

Enhanced Aqueous Suzuki–Miyaura Coupling Allows Site-Specific Polypeptide ¹⁸F-Labeling

Zhanghua Gao, Véronique Gouverneur, and Benjamin G. Davis*

Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford OX1 3TA, U.K.

S Supporting Information

ABSTRACT: The excesses of reagents used in protein chemistry are often incompatible with the reduced or even inverse stoichiometries used for efficient radiolabeling. Analysis and screening of aqueous Pd(0) ligand systems has revealed the importance of a guanidine core and the discovery of 1,1-dimethylguanidine as an enhanced ligand for aqueous Suzuki–Miyaura cross-coupling. This novel Pd catalyst system has now allowed the labeling of small molecules, peptides, and proteins with the fluorine-18 prosthetic [¹⁸F]4-fluorophenylboronic acid. These findings now enable site-specific protein ¹⁸F-labeling under biologically compatible conditions using a metal-triggered reaction.

Peptides and proteins have been increasingly applied as substrates for positron emission tomography (PET) tracers, but such tracers remain difficult to create.^{1–6} Their application requires practical methods to incorporate positron emission nuclides (such as ¹⁸F, ¹¹C, ⁶⁸Ga). The most common radionuclide used in PET imaging is ¹⁸F. The relatively long half-life (*t*_{1/2} = 109 min) allows for chemical synthesis, and a low positron energy offers superior imaging resolution.⁷ Protein-labeling using prosthetic ¹⁸F-synthons has been developed and applied to a variety of substrates, generally targeting either lysine^{8–11} or cysteine^{12–14} residues. Some variants employ heterobifunctional cross-linkers to Lys/Cys, allowing a two-step process combining other modification chemistries^{15–21} or even complexation.^{22,23} However, these protein-labeling techniques can result in nonspecific labeling, which may affect the biological activity and typically generate mixtures of differently labeled proteins as product. Site-specific labeling of proteins is highly desirable because a homogeneous product allows unambiguous biophysical and activity-based characterization, leading to reproducibility in labeling and imaging. It also allows labeling to be precisely targeted to functionally benign sites in a given protein substrate; this is of particular relevance to proper therapeutic protein evaluation. In particular, site-specific installation of unnatural amino acids can provide diverse chemical functionality (a “tag”) for subsequent reaction (“modification”) that will allow protein-labeling. A few approaches have been developed; these include the use of expressed protein ligation to install an aminoxy group labeled by oxime chemistry²⁴ (used at the C-terminus) and the use of an unnatural alkynyl amino acid “tag” modified by triazole chemistry (in low radiochemical yield (RCY)).²⁵

Using a “tag-and-modify” approach²⁶ in our research in protein post-translational modification, we have demonstrated efficient Suzuki–Miyaura coupling (SMC) on proteins under biologically compatible conditions.^{27–31} Suitable aryl iodide-containing “tag” residues may be site-specifically introduced into proteins either by chemical modification²⁷ or in a genetically encoded manner^{28,29,31} with great site flexibility, making this a useful strategy for metal-mediated protein-labeling. The efficiency, directness, and chemoselectivity as well as the low toxicity of palladium²⁹ encouraged us to expand the application scope of SMC protein modification into highly challenging site-specific ¹⁸F-protein-labeling. Here we describe the development of an enhanced aqueous Pd ligand system that now enables Pd-mediated protein and peptide ¹⁸F-labeling.

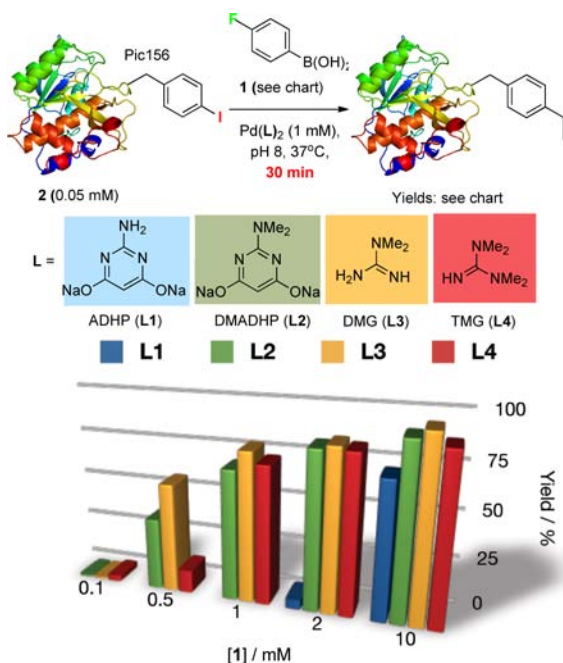
Due to the metabolic stability³² of aryl fluorides as well as the structural simplicity, we designed 4-fluorophenylboronic acid (**1**) as a relevant “minimal” boronic acid prosthetic for such labeling. Despite the power of the SMC reaction, to our knowledge, **1** has not previously been used as an ¹⁸F-prosthetic; a successful SMC between [¹⁹F]**1** and a model protein was disclosed in our previous research.²⁷ Using a phosphine-free, water-soluble ligand, 2-amino-4,6-dihydroxy-pyrimidine (ADHP, **L1**), complete coupling of **2** (0.03 mM) was achieved by using a 50-fold excess of (**L1**)₂Pd(OAc)₂ and a 500-fold excess of **1**. (**L1**)₂Pd(OAc)₂ has also recently been successfully used in small-molecule ¹⁸F-labeling, catalyzing the SMC of [¹⁸F]2-bromo-4-fluorophenol and phenylboronic acid in quantitative yield.³³ Despite these encouraging precedents, SMC coupling of an ¹⁸F-prosthetic, such as **1**, presents particular and striking challenges. In no-carrier-added ¹⁸F-radiochemistry, the radioactive material is generally the least abundant component in a reaction (and the key species on which RCY is based). Such labeling with a nonabundant reagent (perhaps even under reverse stoichiometry) is not addressed well by standard protein chemistry methods; this has largely prevented effective bioconjugations in which neither moiety can be used in great excess. Limitations in current ¹⁸F-labeling methods include elevated temperature,¹⁷ multistep processes,^{15–23} and the use of organic solvent.^{12,18} Additionally, the half-life of ¹⁸F, although longer than for other PET isotopes, still encourages short reaction times (<1 h typically). Moreover, protein substrates often place strong limits on concentrations. However, we considered that the apparent efficiency and utility of the SMC might uniquely tackle these combined constraints of rapid aqueous reaction under low concentrations with nonabundant reagent.

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To probe the limits of the SMC, and so test its possible utility under such stringent conditions, we explored key parameters. The impact of boronic acid concentration ($[1]$) was tested first using **L1** as the ligand, and a model 3-layer- α/β -Rossmann-fold protein subtilisin from *Bacillus lentus* (SBL) containing the haloaromatic amino acid Pic²⁷ SBL-156ArI (**2**) as the substrate (Scheme 1). The reaction was conducted under key constraints:

Scheme 1. Suzuki–Miyaura Coupling of 1 with Model Proteins SBL-ArI (2) under Constrained Conditions, Identifying the Guanidine Catalyst “Core” and More Effective Ligand System L3^a



^aPd source added as precatalyst from Pd(II)OAc₂ (see SI for details).

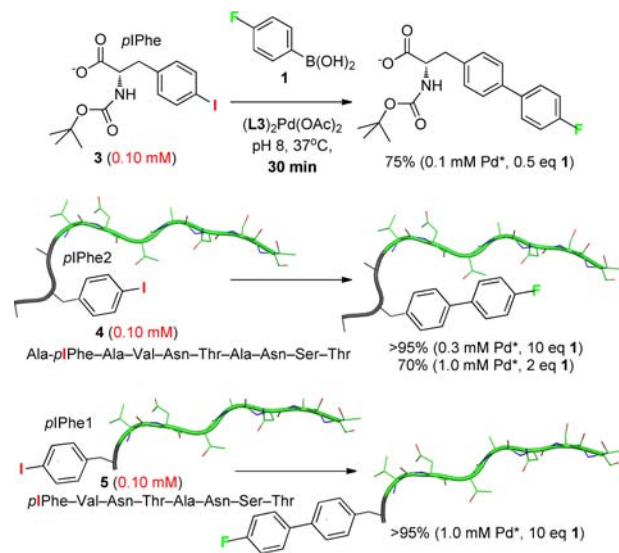
0.05 mM protein at fixed temperature (37 °C) and short reaction time (30 min) in the absence of any organic solvents. This revealed a striking dependency of coupling yield (judged with respect to the protein) upon boronic acid concentration to the extent that, when $[1]$ was reduced from 10 mM to 2 mM, protein conversion decreased from 72% to 5%.

We then looked for a more active Pd catalyst system. The dimethylated analogue of **L1**, 2-dimethylamino-4,6-dihydroxypyrimidine (**L2**), has recently been reported as a variant ligand for Pd(0)-mediated reactions on proteins.³⁴ Although structural information on the new aqueous Pd catalyst systems does not yet exist, our developmental studies³⁰ have suggested that it is the central guanidine moiety that coordinates Pd. To this end we also considered “minimal” ligand structures, dimethylguanidine (**L3**) and tetramethylguanidine (**L4**). Importantly, both are widely available and water-soluble.^{35,36} Moreover, their lack of toxicity opens up future possibilities for *in vivo* application. We were pleased to find all three ligand variants, **L2**, **L3**, and **L4**, afforded increased catalytic activity at lower boronic acid concentration. With $[1] = 1.0$ mM, all three ligands offered >60% protein conversion, conditions under which essentially no coupling product was observed from **L1**; this emphasized the critical need for novel ligands in such low concentrations and excesses. When $[1]$ was further decreased to 0.5 mM (only 10 equiv to protein), **L3** stood out by affording a 58% conversion, followed by **L2**

(38%) and **L4** (<15%). Moreover, variation of boronic acid (Ph, *p*-CN, *p*-Me, *p*-MeO, *o,p*-diOMe, furyl; Table S3) confirmed generality (65–90% yield) of these low substrate conditions toward other substrates.

To test this enhanced catalytic system in a lowered or reversed stoichiometry ($[^{18}\text{F}]\mathbf{1} < [\text{ArI}]$) more relevant to radiochemical conditions, the model unnatural amino acid aryl iodide *p*-iodo-*L*-phenylalanine residue (Boc-*p*IPhe, **3**) was subjected to SMC with **1** (0.5 equiv to **3**) at low concentrations ($[3] = 0.10$ mM, $[1] = 0.05$ mM) (Scheme 2). Notably, optimal reactions required

Scheme 2. SMC of 1 with Model Haloaromatic Small Biomolecules under Low or Reversed Stoichiometry



low Pd loading of 0.1 mM (2 equiv to **1**). Higher catalyst amounts led to decreased yield, possibly due to the decomposition of **1** or the opening of another Pd-mediated reaction manifold (see SI for more details.) Thus, 75% SMC yield was obtained with only 0.10 mM (**L3**)₂Pd(OAc)₂ catalyst loading (Scheme 2), and >70% yields were obtained from all three enhanced ligands under these conditions (see SI). Based on the above, enhanced ligand **L3** was selected for more complex peptide and protein substrates.

To further evaluate the utility of SMC coupling, a decamer peptide (Ala-*p*IPhe-Ala-Val-Asn-Thr-Ala-Asn-Ser-Thr, **4**) containing haloaromatic unnatural residue *p*-iodo-*L*-phenylalanine was designed, constructed, and tested. Peptide **4** contains the “tag” amino acid *p*IPhe inserted between two alanine residues in an *N*-terminal cap, followed by a VNTANST sequence as the C-terminal. VNTANST, namely the Comprehensive Carcinoma Homing Peptide (CCHP), has been suggested as a broad cancer-targeting mini-peptide.³⁷ Upon reoptimization with fixed temperature and reaction time (37 °C, 30 min), the quantitative SMC conversion (>95%) of **4** (0.1 mM) was achieved with 1.0 mM **1** and $[\text{Pd}]$ no lower than 0.3 mM. Even at lower boronic acid concentration (0.2 mM, 2 equiv), 70% conversion was possible ($[\text{Pd}] = 1.0$ mM). To explore positional dependency in a peptide, we also tested a variant of the CCHP in which the “tag” amino acid *p*IPhe was found directly at the *N*-terminus (*p*IPhe-Val-Asn-Thr-Ala-Asn-Ser-Thr, **5**); >95% conversion was also possible.

With these promising small-molecule and peptide reactions in hand, a final non-radioactive (cold) optimization of protein SMC

was carried out at the very low boronic acid:protein ratio of 2. As for all of the reactions thus described, only reaction times of 30 min were considered to be of realistic utility in analogous hot chemistry given the half-life of ^{18}F (109 min). By varying the pH, buffer, concentration of Pd catalyst, and concentration of protein, reaction was seen under these unprecedentedly stringent conditions; at pH 8, with 2 mM $(\text{L3})_2\text{Pd}(\text{OAc})_2$ and 0.2 mM $[\text{SBL-ArI}]$, 10% conversion of protein was obtained. Although low in synthetic terms, this first observable Pd-mediated protein F-labeling under such very dilute and time-restricted conditions paves the way for detectable (and so useful) protein ^{18}F -radiolabeling.

The radiochemical synthesis (Scheme 3a) of our chosen, novel ^{18}F -prosthetic reagent $[\text{F}^{18}]\text{1}$ exploited $[\text{F}^{18}]\text{4}$ -fluoroiodobenzene ($[\text{F}^{18}]\text{7}$) as a known ^{18}F -intermediate.^{38–41} Thus, di(4-iodophenyl)iodonium triflate (**6**) was treated with Kryptofix 2.2.2-potassium $[\text{F}^{18}]\text{fluoride}$ to give $[\text{F}^{18}]\text{7}$.^{38–42} Since a free

boronic acid was of high importance to execute the desired SMC rapidly, tetrahydroxydiborane (**8**) was employed to allow direct $\text{C}-\text{B}(\text{OH})_2$ formation, thereby avoiding protecting group manipulation. Despite an array of reported possible conditions for borylations,^{43–45} KOAc and DMSO were found to be critical to success as base and solvent, as originally noted by Miyaura in “cold” borylations using bis(pinacolato)diboron⁴⁶ (see SI for more details). Thus, $[\text{F}^{18}]\text{7}$ was converted to $[\text{F}^{18}]\text{1}$ by treatment with **8** in DMSO at 90 °C in the presence of $\text{Pd}(\text{dppf})\text{Cl}_2$ and potassium acetate. The two-step radiosynthesis gave excellent radiochemical purities (RCPs) at each step (RCP >95%) and fair yields, giving an overall 5–10% decay-corrected RCY (specific activity 9.7 GBq/ μmol ; 3.1–14.4 GBq/ μmol , $n = 3$). After solid-phase extraction purification, the prosthetic reagent $[\text{F}^{18}]\text{1}$ was concentrated and reformulated in aqueous-only pH 8 phosphate buffer for ready “hot” SMC. It is important to note that the boronic acid moiety valuably allowed flexible use of $[\text{F}^{18}]\text{1}$ in both organic and aqueous solvents. In addition, to extend accessibility of our method, we also successfully developed one-pot methods that allowed direct access to $[\text{F}^{18}]\text{1}$ from **6** (see SI).

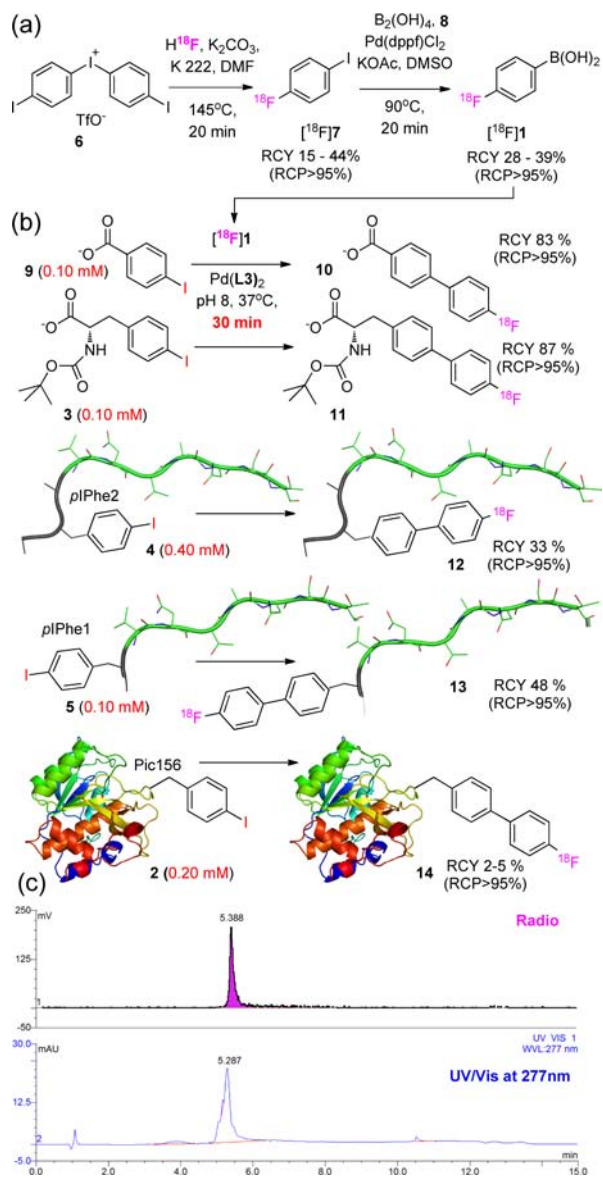
^{18}F -SMC was first demonstrated by the labeling of two small molecules, **3** and 4-iodobenzoic acid (**9**) (Scheme 3b). Thus, **3** or **9** (0.1 mM) was mixed with $[\text{F}^{18}]\text{1}$ and 1 mM $(\text{L3})_2\text{Pd}(\text{OAc})_2$ under low concentration conditions in pH 8 buffer and shaken at 37 °C for 30 min to give RCY = 87% or 83%, respectively. To our knowledge, these are the lowest substrate concentrations used in Pd-catalyzed ^{18}F chemistry;^{47,48} as a result, purification was greatly simplified due to the very low quantity of remaining reagents, starting material, and potential side products present in the final product mixture (<5 nmol).

^{18}F -SMC also proved successful on the Carcinoma Homing VNTANST peptide **4** and with the peptide variant **5** bearing a direct *N*-terminal tag; reaction of $[\text{F}^{18}]\text{1}$ under optimized conditions ($[\text{4}]$ or $[\text{5}] = 0.1\text{--}0.4$ mM, $[(\text{L3})_2\text{Pd}(\text{OAc})_2] = 2.0$ mM, 37 °C, 30 min) gave ^{18}F -labeled peptide **12** in 33% RCY (RCP >95%, $n = 9$) and **13** in 48% RCY (RCP >95%, $n = 4$).

Finally, protein-labeling was carried out by incubating the reaction mixture (SBL-ArI (**2**), reformulated $[\text{F}^{18}]\text{1}$ (10–20 MBq), and $(\text{L3})_2\text{Pd}(\text{OAc})_2$ in pH 8 phosphate buffer) at 37 °C for just 30 min. Palladium catalyst was scavenged by 3-mercaptopropionic acid²⁸ before the protein product **14** was purified by size-exclusion chromatography down to levels as low as 1 ppm Pd (as measured by ICP-OES, see SI). Even under these conditions, detection of radiolabeled protein was confirmed by HPLC (Scheme 3c) with a RCY of ~2–5% from boronic acid **1** (decay-corrected);⁴⁹ addition of 2 equiv of carrier $[\text{F}^{19}]\text{1}$ did not improve the yield.

This first Pd-catalyzed incorporation of ^{18}F into a protein using a direct method is an encouraging first step in a new strategy in ^{18}F -protein-labeling and is another, rare, metal-mediated example (see SI). The $^{18}\text{F}[\text{1}]$ aryl boronic acid may also find use in other powerful synthetic methods, and these are being explored currently. Further improvement of the RCY would help the clinical application of this methodology. Nevertheless, despite the low yield, the current research has demonstrated the power of Pd-catalyzed cross-coupling reactions under extreme conditions (biomacromolecules, biocompatible aqueous context, low concentrations and excesses, and short time frames dictated by isotope half-life) to allow detectable ^{18}F -protein-labeling. This was enabled by the discovery of the enhanced, readily available Pd ligand **L3**. It is an effective Pd ligand for aqueous Suzuki–Miyaura coupling at low substrate concentrations (0.10–0.20 mM), allowing “hot SMC” coupling of small molecules and

Scheme 3. (a) Radiosynthesis of $[\text{F}^{18}]\text{1}$ and (b) Its Use in the Direct ^{18}F -Labeling of Small Molecules, Peptide, and Protein; (c) HPLC (Radio or UV/Vis Detection) of Labeled Protein **14**



peptides in radiochemical yields of up to 87%. This discovery further expands the application scope of aqueous Pd catalysis to radiobiology.

■ ASSOCIATED CONTENT

📄 Supporting Information

Details of experimental procedures of nonradioactive reaction optimization, ^{18}F -radiolabeling, and preparation of aqueous Pd catalysts and substrates. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

ben.davis@chem.ox.ac.uk

Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Wester, H. J.; Schottelius, M. In *PET Chemistry: The Driving Force in Molecular Imaging*; Schubiger, P. A., Lehmann, L., Friebe, M., Eds.; Springer: Berlin, 2007; pp 79–111.
- (2) Reubi, J. C.; Maecke, H. R. *J. Nucl. Med.* **2008**, *49*, 1735–1738.
- (3) Lee, S.; Xie, J.; Chen, X. *Chem. Rev.* **2010**, *110*, 3087–3111.
- (4) Tolmachev, V.; Stone-Elander, S. *Biochim. Biophys. Acta* **2010**, *1800*, 487–510.
- (5) Wu, Z.; Kandeel, F. *Curr. Pharm. Biotechnol.* **2010**, *11*, 572–580.
- (6) Smith, T. A. D. *J. Labeled Compds. Radiopharm.* **2012**, *55*, 281–288.
- (7) Ametamey, S. M.; Honer, M.; Schubiger, P. A. *Chem. Rev.* **2008**, *108*, 1501–1516.
- (8) Cai, W.; Olafsen, T.; Zhang, X.; Cao, Q.; Gambhir, S. S.; Williams, L. E.; Wu, A. M.; Chen, X. *J. Nucl. Med.* **2007**, *48*, 304–310.
- (9) Kostikov, A. P.; Chin, J.; Orchowski, K.; Schirrmacher, E.; Niedermoser, S.; Jurkschat, K.; Iovkova-Berends, L.; Wängler, C.; Wängler, B.; Schirrmacher, R. *Nat. Protoc.* **2012**, *7*, 1956–1963.
- (10) Lang, L.; Eckelman, W. C. *Appl. Radiat. Isot.* **1997**, *48*, 169–173.
- (11) Lang, L. X.; Eckelman, W. C. *Appl. Radiat. Isot.* **1994**, *45*, 1155–1163.
- (12) Jeon, J.; Shen, B.; Xiong, L.; Miao, Z.; Lee, K. H.; Rao, J.; Chin, F. T. *Bioconjugate Chem.* **2012**, *23*, 1902–1908.
- (13) Wängler, B.; Kostikov, A. P.; Niedermoser, S.; Chin, J.; Orchowski, K.; Schirrmacher, E.; Iovkova-Berends, L.; Jurkschat, K.; Wängler, C.; Schirrmacher, R. *Nat. Protoc.* **2012**, *7*, 1964–1969.
- (14) Wuest, F.; Berndt, M.; Bergmann, R.; van den Hoff, J.; Pietzsch, J. *Bioconjugate Chem.* **2008**, *19*, 1202–1210.
- (15) Chang, Y. S.; Jeong, J. M.; Lee, Y.-S.; Kim, H. W.; Rai, G. B.; Lee, S. J.; Lee, D.; Chung, J.; Lee, M. C. *Bioconjugate Chem.* **2005**, *16*, 1329–33.
- (16) Cheng, Z.; De Jesus, O. P.; Namavari, M.; De, A.; Levi, J.; Webster, J. M.; Zhang, R.; Lee, B.; Syud, F. A.; Gambhir, S. S. *J. Nucl. Med.* **2008**, *49*, 804–813.
- (17) Namavari, M.; Padilla De Jesus, O.; Cheng, Z.; De, A.; Kovacs, E.; Levi, J.; Zhang, R.; Hoerner, J.; Grade, H.; Syud, F.; Gambhir, S. M. *Mol. Imaging Biol.* **2008**, *10*, 177–181.
- (18) Ramenda, T.; Kniess, T.; Bergmann, R.; Steinbach, J.; Wuest, F. *Chem. Commun.* **2009**, 7521–7523.
- (19) Gill, H. S.; Marik, J. *Nat. Protoc.* **2011**, *6*, 1718–1725.
- (20) Sachin, K.; Jadhav, V. H.; Kim, E.-M.; Kim, H. L.; Lee, S. B.; Jeong, H.-J.; Lim, S. T.; Sohn, M.-H.; Kim, D. W. *Bioconjugate Chem.* **2012**, *23*, 1680–1686.
- (21) Liu, S.; Hassink, M.; Selvaraj, R.; Yap, L.-P.; Park, R.; Wang, H.; Chen, X.; Fox, J. M.; Li, Z.; Conti, P. S. *Mol. Imaging* **2013**, *12*, 121–8.
- (22) Ting, R.; Adam, M. J.; Ruth, T. J.; Perrin, D. M. *J. Am. Chem. Soc.* **2005**, *127*, 13094–13095.
- (23) Laverman, P.; McBride, W. J.; Sharkey, R. M.; Eek, A.; Joosten, L.; Oyen, W. J. G.; Goldenberg, D. M.; Boerman, O. C. *J. Nucl. Med.* **2010**, *51*, 454–461.
- (24) Flavell, R. R.; Kothari, P.; Bar-Dagan, M.; Synan, M.; Vallabhajosula, S.; Friedman, J. M.; Muir, T. W.; Ceccarini, G. *J. Am. Chem. Soc.* **2008**, *130*, 9106–9112.
- (25) Boutureira, O.; D'Hooge, F.; Fernandez-Gonzalez, M.; Bernardes, G. J. L.; Sanchez-Navarro, M.; Koeppe, J. R.; Davis, B. G. *Chem. Commun.* **2010**, 46, 8142–8144.
- (26) Chalker, J. M.; Bernardes, G. J. L.; Davis, B. G. *Acc. Chem. Res.* **2011**, *44*, 730–741.
- (27) Chalker, J. M.; Wood, C. S. C.; Davis, B. G. *J. Am. Chem. Soc.* **2009**, *131*, 16346–7.
- (28) Spicer, C. D.; Davis, B. G. *Chem. Commun.* **2011**, 47, 1698–1700.
- (29) Spicer, C. D.; Triemer, T.; Davis, B. G. *J. Am. Chem. Soc.* **2012**, *134*, 800–803.
- (30) Dumas, A.; Spicer, C. D.; Gao, Z.; Takehana, T.; Lin, Y. A.; Yasukohchi, T.; Davis, B. G. *Angew. Chem., Int. Ed.* **2013**, *52*, 3916–3921.
- (31) Spicer, C. D.; Davis, B. G. *Chem. Commun.* **2013**, 49, 2747–2749.
- (32) Knust, E. J.; Kupfernagel, C.; Müller-Platz, C.; Stöcklin, G. *J. Fluorine Chem.* **1980**, *16*, 535–536.
- (33) Gao, Z.; Lim, Y. H.; Tredwell, M.; Li, L.; Verhoog, S.; Hopkinson, M.; Kaluza, W.; Collier, T. L.; Passchier, J.; Huiban, M.; Gouverneur, V. *Angew. Chem., Int. Ed.* **2012**, *51*, 6733–6737.
- (34) Li, N.; Lim, R. K. V.; Edwardraja, S.; Lin, Q. *J. Am. Chem. Soc.* **2011**, *133*, 15316–15319.
- (35) Li, S.; Lin, Y.; Cao, J.; Zhang, S. *J. Org. Chem.* **2007**, *72*, 4067–4072.
- (36) Pd complexes of more alkylated L (e.g., 1,1,3,3-tetramethyl-2-butylguanidine) caused precipitation and were not tested further.
- (37) Cutrera, J.; Dibra, D.; Xia, X.; Hasan, A.; Reed, S.; Li, S. *Mol. Therapy* **2011**, *19*, 1468–1477.
- (38) Cardinale, J.; Ermert, J.; Coenen, H. H. *Tetrahedron* **2012**, *68*, 4112–4116.
- (39) Ross, T. L.; Ermert, J.; Hocke, C.; Coenen, H. H. *J. Am. Chem. Soc.* **2007**, *129*, 8018–8025.
- (40) Wüst, F. R.; Kniess, T. *J. Labeled Compds. Radiopharm.* **2003**, *46*, 699–713.
- (41) Wüst, F. R.; Kniess, T. *J. Labeled Compds. Radiopharm.* **2004**, *47*, 457–468.
- (42) Iodobenzene, released from the decomposition of precursor **6**, presents as the major impurity after solid-phase purification.
- (43) Marcuccio, S. M.; Rodopoulos, M.; Weigold, H. (CSIRO, Australia). PCT WO9912940A1, 1999.
- (44) Molander, G. A.; Trice, S. L. J.; Dreher, S. D. *J. Am. Chem. Soc.* **2010**, *132*, 17701–17703.
- (45) Molander, G. A.; Trice, S. L. J.; Kennedy, S. M.; Dreher, S. D.; Tudge, M. T. *J. Am. Chem. Soc.* **2012**, *134*, 11667–11673.
- (46) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508–7510.
- (47) An SMC example involving ^{18}F -radiochemistry, 60 mM arylboronic acid was used to couple $[\text{F}^{18}]$ **7**. See the following reference.
- (48) Steiniger, B.; Wuest, F. R. *J. Labeled Compds. Radiopharm.* **2006**, *49*, 817–827.
- (49) The decayed protein sample was analyzed by LCMS, showing the presence of starting material.