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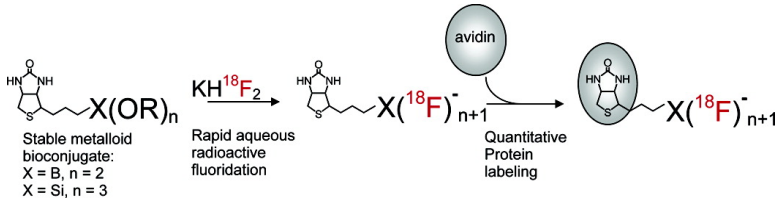

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Arylfluoroborates and Alkylfluorosilicates as Potential PET Imaging Agents: High-Yielding Aqueous Biomolecular ^{18}F-Labeling

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Positron (β^+) emission tomography (PET) permits high-resolution localization of biological phenomena, provided that suitably labeled ligands can specifically recognize molecular markers of the diseased state.[1] Larger biomolecules such as peptides and oligonucleotides will be valuable for addressing this.

^{18}F, with a half-life of 110 min, provides high specific radioactivity when introduced efficiently. Nevertheless, the chemistry of fluorine limits labeling strategies involving C−F bond formation. Reactive F^+ reagents (e.g. F_2, DAST) are difficult to prepare with high specific activity and moreover are too reactive for direct labeling of biomolecules. Anionic F^-, while readily prepared with high specific activity, is a poor nucleophile in aqueous media in which most large biomolecules must be stored. Current labeling strategies involve the generation of small radioactive precursors that are prepared in polar aprotic solvents at 70−140 °C via nucleophilic displacement by $^{18}F^-$ under scrupulously dry conditions. Coupling these precursors to biomolecules requires additional transformations. In all cases to date, ^{18}F is introduced in the first of several chemical steps.[2]

Following synthesis, rapid decay and safety concerns limit the ability to correlate radiochemical yields with the imaging agent's biological activity, which might be compromised by improper coupling or by contaminants carried over from prior steps.[3] Ideally, a large-molecule imaging agent would be synthesized as a shelf-stable precursor that could be rapidly labeled in a single high-yielding step under aqueous conditions via simple addition of $^{18}F^-$. Indeed, the advantage of such a strategy is recognized in the case of DOTA conjugates that are labeled by ^{64}Cu wash-in.[4] To date, no such strategy has been described for ^{18}F labeling.[5]

Here we describe two new classes of biomolecule precursors that acquire ^{18}F highly efficiently in one step in aqueous or mixed-solvent media: a pinacol phenylboronate diester (**1**) and an alkyltriethoxysilane (**2**) (Figure 1). Each metalloid was conjugated to biotin via an amide bond[13] (see Supporting Information for synthetic characterization) to investigate protein targeting to avidin.

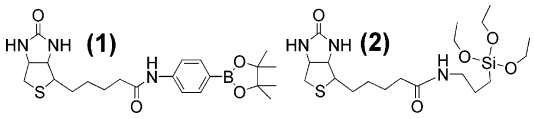

Figure 1. Biotinylated *p*-aminophenylboronylpinacolate (**1**) and biotinylated (aminopropyl)triethoxysilane (**2**).

Fluoride treatment of **1** and **2** gave trifluoroborate and tetrafluorosilicate "ate" salts that were captured by the protein avidin. Thus, this labeling should apply to other ligands (e.g. peptides, aptamers, antibodies) that recognize various protein targets.

An aqueous volume of 5 μL (440 μCi from unfixed target water) of 100 mM or 130 mM KHF_2 (3.3 or 4.4 equiv of $^{19}F^-$) in 200 mM NaOAc, pH 4.5, was added to 60 mM solutions of **1**, **2**, or unmodified biotin that had been dissolved in 5 μL of DMF, MeOH, MeCN, DMSO, or H_2O. For reaction at pH 7.5, 200 mM HEPES was used as a buffer in water. After 1 h at room temperature, reactions were quenched by addition of 200 μL of 300 mM $NaHCO_3$, pH 7.5. The reaction was transferred to 15 μL of washed, polydisperse avidin magnetic particles (AMPs) with an experimentally verified capacity for binding 525 pmol of biotin (see Supporting Information) in 300 μL total volume. After 20 min, the AMPs were magnetized, washed twice with 200 μL of 300 mM carbonate and thrice with 200 μL of 1 M NaCl, 10 mM TrisHCl, pH 7.5, and 1 mM EDTA (total wash time ~40 min), resuspended in 5 μL of water, and transferred to a silica TLC plate, which was affixed with clear tape and subjected to autoradiography as shown in Figure 2.

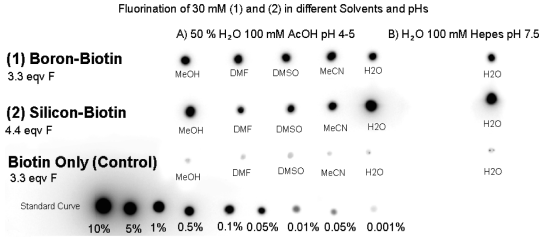

Figure 2. Autoradiogram of the washed AMPs: first row, boron **1**; second row, silicon **2**; third row, unmodified biotin control. Spots 1−6 (left to right): 50% MeOH, 50% DMF, 50% DMSO, 50% MeCN, 100% H_2O, at pH 4.5, then 100% H_2O at pH 7.5. Bottom: Standard curve representing percentage of total starting radioactivity. With a fluoridation efficiency of 90%, the density on the AMPs should be 0.15% (vide infra). Contrast is increased to show radiographic location of control samples in third row.

Fluoridation is seen only for compounds **1** and **2**, whereas negligible fluoride carry-over is found on the AMPs that bound unmodified biotin treated in the same way. Yields varied little with various cosolvents. If one assumes that all avidin is bound ($K_d < 10^{-12}$ M) and no biotin dissociates during washing ($k_d < 10^{-5}$ min^{-1}), the ratio of AMP-bound counts to total counts must equal the ratio of biotin binding capacity (525 pmol) to the total biotin (3×10^5 pmol) multiplied by the fluoridation efficiency. To correlate autoradiographic density with the fraction of F^- spotted or incorporated, serially diluted quantities of $^{18}F^-$ used in fluoridation were applied to the plate. Fluoridation efficiencies were variable but approached 100% for **2** and 80% for **1** (see Supporting Information), consistent with yields for such "ate" complexes obtained at higher fluoride and organometalloid concentrations.[6,9] ^{19}F NMR and HRMS spectra verified a F:B ratio of 3:1 for the "ate" salt of **1**. For that of **2**, ^{19}F NMR shifts are given and low-resolution ESI indicated a F:Si ratio of 4:1 (see Supporting Information).

Whereas the Si−F and B−F bonds are among the strongest bonds known, and although alkyltetrafluorosilicates and aryltrifluoroborates can be precipitated from water, to the best of our knowledge, no study has examined the hydrolytic decomposition rate of either an alkyltetrafluorosilicate or an aryltrifluoroborate. The only relevant work was an investigation of the hydrolysis of tetrafluoroborate

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which proceeds very slowly ($t_{1/2} \approx 168$ h, 100 °C).[14] To that end, we sought to measure the hydrolytic stability of the "ate" complexes here at ~21 °C. To do this we first verified that significantly less fluoridation occurs at dilute conditions: 1.5−2 mM KHF_2 (3−4 equiv of F^-) and 1 mM **1** or **2** (~3−10-fold over background, data not shown). Therefore, if the "ate" were to hydrolyze at high dilution, no significant back reaction (i.e. refluoridation) would occur. ^{18}F-labeled **1** and **2** were diluted to 0.3 mM into carbonate buffer for periods of time prior to avidin capture. In addition, the same were diluted 100-fold into 200 mM $KH^{19}F_2$. As fluoridation is both kinetically and thermodynamically favorable at 200 mM KHF_2, this isotope exchange experiment ensured that any dissociated ^{18}F would be replaced by ^{19}F. The autoradiogram of AMP capture of **1** and **2** after dilution is shown in Figure 3. Quantitative analysis followed as above and as detailed further in the Supporting Information.

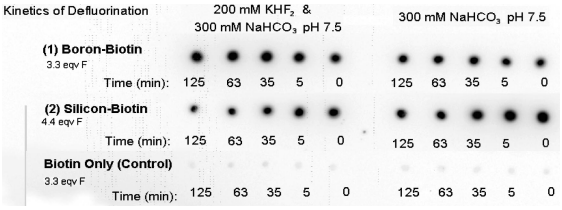

Figure 3. Stability assay of "ate" salts of **1** and **2**: first column, samples are diluted into KHF_2 at pH 7.5; second column, samples are simply diluted into HCO_3^- buffer; first row, boron; second row, silicon; third row, unmodified biotin. Incubation times prior to AMP capture are noted.

In the case of the tetrafluorosilicate, signal intensity dissipates over time. Data were fit to a first-order function that returned a rate constant of 0.01 min^{-1} in the presence of KHF_2 and 0.008 min^{-1} in the absence, suggesting modest stability (see Supporting Information for data fits). For the trifluoroborate, no decomposition was observed, suggesting considerable stability in aqueous media.

For any labeling method to be used in imaging, the radioisotope must be readily incorporated onto the imaging agent, which in turn must be kinetically stable upon humoral dilution. To test this, "ates" of **1** and **2** were incubated in either serum or whole blood for 1 h prior to AMP capture. No time-dependent loss of ^{18}F was observed (see Supporting Information).

Conclusions and Discussion. Use of a short-lived isotope requires that radiopharmaceutical synthesis be kinetically and thermodynamically favorable at the time of preparation, and that the product be at least kinetically stable following injection. To that end, we contemplated the varied chemistries of organosilicon and organoboron[6] that have found use respectively in protecting groups and biochip fabrication,[7] Suzuki chemistries,[8,9] and fluoride sensors.[10] Both compositions form stable linkages to alcohols yet react rapidly and quantitatively with F^- to give tetrafluorosilicates[11] and trifluoroborates that are sufficiently stable to be precipitated from aqueous media.[6,12] Organoboron and organosilicon bioconjugates fluoridate in a single, rapid, and high-yielding step at pH 4−7 in aqueous solvents and at temperatures that are unlikely to denature the biomolecule. Their use should obviate multistep synthetic transformations that normally follow radioisotope incorporation and which require cumbersome robotic shielding. The aryltrifluoroborate is appreciably more stable than the alkyltetrafluorosilicate and should thus prove useful in developing stable biomolecule precursors for imaging. The alkyltetrafluorosilicate was moderately stable in aqueous media and decomposed with a rate that is on par with that of ^{18}F decay. Thus, the utility of an alkyltetrafluorosilicate would be limited to ligands that associate very quickly with physiological targets. Despite its limited stability, the triethoxysilane was studied because of its extensive use in biochips. It reasons that biomolecules affixed to solid supports via a siloxy linkage can be simultaneously released from the chip and labeled by ^{18}F.

Due to safety concerns in *this* work, carrier ^{19}F was added to trace ^{18}F (<100 pmol) to show high fluoridation efficiency. Nevertheless, this approach should deliver specific activities of >1 Ci/μmol suitable for PET imaging *without* adding carrier: typically, "no carrier added" ^{18}F preparations have specific activities of ~5 Ci/μmol, which contain significant quantities of carrier ^{19}F. Thus, reaction of 1 Ci (200 nmol total $^{18/19}F$) with 0.25 or 0.33 equiv of metalloid will yield "ates" with specific activities of 16−20 Ci/μmol. Since B or Si acquires three or four atoms of F^- respectively, this method, like no other, *increases* the effective specific activity of the source ^{18}F by 3 or 4 times (see Supporting Information). Of note, other boron derivatives used in F^- sensing may improve radiochemical yields,[10] particularly in cases where large molecules may not be soluble at 30 mM. Smaller ^{18}F-"ate" precursors could be labeled first and then coupled to biomolecules as with traditional precursors. Finally, as avidin itself has been used in imaging,[15] biotins **1** and **2** should be of use with avidin-fusion proteins.

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Supporting Information Available: Full references list; experimental and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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