ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Development and Preliminary Evaluation of TFIB, a New Bimodal Prosthetic Group for Bioactive Molecule Labeling

Emilie M. F. Billaud,[§,∥,⊥] Aurélien Vidal,[†,§,∥,⊥] Amélie Vincenot,[∥,⊥] Sophie Besse,[∥,⊥] Bernadette Bouchon,[∥,⊥] Eric Debiton,[∥,⊥] Elisabeth Miot-Noirault,[∥,⊥] Imen Miladi,[∥,⊥] Latifa Rbah-Vidal,[‡,∥,⊥] Philippe Auzeloux,[∥,⊥,#] and Jean-Michel Chezal*[,∥,⊥,#]

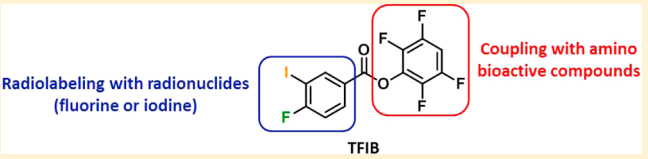
[∥]Clermont Université, Université d'Auvergne, Laboratoire d'Imagerie Moléculaire et Thérapie Vectorisée, BP 10448, F-63000 Clermont-Ferrand, France

[⊥]INSERM, U990, F-63005 Clermont-Ferrand, France

[#]Centre Jean Perrin, F-63011 Clermont-Ferrand, France

Supporting Information

ABSTRACT: The new readily available prosthetic group, tetrafluorophenyl 4-fluoro-3-iodobenzoate (**TFIB**), designed for both molecular imaging and targeted radionuclide therapy purposes was radiolabeled either with fluorine or iodine radionuclides with excellent radiochemical yields and purities. These radiolabeled tags were conjugated to *N*,*N*-diethylethylenediamine to give melanin-targeting radiotracers [^{125}**I**]**9** and [^{18}**F**]**9**, which were successfully evaluated by PET and gamma scintigraphic imaging in B16F0 pigmented melanoma-bearing C57BL/6J mice. Then, radiolabeled [^{125}**I**]/[^{18}**F**]**TFIB** was used to tag tumor-targeting peptides (i.e., $PEG_3[c(RGDyK)]_2$ and NDP-MSH targeting $\alpha_v\beta_3$ integrin and MC1R receptors, respectively) in mild conditions and with good radiochemical yields (47−83% d.c.) and purities (>99%). The resulting radiolabeled peptides were assessed both *in vitro* and by PET imaging in animal models.



KEYWORDS: *Bioconjugation, prosthetic group, radiochemistry, radiofluorination, radioiodination, molecular imaging*

Iodine radioisotopes are the radiohalides most widely used for the labeling of small organic compounds, peptides, antibodies, oligonucleotides, or nanoobjects. This is due to the remarkable physical properties of several iodine radioisotopes (e.g., ^{125}I, ^{123}I, ^{124}I, and ^{131}I), which allow a broad range of applications from biochemical research to nuclear medicine.[1] For the radioiodination of compounds with no aromatic group or that are sensitive to radiolabeling conditions, the most useful technique remains the use of iodine-containing prosthetic groups. Accordingly, a variety of radioiodinated bifunctional agents have been developed that exploit conjugation strategies such as acylation, hydrazone, imidate, or carbamate formation or thiol alkylation.[2−4] Among them, the best known and most extensively studied labeling agents are two activated esters, *N*-succinimidyl 3-(4-hydroxy-3-iodophenyl) propionate[5] (also called Bolton−Hunter reagent) and *N*-succinimidyl 3-iodobenzoate (**SIB**)[6] (Figure 1). In the growing field of personalized cancer medicine, tumor-targeted radioiodinated agents are promising tools for multimodal or theranostic purposes.

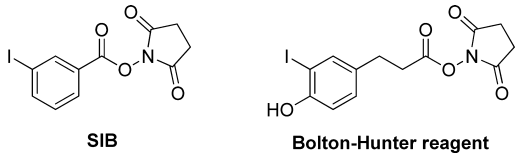


Figure 1. Examples of common iodinated aromatic prosthetic groups.

According to the iodine radioisotope used, they can enable imaging by single-photon emission computed tomography (SPECT, with ^{123}I) or positron emission tomography (PET, with ^{124}I) for the diagnosis and/or stratification of patients, followed by targeted radionuclide therapy with ^{131}I. PET, the most sensitive functional imaging technique, is preferred. However, the physical properties of ^{124}I present some limitations[7] ($t_{1/2}$ = 4.2 d; β^+ 1.5 and 2.1 MeV, 22%) that reduce the spatial resolution of PET images and so lead to high patient dosimetry.

We set out to develop an isostructural prosthetic group allowing both fluorine and iodine radiolabeling. Such a prosthetic reagent could facilitate clinical transfer of new tandem PET and therapeutic radiopharmaceuticals for the following reasons: first, ^{18}F ($t_{1/2}$ = 109.8 min) is currently the PET radionuclide of choice given its favorable nuclear and chemical properties, including its decay process (97%, β^+ emission) and low positron energy (635 keV). Second, ^{18}F and ^{131}I are inexpensive, do not suffer from production limitations, and are currently available in pharmaceutical grade, unlike other pairs of PET-therapy radioisotopes such as ^{64}Cu−^{67}Cu, ^{44}Sc−^{47}Sc, ^{86}Y−^{90}Y, or ^{71}As−^{77}As.[8] Finally, the radioiodinated or radiofluorinated conjugates will possess

Received: October 18, 2014
Accepted: November 24, 2014
Published: November 24, 2014

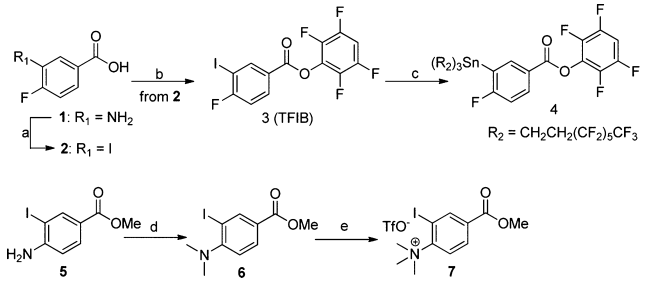
   dx.doi.org/10.1021/ml500423v | *ACS Med. Chem. Lett.* 2015, 6, 168−172

similar physicochemical properties (e.g., lipophilicity, steric hindrance, polarizability, etc.), a key parameter when developing tumor receptor-targeting ligands.

Nevertheless, the "ideal" prosthetic group has to meet certain criteria: (i) it should be a small organic compound with a low steric hindrance to avoid as far as possible any modifications of the pharmacological properties of the coupled bioactive compound; (ii) the radiolabeled prosthetic group precursors must be readily available for widespread use; they must be synthesizable in very few steps starting from commercially available reagents, with fast and reliable protocols; (iii) the radiolabeled prosthetic group must be obtainable promptly in high radiochemical yield, purity, and specific activity; (iv) the coupling reaction must be fast and use mild reaction conditions, with high selectivity, high yield, and easy purification methods; and (v) conjugates must be stable toward *in vivo* dehalogenation.

In this context and on the basis of our earlier work on the development of (hetero)aromatic benzamides for PET imaging and targeted radionuclide therapy of melanoma,[9,10] we designed the first bimodal fluorinated and iodinated prosthetic group, the tetrafluorophenyl 4-fluoro-3-iodobenzoate (**3** or **TFIB**, Scheme 1), as a suitable acylating agent for the labeling

Scheme 1. Preparation of TFIB and Radiolabeling Precursors[a]



[a]Reagents and conditions. (a) HCl, $NaNO_2$, KI, 0 °C, 0.5 h then RT 3 h, 86%; (b) 1. $(COCl)_2$, DMF, CH_2Cl_2, 0 °C, 0.5 h, then RT, 17 h; 2. TFP, NEt_3, CH_2Cl_2, RT 4 h, 63%; (c) 1. LiCl, Zn, THF, dibromoethane, Me_3SiCl, Xantphos, $CoCl_2$, 50 °C, 3 h; 2. tris(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)tin bromide, THF, 50 °C, 72 h, 28%; (d) paraformaldehyde, $NaBH_3CN$, AcOH, RT, 4 h, 99%; (e) MeOTf, Et_2O, RT, 4 d, 49%.

of a broad variety of compounds bearing a primary amine function. Here we present preliminary results of the synthesis, radiolabeling, and biological applications of **TFIB**, highlighting its potential for both PET imaging and targeted radionuclide therapy applications.

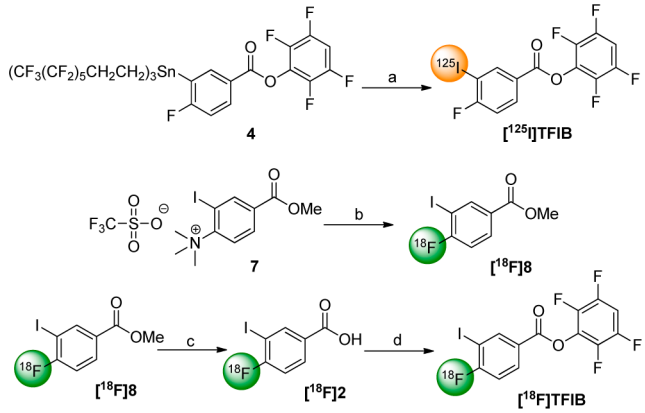
TFIB was prepared in two steps, starting from commercial 3-amino-4-fluorobenzoic acid (**1**, Scheme 1): diazotization–iodination giving compound **2** followed by esterification with 2,3,5,6-tetrafluorophenol (TFP) using an acyl chloride intermediate.

It should be noted that the tetrafluorophenyl ester group was chosen as the activated ester function for its high stability under basic conditions, frequently used for amide bond formation.[11,12] **TFIB** was thus obtained with 54% overall yield.

For radioiodination, we decided to use a (perfluoro)tin derivative as precursor (**4**, Scheme 1). It allows high specific activity radiolabeling under mild conditions and can be easily separated from radioiodinated **TFIB** using FluoroFlash solid-phase extraction cartridges (F-SPE), avoiding time-consuming purification by HPLC.[13] Precursor **4** was synthesized from **TFIB** by treating (perfluoro)tin bromide with an organozinc intermediate[14] generated *in situ* using a cobalt-Xantphos-catalyzed LiCl-mediated protocol.[15,16] Precursor **4** was thus prepared in a one-pot two-step procedure starting from **TFIB**, with 28% overall yield.

A three-step radiolabeling strategy starting from methyl ester **7** (Schemes 1 and 2) was carried out and based, for the

Scheme 2. Radiosyntheses of [^{125}I/^{18}F]TFIB[a]



[a]Reagents and conditions. (a) [^{125}I]NaI, AcOH, iodogen, MeOH, RT, 40 min, 71%; (b) [^{18}F]KF, K_{222}, MeCN, 80 °C, 4 min; (c) 1. NaOH 0.5 N, 50 °C, 2 min; 2. HCl 0.5 N; (d) DCC, TFP, 40 °C, 5 min, overall RCY 32% (d.c.).

radiofluorination precursor, on a trimethylammonium triflate salt as leaving group for fluorine-18 aromatic nucleophilic substitution. Indeed, this salt is highly soluble in organic solvents and tends to be more reactive than compounds with halogen or nitro leaving groups.[17,18] It can also be readily purified using a C18 solid-phase extraction cartridge (C18-SPE). Precursor **7** was prepared in two steps, starting from commercial methyl 4-amino-3-iodobenzoate (**5**): reductive amination, in the presence of paraformaldehyde and sodium cyanoborohydride,[19] yielded quantitatively the derivative **6**, which was then methylated with methyl trifluoromethanesulfonate to give the quaternary ammonium triflate salt **7** in 49% overall yield.

High specific activity radioiodination of **TFIB** was carried out by iodo-demetalation of organotin **4** with iodine-125 ($t_{1/2}$ = 59.9 d; γ 35 keV, Scheme 2).

It should be noted that this radioisotope was used as a radiolabeling model for iodine-131 ($t_{1/2}$ = 8.0 d; β^- 606 keV, 90%, γ 365 and 637 keV, 89%) for first preclinical developments. Precursor **4** was treated with [^{125}I]NaI in the presence of the gentle oxidizing agent iodogen and acetic acid, at RT. [^{125}I]**TFIB** was then purified by F-SPE (F-SPE separation depends on the fluorine content of each loaded compound and on the fluorophilicity of the solvents used) and formulated on a C18-SPE. Radiolabeled prosthetic group [^{125}I]**TFIB** was finally obtained in MeCN with 60 min total preparation time, 71% overall radiochemical yield (RCY), radiochemical purity (RCP) > 99%, and a specific activity (SA) of 30–35 GBq·μmol^{-1}. [^{125}I]**TFIB** in MeCN was radiochemically stable at RT for at least 4 d after preparation.

Fully automated three-step radiosynthesis of [^{18}F]**TFIB** was developed on a Raytest SynChromR&D bireactor module (Scheme 2). Nucleophilic substitution of compound **7** by the

dx.doi.org/10.1021/ml500423v | ACS Med. Chem. Lett. 2015, 6, 168–172

Scheme 3. General Synthesis Pathway for Coupling Reactions between TFIB and the Three Compounds of Interest Chosen

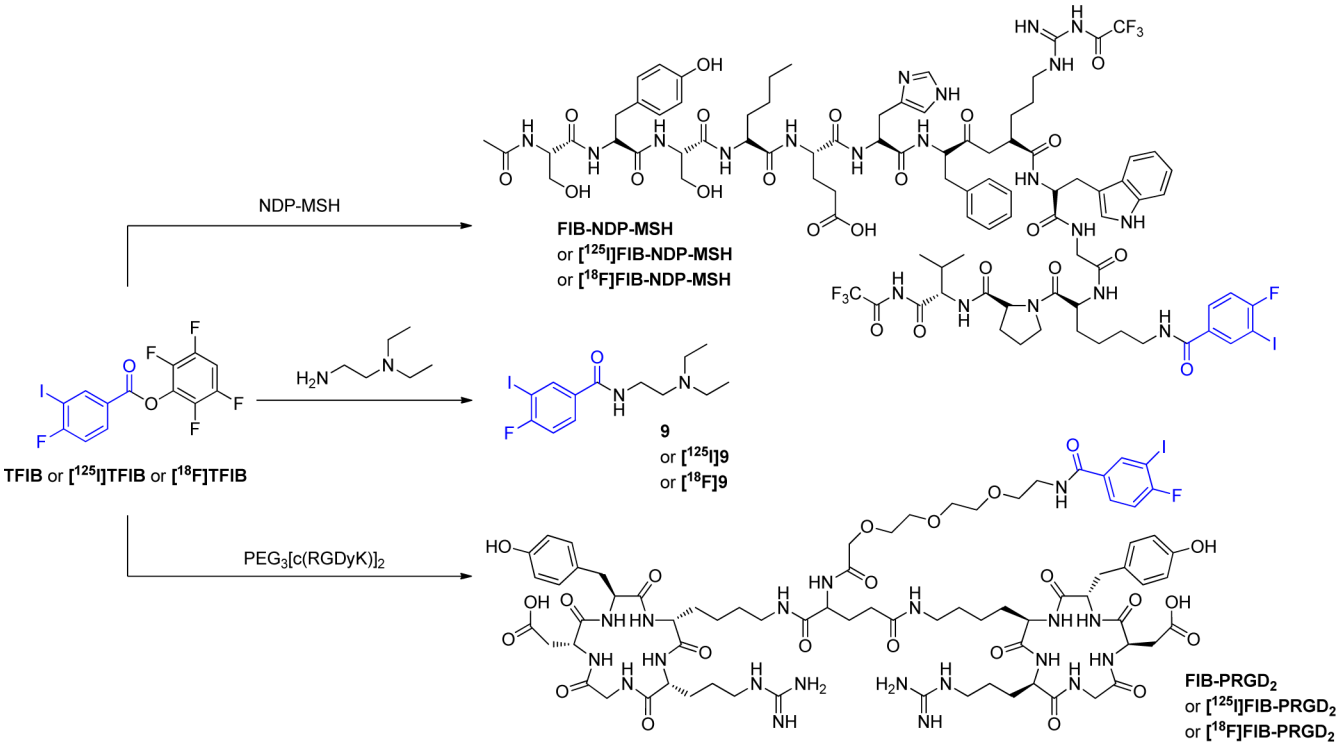


Table 1. Labeling Conditions and Radiochemical Data for Radiohalogenated 9, FIB-NDP-MSH, and FIB-PRGD$_2$

compd	volume of [X]TFIB/MeCN solution	amount of amine reagent (solvent/volume)	reaction temperature/time	purification	preparation time	RCY[a]	RCP[b]
[^{125}I]**9**	2 mL	10 μL	RT/10 min	C18-SPE	15 min	92%	>99%
[^{18}F]**9**	2 mL	10 μL (MeCN/1 mL)	RT/3 min	C18-SPE	8 min	90%	>99%
[^{125}I]**FIB-NDP-MSH**	33 μL	100 μg (borate buffer pH 8.0/100 μL)	45 °C/30 min	HPLC	60 min	83%	>99%
[^{18}F]**FIB-NDP-MSH**	1.2 mL	1.5 mg (borate buffer pH 8.0/1.5 mL)	50 °C/10 min	HPLC	25 min	57%	>99%
[^{125}I]**FIB-PRGD$_2$**	33 μL	100 μg (borate buffer pH 7.6/100 μL)	45 °C/30 min	HPLC	60 min	78%	>99%
[^{18}F]**FIB-PRGD$_2$**	1.2 mL	1.5 mg (borate buffer pH 7.6/1.5 mL)	50 °C/15 min	HPLC	31 min	47%	>99%

[a]Radiochemical yields are decay corrected. [b]Radiochemical purity determined by analytical HPLC.

dry [^{18}F]KF, K_{222} complex (5−10 GBq) at 80 °C in MeCN gave [^{18}F]**8** in 4 min (65−85% decay-corrected, d.c.). Subsequent saponification with a 0.5 N aqueous NaOH solution at 50 °C for 2 min provided [^{18}F]**2** as a sodium carboxylate salt (99% d.c.). Acidification of the media with a 0.5 N aqueous HCl solution was then necessary to trap intermediate [^{18}F]**2** on a C18-SPE cartridge. After elimination of hydrophilic and charged compounds with H_2O elution, [^{18}F]**2** was eluted in the second reactor with MeCN. The solution was further azeotroped at 100 °C for 5 min in the presence of Bu_4NOH (0.1 M/MeOH). Esterification of [^{18}F]**2** with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and TFP in MeCN at 40 °C for 5 min then yielded [^{18}F]**TFIB** (68−78% d.c.). Semipreparative HPLC purification followed by formulation on a C18-SPE cartridge provided [^{18}F]**TFIB** in 61 min with 32% overall RCY (d.c.), RCP > 99%, and SA of 30−55 GBq·μmol^{-1}. [^{18}F]**TFIB** was obtained in MeCN ready for coupling, and the solution was radiochemically stable at RT for at least 3 h after preparation.

To validate our approach, we tested three [^{125}I/^{18}F]**TFIB** coupling reactions (Scheme 3 and Table 1). We first investigated the reaction between [^{125}I/^{18}F]**TFIB** and *N*,*N*-diethylethylenediamine, providing [^{125}I/^{18}F]**9**, a benzamide analogue of previously studied melanin-targeting ligands.[9] [^{125}I/^{18}F]**9** was obtained in a very short time (8−15 min), with excellent RCY (>90%, d.c.) and RCP (>99%). We then investigated coupling reactions between [^{125}I/^{18}F]**TFIB** and well-known tumor-targeting peptides NDP-MSH[20] (targeting melanocortin type 1 receptor (MC1R), overexpressed by most melanoma cells) and PEG3[c(RGDyK)]$_2$[21] (targeting $\alpha_v\beta_3$ integrin, overexpressed by numerous tumor cells), affording [^{125}I/^{18}F]**FIB-NDP-MSH** and [^{125}I/^{18}F]**FIB-PRGD$_2$**, respectively. For these reactions, carried out in mild conditions, many parameters were optimized, such as the pH, the temperature and duration of reaction, the total volume of solvent, and the ratio between the volumes of acetonitrile (**TFIB**) and buffer (peptide). These coupling reactions provided the radiolabeled peptides in a short time (25−60 min) with good RCY (47−83%, d.c.) and RCP (>99%). Chemical structures of conjugated

dx.doi.org/10.1021/ml500423v | ACS Med. Chem. Lett. 2015, 6, 168−172

peptides were confirmed by MALDI-ToF mass spectrometry for their nonradioactive counterparts.

The *in vivo* stability of [^{125}I/^{18}F]**9** and its affinity for melanin pigment were investigated *in vivo* by γ scintigraphic and PET imaging in primary melanoma-bearing mice. For [^{125}I/^{18}F]**9**, tumor uptakes and good clearances from nonpigmented tissues were noted (Tables 3 and S1, Supporting Information), giving high-contrast PET images ([^{18}F]**9**, Figure 2). Moreover, the

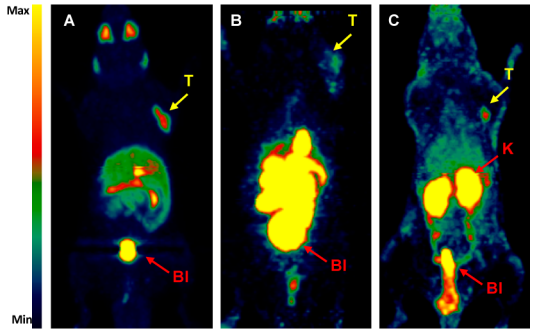


Figure 2. *In vivo* PET maximum-intensity-projection images at 1 h after injection. (A) [^{18}F]**9** biodistribution in B16F0 primary melanoma-bearing C57BL/6J mice. (B) [^{18}F]**FIB-NDP-MSH** biodistribution in B16F10 primary melanoma-bearing C57BL/6J mice. (C) [^{18}F]**FIB-PRGD$_2$** biodistribution in U87-MG primary glioblastoma-bearing nude mice. Arrows indicate the tumor (T), kidneys (K), and bladder (Bl).

tumor uptake values for [^{125}I]**9** and [^{18}F]**9** were quite similar and consistent at 1 and 3 h p.i. In addition, no radiodefluorination and only a slight radiodeiodination were observed, indicating that [^{125}I/^{18}F]**9** was quite stable *in vivo*. To investigate the influence of the prosthetic group on the biological properties of both peptides, *in vitro* competitive receptor-binding assays were carried out (see Table 2). The

Table 2. Receptor-Binding Affinities (IC$_{50}$) of Reference Peptides and FIB Conjugates

compd	MC1 receptor IC$_{50}$ (pM)[a]	$\alpha_v\beta_3$ receptor IC$_{50}$ (nM)[b]
[^{125}I]**NDP-MSH**	71 ± 4	
FIB-NDP-MSH	21 ± 1	
[^{125}I]**echistatin**		1.40 ± 0.01
FIB-PRGD$_2$		1.10 ± 0.60

[a]B16F10 murine melanoma cells. [b]U87-MG human glioblastoma cells.

calculated IC$_{50}$ value for the binding affinity to MC1R of **FIB-NDP-MSH** was found to be lower than for the reference NDP-MSH. The same trend was observed for the binding affinity to $\alpha_v\beta_3$ integrin of **FIB-PRGD$_2$** compared with the reference echistatin.

These results indicate that the 4-fluoro-3-iodobenzamide moiety does not interfere with the binding properties of the two bioactive peptides. *In vivo* PET imaging evaluation of [^{18}F]**FIB-NDP-MSH** and [^{18}F]**FIB-PRGD2** was conducted in primary melanoma-bearing mice and in primary glioblastoma-bearing mice, respectively. Considering tumor uptake (see Table 3), results with [^{18}F]**FIB-NDP-MSH** are similar to those described for NDP-MSH radiolabeled with [^{125}I]**SIB** or *para*-iodo *N*-succinimidyl 4-iodobenzoate [^{125}I]**PIB**.[22,23]

For [^{18}F]**FIB-PRGD2** we noted a slightly higher tumoral uptake in $\alpha_v\beta_3$ integrin positive tumors than in those determined with PRGD2 labeled with other ^{18}F-prosthetic groups.[24,25] No defluorination was observed for either radiopeptide between 1 and 3 h p.i. The observed predominant renal-urinary clearance route is also consistent with published results.[16,17] These preliminary *in vivo* PET imaging results suggest that the 4-fluoro-3-iodobenzamide moiety do not appear to interfere with the biological activity of both tested peptides.

In conclusion, we have developed a new prosthetic group that can be selectively radiofluorinated or radioiodinated with excellent radiochemical yields and purities. These radiolabeled acylating agents were first validated on a pigmented melanoma model through a melanin-binding benzamide approach and then successfully applied to the labeling and PET imaging of tumor-targeting peptides. These preliminary results encourage us to develop a more convenient procedure for the radiofluorination of **TFIB** (i.e., reducing the number of steps and/or the overall radiolabeling time) as well as to implement these findings in an extensive preclinical study involving PET imaging stratification and targeted radionuclide therapy applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for (radio)chemical synthesis, receptor-binding assays, *in vivo* imaging, compound characterization data, and HPLC (radio)chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: j-michel.chezal@udamail.fr.

Present Addresses

[†]GIP Arronax, 1 rue aronnax, BP10112, F-44817 Saint-Herblain, France.

[‡]Université de Nantes, UFR sciences Pharmaceutiques et biologiques, 9 rue Bias, BP53508, F-44035 Nantes Cedex, France. Institut de Recherche en Santé IRS UN, Unité

Table 3. PET Quantitative Analysis of Tumors and Selected Tissues at 1 and 3 h after i.v. Injection of Radiotracer in Mice

compd	time	tumor[d]	muscle[d]	bone[d]	TMR[e]
[^{18}F]**9**[a]	1 h	6.8 ± 1.9	1.1 ± 0.6	0.9 ± 0.1	6.5 ± 2.4
	3 h	6.9 ± 0.5	0.4 ± 0.1	0.5 ± 0.1	15.5 ± 1.5
[^{18}F]**FIB-NDP-MSH**[b]	1 h	1.4 ± 0.9	0.3 ± 0.2	0.4 ± 0.2	4.0 ± 0.8
	3 h	0.7 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	4.3 ± 1.2
[^{18}F]**FIB-PRGD2**[c]	1 h	7.3 ± 1.0	1.6 ± 0.7	1.7 ± 0.8	5.3 ± 2.5
	3 h	4.3 ± 1.0	0.9 ± 0.2	1.5 ± 0.9	4.9 ± 0.1

[a]B16F0 primary melanoma-bearing C57BL/6J mice. [b]B16F10 primary melanoma-bearing C57BL/6J mice. [c]U87-MG primary glioblastoma-bearing nude mice. [d]Data are expressed as percentage of injected dose per gram of tissue (%ID·g^{-1}) ± SD. [e]Tumor-to-muscle ratio.

INSERM 892-CNRS 6299 Centre de Recherche en Cancérologie Nantes-Angers, 8 quai Moncousu, BP70721, F-44007 Nantes Cedex, France.

Author Contributions

[§]These authors contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by CLARA (Cancéropôle Lyon Auvergne Rhône-Alpes), the French Directorate-General for Research and Innovation, the Auvergne Regional Council, the Puy-de-Dôme General Council, Clermont Community, the European Union, the European Regional Development Fund, the French Ligue contre le cancer, and the Bullukian Foundation.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Cyclopharma Laboratories for helpful technical supports and for radionuclide providing. The technical assistance of S. Tarrit is also gratefully acknowledged.

■ ABBREVIATIONS

HPLC, high pressure liquid chromatography; RT, room temperature; MALDI-TOF, matrix-assisted laser desorbtion/ionization time-of-flight; iodogen, 1,3,4,6-tetrachloro-3α,6α-diphenylglucoluril; Xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; K_{222}, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane

■ REFERENCES

(1) Adam, M. J.; Scott, D. J. Radiohalogens for imaging and therapy. *Chem. Soc. Rev.* **2005**, *34* (2), 153−163.

(2) Wilbur, D. S. Radiohalogenation of proteins: an overview of radionuclides, labeling methods, and reagents for conjugate labeling. *Bioconjugate Chem.* **1992**, *3* (6), 433−470.

(3) Dong, S.; Moroder, L.; Budisa, N. Protein iodination by click chemistry. *ChemBioChem* **2009**, *10* (7), 1149−1151.

(4) Avory, M. Iodine Radiolabelling Method. WO 2011070136, 2011.

(5) Bolton, A. E.; Hunter, W. M. The labeling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem. J.* **1973**, *133* (3), 529−539.

(6) Vaidyanathan, G.; Zalusky, M. R. Preparation of N-succinimidyl 3-[*I]iodobenzoate: an agent for the indirect radioiodination of proteins. *Nat. Protoc.* **2006**, *1* (2), 707−713.

(7) Glaser, M.; Luthra, S. K.; Brady, F. Applications of positron-emitting halogens in PET oncology (Review). *Int. J. Oncol.* **2003**, *22* (2), 253−267.

(8) Zimmermann, R. G. Why are investors not interested in my radiotracer? The industrial and regulatory constraints in the development of radiopharmaceuticals. *Nucl. Med. Biol.* **2013**, *40* (2), 155−166.

(9) Chezal, J. M.; Dollé, F.; Madelmont, J. C.; Maisonial, A.; Miot-Noirault, E.; Moins, N.; Papon, J.; Kuhnast, B.; Tavitian, B. Labelled Analogues of Halobenzamides as Multimodal Radiopharmaceuticals and Their Precursors. WO 2009095872, 2009.

(10) Billaud, E. M. F.; Rbah-Vidal, L.; Vidal, A.; Besse, S.; Tarrit, S.; Askienazy, S.; Maisonial, A.; Moins, N.; Madelmont, J. C.; Miot-Noirault, E.; Chezal, J. M.; Auzeloux, P. Synthesis, radiofluorination, and *in vivo* evaluation of novel fluorinated and iodinated radiotracers for PET imaging and targeted radionuclide therapy of melanoma. *J. Med. Chem.* **2013**, *56* (21), 8455−8467.

(11) Wilbur, D. S.; Hamlin, D. K.; Srivastava, R. R.; Burns, H. D. Synthesis and radioiodination of *N*-Boc-*p*-(tri-n-butylstannyl)-L-phenylalanine tetrafluorophenyl ester: preparation of a radiolabeled phenylalanine derivative for peptide synthesis. *Bioconjugate Chem.* **1993**, *4* (6), 574−580.

(12) Lockett, M. R.; Phillips, M. F.; Jarecki, J. L.; Peelen, D.; Smith, L. M. A tetrafluorophenyl activated ester self-assembled monolayer for the immobilization of amine-modified oligonucleotides. *Langmuir* **2008**, *24* (1), 69−75.

(13) Zhang, W.; Curran, D. P. Synthetic application of fluorous solid-phase extraction (F-SPE). *Tetrahedron* **2006**, *62* (51), 11837−11865.

(14) Donovan, A.; Forbes, J.; Dorff, P.; Schaffer, P.; Babich, J.; Valliant, J. F. A new strategy for preparing molecular imaging and therapy agents using fluorine-rich (fluorous) soluble supports. *J. Am. Chem. Soc.* **2006**, *128* (11), 3536−3537.

(15) Jin, M. Y.; Yoshikai, N. Cobalt-Xantphos-catalyzed, LiCl-mediated preparation of arylzinc reagents from aryl iodides, bromides, and chlorides. *J. Org. Chem.* **2011**, *76* (7), 1972−1978.

(16) Krasovskiy, A.; Malakhov, V.; Gavryushin, A.; Knochel, P. Eficient synthesis of functionalized organozinc compounds by the direct insertion of zinc into organic iodides and bromides. *Angew. Chem., Int. Ed.* **2006**, *45* (36), 6040−6044.

(17) Banister, S.; Roeda, D.; Dollé, F.; Kassiou, M. Fluorine-18 chemistry for PET: A concise introduction. *Curr. Radiopharm.* **2010**, *3* (2), 68−80.

(18) Olberg, D. E.; Arukwe, J. M.; Grace, D.; Hjelstuen, O. K.; Solbakken, M.; Kindberg, G. M.; Cuthbertson, A. One step radiosynthesis of 6-[^{18}F]fluoronicotinic acid 2,3,5,6-tetrafluorophenyl ester [^{18}F]F-Py-TFP): a new prosthetic group for efficient labeling of biomolecules with fluorine-18. *J. Med. Chem.* **2010**, *53* (4), 1732−1740.

(19) Zhang, X.; He, Y.; Liu, S.; Yu, Z.; Jiang, Z. X.; Yang, Z.; Dong, Y.; Nabinger, S. C.; Wu, L.; Gunawan, A. M.; Wang, L.; Chan, R. J.; Zhang, Z. Y. Salicylic acid based small molecule inhibitor for the oncogenic Srchomology-2 domain containing protein tyrosine phosphatase-2 (SHP2). *J. Med. Chem.* **2010**, *53* (6), 2482−2493.

(20) Cheng, Z.; Zhang, L.; Graves, E.; Xiong, Z.; Dandekar, M.; Chen, X.; Gambhir, S. S. Small-animal PET of melanocortin 1 receptor expression using a ^{18}F-labeled alpha-melanocyte-stimulating hormone analog. *J. Nucl. Med.* **2007**, *48* (6), 987−994.

(21) Chin, F. T.; Shen, B.; Liu, S.; Berganos, R. A.; Chang, E.; Mittra, E.; Chen, X.; Gambhir, S. S. First experience with clinical-grade ([^{18}F]FPP(RGD$_2$): an automated multi-step radiosynthesis for clinical PET studies. *Mol. Imaging Biol.* **2012**, *14* (1), 88−95.

(22) Garg, P. K.; Alston, K. L.; Welsh, P. C.; Zalutsky, M. R. Enhanced binding and inertness to dehalogenation of alpha-melanotropic peptides labeled using *N*-succinimidyl 3-iodo benzoate. *Bioconjugate Chem.* **1996**, *7* (2), 233−239.

(23) Chen, J.; Giblin, M. F.; Wang, N.; Jurisson, S. S.; Quinn, T. P. In vivo evaluation of ^{99m}Tc/^{188}Re-labeled linear alpha-melanocyte stimulating hormone analogs for specific melanoma targeting. *Nucl. Med. Biol.* **1999**, *26* (6), 687−693.

(24) Wu, Z.; Li, Z. B.; Cai, W.; He, L.; Chin, F. T.; Li, F.; Chen, X. ^{18}F-labeled mini-PEG spacered RGD dimer (^{18}F-FPRGD2): synthesis and microPET imaging of $\alpha_v\beta_3$ integrin expression. *Eur. J. Nucl. Med. Mol. Imaging* **2007**, *34* (11), 1823−1831.

(25) Li, W.; Lang, L.; Niu, G.; Guo, N.; Ma, Y.; Kiesewetter, D. O.; Shen, B.; Chen, X. *N*-succinimidyl 4-[^{18}F]-fluoromethylbenzoate-labeled dimeric RGD peptide for imaging tumor integrin expression. *Amino Acids* **2012**, *43* (3), 1349−1357.