ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 9324–9326

www.rsc.org/chemcomm

COMMUNICATION

Rapid aqueous [^{18}F]-labeling of a bodipy dye for positron emission tomography/fluorescence dual modality imaging†

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Received 26th May 2011, Accepted 2nd July 2011
DOI: 10.1039/c1cc13089g

We report the rapid nucleophilic [^{18}F]-radiolabeling of a bodipy dye in aqueous solutions. This radiolabeled dye, whose biodistribution and clearance has been studied in mice, is stable *in vivo* and can be used as a positron emission tomography/fluorescence dual modality imaging agent.

[^{18}F]-Positron emission tomography (PET) is a powerful imaging technique[1] which provides *in vivo* information on the distribution of radiolabeled biomolecules. Despite numerous advantages, this technique remains affected by two major limitations. First of all, the short-lived ^{18}F-radionuclide needs to be incorporated into molecules as expediently as possible. However, ^{18}F is typically prepared by proton bombardment of [^{18}O]-water and is thus obtained as the anion in an aqueous/non-nucleophilic form.[2] A second limitation of PET imaging relates to the relatively low spatial resolution (1–2 mm) provided by this technique.[2] To tackle the first challenge, a great deal of effort has been devoted to the development of aqueous fluorination protocols based on fluorophilic elements such as silicon and aluminum.[3] Another elegant approach was developed by Perrin who showed that electron deficient arylboronic acid or esters can be radiofluorinated in aqueous solutions to afford [^{18}F]-labeled aryltrifluoroborates. The second challenge can be addressed by combining PET imaging with a second imaging technique such as fluorescence which offers much higher spatial and temporal resolution.[4] This approach necessitates the synthesis of PET/fluorescence dual modality agents,[5] which is typically achieved by introduction of a fluorophore and a radiolabeled component as two separate entities.[6]

In search of an integrated solution to these two problems, we were drawn by the appealing properties of bodipy dyes which have been used for the fluorescent labelling of biomolecules.[7] In addition to being very stable, such dyes have high quantum yields and their emission can be tuned into the NIR by simple variation of the molecular structure.[7] They also typically possess a BF_2 unit which could in principle provide a site for [^{18}F]-radiofluorination. To explore this possibility, we have now embarked on a project aimed at the radiofluorination of bodipy dyes for biological imaging. In this paper, we provide an original illustration of this approach.

As part of our work[8] on the reactivity of the B–F bond of bodipy dyes,[9] we recently observed that the hydroxo derivative **1**-OH could be easily converted into the corresponding fluoride **1**-F by simple reaction with KHF_2 in THF (Fig. 1).[10] Encouraged by this observation, we decided to determine if similar reactions could also be implemented in aqueous solution. To this end, we targeted the cationic derivative [**2**-OH]$^+$ as a triflate salt. This compound, which has been thoroughly characterized by NMR spectroscopy and mass spectrometry, was synthesized by alkylation of the dimethylamino precursor (Scheme 1).[11] Owing to its cationic nature,[12] this derivative can be dissolved in water at 10^{-3}–10^{-2} M concentrations. Under these conditions, however, [**2**-OH]$^+$ does not react with KHF_2, presumably because of the strength of the B–OH bond. Fortunately, we found that conversion into [**2**-F]$^+$ could be achieved by addition of KHF_2 to a deutero hydrochloric acid (DCl) solution (0.95 M) in MeOD/D_2O (1/1 vol.) (Scheme 1).

This reaction, which can be monitored by the appearance a ^{19}F NMR signal at −173 ppm, is complete in less than 2 min (see Supporting Information†). The triflate salt of [**2**-F]$^+$ has been fully characterized. The photophysical properties of this derivative are typical of other bodipy dyes. It features a broad absorption band at 506 nm and an emission band centered at 528 nm (Φ = 14.3% in CH_2Cl_2). Encouraged by these synthetic and spectroscopic results, we investigated the radiofluorination of [**2**-OH]$^+$ in aqueous solution. The optimized radiolabeling procedure is described below: 30 mCi of [^{18}F]-fluoride (100 μL unfixed target water) was directly added

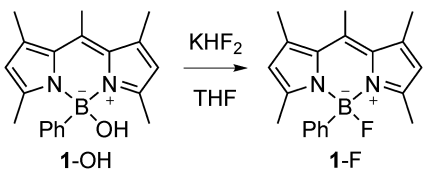

Fig. 1 The conversion of **1**-OH to **1**-F by simple reaction with KHF_2.

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† Electronic supplementary information (ESI) available: Experimental and characterization data. See DOI: 10.1039/c1cc13089g
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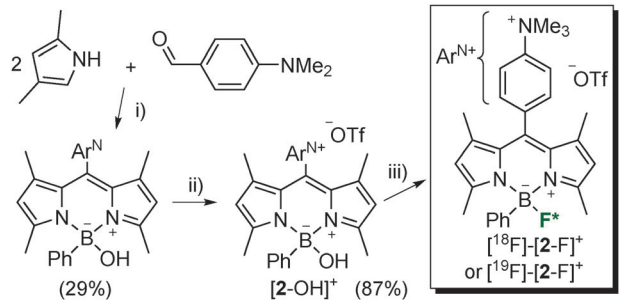

Scheme 1 Synthesis of bodipy $[\mathbf{2}\text{-OH}]^+$. Ar^N = $[4\text{-}(Me_2N)\text{-}C_6H_4]$; Ar^{N+} = $[4\text{-}(Me_3N)\text{-}C_6H_4]^+$. F* = ^{18}F or ^{19}F. Reagents and reaction conditions: (i) *p*-chloranil, Et_3N, and $PhBCl_2$ in CH_2Cl_2 followed by aqueous workup; (ii) MeOTf in CH_2Cl_2; (iii) For $[^{19}F]$–$[\mathbf{2}\text{-F}]^+$: KHF_2, 0.95 M DCl in MeOD/D_2O (1/1 vol.); For $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$: $^{18}F^-/KHF_2$, H_2O/MeOH, pH = 2–3.

to 5 μL of KHF_2 (0.1 mol L^{-1}). The mixture was heated at 70 °C for 10 min to ensure a complete homogenization. After cooling down to room temperature, compound $[\mathbf{2}\text{-OH}]^+$ (500 μg, 0.85 μmol in 100 μL MeOH) was added and the labeling was performed at room temperature for 15 min in the 2–3 pH range (Scheme 1). After dilution with 800 μL of water, the crude mixture was loaded onto a reverse phase HPLC and $[^{18/19}F]$–$[\mathbf{2}\text{-F}]^+$ was obtained in 22 ± 3% yield (decay corrected, based on separation, n = 4). The specific activity of the final product was calculated to be 25 ± 4 mCi/μmol by comparing its UV absorption with the standard titration curve. The identity of $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ was confirmed by the co-injection with the non-radiolabeled standard (Fig. 2). To broaden the scope of our approach, we have also investigated the formation of $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ under no carrier added conditions. We first tested the reaction of $[\mathbf{2}\text{-OH}]^+$ with azeotropically dried $[^{18}F]$-TBAF in acetonitrile, which, however, did not afford any detectable yield of the target radiolabeled compound. The failure of this reaction to proceed can be assigned to the stability of the B–OH bond in $[\mathbf{2}\text{-OH}]^+$ as well as the presence of residual water in the $[^{18}F]$-TBAF and/or the acetonitrile.

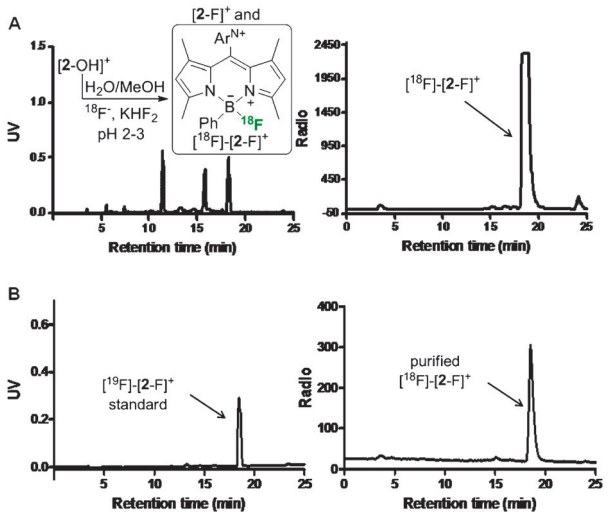

Fig. 2 (A) HPLC traces showing the formation of $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$; (B) HPLC trace obtained for the non-radiolabeled standard $[\mathbf{2}\text{-F}]^+$ and the radio trace obtained for purified $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$.

To circumvent this difficulty, we decided to repeat the reaction in the presence of an activating/water scrubbing agent such as TMSOTf. With this in mind, $[\mathbf{2}\text{-OH}]^+$ (500 μg, 0.85 μmol in 100 μL MeCN) was pretreated with TMSOTf (20 eq.) and then subsequently mixed with a MeCN solution (100 μL) of azeotropically dried $[^{18}F]$-TBAF (10 mCi). Gratifyingly, this reaction, which was allowed to proceed for 5 min at 60 °C, afforded $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ (specific activity ≥ 1.4 Ci/μmol) in 61% yield as indicated by HPLC.

Although B–F bonds are among the strongest bonds known,[13] the stability of ^{18}F–B compound is very critical as free $[^{18}F]$-fluoride ions could bind to the bones giving rise to unwanted and interfering background signals.[14] We first studied the stability of $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ in PBS buffer at pH 7.5 over a period of several hours. HPLC analysis carried out at different time intervals indicated that the concentration of intact $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ decreased from about 99% after 1 h, to 97% after 3 h and 95% after 6 h thus pointing to the remarkable resistance of this derivative to hydrolysis at physiological pH (see Supporting Information†). In order to validate our approach and demonstrate its potential for *in vivo* PET imaging, $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ (prepared *via* the aqueous route) was injected into normal nude mice that were imaged using a microPET scanner 1 h, 2 h, and 4 h post injection. We did not observe bone uptake even at 4 h post injection, which indicates that the hydrolytic release of free $[^{18}F]$-fluoride from $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ is essentially negligible on the time scale of the ^{18}F-nuclear decay (Fig. 3). More interestingly, we observed a clear accumulation of the radiolabeled probe primarily in the liver and kidneys but also in the gall bladder 2 h post injection. Bearing in mind that the probe $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$ lacks any specific targeting functionalities, its accumulation in these organs constitutes a normal phenomenon, in line with its hydrophobic and cationic nature.

Next, we endeavoured to confirm the dual modality potential of the probe. To this end, the animal was euthanized and selected organs were harvested for *ex vivo* fluorescence and microPET imaging (Fig. 4). The fluorescence images were obtained by irradiation of the organs at λ = 500 nm. This wavelength was chosen because it falls within the absorption band of $[\mathbf{2}\text{-F}]^+$ (see Supporting Information†). The fluorescence image was reconstructed based on the emission intensity measured at λ = 580 ± 20 nm, a wavelength which is within

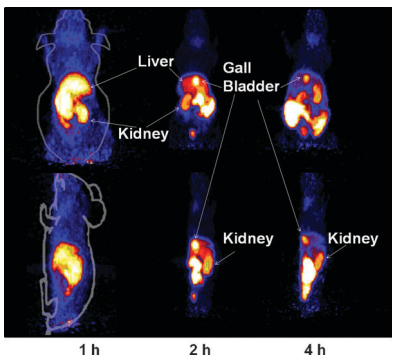

Fig. 3 PET images of a mouse injected with purified $[^{18}F]$–$[\mathbf{2}\text{-F}]^+$. No detectable bone uptake is observed up to 4 h post-injection. All images shown are 2D projection instead of a single slice of the scan.

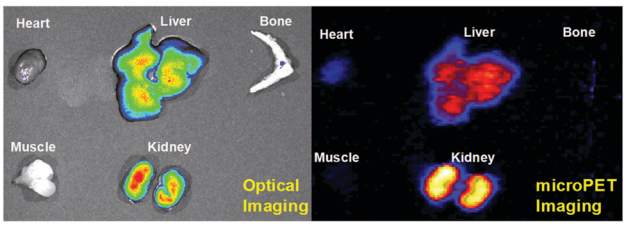

Fig. 4 Representative *ex vivo* fluorescence (*left*) and microPET (*right*) imaging of dissected organs of a nude mouse. The animal was sacrificed after the microPET scan taken 4 h post injection.

the fluorescence band of $[\mathbf{2}\text{-F}]^+$ (see Supporting Information†). As shown in Fig. 4, the *ex vivo* microPET and fluorescence imaging correlate extremely well with each other, showing accumulation of the probe in the liver and kidneys. These images are thus in perfect agreement with the *in vivo* PET studies which showed accumulations in the same organs. The heart and representative bones and muscles showed minimal uptake. Finally, the observed fluorescence from the liver and kidneys provide a further confirmation that $[\mathbf{2}\text{-F}]^+$ is stable *in vivo*. Indirectly, these results indicate that $[\mathbf{2}\text{-F}]^+$ is resistant to oxidative degradation reactions which can sometimes affect organoboron species.[15]

In summary, we report the synthesis of a novel [^{18}F]-PET/fluorescence dual modality agent in which both the positron emitting and fluorescence properties are confined to a unique molecular compartment. Moreover, the radiosynthesis of this novel dual modality imaging agent can be carried in the matter of minutes in aqueous solutions using the target [^{18}O]-water/[^{18}F]-fluoride solution. These conditions are attractive because they do not involve the potentially tedious [^{18}F]-fluoride drying steps inherent to many nucleophilic radiofluorination protocols.[16] We are currently exploring the upper limit of our approach by working under more concentrated conditions with [^{18}F]-fluoride solutions of higher specific activity. We are also pursuing the radiofluorination of bodipy dyes that emit in the NIR region.

This work was supported by the USC Department of Radiology; the Department of Energy (DE-SC0002353), the National Cancer Institute (P30CA014089), the National Science Foundation (CHE-0952912) and the Welch Foundation (A-1423).

References

1 S. Purser, P. R. Moore, S. Swallow and V. Gouverneur, *Chem. Soc. Rev.*, 2008, **37**, 320–330; P. W. Miller, N. J. Long, R. Vilar and A. D. Gee, *Angew. Chem., Int. Ed.*, 2008, **47**, 8998–9033; F. Dolle, D. Roeda, B. Kuhnast and M.-C. Lasne, *Fluorine and Health*, 2008, **3**, 65.
2 B. N. Ganguly, N. N. Mondal, M. Nandy and F. Roesch, *J. Radioanal. Nucl. Chem.*, 2009, **279**, 685–698.
3 L. Mu, A. Höhne, P. A. Schubiger, S. M. Ametamey, K. Graham, J. E. Cyr, L. Dinkelborg, T. Stellfeld, A. Srinivasan, U. Voigtmann and U. Klar, *Angew. Chem., Int. Ed.*, 2008, **47**, 4922–4925; A. Höhne, L. Mu, M. Honer, P. A. Schubiger, S. M. Ametamey, K. Graham, T. Stellfeld, S. Borkowski, D. Berndorff, U. Klar, U. Voigtmann, J. E. Cyr, M. Friebe, L. Dinkelborg and A. Srinivasan, *Bioconjugate Chem.*, 2008, **19**, 1871–1879; E. Schirrmacher, B. Wängler, M. Cypryk, G. Bradtmöller, M. Schäfer, M. Eisenhut, K. Jurkschat and R. Schirrmacher, *Bioconjugate Chem.*, 2007, **18**, 2085–2089; R. Schirrmacher, G. Bradmoeller, E. Schirrmacher, O. Thews, J. Tillmanns, T. Siessmeier, H. G. Bucholz, P. Bartenstein, B. Waengler, C. M. Niemeyer and K. Jurkschat, *Angew. Chem., Int. Ed.*, 2006, **45**, 6047–6050; W. J. McBride, R. M. Sharkey, H. Karacay, C. A. D'Souza, E. A. Rossi, P. Laverman, C.-H. Chang, O. C. Boerman and D. M. Goldenberg, *J. Nucl. Med.*, 2009, **50**, 991–998; W. J. McBride, C. A. D'Souza, R. M. Sharkey, H. Karacay, E. A. Rossi, C.-H. Chang and D. M. Goldenberg, *Bioconjugate Chem.*, 2010, **21**, 1331–1340.
4 R. Y. Tsien, *Nat. Cell Biol.*, 2003, **4**, SS16–SS21.
5 F. Ducongé, T. Pons, C. Pestourie, L. Hérin, B. Thézé, K. Gombert, B. Mahler, F. Hinnen, B. Kühnast, F. Dollé, B. Dubertret and B. Tavitian, *Bioconjugate Chem.*, 2008, **19**, 1921–1926.
6 R. Ting, T. A. Aguilera, J. L. Crisp, D. J. Hall, W. C. Eckelman, D. R. Vera and R. Y. Tsien, *Bioconjugate Chem.*, 2010, **21**, 1811–1819.
7 A. Loudet and K. Burgess, *Chem. Rev.*, 2007, **107**, 4891–4932; G. Ulrich, R. Ziessel and A. Harriman, *Angew. Chem., Int. Ed.*, 2008, **47**, 1184–1201.
8 T. W. Hudnall and F. P. Gabbaï, *Chem. Commun.*, 2008, 4596–4597.
9 C. Bonnier, W. E. Piers, M. Parvez and T. S. Sorensen, *Chem. Commun.*, 2008, 4593–4595; C. Bonnier, W. E. Piers, A. Al-Sheikh Ali, A. Thompson and M. Parvez, *Organometallics*, 2009, **28**, 4845–4851.
10 T. W. Hudnall, T.-P. Lin and F. P. Gabbaï, *J. Fluorine Chem.*, 2010, **131**, 1182–1186.
11 X. Qi, S. K. Kim, E. J. Jun, L. Xu, S.-J. Kim and J. Yoon, *Bull. Korean Chem. Soc.*, 2007, **28**, 2231–2234.
12 M. Tasior, J. Murtagh, D. O. Frimannsson, S. O. McDonnell and D. F. O'Shea, *Org. Biomol. Chem.*, 2010, **8**, 522–525; T. W. Hudnall and F. P. Gabbaï, *J. Am. Chem. Soc.*, 2007, **129**, 11978–11986; C.-W. Chiu, Y. Kim and F. P. Gabbaï, *J. Am. Chem. Soc.*, 2009, **131**, 60–61.
13 C. R. Wade, A. E. J. Broomsgrove, S. Aldridge and F. P. Gabbaï, *Chem. Rev.*, 2010, **110**, 3958–3984.
14 R. Ting, C. Harwig, U. auf dem Keller, S. McCormick, P. Austin, C. M. Overall, M. J. Adam, T. J. Ruth and D. M. Perrin, *J. Am. Chem. Soc.*, 2008, **130**, 12045–12055; U. auf dem Keller, C. L. Bellac, Y. Li, Y. Lou, P. F. Lange, R. Ting, C. Harwig, R. Kappelhoff, S. Dedhar, M. J. Adam, T. J. Ruth, F. Bénard, D. M. Perrin and C. M. Overall, *Cancer Res.*, 2010, **70**, 7562–7569.
15 G. C. Van de Bittner, E. A. Dubikovskaya, C. R. Bertozzi and C. Chang, *J. Proc. Natl. Acad. Sci. U.S.A Early Ed.*, 2010, 1–6.
16 L. Cai, S. Lu and V. W. Pike, *Eur. J. Org. Chem.*, 2008, 2853–2873.

