Radiopharmaceuticals

# Synthesis of [18F]BODIPY: Bifunctional Reporter for Hybrid Optical/Positron Emission Tomography Imaging**

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Fluorescence-based intravital imaging approaches have been essential for studying the function and distribution of biologically active small molecules and their molecular targets in vivo.[1] While optical imaging enables visualization over a broad scope of spatial resolution ranging from subcellular to whole-body anatomical imaging, its application is constrained by the optical transparency of tissues. Optical imaging modalities are thus better suited for small-animal imaging rather than for translational applications.[2] In contrast, positron emission tomography (PET) is used routinely for clinical applications but its use is limited primarily to macroscopic spatial and temporal imaging. A strategy that would enable seamless switching between fluorescence and PET imaging would thus be highly desirable for several reasons. First, the ability to visualize PET probes at the microscopic level would allow more rapid validation of the former in vivo and consequently expedite the development of novel PET probes. Second, for macroscopic hybrid imaging,[3] it would be possible for optical and PET signals to be superimposed onto structural information derived from magnetic resonance imaging (MRI) or computed tomography (CT). Likewise, the ability to image in more than one or two channels would be useful for visualizing underlying physiological events. Third, hits from microscopic screens of small-molecule fluorochrome adducts could be more rapidly translated into clinically relevant applications.

To date, the development of hybrid optical/PET probes has been hindered by various methodological limitations. As a result, a synthetic strategy that enables general access to such compounds has yet to be established. For any approach to be successful, however, two critical factors must be considered: 1) the short half-lives of clinically relevant PET radioisotopes, namely 18F (110 min) and 11C (20 min), both of which (ideally) require the introduction of the nuclide during the final synthetic steps; and 2) the pharmacodynamic changes that result from addition of the imaging tag(s). Because of these limiting factors, previous strategies have

often relied on the use of distinct labels for each imaging modality. For example, PET ligands and fluorochromes have been used simultaneously on nanoparticles.[3] However, these methods result in chemically distinct compounds with non-identical pharmacological properties.

Herein we introduce a generic and widely applicable approach that enables a hybrid PET/fluorescence molecular tomography (FMT) imaging agent, based on boron dipyrromethene (BODIPY), to be accessed through the 19F/18F exchange of one of the fluorides within the canonical BF_2 dipyrromethene core **1**, a motif that is shared by most BODIPY dyes. This efficient method for incorporating a PET tracer thus evades the above-mentioned bottleneck in drug testing. We believe that this novel approach, which uses hybrid fluorescence/18F tags, will accelerate not only the development of imaging agents, but perhaps also the screening of therapeutic molecules.
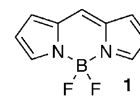
BODIPY dyes represent a unique class of fluorescent small molecules, and have recently received recognition for their extraordinary versatility as fluorescent tags in biological imaging applications.[4] The most widely appreciated characteristics of BODIPY dyes include their good photostability, high brightness, compatibility with biological media, and their broad tunable color range which covers the green to near-infrared spectrum.[5] One particular advantage of BODIPY dyes over the majority of other commonly used fluorescent dyes is the neutral nature of their scaffold. This feature is critical for the efficient penetration of cell membranes, and as such BODIPY dyes remain one of the few fluorophore classes suited to intracellular imaging within living cells.

These attractive features of the BODIPY dyes, combined with the presence of the canonical BF_2 group within their central core, highlighted this fluorochrome class as a promising candidate for the development of hybrid 18F PET/FMT imaging agents through a 19F/18F exchange. This strategy would not only allow the wide variety of analogues already commercially available to be exploited, but also allows the desirable characteristics of the BODIPY dyes to be retained. Furthermore, this exchange system could potentially even allow the seamless conversion of BODIPY-tagged small-molecule probe compounds, already validated for fluorescent imaging, into PET imaging probes.

Inspired by the recent report by Hudnall and Gabbaï, which described the development of a BODIPY-based fluorogenic sensor for fluoride ions, we chose to explore the use of a 4-dimethylaminopyridine (DMAP) BODIPY intermediate (**3**; Scheme 1) as an activated reagent for the efficient incorporation of 18F.[6]

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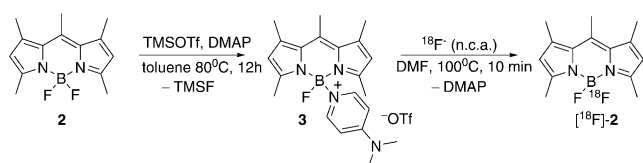
Scheme 1. Synthesis of [¹⁸F]-BODIPY ([¹⁸F]-**2**) through DMAP-activated BODIPY **3**. n.c.a = no carrier added.

While the reported compound **3** was readily accessible following the published procedure, efficient incorporation of ¹⁸F to yield 4-[¹⁸F]-1,3,5,7,8-pentamethyl BODIPY ([¹⁸F]-**2**) could only be accomplished following microwave heating of the reaction mixture (Scheme 1). Optimization of the reaction conditions subsequently allowed access to the desired ¹⁸F-labeled [¹⁸F]-**2** in $(67.6 \pm 22.9)\%$ radiochemical yield (Figure 1a). We next analyzed the stability of [¹⁸F]-**2** under
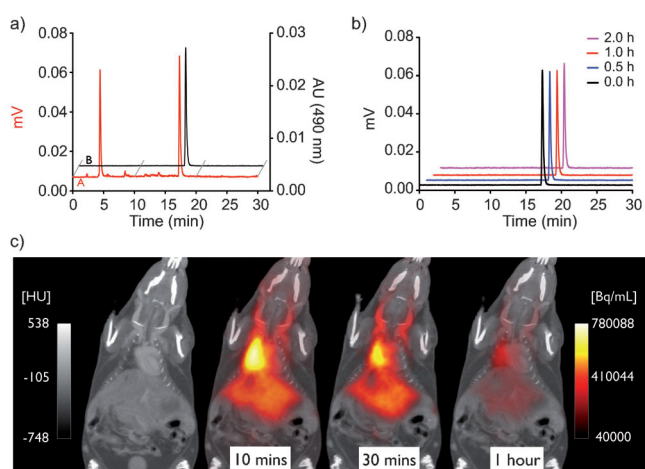
Figure 1. a) HPLC analyses of the reaction mixture containing the crude (red) and the HPLC-purified ¹⁸F BODIPY (black). b) Stability analyses of HPLC-purified [¹⁸F]-**2** in PBS buffer (pH 7.4) at 37°C (individual traces are decay corrected). c) In vivo images of [¹⁸F]-**2** showing its systemic distribution, and rapid excretion within an hour, with no measurable release of free ¹⁸F⁻. (left scale Hounsfield units [HU], right scale Becquerel mL⁻¹ [Bq/mL]).

physiological conditions to determine its applicability for pilot in vivo studies. As shown in Figure 1b, no radioactivity was released, even after 2 hours at 37°C in PBS buffer, thus validating the excellent hydrolytic stability of the BODIPY core under physiological conditions. We verified these findings by assessing the in vivo stability of [¹⁸F]-**2**. The PET/CT scan (Figure 1c) demonstrates that [¹⁸F]-**2**, as expected for a nontargeted small molecule, does not exhibit tissue-specific labeling and is eliminated within 1 hour. BODIPY dyes have been used previously for in vivo imaging.[7] While some degree of metabolic modification is expected for virtually all small molecules including BODIPY-labeled compounds, it is important to note that the BF₂ core appears to be metabolically stable, as no measurable uptake of radioactivity in the skeleton was detected, which would sensitively indicate the release of free ¹⁸F⁻.[8]

While it was possible for us to synthesize [¹⁸F]-**2** successfully, we found that the specific reaction conditions necessary to do so had several shortcomings, which consequently limited the applicability of this approach. In particular, prolonged heating in the presence of the reactive reagents (to enable access to the DMAP BODIPY intermediate), and the conditions required for efficient DMAP/¹⁸F exchange, are most likely to be incompatible with many common functional groups. This is especially true of more sensitive moieties, such as those typically present in complex biologically active small molecules or in activated BODIPY dyes, such as *N*-hydroxysuccinimide-ester-bearing analogues. Reaction conditions that are compatible with the latter, however, are highly desirable since it would allow direct conversion of commercially available activated BODIPY esters into PET/FMT agents and consequently their immediate use for tagging amine-bearing small molecules or proteins. Moreover, upon heating, we observed that BODIPY dyes undergo a metathesis reaction in the presence of acid, where ¹⁹F is liberated from decomposed starting material and subsequently replaces ¹⁸F in [¹⁸F]-**2** (Scheme 2). Following prolonged heating, this undesired side reaction results in low specific activity of the target compound.
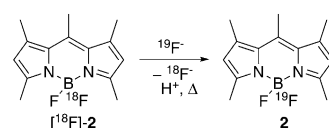
Scheme 2. Acid- and heat-catalyzed ¹⁸F/¹⁹F metathesis.

During the course of our synthetic efforts, using **2** as a model substrate, we found that TMSOTf efficiently abstracted one fluoride from the BODIPY core at 0°C within less than 5 minutes. This step yielded the activated BODIPY triflate **4** in near quantitative yields (Scheme 3).[9]
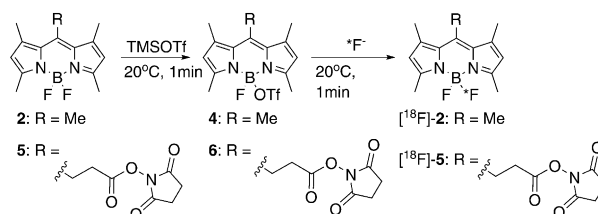
Scheme 3. One-pot synthesis of ¹⁸F-labeled BODIPY dyes through activated BODIPY triflates.

The subsequent addition of free fluoride to **4** instantly and cleanly converted **4** into its starting material **2**, thus demonstrating the easily reversible, rapid, and efficient incorporation of fluoride. Importantly, we discovered that upon the addition of a mild, non-nucluophilic, organic base such as diisopropylethylamine (DIPEA) or 2,6-lutidine, **4** remained stable in solution at room temperature for a prolonged time. In contrast, under unbuffered acidic conditions a metathesis reaction was seen to occur over time, thus causing the

formation of **2** and decomposition by-products. Thus, the efficient conversion of **4** into **2**, under unbuffered conditions, consequently required immediate reaction with free fluoride after activation, while acidification of base-buffered **4** was required for the fluorination to proceed efficiently. The latter reaction conditions therefore provide an additional level of reaction control. Furthermore, we found that addition of *tert*-butyl alcohol after TMSOTf activation efficiently quenched excess TMSOTf and reduced subsequent generation of TMS^{18}F to a minimum.

We next explored the applicability of the above method to more sensitive molecules such as the BODIPY *N*-hydroxysuccinimide (NHS) ester **5**, which proved incompatible with the previously described DMAP-based approach. As expected, the NHS ester was not only stable under the new reaction conditions but also allowed clean TMSOTf activation and efficient re-fluorination following the simple one-pot strategy.

Following its validation under cold conditions, we next tested the applicability of the new strategy to the ^{18}F labeling of the model substrate **2**. We thus found that the methodology developed under nonradioactive conditions could readily be adapted to radiochemical synthesis. We observed, however, that a four- to fivefold molar excess of TMSOTf was required for high radiochemical yields. We speculate that this is a consequence of the relatively small quantities of reagents needed for the radiosynthesis, which renders the reaction more sensitive to both residual water (present in the reaction vessel) and to the dried ^{18}F starting material. Optimization of the reaction conditions allowed clean conversion of **2** into [^{18}F]-**2** within 2 minutes in 67% radiochemical yield with a specific activity of 0.96 Ci/umol (see the Experimental Section and Supporting Information). Following the same strategy, [^{18}F]-**5** was accessed by solid-phase extraction in 73% radiochemical yield, with excellent purity (see the Supporting Information). To subsequently demonstrate efficient biomolecule labeling, we selected Trastuzumab (Herceptin©, Genentech) as a clinically relevant model system.[10] Trastuzumab is a monoclonal antibody that binds to the HER2/neu receptor and is routinely used for the treatment of HER2-positive breast cancer.

Incubation of a PBS-solution of Trastuzumab with [^{18}F]-**5** for 15 minutes at room temperature and subsequent centrifugal ultrafiltration dialysis, demonstrated that the monoclonal antibody could be efficiently labeled using our PET/FMT ligand (Figure 2).

In summary, we have developed an easily amenable and efficient approach for converting BODIPY dyes into hybrid PET/FMT imaging agents. We believe this approach is well suited for the labeling of small molecules as well as biological macromolecules. Given the stability of the BODIPY scaffold in vivo, small BODIPY NHS esters or similar BODIPY dyes, functionalized with maleimides and click-tags, represent promising reagents that can now be readily converted into
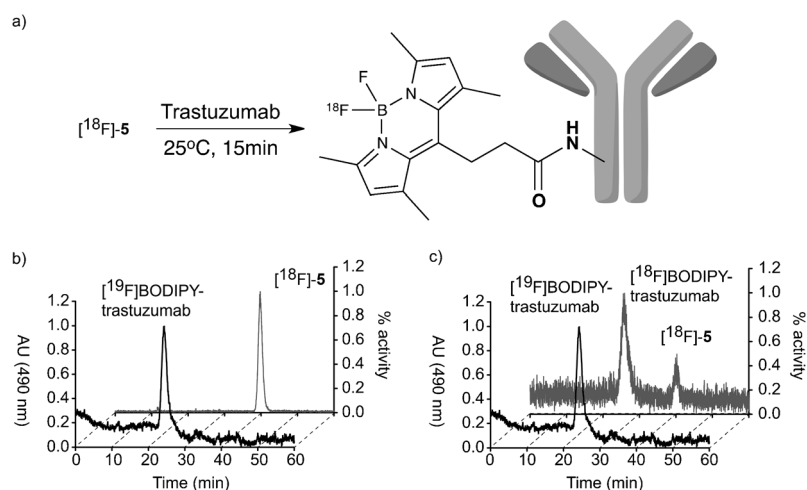
Figure 2. a) Labeling of Trastuzumab with [^{18}F]-**5**. b) Reference HPLC traces of Trastuzumab labeled with cold **5** (front) and free [^{18}F]-**5** (back). c) HPLC traces of the reaction mixture.

[^{18}F]-PET probes and used to modify biologically relevant molecules for biomedical imaging.

Experimental Section

General procedure: At room temperature 5 μL of a 250 mM solution (1.25 μmol, 5 mol equivalents) of trimethylsilyl triflate in acetonitrile were added to 0.25 μmol 4,4-difluoro-1,3,5,7,8-pentamethyl-bodipy (**2**) in 25 μL acetonitrile/dichloromethane (2:1). After 1 min *tert*-butyl alcohol (5 μL, 250 mM in acetonitrile) was added to quench the residual trimethylsilyl triflate, and the 2,6-lutidine (5 μL, 250 mM in acetonitrile) was added to stabilize the BODIPY triflate intermediate **4**. 1.6 μL (0.01 μmol) of this solution was combined with a solution of azeotropically dried ^{18}F/TBAB (15 μL, 0.9 (33.3 MBq) ± 0.3 mCi) in acetontrile, which had been pretreated with triflic anhydride (10 μL, 100 mM in acetonitrile) to remove residual water, and then *tert*-butanol (5 μL, 250 mM in acetonitrile) to quench excess triflic anhydride and to acidify the reaction mixture (as result of the generated triflic acid). The reaction was complete after stirring for 1 min at room temperature. Radiochemical yield was determined by radio-HPLC and radio-TLC (10:1 acetonitrile/toluene, ITLC; R_f (^{18}F$^-$) = 0.0; R_f (4-[^{18}F]-1,3,5,7,8-pentamethyl bodipy **[^{18}F]2**) = 0.91) dcRCY 66.7% (HPLC), 67.9% (TLC) with an specific activity of 0.96 Ci/μmol.

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