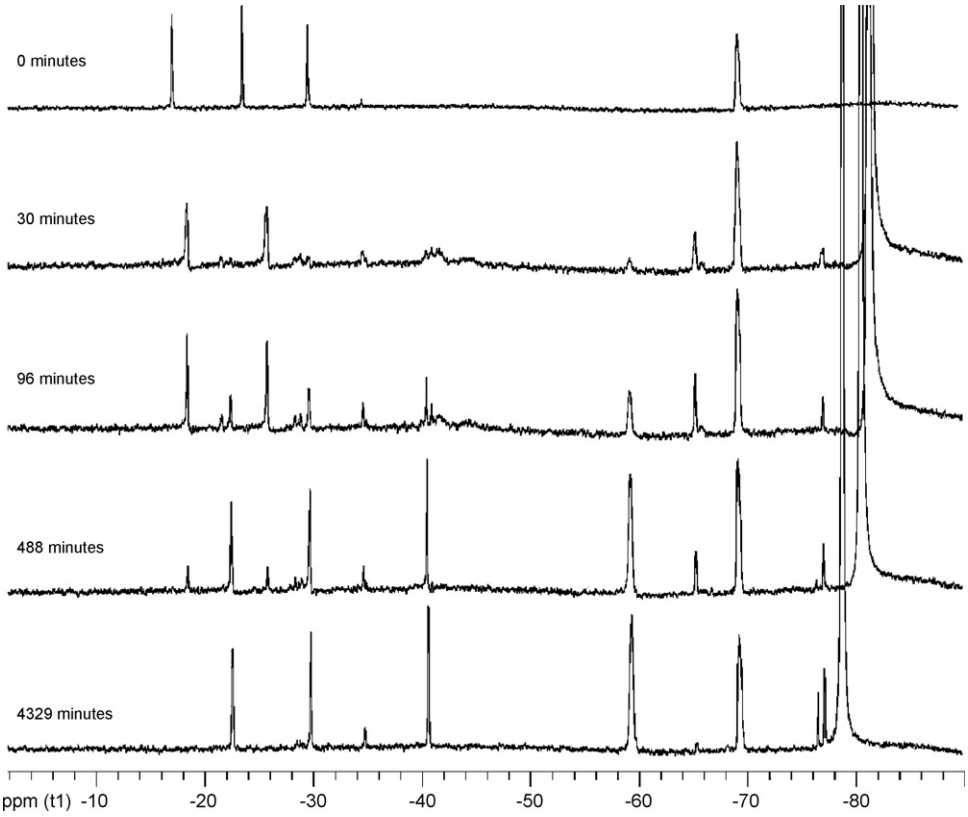


Fig. 1. ^{19}F NMR kinetic analysis of the conversion of the boronate ester to the aryltrifluoroborate referenced to neat trifluoroacetic acid as the external reference ($\delta = 0.00$, -78.3 ppm relative to $CFCl_3$) and 2,2,2-trifluoroethanol as the internal reference not shown ($\delta = -0.60$, -78.9 ppm relative to $CFCl_3$).

the tetraphenylpinacol ester, along with the greasy BODIPY, was insoluble in water at concentrations greater than 0.1 mM.

As our goal has been to establish conditions for the quantitative synthesis of an aryltrifluoroborate in order to ultimately address its aqueous stability, we were content to observe clean conversion to the aryltrifluoroborate in a reasonable period of time under the chosen conditions. Following complete reaction in the NMR tube, the reaction contents were concentrated and loaded onto a 0.5 cm silica column which afforded a relatively clean separation of the aryltrifluoroborate from the tetraphenylpinacol ester (**2**). Nevertheless, a small amount of deboronylated compound was observed by ^{19}F NMR. This contaminant was subsequently removed by preparative TLC (Silica Gel 60 F_{254} Glass TLC plate from EMD Chemicals run in 20:80 MeOH:$CHCl_3$) which afforded a very pure sample following elution into MeOH. Indeed, we continued to use this solvent system to analytically demonstrate the extent of fluoridation in the crude reaction after 72 h as well as the purity of the resulting aryltrifluoroborate. Such is shown by fluorescent excitation at 365 nm on an analytical TLC which was photographed for presentation in Fig. 2.

If aryltrifluoroborates are to be contemplated for PET-imaging, then one would not only need to resolve the aryltrifluoroborate from the tetraphenylpinacolate ester precursor (**2**) but from any free fluoride as well. To that end, both ^{1}H NMR and ^{19}F NMR analyses were run on the TLC-purified sample to demonstrate purity with respect to both starting material (**1**) and free fluoride. These are shown in Fig. 3. Of note, in the ^{1}H NMR spectrum are two broad singlets at 1.25 and 0.95 ppm, which were found to derive from the gum-backing of the TLC plate (independently confirmed by running a dummy plate and eluting accordingly—data not shown). In addition, HRMS on this sample confirmed the identity of the expected aryl trifluoroborate (see Section 4).

With these data in hand, we sought to generate an [^{18}F]-labeled aryltrifluoroborate that would ultimately allow us to investigate its stability under aqueous conditions (*vide infra*). To do this, 2.8 μmole of (**2**) was dissolved in 24.6 μL MeOH to which was added, 2 μL of water containing 1 mCi of carrier-free [^{18}F]-fluoride at pH 2–3, followed by 1.4 μL of a 4 M [^{19}F]-KHF_2 solution (11.2 μmole total fluoride, or 4 equivalents) to afford a specific activity of 89 μCi/μmole, at $t = 0$ (10:15 a.m.) [10]. Upon the addition of the [^{19}F]-KHF_2 solution, a precipitate was observed which had not been observed previously. On account of this anomalous partial solubility, an additional 40 μL of MeOH was added to solubilize all components such that the final concentrations of (**2**) and free fluoride were, respectively, 41 and 164 mM at the start of the reaction. The reaction was allowed to proceed at 37 °C for 160 min (2.66 h). The entire reaction was applied, via a micropipette tip, to a standard TLC plate approximately 8 cm in width. After air-drying the plate for approximately 5 min, the plate was resolved as noted above (run approximately 4 cm.). Following resolution, a visible light photograph was taken of the plate shown in Fig. 4.

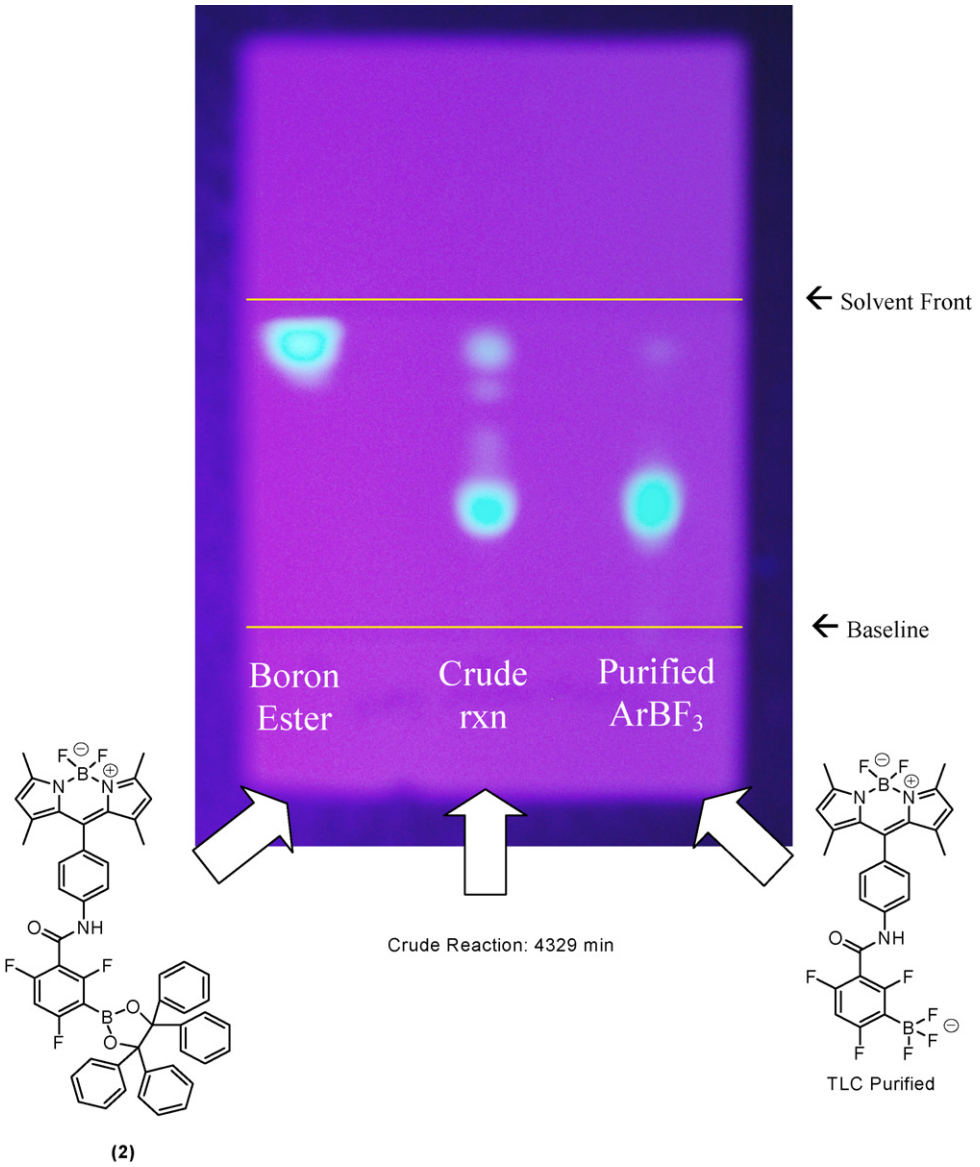


Fig. 2. TLC analysis of starting material, crude reaction after 72 h, and purified aryltrifluoroborate visualized at 365 nm after running in 20% MeOH/$CHCl_3$. Photograph was taken with a common digital camera.

The silica containing the trifluoroboroate was scraped from the plate and was found to contain 70 μCi at $t = 195$ min from which the product was eluted successively into three volumes of 166 μL MeOH, which were then pooled and found to contain 56 μCi at $t = 200$ min to give a final radiochemical yield of 16.8% (time adjusted), calculated as the fraction of (cpm-[^{18}F]-trifluoroborate)/(cpm-[^{18}F]-fluoride). The remaining starting material (**2**) was also purified accordingly and was found to contain less than 4 μCi. At $t = 200$ min, the final specific activity of the isolated aryltrifluoroborate was calculated to be 89 μCi/μmole. Accordingly, the total chemical yield with respect to (**2**) was estimated from the time-adjusted specific activity of the aryltrifluoroborate (thrice that of the carrier-added fluoride, time adjusted) to give a yield of 22% (i.e. 0.63 μmole aryltrifluoroborate/2.8 μmole starting material); the concentration of the purified aryltrifluoroborate was thus estimated at 0.125 mM. ^{19}F NMR analysis of the same sample suggested a concentration of approximately 0.2 mM calculated from the relative integration against a known concentration of 2,2,2-trifluoroethanol (data not shown).

A second TLC plate was run and visualized by UV fluorescence and autoradiography to assess the purity as well as the stability of the purified, radiolabeled aryltrifluoroborate under physiologically buffered (aqueous phosphate pH 7.4) conditions. Fig. 5 nicely demonstrates the facile and high degree of resolution of the aryltrifluoroborate from both starting material and free fluoride, as well as its overall purity (lanes 1–3). The small amount of label that is found in the starting material (**2**), which runs at the solvent front, may either be the suspected monofluoro-tetraphenylpinacoloborate, or some other unidentified impurity derived from the silica plate.

In order to demonstrate stability of the aryltrifluoroborate to solvolytic defluoridation, an ‘‘isotopic exchange’’ experiment was employed to probe the time-dependent solvolytic exchange

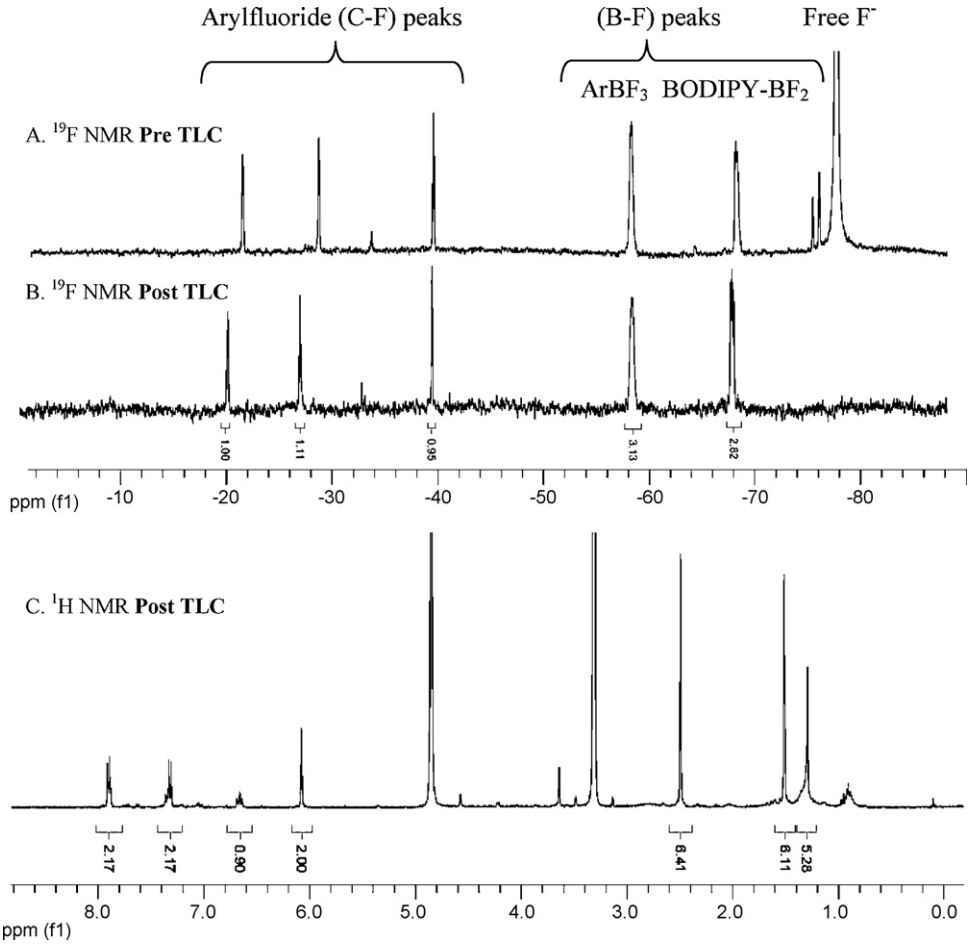


Fig. 3. NMR analysis of the aryltrifluoroborate. (A) Top, ^{19}F NMR before TLC chromatography; (B) middle, ^{19}F NMR (methanol-d_4) following TLC purification; (C) bottom, ^{1}H NMR (deuterated methanol-d_4) following TLC purification.

of the boron-bound fluoride atoms in the presence of large excess of [^{19}F]-fluoride and at very low concentration of [^{18}F]-labeled aryltrifluoroborate. To do this, 10 μL of the above 0.125 mM solution of aryltrifluoroborate was added to 300 μL of aqueous 100 mM [^{19}F]-KF in 100 mM phosphate at pH 7.4 and allowed to sit for increasing amount of time. After being allowed to exchange bound [^{18}F]-fluoride for free [^{19}F]-fluoride for varying time periods, 0.5 μL of each chase reaction was spotted on the TLC plate (lanes 4–9) along with samples representing the purified starting material (**2**), the crude reaction and the purified aryltrifluoroborate (lanes 1–3). After air-drying for approximately 5 min (it is assumed that the silica matrix of the TLC plate, into which the samples had dried did not catalyze significant exchange), the TLC plate was resolved as before, and visualized by UV-fluorescence and by autoradiography. Impressively, the $ArBF_3$ is cleanly separated (R_f of 0.5) from both free fluoride and precursor boronate ester as featured in Fig. 5.

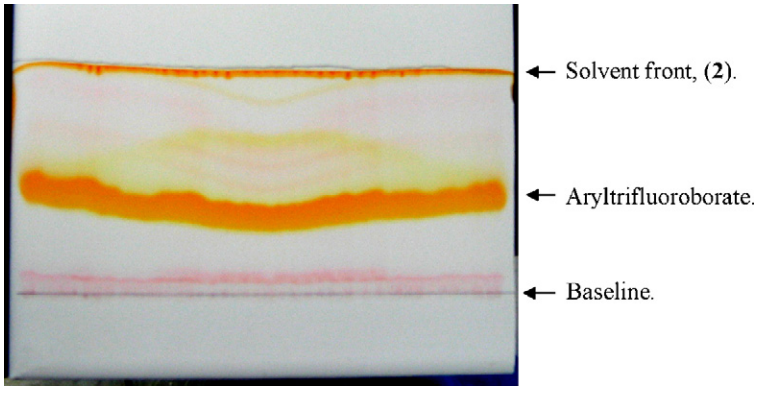


Fig. 4. Visible light photograph of the TLC plate that was run in 20% MeOH and 80% $CHCl_3$ to resolve the aryltrifluoroborate from starting material (**2**).

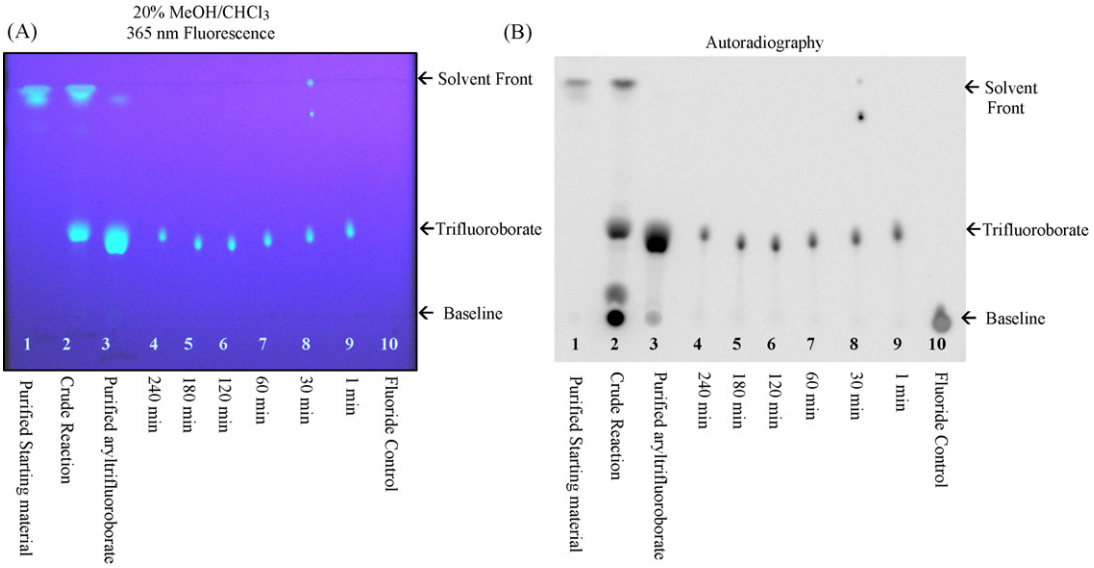


Fig. 5. (A) Fluorescent visualization of the repurified starting material (**2**) following [^{18}F]-fluoridation, the crude reaction and the purified aryltrifluoroborate in the first three lanes respectively from the left. Lanes 4–9 represent the aryltrifluoroborate following time-dependent incubation in excess [^{19}F]-fluoride. (B) Autoradiographic analysis of the same plate. Only in panel B (lane 10), does one see a control spot for free [^{18}F]-fluoride, by autoradiography.

The isotopic wash-out experiment (lanes 4–9) is based on the hypothesis that if the fluoride atoms of an aryltrifluoroborate were solvolytically labile, then any solvolytic loss of fluoride would result in the unstable difluoroborane, which would either hydrolyze completely to the boronic acid, or reform as the unlabeled [^{19}F]-$ArBF_3$ in the presence of such a large excess of [^{19}F]-anion such that over time one would observe a time-dependent emergence of a spot corresponding to free [^{18}F]-fluoride and a concomitant disappearance of the spot corresponding to the aryltrifluoroborate on the autoradiographic image of the TLC.

By eye it is difficult to appreciate any time-dependent defluoridation in lanes 4–9 of Fig. 5B. However, by quantifying and normalizing the autoradiographic density at each time point for both free fluoride and for aryltrifluoroborate, we in fact observed a very small amount of time-dependent fluoride exchange. The rate equation by which the labeled aryltrifluoroborate exchanges its fluoride with aqueous [^{19}F]-fluoride is necessarily first-order because: (i) the kinetic isotope effect between [^{18}F]-fluoride and [^{19}F]-fluoride is taken to be negligible [12], (ii) on average the aryltrifluoroborate has only one boron-attached [^{18}F]-fluorine atom, (iii) the rate of nuclear [^{18}F]-decay is independent of whether the fluoride atom is bound to boron or free in solution and (iv) the concentration of [^{19}F]-fluoride in solution (100 mM) is much greater than that of the radiolabeled aryltrifluoroborate ($\sim$4 μM).

We calculated the solvolysis rate from the relative autoradiographic density corresponding to the aryltrifluoroborate spot compared to the total autoradiographic density that includes both the aryltrifluoroborate spot and free fluoride spot. Because all samples were prepared using the same [^{18}F]-fluoride sample, the nuclear decay process does not enter into our calculation of the rate constant for the exchange reaction. In addition, we assumed that the rate of fluoride loss in the methanolic stock solution over the period of 4 h was negligible since there was little free fluoride seen in lane 3. Moreover, even

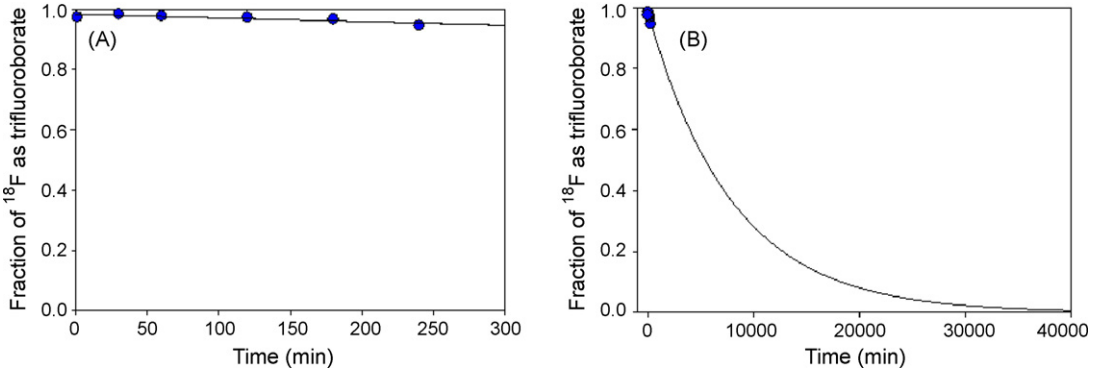


Fig. 6. (A) Plot of relative autoradiographic density of the aryltrifluoroborate spot in the autoradiogram as a function of all cpm ([^{18}F]-$ArBF_3$ + free [^{18}F]-fluoride) vs. time: data are scaled 300 min. (B) Data were fit to the function $y = Ae^{-k_{obs}t}$ with a curve showing decay to zero: $k_{obs} = 0.00012 \pm 0.00004$, $A = 0.984 \pm 0.005$, $R^2 = 0.7341$.

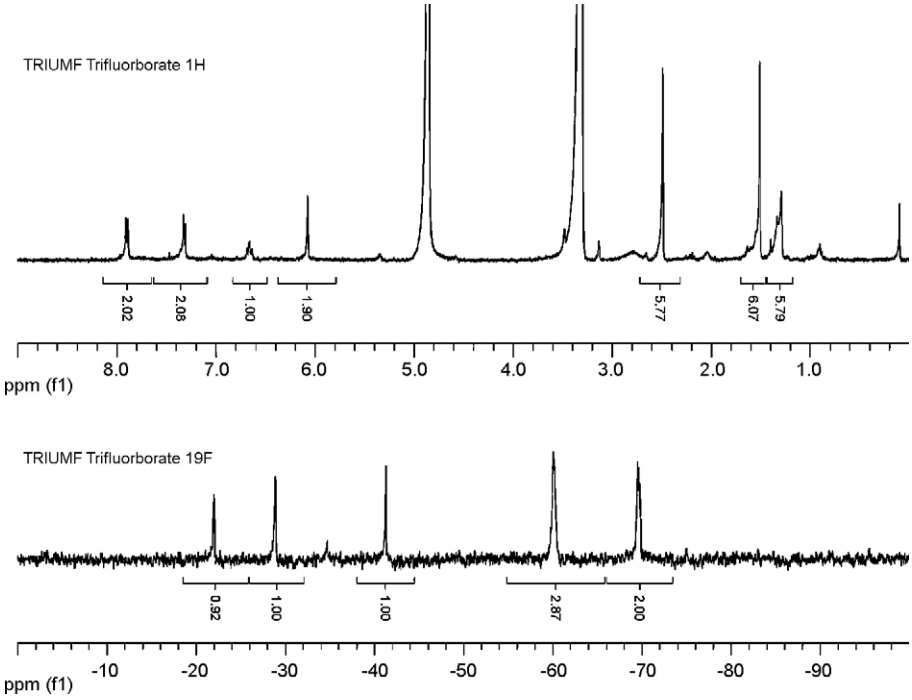


Fig. 7. (Above) Post-decay ^{1}H NMR spectrum of the aryltrifluoroborate in deuterated methanol, (below) ^{19}F NMR of the same sample.

after a 72 h decay, no free fluoride is detected in the NMR in Fig. 7 suggesting that the presence of a less polar solvent significantly depresses the rate of defluoridation.

Experimentally, the rate constant for fluoride exchange can be estimated by either linear approximation using the ''method of initial rates'' or a non-linear least square fit to the exponential equation: $y = Ae^{-k_{obs}t}$, where y is the fraction of [^{18}F]-trifluoroborate and t is time. Fitting the data to the exponential equation that must approach zero at infinity, we obtained a rate constant for defluorination of: $k_{obs} = 1.2 \pm 0.4 \times 10^{-4}$ min^{-1}. The method of initial rates rendered virtually the same value. This rate constant dictates a half-life of 92.5 ± 29 h, a value well over 30 times that for the decay of [^{18}F]-fluoride. Fig. 6 shows a negligible extent of defluoridation over 300 min, a period of time longer than 2.5 half-lives of [^{18}F]-fluoride decay, as well as the full fit of the first-order rate equation to a time course that approaches 100% defluoridation at infinity.

Since the above analysis consumed approximately 65 μL of the 500 μL solution of the purified aryltrifluoroborate in methanol, the remainder was allowed to decay for 72 h and then analyzed by ESI-MS (negative ion mode: 564.16 exp, 564.4 found, data not shown), and by both ^{1}H and ^{19}F NMR to verify that the radioactive sample was indeed the expected aryltrifluoroborate as demonstrated in Fig. 7 which shows identical spectra to those obtained in the non-radioactive preparation.

3. Conclusions and significance

Herein, we describe an arylboronic acid with several physical/fluorescent properties that allow confirmation of the synthesis, purity and identity of the corresponding aryltrifluoroborate. For this study, we chose to include three [^{19}F]-atoms on the phenylboronic acid to enable us to follow its conversion to the aryltrifluoroborate by ^{19}F NMR. The BODIPY fluorophore provided fluorescent evidence as to the identities, purities and chromatographic mobilities of both the starting material (**2**) and fluoridated product. This approach then allowed us to also correlate these physical data with the ephemeral radioactive decay that provided an autoradiographic trace of the chromatographic mobility of the desired aryltrifluoroborate. Using the autoradiographic data in conjunction with an isotopic exchange experiment, we were able to estimate a rate constant for the defluoridation of the pure aryltrifluoroborate.

When we initially approached the question of defluoridation, we had hoped to use ^{19}F NMR. However, at 0.5 mM aryltrifluoroborate, a concentration which provided a reasonable detection of [^{19}F]-fluorine by NMR, we were unable to observe any appreciable amount of defluoridation over 72 h (data not shown) in part because at this concentration, the trifluoroborate was sparingly soluble in water and the presence of methanol seems to greatly retard the rate of solvolysis.

Thus, we chose to employ an isotopic exchange experiment that involved placing trace amounts of [^{18}F]-trifluoroborate in a large excess of [^{19}F]-fluoride. In this manner, we could measure the rate of fluoride exchange independently of an unknown equilibrium constant for aryltrifluoroborate formation. This experiment measures the rate of loss of a single fluorine atom, as anionic fluoride, from the parent aryltrifluoroborate to afford an aryldifluoroborane. As aryldifluoroboranes are considered to be highly unstable in aqueous conditions, the resulting difluoroborane in this case went undetected, as anticipated. Such a fleeting intermediate must have partitioned to either the fully hydrolyzed arylboronic acid/arylborate, or back to the aryltrifluoroborate, which, as a product of recapture, is unlabeled due to

the large excess of [^{19}F]-fluoride present in the isotopic wash-out experiment.

From this experiment, it is impossible to know whether the aryldifluoroborane completely hydrolyzed to the unlabeled arylboronic/arylborate or reacted with 100 mM aqueous [^{19}F]-fluoride at pH 7.5 to regenerate the unlabeled aryltrifluoroborate isotopologue. What is important to emphasize is that the excess of [^{19}F]-fluoride in the chase solution prevented any recapture of released [^{18}F]-fluoride by boron when the TLC plate dried just prior to resolution. Irrespective of the subsequent fate of the resulting aryldifluoroborane, this experiment accurately measures the rate of loss of an atom of fluoride during a single rate-limiting step. Provided that the presence of 100 mM free fluoride does not catalyze solvolysis, the same kinetic stability should be observed under physiological conditions (i.e. *in vivo*) such that this trifluoroborate should retain its integrity as it clears from the blood stream to the bladder without solvolytic loss of [^{18}F]-fluoride to the bone.

For this aryltrifluoroborate, the rate of defluoridation is extremely slow. Although, we took advantage of the three ^{19}F-fluorine atoms on the phenyl ring for ^{19}F NMR analysis, these atoms may not have been naive spectators in either the conversion of (**2**) to the aryltrifluoroborate or in the fluoride exchange reaction. It is reasonable to suspect that the electron-withdrawing nature of the three [^{19}F]-fluorine atoms may help stabilize the aryltrifluoroborate with respect to solvolytic defluoridation. Nevertheless, these data and experiments represent an experimental framework for evaluating the effects of various substituents on the solvolysis of aryltrifluoroborates, a reaction that has not to date been quantitatively and deliberately investigated.

The implications for having identified a very stable aryltrifluoroborate may be two-fold. Firstly, if defluoridation is invoked as the necessary first step in a Suzuki-Miyaura coupling, one will have to reconcile the extreme sluggishness of the defluoridation with the general finding that other trifluorophenyltrifluoroborates are readily coupled in this reaction. If indeed the Suzuki-Miyaura coupling reaction requires moderate solvolytic lability of the aryltrifluoroborate, conversely, the successful application of [^{18}F]-trifluoroborates to PET imaging can tolerate little if any lability. In this regard, the aryltrifluoroborate we have prepared using carrier added [^{18}F]-fluoride should find potential utility in the generation of [^{18}F]-labeled PET imaging agents.

In this case, it is noteworthy that excess carrier fluoride was added such that boron was limiting (four equivalents of fluoride to one equivalent of boron). As long as fluoride is at least four times in excess of boron, the specific activity of the resulting aryltrifluoroborate will be directly proportional to that of the source fluoride and is independent of the concentration of boronic ester used. In other words, addition of more arylboronic acid will result in the production of more aryltrifluoroborate, but the specific activity (cpm/mole $ArBF_3$) will remain the same. Consequently, the only requirement is that the resulting aryltrifluoroborate be separated from the boronic acid/ester precursor—a condition that was met in this case by the TLC separation.

To that end, one might ask whether a carrier-added synthesis of an [^{18}F]-fluoride labeled aryltrifluoroborate be expected to generate a specific activity greater than 1 Ci/μmole, which would be required for PET imaging of excellular receptors (cancer). Although, true carrier-free [^{18}F]-fluoride has a specific activity of 1720 Ci/μmole, in practice the specific activity in no-carrier added [^{18}F]-fluoride is less than 100 Ci/μmole due to environmental contamination with [^{19}F]-fluoride. Because in practice 1 Ci contains at least 10 nmole fluoride ion ([^{18}F] and [^{19}F]) one could easily envision adding up to 990 nmole of additional [^{19}F]-fluoride to a radiochemical synthesis that would still ensure a requisite specific activity of 1 Ci/μmole.

In this report, we condensed 1 mCi contained in 11.4 μmole of fluoride in 28–68 μL of volume. It is readily conceivable that we could have performed the same reaction in one-tenth the volume (e.g. 4 μL) using 1 Ci. Although for safety reasons, we did not work with this quantity of radioactivity, had we done so, we would have generated an aryltrifluoroborate with a specific activity of 3 Ci/μmole during the initial time points of the labeling reaction. Following a 3 h synthesis, nuclear decay would have reduced the specific activity to the requisite minimum of 1 Ci/μmole.

The idea of using boronic acids as captors of aqueous [^{18}F]-fluoride is an attractive one given the simplicity of working with aqueous [^{18}F]-fluoride and a precursor boronate ester. Here, we have conclusively demonstrated that the resulting [^{18}F]-trifluoroborate is both easily separated from both starting material and free [^{18}F]-fluoride and stable to solvolytic defluoridation. Although, it was necessary in this study to add carrier [^{19}F]-fluoride because of the reaction volume and low level radioactivity that we used, a salient advantage of using of boron is that the specific activity of the resulting aryltrifluoroborate increases three-fold over that of the aqueous fluoride because three fluoride atoms are condensed to form one aryltrifluoroborate. In the future, specific activities sufficient for imaging (∼1 Ci/μmole) will be attained by using smaller reaction volumes and greater amounts of radioactivity. Use of a microreactor that should ultimately enable a no-carrier added synthesis of an [^{18}F]-labeled aryltrifluoroborate. As compound **1** can be readily conjugated to biologically active molecules, the development of [^{18}F]-labeled aryltrifluoroborate PET agents for imaging cancer in mice is currently underway and will be reported in due time.

4. Experimental

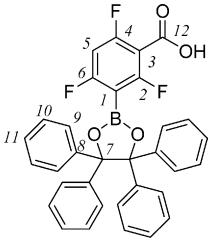
4.1. General methods

Chemicals were purchased from Sigma–Aldrich or Acros Organic. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Analytical and preparative thin layer chromatographies were run on Silica Gel 60 F_{254} Glass TLC plates from EMD Chemicals. All ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 or 400 MHz instrument. Chemical shifts are reported using the δ scale in ppm and all coupling constants (J) are reported in

hertz (Hz). Unless specified, ^{1}H NMR spectra are referenced to the tetramethylsilane peak ($\delta = 0.00$), and ^{19}F NMR spectra are referenced to NEAT trifluoroacetic acid ($\delta = 0.00$, −78.3 ppm relative to $CFCl_3$). Due to the presence of ^{19}F contaminations in the NMR spectrometer probe, baseline corrections for samples less than 20 mM in [^{19}F]-anion concentration had to be adjusted by multipoint linear baseline correction using MestReC 4.9.9.9. This correction did not affect the absolute chemical shifts or integration ratios of ^{19}F signals. Mass spectrometry was performed at the Mass Spectrometry lab of the University of British Columbia (U.B.C.) Chemistry Department.

2,4,6-Trifluoro-3-(4,4,5,5-tetraphenyl-1,3,2-dioxaborolan-2-yl)benzoic acid (**1**). 2,4,6 Trifluorobenzoic acid (0.51 g, 2.9 mmole) was added to a stirring solution of THF (30 mL) that was cooled with a acetone/CO_2(s) bath under N_2(g) flow. Following the cooling period, 4 mL of a 1.6 M solution of *n*-butyl lithium (nBuLi) in hexanes (6.4 mmole, 2.2 equivalents) was added through a syringe to the solution over a period of 5 min. The lithiation reaction was allowed proceed for 12 min before trimethylborate was added (800 μL, 7 mmole) and the reaction stirred for 1 h. After this period, the reaction was quenched with anhydrous HCl in dioxane followed by 7 mmole of benzopinacol dissolved in THF. The reaction was then warmed to room temperature where it could be stored overnight. Fifty milliliters of toluene was added to the crude reaction and the reaction was immediately concentrated to an oil. The resulting solid was triturated in a 50/50 solution of EtOAc/hexanes and the precipitates were filtered off. The EtOAc/hexane soluble material was dried, solubilized in 50 mL of EtOAc and suspended over a 50 mL saturated sodium bicarbonate solution. Water soluble impurities were extracted, discarded and the EtOAc layer was washed again with one wash of 50 mL saturated aqueous sodium bicarbonate, two washes of 50 mL of deionized water, and finally with 30 mL of a 1.5 M solution of HCl. The EtOAc layer was concentrated to a solid.

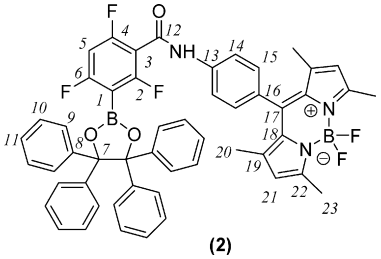
The concentrated product was loaded onto a 1 cm silica column and eluted with 10% EtOAc/hexanes. Product elution was monitored by TLC. The appropriate fractions were concentrated to give 864 mg of (**1**) as a white solid in 54% yield.



(1)

^{1}H NMR (CD_2Cl_2, 300 MHz): 7.25–7.21 (*m*, 8H, C**H**^{9}), 7.12–7.10 (*m*, 12H, C**H**^{10,11}), 6.92 (*t*, $J = 10.5$ Hz, 1H, C**H**^{5}). ^{19}F NMR (CD_2Cl_2, 300 MHz): −12.672 (1F), −16.760 (1F), −23.622 (1F). HRMS (ESI) calculated for $C_{33}H_{22}BF_3NaO_4^+$ $(M+Na)^+$: 573.14554 *m*/*z*, found: 573.1451.

N-4-aminophenyl di(dimethylpyrro)methene boron difluoride (2,4,6-trifluoro-3-(4,4,5,5-tetraphenyl-1,3,2-dioxaborolan-2-yl)phenyl)methanone (**2**). Reagents were added to a 4 mL vial containing 3.55 mL of DMF in the following order: amino phenyl di(dimethylpyrro)methene boron difluoride (BODIPY) [11], 25.8 mg, 0.076 mmole), pyridine (33.2 μL, 0.41 mmole), HOBt monohydrate (13.5 mg, 0.10 mmole, (**1**) (50 mg, 0.09 mmole)) and EDC (19.4 mg, 0.10 mmole). This reaction was left for 16 h after which it was concentrated to oil and loaded onto a 0.5 cm silica column. The product, compound (**2**) was eluted with 80% $CHCl_3$/hexanes (v/v). Pooling the appropriate fractions gave a sample of (**2**) contaminated with the protodeborinated product; 2,4,6-trifluorobenzamide-BODIPY. Instead, the impurity was removed on a preparative TLC plate run on a Silica Gel 60 F_{254} Glass TLC plate from EMD Chemicals with 40% EtOAc/hexanes as the running solvent. The fluorescent bands were run ∼10 cm up the plate, the top 366 nm fluorescent band which corresponded to (**2**) was scraped from the plate, dissolved in CH_2Cl_2 and filtered through sea sand on top of glass wool. A non-fluorescent aliphatic impurity coming from the EMD silica plates was present in the ^{1}H NMR. This impurity was isolated through a preparative TLC plate run on a blank sample, did not react with fluoride, and was not fluorescent.



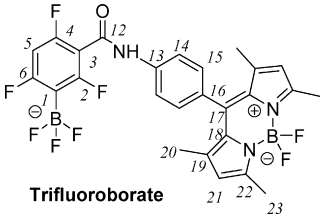
(2)

^{1}H NMR (CD_2Cl_2, 400 MHz): 7.88 (*s*, 1H, N**H**), 7.84 (*d*, $J = 8.4$ Hz, 2H, C**H**^{14}), 7.35 (*d*, $J = 8.4$ Hz, 2H, C**H**^{15}), 7.26–7.24 (*m*, 8H, C**H**^{9}), 7.14–7.11 (*m*, 12H, C**H**^{10,11}), 6.97 (*t*, $J = 9.2$ Hz, 1H, C**H**^{5}), 6.05 (*s*, 2H, C**H**^{21}), 2.53 (*s*, 6H, C**H**^{23}), 1.50 (*s*, 6H, C**H**^{20}). Prep TLC impurity: 1.55 (*s*), 1.28 (*s*), 0.92–0.86 (*m*). ^{19}F NMR (CD_2Cl_2, 300 MHz): −17.41 (s, 1F, C**F**), −23.87 (s, 1F, C**F**), −29.90 (s, 1F, C**F**), −69.46 (1:1:1:1 q, $J = 33$ Hz, 2F, B**F**$_2$). HRMS (ESI) calculated for $C_{52}H_{39}B_2F_5N_3O_3^-$ $(M-H)^-$: 870.31032 *m*/*z*, found: 870.3108.

Potassium *N*-4-aminophenyl di(dimethylpyrro)methene boron difluoride (2,4,6-trifluoro-3-(trifluoroborate)phenyl)-methanone. A ^{1}H NMR tube containing 450 μL of MeOH was charged with (**2**) (0.87 mg, 1 μmole) and 50 μL of 1 M HOAc/NaOAc pH 4.5. Twenty five microliters of 4 M KHF_2 (200 μmole fluoride) was added. The kinetics of aryltrifluoroborate formation was monitored by ^{19}F NMR.

Following completion of the reaction, the reaction was concentrated and loaded onto a 0.5 cm silica column. The aryltrifluoroborate was eluted with 10% MeOH/$CHCl_3$ (v/v). Pooling the appropriate fractions gave a sample of the aryltrifluoroborate contaminated with the protiodeboronated product: 2,4,6-trifluorobenzamide-BODIPY. This contamination was removed on a preparative TLC plate run on a Silica Gel 60 F_{254} Glass TLC plate from EMD Chemicals with 20% MeOH/$CHCl_3$ as the running solvent. The bottom band that corresponded to the aryltrifluoroborate was scraped off the

plate, dissolved in CH_2Cl_2 and filtered through sea sand on top of glass wool.



^{1}H NMR (MeOH-d_4, 400 MHz): 7.99 (*d*, *J* = 8.4 Hz, 2H, C**H**^{14}), 7.36 (*d*, *J* = 8.8 Hz, 2H, C**H**^{15}), 6.66 (*t*, *J* = 9.6 Hz, 1H, C**H**^{5}), 6.08 (*s*, 2H, C**H**^{21}), 2.49 (*s*, 6H, C**H**^{20}), 1.51 (*s*, 6H, C**H**^{20}). Prep TLC impurity: 1.23 (*s*), 1.28 (*s*), 0.97–0.86 (*m*). ^{19}F NMR (MeOH-d_4, 300 MHz): −20.93 (s, 1F, C**F**), −27.78 (s, 1F, C**F**), −40.26 (s, 1F, C**F**), −59.15 (br, 3F, B**F**$_3$), −68.64 (1:1:1:1 q, *J* = 34.8 Hz, 2F, B**F**$_2$). HRMS (ESI) calculated for $C_{26}H_{20}B_2F_8N_3O^-$ (M)$^-$: 564.1665 *m*/*z*, found: 564.1675.

4.2. Fluoride exchange kinetics and autoradiography

Ten microliters of the purified [^{18}F]-labeled aryltrifluoroborate estimated at 0.125 mM solution (1.12 μCi at the start of the exchange reaction *t* = 0 min, and 0.25 μCi at *t* = 240 min at the end of the exchange reaction) was added to 300 μL of aqueous 100 mM [^{19}F]-KF in 100 mM phosphate at pH 7.4 and allowed to sit for increasing amount of time over the period of 4 h. 0.5 μL (∼367 pCi at *t* = 4 h) of each chase reaction was spotted on the TLC plate (lanes 4–9). After air-drying for approximately 5 min the TLC plate was resolved as before, and a storage phosphor screen was exposed overnight to collect the positron decay from the TLC plate. The screen was then scanned at 50 μm resolution using a Typhoon 9200 Phosphorimager. The screen was normalized for background and the autoradiographic densities for each spots corresponding to the aryltrifluoroborate and for each spot corresponding to free fluoride were calculated using ImageQuant 5.0. The density of the fluoride was approximately two-fold over background and that for the trifluoroborate was 40-fold over background. For example, a typical data set was found to contain: 4,243,009 pixel counts for the aryltrifluoroborate, 243,529 pixel counts for free fluoride and 135,324 pixel counts for a background area of the same size. The background value was subtracted from each value (aryltrifluoroborate and free fluoride) and the densities at each time point were related to total density for each time point. The fractional density corresponding to the aryltrifluoroborate, at each time point, was then fit either to a line (method of initial rates) or to a first-order exponential decay function using SigmaPlot 10.0. The extent of fluoride loss by the aryltrifluoroborate during the period of up to 4 h in MeOH at 0.125 mM was taken to be negligible however if any fluoride had exchanged during this period, then this experiment actually over-estimates the rate of fluoride exchange.

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