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Single Photon Emission Computed Tomography/Positron Emission Tomography Imaging and Targeted Radionuclide Therapy of Melanoma: New Multimodal Fluorinated and Iodinated Radiotracers

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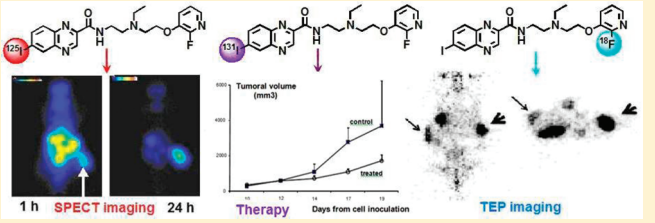
Supporting Information

ABSTRACT: This study reports a series of 14 new iodinated and fluorinated compounds offering both early imaging (^{123}I, ^{124}I, ^{18}F) and systemic treatment (^{131}I) of melanoma potentialities. The biodistribution of each ^{125}I-labeled tracer was evaluated in a model of melanoma B16F0-bearing mice, using in vivo serial γ scintigraphic imaging. Among this series, [^{125}I]**56** emerged as the most promising compound in terms of specific tumoral uptake and in vivo kinetic profile. To validate our multimodality concept, the radiosynthesis of [^{18}F]**56** was then optimized and this radiotracer has been successfully investigated for in vivo PET imaging of melanoma in B16F0- and B16F10-bearing mouse model. The therapeutic efficacy of [^{131}I]**56** was then evaluated in mice bearing subcutaneous B16F0 melanoma, and a significant slow down in tumoral growth was demonstrated. These data support further development of **56** for PET imaging (^{18}F, ^{124}I) and targeted radionuclide therapy (^{131}I) of melanoma using a single chemical structure.



INTRODUCTION

Every year, 2−3 million new skin cancers are diagnosed worldwide. Melanoma represents only 132 000 of these tumors[1] but accounts for 79% of all cutaneous neoplasm-related deaths.[2] The incidence of melanoma has spiked dramatically over the past few years,[3,4] and this cancer is rapidly becoming a major public health problem in the industrialized world. For localized lesions, where tumor thickness is <1.5 mm (stage I or II), melanoma can be cured by total resection. Unfortunately, this cancer displays a strong tendency to metastasize. For stages III or IV, median survival rate is around 6 months and the success rate only reaches 5% survival at 5 years, highlighting a pressing need for efficient therapies to treat disseminated melanoma.[1,5,6] Early detection is crucial for prognosis, and there have been significant advances in diagnosis such as dermoscopy of the primary tumor.[7] Compared to the diagnostic imaging techniques classically used for melanoma (CT, MRI, and ultrasound scan), noninvasive whole-body functional scintigraphic imaging would provide a valuable single specific examination for initial staging or patient follow-up. PET/CT technology using fluorodeoxyglucose (FDG) labeled with ^{18}F (half-life 1.83 h; β^+ emitter) allows significantly greater sensitivity, spatial resolution, and quantification for the diagnosis of advanced melanoma.[2,8] Unfortunately, this cancer can spread widely and unpredictably throughout the body and [^{18}F]FDG PET/CT is inappropriate for the diagnosis of early stage disease[9] for the detection of regional metastases[9c] and for the discrimination between melanoma metastases and other tumor types. Moreover, the use of this nonspecific radiotracer may be limited by the risk of false-positives due to abnormal uptake by inflammatory areas for example. [^{18}F]FDG PET/CT is also very unlikely to identify metastases in organs presenting a high physiological background stemming from [^{18}F]FDG metabolism such as brain or liver.[10] The use of [^{18}F]FDG is also limited for the treatment follow-up in cases of pretreated metastases with slow metabolism or hypometabolic areas.[11] The most powerful predictor of outcome remains the Breslow thickness for stage I

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and II melanoma or the highly sensitive sentinel lymph node biopsy in cases involving tumors ≥1 mm depth.[12]

In this context, there is a pressing need for agents with specific affinity for melanoma suitable for both early imaging and systemic treatment. Matched-pair radiotracers for melanoma imaging and radionuclide therapy have already been investigated and offer promising potential for accurate patient staging and specific dosimetry studies useful for treatment planning or monitoring response to targeted radionuclide therapy. Most often, this approach has been applied to peptide analogues of α-melanocyte stimulating hormone (α-MSH), which specifically target the melanocortin-1 receptors (MC1R) overexpressed in over 80% of human metastatic melanomas and such matched-pair radiotracers labeled with ^{99m}Tc (half-life 6.01 h; γ emitter)/^{188}Re (half-life 17.01 h; β^- emitter) or ^{111}In (half-life 2.80 d; γ emitter)/^{90}Y (half-life 2.67 d; β^- emitter) have posted very promising results.[13,14] Moreover, associating PET imaging with radionuclide therapy has been also investigated using positron-emitters such as ^{86}Y (half-life 14.74 h), ^{68}Ga (half-life 1.13 h), or ^{64}Cu (half-life 12.70 h).[15] However, the radiotherapy setting could be compromised by the high and nonspecific kidney uptake of these peptides that can result in a restricting renal toxicity. Moreover, for imaging and therapeutic applications, the labeling of the same peptide with similar but not fully identical radionuclides may lead to significant differences in biodistribution profiles.[14b,16] To overcome this problem, peptides radiolabeled with ^{203}Pb (half-life 51.93 h; γ emitter)/^{212}Pb (half-life 10.64 h; β^- emitter) have been assessed for SPECT imaging and α-therapy applications.[17] Unfortunately, around a third of the radioactivity escaped the chelator due to superionization reactions occurring when ^{212}Pb decays into ^{212}Bi (half-life 60.54 min; β^- and α emitter).[18]

Other specific relevant targets for melanoma that have been already studied include melanins. Although many compounds are known to interact with these natural pigments, only a few have successfully been used as carriers for radioisotopes.[19] Among the benzamide family, compounds such as *N*-(2-diethylaminoethyl)-2- and 4-iodobenzamide (**1a** (BZA), **b** (BZA2), Figure 1) and their ^{123}I-radiolabeled (half-life 13.22 h; γ emitter) derivatives gave promising results for SPECT imaging of melanoma lesions.[20] More recently, various preclinical studies have demonstrated that radiofluorinated benzamide derivatives were also of great interest for specific PET imaging of melanotic tumors (**2**, Figure 1).[21] At the same time, our team identified a new quinoxaline derivative of **1** (*N*-(2-diethylaminoethyl)-6-iodoquinoxaline-2-carboxamide, **3** (ICF01012), Figure 1) with favorable pharmacokinetic properties for ^{131}I (half-life 8.03 d; β^- emitter) radionuclide therapy.[22] [^{131}I]**3** has shown antitumoral efficacy in melanoma-bearing mice after iv administration of 2 × 18.5 MBq.[23] Moreover, the rapid and specific tumor uptake of this radiotracer suggests that this class of compounds has great potential applications for PET imaging. One possible approach would be to replace the iodine atom on the molecular scaffold of **3** with a fluorine atom. Indeed, ^{18}F is currently the most attractive positron-emitting halogen radionuclide for radiopharmaceutical chemistry and PET imaging due to its well-adapted physical and nuclear characteristics. However, this strategy may affect tracer biodistribution given that the aromatic iodine/fluorine replacement influences especially for electronic density distribution in aromatic systems.[24] An alternative could be to design iodinated and fluorinated analogues of our lead tracer **3**. The expected performances of these new radiotracers should extend

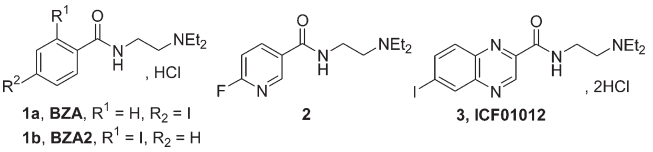


Figure 1. Tracers with specific affinity for melanins.

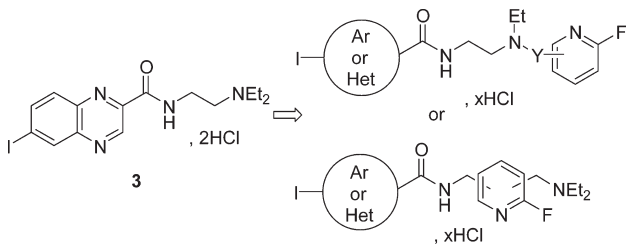


Figure 2. Overall strategy for the SAR study of **3**.

to both specific SPECT (^{123}I) or PET (^{18}F or ^{124}I, half-life 4.17 d) imaging as well as efficient targeted radionuclide therapy (^{131}I) of melanoma.[25]

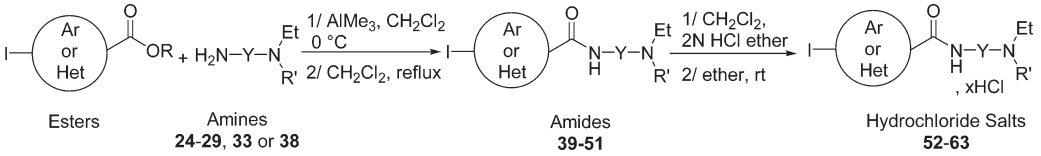
Herein, we report on: (i) the chemical synthesis of 14 new specific iodinated and fluorinated compounds, (ii) the radiolabeling with ^{125}I (half-life 59.41 d; γ emitter) of each tracer followed by tumor uptake evaluation in B16F0 melanoma-bearing mice using in vivo serial γ scintigraphic imaging, (iii) selection of the tracer with the kinetic profile best geared to performing a PET imaging assay after labeling with ^{18}F from the corresponding precursor, and finally (iv), a detailed in vivo biodistribution study achieved by autoradiography, followed by a targeted radionuclide therapy assay after labeling of the same selected compound with ^{131}I.

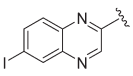
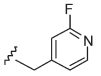
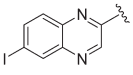
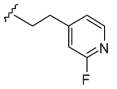
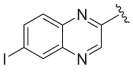
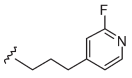
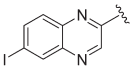
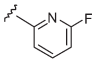
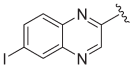
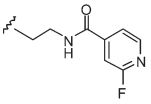
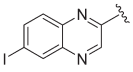
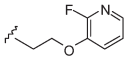
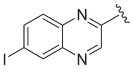
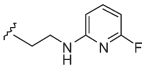
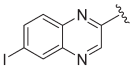
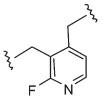
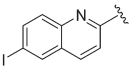
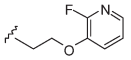
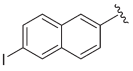
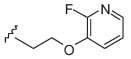
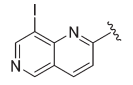
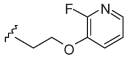
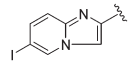
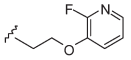
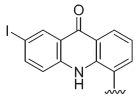
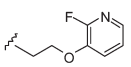
RESULTS AND DISCUSSION

Chemistry. The tracer **3** (Figure 1) was chosen as a lead compound for our structure−activity relationship study (SAR). As a first approach step, we decided to focus on the incorporation of fluorinated groups into the carboxamide side chain. As depicted in Figure 2, and with the aim of improving the C−F bond stability, we designed compounds wherein the fluorine atom was incorporated as a 2- or 6-fluoropyridine core in the *N*, *N*-diethylethylenediamine framework of our lead tracer **3**. Indeed, this strategy should allow nucleophilic heteroaromatic radiofluorinations of corresponding halogeno- or nitro- precursors without requiring of an additional electron-withdrawing substituent in the aromatic ring.[26] Fluoropyridines were introduced on the tertiary amine either directly or in combination with various linkers. To complete our pharmacomodulation study on the carboxamide side chain, we also considered the introduction of a 2-fluoropyridine moiety between the amidic function and the tertiary amine. When possible, final amide tracers **39**−**51** (Table 1) were obtained by coupling iodinated (hetero)aromatic esters with corresponding primary amines, mainly synthesized via protective phthalimide intermediates. For pharmacological studies, final compounds were converted, when possible, into their hydrochloride salts to improve solubility in water.

Synthesis of Amines. The synthetic pathways opening access to amines **24**−**29** obtained from phthalimides **15**−**18**, **21**, and **23** are outlined in Scheme 1. Protected intermediates **15**−**17** were prepared from 2-fluoro-4-methylpyridine (**4**)[27] via alcohols

Table 1. Synthesis of 2-Fluoropyridine Analogues 39−51 and their Corresponding Hydrochloride Salts 52−63



Entry	Ester	R	Cpd	Amine Y	Amine R'	Amide Cpd (yield %)[a]	Hydrochloride Salt Cpd (yield %)[b]	x
1		Et	**24**	-CH_2CH_2-		**39** (38)	**52** (64)	2
2		Et	**25**	-CH_2CH_2-		**40** (68)	**53** (78)	2
3		Et	**26**	-CH_2CH_2-		**41** (76)	**54** (84)	2
4		Et	**27**	-CH_2CH_2-		**42** (61)	-	-
5		Et	**28**	-CH_2CH_2-		**43** (63)	**55** (84)	2
6		Et	**29**	-CH_2CH_2-		**44** (94)	**56** (91)	2
7		Et	**33**	-CH_2CH_2-		**45** (97)	**57** (80)	2
8		Et	**38**		Et	**46** (57)	**58** (90)	2
9		Et	**29**	-CH_2CH_2-		**47** (96)	**59** (81)	2
10		Me	**29**	-CH_2CH_2-		**48** (60)	**60** (79)	1
11		Et	**29**	-CH_2CH_2-		**49** (96)	**61** (87)	2
12		Et	**29**	-CH_2CH_2-		**50** (58)	**62** (91)	2
13		Et	**29**	-CH_2CH_2-		**51** (89)	**63** (79)	1

[a] Yields refer to the products isolated after chromatography. [b] Yields refer to the products isolated after filtration.

7, **9**, and **11** respectively. Briefly, oxidation of derivative **4**[27] by potassium permanganate in refluxing water followed by esterification of acid **5** with ethyl chloroformate in the presence of catalytic quantities of 4-dimethylaminopyridine (DMAP) provided ester **6**, which was reduced in primary alcohol **7** using lithium aluminum hydride (LAH) at −50 °C. For the syntheses of **9** and **11**, treatment of picoline **4**[27] by freshly prepared lithium diisopropylamide (LDA) at −80 °C, followed by trapping of the

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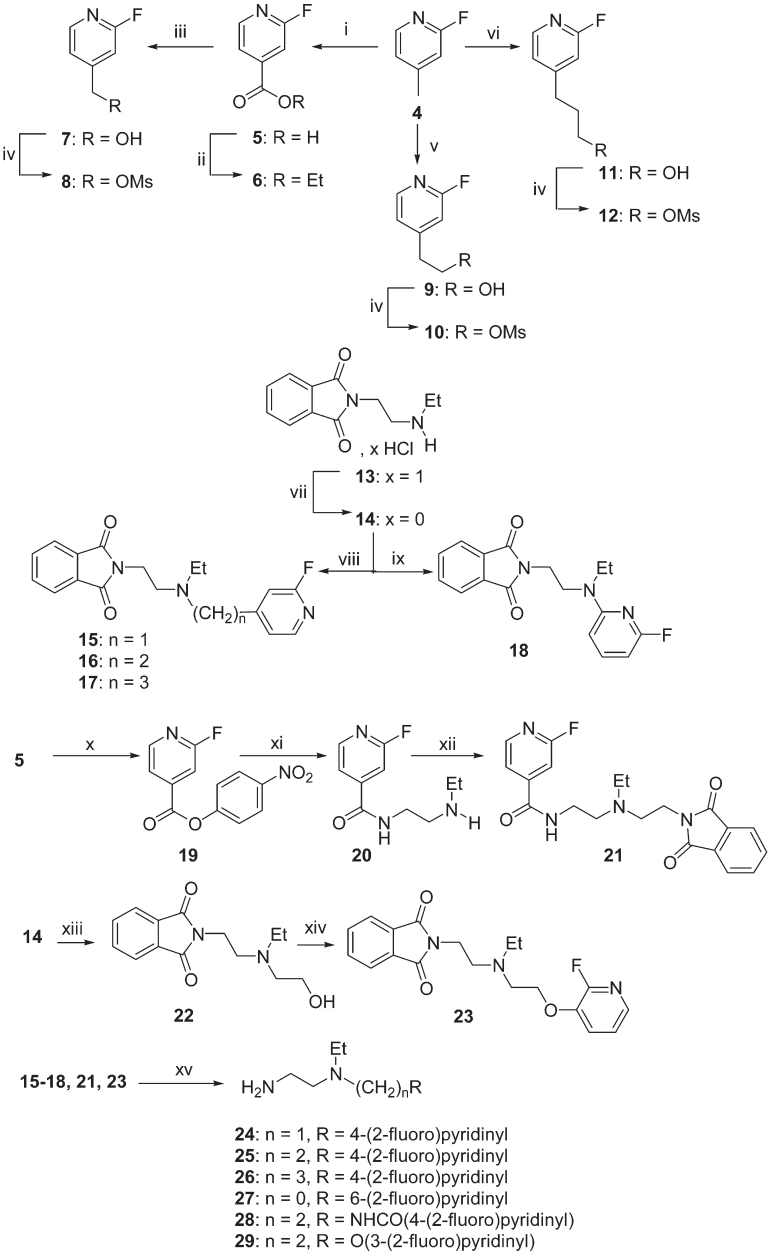
Scheme 1. Synthesis of Amines 24−29[a]



[a] Reagents and conditions: (i) $KMnO_4$, H_2O, reflux, 13 h; (ii) (a) ethyl chloroformate, NEt_3, CH_2Cl_2, 0 °C, 10 min, (b) DMAP, 0 °C, 4 h; (iii) $LiAlH_4$, THF, −50 °C, 15 min; (iv) NEt_3, methanesulfonyl chloride, EtOAc, 0 °C, 10−30 min; (v) (a) LDA, THF, −80 °C, 1 h, (b) *p*-formaldehyde, −80 °C, 1 h then rt, 12 h; (vi) (a) LDA, THF, −80 °C, 1 h, (b) ethylene oxide, −80 °C, 1 h then rt, 12 h; (vii) 5% aq Na_2CO_3, CH_2Cl_2, rt; (viii) compound **8**, NEt_3, DMF, 90 °C, 3 h/compound **10** or **12**, NEt_3, EtOH, 70 °C, 24 h; (ix) 2,6-difluoropyridine, NEt_3, DMF, 130 °C, 16 h; (x) (a) $SOCl_2$, CH_2Cl_2, DMF, reflux, 3.5 h, (b) *p*-nitrophenol, NEt_3, THF, 50 °C, 12 h; (xi) *N*-ethylethylenediamine, THF, rt, 24 h; (xii) *N*-2-bromoethylphthalimide, NEt_3, CH_3CN, reflux, 63 h; (xiii) 2-bromoethanol, NEt_3, EtOH, reflux, 60 h; (xiv) 2-fluoro-3-hydroxypyridine,[30] PPh_3, DIAD, THF, rt, 24 h; (xv) $NH_2NH_2.H_2O$, EtOH, rt or reflux, 4.5−24 h.

resulting lithium anion with either *p*-formaldehyde or ethylene oxide, provided the desired alcohols **9** and **11**, respectively. Alcohols **7**, **9**, and **11** were then converted into their mesylate derivatives **8**, **10**, and **12** by following the procedure described by Pesti et al.[28] Nucleophilic displacement of mesylates **8**, **10**, and **12** with *N*-[2-(ethylamino)ethyl]phthalimide (**14**) obtained from its corresponding hydrochloride salt **13**,[29] provided the key phthalimide derivatives **15**−**17**, respectively. Despite various sets of conditions used to optimize the nucleophilic substitution, compound **16** can not be synthesized without the concomitant and predominant elimination reaction leading to 2-fluoro-4-vinylpyridine (35%). Reaction between phthalimide **14** and commercially available 2,6-difluoropyridine yielded compound **18**. The preparation of phthalimide **21** started from 2-fluoroisonicotic acid (**5**). This acid was first treated with thionyl chloride ($SOCl_2$) in refluxing dichloromethane to provide the corresponding unstable carbonyl chloride intermediate, which was rapidly converted into *p*-nitrophenyl 2-fluoroisonicotinate (**19**)

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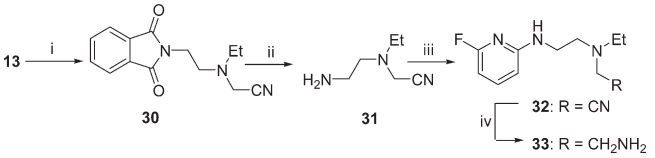
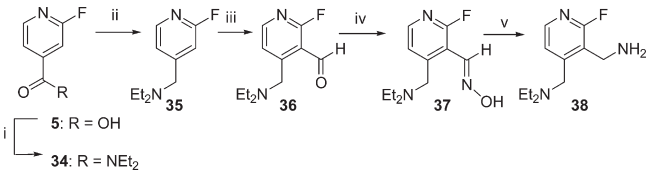
Scheme 2. Synthesis of Amine 33[a]



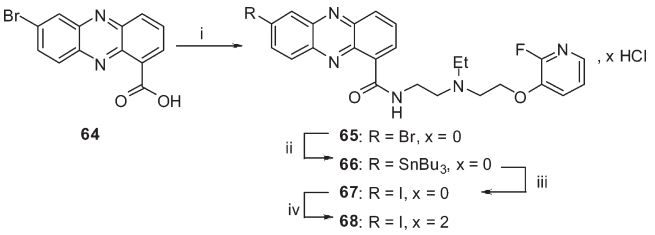
[a] Reagents and conditions: (i) $BrCH_2CN$, K_2CO_3, CH_3CN, rt, 48 h; (ii) $NH_2NH_2 \cdot H_2O$, EtOH, rt, 14 h; (iii) 2,6-difluoropyridine, NEt_3, DMSO, 80 °C, 8 h; (iv) $LiAlH_4$, THF, 0 °C, 2 h.

Scheme 3. Synthesis of Amine 38[a]



[a] Reagents and conditions: (i) (a) $SOCl_2$, CH_2Cl_2, DMF, reflux, 3 h; (b) *N*,*N*-diethylamine, THF, 0 °C then rt, 12 h; (ii) (a) BH_3, THF, 50 °C, 1 h; (b) MeOH, 1.0 M aq. NaOH, 0 °C then 50 °C, 5 h; (iii) (a) LDA, THF, −78 °C, 7 h, (b) DMF, −78 °C, 5 min; (iv) NH_2OH. HCl, KOAc, H_2O, MeOH, rt, 4 h; (v) Zn, AcOH, rt, 24 h.

Scheme 4. Synthesis of Phenazine 68[a]



[a] Reagents and conditions: (i) (a) $SOCl_2$, reflux, 1 h, (b) **29**, CH_2Cl_2, 0 °C then rt, 24 h; (ii) Sn_2Bu_6, $Pd(PPh_3)_4$, toluene, reflux, 17 h; (iii) (a) I_2, $CHCl_3$, rt, 18 h; (iv) (a) CH_2Cl_2, 2N HCl Et_2O, 10−15 min, (b) Et_2O, rt, 14 h.

using *p*-nitrophenol and triethylamine (NEt_3) at 61% overall yield. The activated ester **19** was then treated with commercially available *N*-ethylethylenediamine, and the resulting fluoroisonicotinamide **20** was alkylated with *N*-(2-bromoethyl)phthalimide in the presence of NEt_3 to afford the desired compound **21**. Phthalimide **23** was obtained via Mitsunobu reaction between 2-fluoro-3-hydroxypyridine[30] and alcohol **22**, prepared by alkylating *N*-[2-(ethylamino)ethyl]phthalimide (**14**) with 2-bromoethanol in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD). In a last step, deprotection of phthalimides **15**−**18**, **21**, and **23** using hydrazine monohydrate in ethanol at various temperatures afforded primary amine intermediates **24**−**29**, respectively.

The sequence depicted in Scheme 2 was used for the preparation of amine **33**. Phthalimide **13**[29] was first alkylated with bromoacetonitrile in the presence of K_2CO_3 to provide compound **30**. Deprotection of **30** gave primary amine **31**, which was rapidly condensed with 2,6-difluoropyridine under basic conditions to yield the nitrile compound **32**. Reducing compound **32** with LAH at 0 °C provided amine **33**.

Scheme 3 illustrates the synthetic pathway of amine **38**. 2-Fluoroisonicotinic acid (**5**) was treated successively with $SOCl_2$ and diethylamine to provide compound **34**. Reduction of the amidic function using borane in anhydrous THF afforded amine derivative **35**. Formylation of the resultant product using C-3 regiospecific lithiation with LDA at −80 °C and subsequent quenching with DMF led to aldehyde **36**. Its instability in the reaction medium means aldehyde **36** has to be extracted rapidly after return back to room temperature. Finally, treatment with hydroxylamine hydrochloride and potassium acetate led to oxime **37**, which was reduced using zinc dust in acetic acid to provide the expected amine **38**.

Synthesis of Amides. A common strategy, outlined in Table 1, was used for the synthesis of amides **39**−**51**. Primary amines **24**−**29**, **33**, and **38** were condensed with ethyl 6-iodoquinoxaline-2-carboxylate[22a] in the presence of trimethylaluminium to give amides **39**−**46** (entries 1−8) in the 38−97% yield range. After a preliminary in vivo evaluation of the radioiodinated tracers, we explored the effect of replacing the iodoquinoxaline moiety of the most potent analogue identified **56** with several

Table 2. Radiochemical Data for [^{125}I]3 Analogues

entry	starting material	temperature (°C) (time, min)	method	product	HPLC retention time (min)	radiochemical yield (%)[a]	radiochemical purity (%)[b]	specific radioactivity (MBq/μmol)
1	**42**	150 (60)	B[c]	[^{125}I]**42**		81	99.8	29.4
2	**52**	130 (20)	A	[^{125}I]**52**	11.6	26	99.9	7.4
3	**53**	130 (60)	A	[^{125}I]**53**	13.4	33	99.6	30.3
4	**54**	130 (45)	A	[^{125}I]**54**	14.4	34	94.2	23.8
5	**55**	130 (27)	A	[^{125}I]**55**	10.6	45	98.3	16.3
6	**56**	120 (45)	A	[^{125}I]**56**	16.5	53	99.7	31.9
7	**57**	150 (60)	B	[^{125}I]**57**		66	98.8	14.8
8	**58**	130 (45)	A	[^{125}I]**58**	15.4	30	98.5	48.0
9	**59**	130 (50)	A	[^{125}I]**59**	15.8	39	99.8	19.1
10	**60**	130 (60)	A	[^{125}I]**60**	11.4	41	99.9	21.2
11	**61**	130 (45)	A	[^{125}I]**61**	11.9	22	99.9	15.2
12	**68**	130 (45)	A	[^{125}I]**68**	15.7	41	99.0	41.9

[a] Radiochemical yields were calculated by dividing the radioactivity in the final product by the initial amount of radioactive sodium iodide. [b] Radiochemical purities were determinated by analytical HPLC, except for compound [^{125}I]**42** and [^{125}I]**57** (TLC analyses). [c] The final conversion step for the synthesis of corresponding hydrochloride salt has not been performed.

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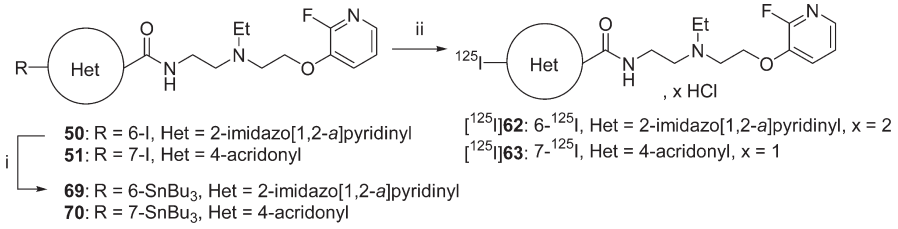
Scheme 5. Synthesis of the Tributyltin Precursors 69 and 70 and Radiolabeling of [^{125}I]62 and [^{125}I]63[a]



[a] Reagents and conditions: (i) Sn_2Bu_6, $Pd(PPh_3)_4$, toluene, reflux, 12 h; (ii) [^{125}I]NaI, CAT.H_2O, EtOH, citrate buffer (pH = 4), H_2O, rt, 30 min for [^{125}I]**62** or [^{125}I]NaI, CAT, EtOH, AcOH 1% EtOH, H_2O, rt, 10 min for [^{125}I]**63**.

Table 3. Tumor Radioactivity Uptake in B16F0 Melanoma-Bearing C57Bl6 Mice of Iodoquinoxaline Analogues [^{125}I]42, [^{125}I]52−58 Compared to [^{125}I]3 at Various Times after iv Injection[a]

		%ID/g tumor B16F0								
entry	compd	1 h	3 h	6 h	24 h	72 h	5 d	7 d	10 d	14 d
1	[^{125}I]**3**	21.8 ± 6.6	26.3 ± 6.6	29.6 ± 8.4	28.0 ± 8.2	12.3 ± 3.7	7.3 ± 3.6	3.4 ± 0.3	1.9 ± 0.4	
2	[^{125}I]**42**	2.0 ± 0.3	2.2 ± 1.0	1.8 ± 0.3	0.9 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1		
3	[^{125}I]**52**	10.3 ± 6.6	12.0 ± 4.8	15.4 ± 6.7	16.0 ± 7.4	10.0 ± 2.6	7.1 ± 1.8	n.d.	1.9 ± 0.9	0.8 ± 0.5
4	[^{125}I]**53**	12.1 ± 4.1	13.3 ± 6.0	14.5 ± 6.6	11.2 ± 2.5	7.7 ± 0.9	5.2 ± 1.7	3.0 ± 1.0	1.6 ± 0.5	1.1 ± 0.3
5	[^{125}I]**54**	9.1 ± 1.8	9.3 ± 0.6	14.1 ± 3.9	16.3 ± 8.9	9.5 ± 5.9	5.1 ± 3.3	2.5 ± 2.1	1.4 ± 1.0	0.7 ± 0.5
6	[^{125}I]**55**	9.6 ± 0.7	8.8 ± 1.6	12.1 ± 3.2	12.1 ± 4.6	9.6 ± 7.1	5.1 ± 3.3			
7	[^{125}I]**56**	12.7 ± 2.3	20.2 ± 4.2	18.8 ± 1.6	18.2 ± 0.6	10.3 ± 1.7	5.2 ± 1.2	3.2 ± 0.4	1.7 ± 0.4	0.6 ± 0.3
8	[^{125}I]**57**	13.1 ± 3.8	13.7 ± 6.1	12.5 ± 3.9	8.6 ± 0.9	5.7 ± 1.5	2.6 ± 0.4	1.5 ± 0.2	0.8 ± 0.1	0.4 ± 0.2
9	[^{125}I]**58**	5.6 ± 2.0	6.5 ± 3.2	6.7 ± 1.9	6.4 ± 1.8	4.1 ± 1.5	2.4 ± 0.7	1.5 ± 0.4	0.8 ± 0.3	0.7 ± 0.1

[a] Radioactive concentration values were calculated from planar scintigraphic images.[40] Mean ± SD (three mice). Absence of value: concentration in the tumor equal to background value.

Table 4. Tumor Radioactivity Uptake in B16F0 Melanoma-Bearing C57Bl6 Mice of Iodo(hetero)aromatic Analogues [^{125}I]59−63, and [^{125}I]68, Compared to [^{125}I]3 and [^{125}I]56 at Various Times after iv Injection[a]

		%ID/g tumor B16F0								
entry	compd	1 h	3 h	6 h	24 h	72 h	5 d	7 d	10 d	14 d
1	[^{125}I]**3**	21.8 ± 6.6	26.3 ± 6.6	29.6 ± 8.4	28.0 ± 8.2	12.3 ± 3.7	7.3 ± 3.6	3.4 ± 0.3	1.9 ± 0.4	
2	[^{125}I]**56**	12.7 ± 2.3	20.2 ± 4.2	18.8 ± 1.6	18.2 ± 0.6	10.3 ± 1.7	5.2 ± 1.2	3.2 ± 0.4	1.7 ± 0.4	0.6 ± 0.3
3	[^{125}I]**59**	10.7 ± 2.3	10.8 ± 2.9	9.7 ± 2.5	6.3 ± 2.5	3.2 ± 0.3	2.3 ± 0.1	1.1 ± 0.6	0.4 ± 0.1	0.2 ± 0.2
4	[^{125}I]**60**	7.9 ± 1.1	8.0 ± 2.8	7.4 ± 1.8	5.9 ± 3.0	nd	2.8	2.1	0.2	0.6
5	[^{125}I]**61**	5.6 ± 1.7	5.5 ± 2.4	5.3 ± 2.4	5.2 ± 2.0	3.3 ± 2.0	1.6 ± 0.7	1.1 ± 0.5	0.5 ± 0.3	0.4 ± 0.3
6	[^{125}I]**62**	13.2 ± 6.0	13.1 ± 5.3	13.4 ± 4.6	15.6 ± 2.7	8.6 ± 1.1	6.4 ± 0.7	3.9 ± 1.2	2.3 ± 0.1	1.6 ± 0.1
7	[^{125}I]**63**	10.7 ± 2.0	13.0 ± 1.9	13.0 ± 2.3	16.0 ± 3.1	8.6 ± 1.7	4.5 ± 1.0	4.3 ± 1.2	2.7 ± 1.3	2.0 ± 0.9
8	[^{125}I]**68**	11.4 ± 1.7	12.3 ± 1.7	13.8 ± 2.5	13.8 ± 2.3	5.8 ± 0.2	3.7 ± 0.6	2.0 ± 0.9	1.2 ± 0.5	0.7 ± 0.4

[a] Radioactive concentration values were calculated from planar scintigraphic images.[40] Mean ± SD (three mice). Absence of value: concentration in the tumor equal to background value. No standard deviation when only one mouse survived. nd: non determined.

iodo(hetero)aromatic rings. Following the same method, amine **29** was condensed with various aromatic or heteroaromatic esters previously prepared in our laboratory[22,31] to provide amides **47**−**51** (entries 9−13) in good to excellent yields (58−96%). The final step was the conversion of amides **39**−**51** into their corresponding hydrochloride salts **52**−**63** via the action of a 2 N HCl/ether solution. In the case of amide **42**, all attempts to form the corresponding hydrochloride salt remained unsuccessful.

The preparation of amide **67**, presented in Scheme 4, required a slightly modified strategy starting from brominated acid **64**, as all attempts to synthesize the iodophenazine acid from corresponding intermediate 2-[(4-iodophenyl)amino]-3-nitrobenzoic acid failed (data not presented). However, intramolecular cyclization of the corresponding brominated nitrobenzoic acid successfully afforded phenazine acid[22b] **64**, which was converted into its carbonyl chloride derivative using refluxing $SOCl_2$. This intermediate was not isolated but directly condensed with amine **29** to give the brominated amide **65** in nearly quantitative yield (95%). The resulting bromo derivative was stannylated using palladium(0)-catalyzed cross-coupling reaction in the presence of freshly prepared tetrakis(triphenylphosphine)-palladium[32] and hexabutylditin to afford the stannane **66**.

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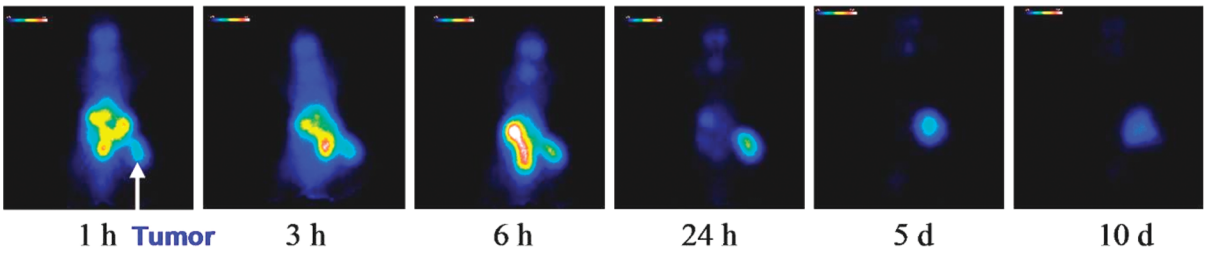

Figure 3. In vivo kinetic of compound [^{125}I]**56** in a B16F0 melanoma-bearing C57Bl6 mouse, illustrated by repeated planar scintigraphic images using a dedicated γ imager for small animals, after iv injection of a 2.7 MBq dose (acquisition time 10 min).

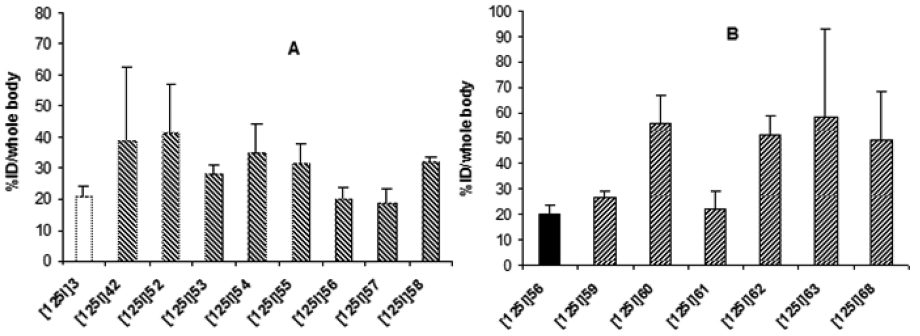

Figure 4. Total activity remaining in the whole body 24 h after iv injection of [^{125}I]-labeled compounds expressed as % of injected activity. Values were calculated from planar scintigraphic images mean ± SD (three mice). (A) [^{125}I]-labeled iodoquinoxaline analogues compared to [^{125}I]**3**. (B) Compounds obtained after pharmacomodulation of the quinoxaline moiety [^{125}I]**59**−**63** and [^{125}I]**68** compared to [^{125}I]**3** and [^{125}I]**56**.

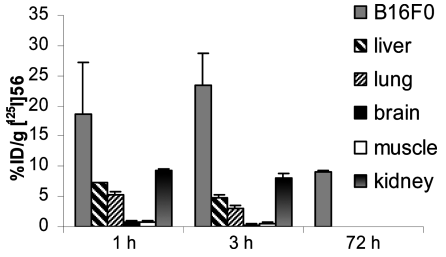

Figure 5. Concentration of [^{125}I]**56** in selected organs after iv injection in B16F0 melanoma-bearing C57Bl6 mice. Means of % ID/g ± SD: two mice, *n* determinations for each compound at each time. Absence of value: organ concentration was equal to background value.

Finally, electrophilic iododestannylation of **66** with diiodine led to the desired iodinated amide **67**, which was converted into its corresponding dihydrochloride salt **68**.

^{125}I Radiochemistry. The radiolabeling at low specific activity of tracers **42**, **52**−**63**, and **68** was performed when possible, using nucleophilic isotopic exchange reaction with no-carrier-added [^{125}I]NaI in acidic medium (citrate buffer or acetic acid) (methods A−B, Table 2) to provide, after purification, the radioiodinated tracers [^{125}I]**42**, [^{125}I]**52**−**61**, and [^{125}I]**68**. Reaction conditions, HPLC retention times, and radiochemical data are reported in Table 2. All attempts at radiolabeling compounds **62** and **63** under these conditions resulted in a complete degradation of the starting materials. To circumvent this problem, we elected to radiolabel **62** and **63** under mild conditions using electrophilic radioiodo-demetalation reaction from corresponding stannane precursors **69** and **70** (Scheme 5), which were obtained by following the procedure developed for compound **66**. This strategy led to radiolabeled compounds [^{125}I]**62** and [^{125}I]**63** in moderate radiochemical yields (18% and 48%, respectively).

Biodistribution Studies. For all ^{125}I-labeled compounds evaluated on melanoma B16F0 bearing mice, scintigraphic images showed significant tumor retention of radioactivity. The tumor uptake values are summarized in Table 3 for iodoquinoxaline derivatives and in Table 4 for iodo(hetero)aromatic derivatives comparatively to our reference [^{125}I]**3**. Except for [^{125}I]**42**, tumor affinity was maintained with all iodoquinoxaline derivatives. For [^{125}I]**52**−**58** (Table 3), a significant tumor radioactivity concentration at 1 h postinjection pi (≥5.6% ID/g) was observed and gradually increased to a maximal tumor accumulation at 3−6 h pi. Tumor radioactivity uptake even reached 20.2% ID/g for [^{125}I]**56** at 3 h pi and continued to be measurable for at least 5 days, as was the case for the kinetic profile of [^{125}I]**3**. Representative serial scintigraphic images produced on melanoma-bearing mice after injection of [^{125}I]**56** (Figure 3) also illustrate rapid clearance from nontarget organs, allowing well-defined images of the tumor. Taken together, these data suggest an overall maintenance of tumor affinity for these new radioiodinated tracers, giving rapid and long-lasting tumor radioactivity uptake. Figure 4A gives values for total remaining whole-body activity at 24 h pi. Of all the iodoquinoxaline analogues studied, [^{125}I]**56** featured among the more rapidly excreted (20% remaining after 24 h), as observed with [^{125}I]**3**. These preliminary results singled out compound **56** as presenting the most favorable kinetic profile for application as a specific radiotracer in both PET imaging and targeted radionuclide therapy of melanoma.

As described in the Chemistry section, we continued our SAR study by replacing the iodoquinoxaline part of the most promising compound **56** by various iodinated (hetero)aromatic rings

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(Table 1, entries 9−13, and Scheme 4). Resulting compounds **59**−**63** and **68** were also labeled with ^{125}I, and their preliminary in vivo biodistributions were studied using the same protocol. Table 4 summarizes the tumor concentration values obtained with this series of radioiodinated (hetero)aromatic tracers. Comparatively to [^{125}I]**3** and [^{125}I]**56**, a high and specific radioactivity uptake in tumor of at 1 h pi (between 5.6% and 13.2% ID/g) was globally observed with these analogues. However, the pharmacomodulation of the quinoxaline moiety did not improve the tumor concentration values previously observed with [^{125}I]**56** (5.5−13.1% ID/g at 3 h pi for [^{125}I]**59**−**63**, and [^{125}I]**68** vs 20.2% ID/g at 3 h pi for [^{125}I]**56**). In addition, tracers [^{125}I]**62** and [^{125}I]**63**, bearing 6-iodoimidazo[1,2-*a*]pyridine-2-yl and 7-iodoacridone-4-yl radicals, respectively, presented a slower general elimination that was less favorable for imaging purposes (Figure 4B).

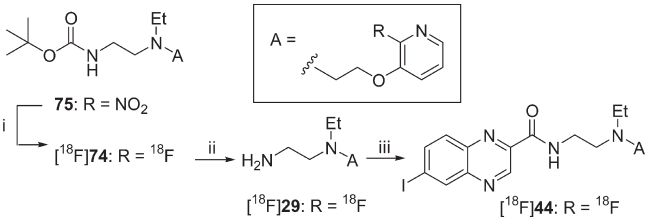
On the basis of these preliminary in vivo biodistribution results obtained by γ imaging, we selected compound **56** as the most promising iodinated and fluorinated tracer for the validation of our concept. To determine [^{125}I]**56** concentrations in nontarget organs more precisely and evaluate the absorbed dose delivered to the tumor tissue during potential treatment with [^{131}I]**56**, the biodistribution of [^{125}I]**56** was studied in all major organs by whole-body autoradiography on animal slices (see Supporting Information for complete data). The highly specific radioactivity accumulation in melanoma tumor was illustrated by a very low concentration in muscle and a rapid release from nontarget tissues. In liver, lung, or kidney, radioactive uptake decreased after 3 h to become very low or even undetectable after 24 h (Figure 5 and Supporting Information). The considerable tumor-to-muscle ratios from 1 to 3 h pi (23 and 37 at 1 and 3 h, respectively) were favorable for PET imaging purposes. The pigmented structures of the eyes (i.e., uvea; see Supporting Information) gave a similar radioactivity uptake profile to tumor. These results were consistent with a melanin binding uptake mechanism previously described for **3**[23] or **1a**.[33] The radioactivity uptake of the throat area visualized by scintigraphic imaging (i.e., 1.5% of ID at 1 h to 0.1% of ID at 10 d, Figure 3) is likely due to a nonspecific concentration, mainly in salivary glands and to a lower extent in thyroid (see Table 2 in Supporting Information). Analyses on collected urine and feces showed an imbalance between urinary and fecal elimination routes, at 38.1% and 36.1%, respectively, for the 0−24 h period and 45.1% and 38.7%, respectively, for the 0−72 h period. The long-lasting tumor radioactivity uptake led to a calculated biological half-life of 38 h for [^{131}I]**56** and a tumor-absorbed dose of 1.12 Gy/MBq of [^{131}I]**56** injected. These values, while lower than those obtained with our reference compound for radionuclide therapy [^{131}I]**3** (49.3 h and 1.71 Gy/MBq injected), were in a range compatible with radionuclide therapy for melanoma.[34]

To validate our concept with the selected tracer **56**, we first performed a PET imaging assay after labeling of corresponding precursor with ^{18}F.

^{18}F Radiochemistry. Initially, we envisaged one-step preparation of [^{18}F]**44** (base form of dihydrochloride salt **56**) starting from precursor **73** (Scheme 6), but there are no reports of ^{18}F incorporation under classical radiofluorination conditions. Therefore, the preparation of [^{18}F]**44** was performed via a three-step two-pot radiosynthesis procedure that was automated with a Zymate laboratory automation system (Zymark Corp.) (Scheme 7), including: (i) ^{18}F incorporation on precursor **75** to give [^{18}F]**74**, (ii) acidic *N*-Boc deprotection of the amine function of [^{18}F]**74**, and (iii) acylation of the radioactive intermediate [^{18}F]**29** to afford [^{18}F]**44**.

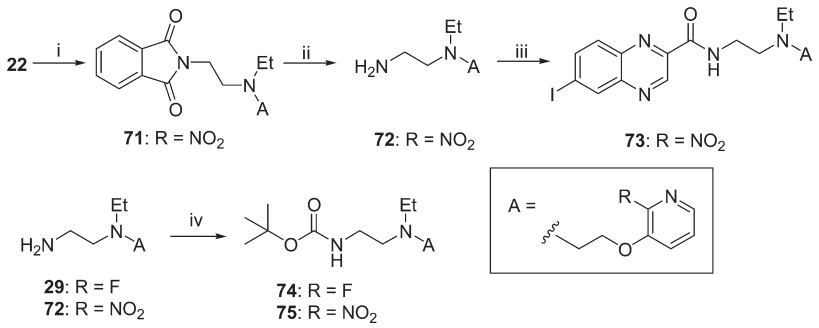
The first radiosynthetic step involved the introduction of ^{18}F via a nitro-for-fluorine heteroaromatic nucleophilic substitution on the pyridine moiety of precursor **75**. This substitution was performed in a mixture of acetonitrile and DMSO at high temperature (145 °C) for 8 min. Contrary to homoaromatic nucleophilic substitution, heteroaromatic nucleophilic substitution does not require an electron withdrawing group to activate the aromatic ring. Only an appropriate leaving group is required as represented by the nitro group on the pyridine moiety of precursor **75**.[26] ^{18}F incorporation affords compound [^{18}F]**74** in

Scheme 7. Radiosynthesis of [^{18}F]44[a]



[a] Reagents and conditions: (i) K[^{18}F]F-K_{222}-carbonate complex, MeCN, DMSO, 145 °C, 8 min; (ii) CH_2Cl_2/TFA (50/1, v/v), 65−75 °C, 4−6 min; (iii) 6-iodoquinoxaline-2-carbonyl chloride,[35] CH_2Cl_2, NEt_3, rt, 10 min.

Scheme 6. Syntheses of Precursors for the Preparation of [^{18}F]44[a]



[a] Reagents and conditions: (i) 2-nitro-3-hydroxypyridine, PPh_3, DIAD, THF, rt, 60 h; (ii) $NH_2NH_2.H_2O$, EtOH, reflux, 12 h; (iii) $AlMe_3$, CH_2Cl_2, 0 °C, 10 min and then ethyl 6-iodoquinoxaline-2-carboxamide,[22a] reflux, 12 h; (iv) di-*tert*-butyl dicarbonate, NEt_3, CH_2Cl_2, rt, 20 h for **74** or 18 h for **75**.

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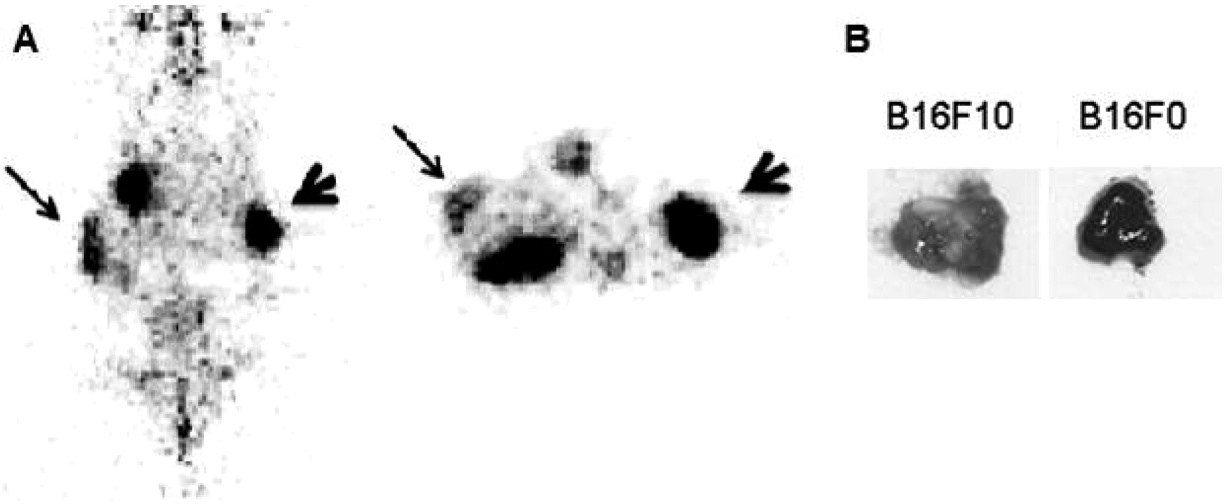
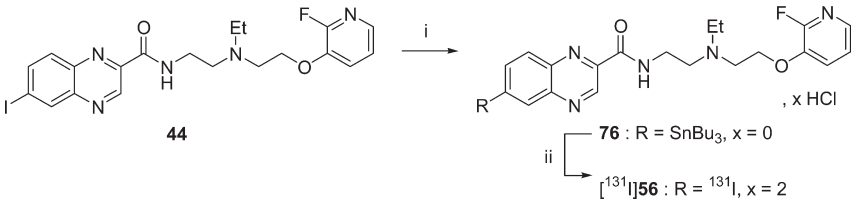


Figure 6. (A) In vivo PET imaging of compound [^{18}F]**44** in a B16F0 (arrowhead) and B16F10 (arrow) melanoma-bearing C57Bl6 mouse, 4 h after iv injection of a 7.4 MBq dose (acquisition time 15 min) illustrated by transaxial and coronal slices. (B) Illustration of the pigmentation difference between B16F0 and B16F10 tumor specimens obtained from mice subcutaneously grafted with these two cell lines.

Scheme 8. Preparation of Precursor 76 and Radiosynthesis of [^{131}I]56[a]



[a] Reagents and conditions: (i) Sn_2Bu_6, $Pd(PPh_3)_4$, toluene, reflux, 4 h; (ii) [^{131}I]NaI, CAT·H_2O, EtOH, citrate buffer (pH = 4), H_2O, rt, 30 min.

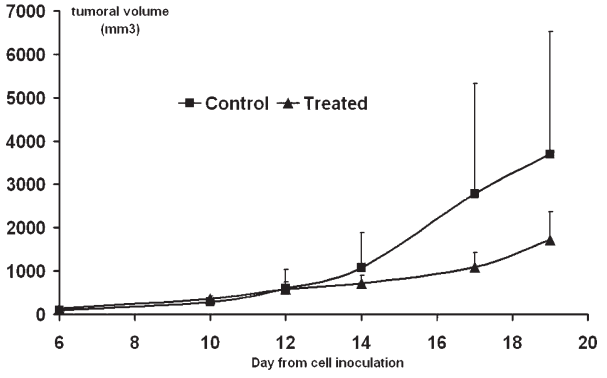


Figure 7. Effect of targeted radionuclide therapy on growth rate of B16F0 murine melanoma tumors in a representative experiment. C57Bl6 mice were inoculated with 3×10^5 melanoma B16F0 cells by dorsal subcutaneous injection at day 0 of the experiment. To monitor tumoral growth, tumor volume in mm^3 was evaluated twice a week. [^{131}I]**56** treatment was administered intravenously at days 6 and 10 ($2 \times$ 18.5 MBq). In comparison with the untreated group (■), [^{131}I]**56** treatment (▲) significantly slowed the B16F0 tumoral growth ($p <$ 0.001).

yields ranging between 20% and 40% based on starting [^{18}F]F$^-$ after Sep-Pak Plus C-18 prepurification (nonisolated decay-corrected yields). The second step corresponded to the quantitative deprotection of the *N*-Boc-protected amine function of [^{18}F]**74** in a CH_2Cl_2/TFA mixture at moderate temperature (65−75 °C) to afford the radioactive intermediate [^{18}F]**29**. The amine function of [^{18}F]**29** was finally acylated using 6-iodoquinoxaline-2-carbonyl chloride[35] in a mixture of CH_2Cl_2/NEt_3 at room temperature for 10 min. Compound [^{18}F]**44** was finally isolated by semipreparative reverse-phase HPLC (eluent H_2O/CH_3CN/TFA: 75/25/0.1 (v/v/v), see Experimental Section) and collected with a retention time of 19−20 min. Acylation yields of [^{18}F]**29** to provide [^{18}F]**44** ranged between 20% and 40% (nonisolated decay-corrected yields). Formulation of pure [^{18}F]**44** for iv injection was performed using a homemade Sep-Pak Plus C-18-based device.

Quality controls on [^{18}F]**44** were performed on an iv injection-ready aliquot of the final solution. The radiotracer preparation was a clear and colorless solution with a measured pH of between 5 and 7. As demonstrated by analytical HPLC analysis, the radiotracer was radiochemically pure (R_t: 2.03 min) and its chemical purity was found to be >95%.

Typically, [^{18}F]**44** can be produced in a 6% to 10% (nonisolated decay-corrected yields) with a specific radioactivity ranging between 0.25 and 0.34 TBq/μmol (calculated from three consecutive HPLC analyses) in 110−130 min.

Preliminary PET Imaging Experiment. Radiotracer [^{18}F]**44** was injected intravenously in C57Bl6 mice bearing two types of melanoma cell lines, identical in all respects including tumor growth rate except for the melanin concentration found in tumors obtained in mice subcutaneously grafted with the two cell lines: B16F0 shows a very high concentration of melanin and its appearance is intense black, while B16F10 has a lower melanin

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content and appears more brownish (Figure 6) Animals were imaged for up to 4 h on a microPET camera, as described in the Experimental Section procedure. Figure 6 features coronal and transaxial sections illustrating a representative image obtained at 4 h after injection. The melanocytic tumors are indicated by arrowheads for B16F0 and arrows for B16F10. The peak uptake in melanocytic tumors is reached around 2 h after injection. At this time, radioactivity uptake in tumor was 8.3 ± 1.7% of ID/cc and 2.1 ± 0.4% of ID/cc (n = 4) for B16F0 and B16F10, respectively. At 4 h, the values remain fairly similar, even though muscle uptake drops from 0.6 ± 0.2% of ID/cc at 2 h to 0.2 ± 0.1% of ID/cc at 4 h, providing a good contrast with surrounding tissues. The difference in terms of melanin content is reflected in the difference in uptake between the two tumors. In vitro assay to determine the tumor melanin content gave a B16F0/B16F10 ratio of around 5 (data not shown), and PET quantification of the difference in uptake gave a ratio of 4.0 ± 0.6 (n = 4). This assay confirmed the biodistribution and kinetics profile previously observed with [^{125}I]**56** (i.e., the dihydrochloride salt of **44**) and thus the utility of combining this tracer specificity with PET technology performances.

^{131}I Radiochemistry. The [^{131}I]-labeled compound **56** was prepared starting from the corresponding stannane precursor **76** (synthesized according to the procedure developed for compound **66**), following a classical electrophilic radioiodo-demetalation reaction, using no-carrier-added [^{131}I]NaI in acidic medium (citrate buffer) in the presence of chloramine-T monohydrate (Scheme 8). [^{131}I]**56** was obtained with a specific activity of 106.9 GBq/μmol and high radiochemical purity (98.5%, analytical-grade HPLC analyses).

Antitumoral Efficacy. To assess the therapeutic efficacy of [^{131}I]**56** in C57Bl6 mice bearing subcutaneous B16F0 melanoma cells, 2 × 18.5 MBq of [^{131}I]**56** were administered at days 6 and 10 in groups of 10 mice (note that in these conditions, [^{131}I]**56** concentration was much lower than the LD_{50}; data not shown). Figure 7 reports tumor growth expressed as tumor volume at each time point. B16F0 tumors in the untreated group (10 mice) showed exponential growth with a tumor doubling time of 2.8 ± 0.3 days, while [^{131}I]**56** inhibited the in vivo growth of B16F0 tumors (doubling time 3.8 ± 0.3 days). These results showed that [^{131}I]**56** treatment significantly slowed B16F0 tumor volume ($p < 0.001$).

CONCLUSION

In an effort to expand the arsenal of radiotracers with high specificity for melanoma lesions via a melanin-targeting strategy, we successfully synthesized 14 new iodinated and fluorinated analogues of our previously described lead compound **3**. All these tracers, containing a 2- or 6-fluoropyridine moiety incorporated in the *N*,*N*-diethylethylenediamine scaffold, were then labeled with ^{125}I to determine their in vivo biodistribution profiles by γ scintigraphic imaging. Even if most of these novel radioiodinated compounds showed significant tumor retention, data derived from this in vivo screening experiment revealed that derivative [^{125}I]**56** presented high, specific, and long-lasting tumoral radioactivity uptake combined with a rapid clearance from nontarget organs of [^{125}I]**56**, allowing well-defined imaging of melanoma tumor. These observations led to the selection of **56** as the most promising candidate for first validation of our concept of a melanoma-specific matched-pair radiotracer, offering both diagnostic (^{18}F) and therapeutic (^{131}I) potentialities. We managed to label this compound with ^{18}F, and very promising results in terms of radioactivity uptake, contrast, and specificity were obtained during preliminary melanoma PET imaging experiments in mice. Finally, selected tracer **56** was labeled with ^{131}I. Data collected during the therapy experiment suggested that [^{131}I]**56** administered after melanoma graft exerted a suppression of the in vivo growth of B16F0 tumors in mice. Therefore, we obtained a first validation of our concept. We consider that iodinated and fluorinated derivative **56** represents a new lead compound, worthy of further investigations toward the ultimate aim of identifying a clinical candidate usefully suitable for PET imaging (^{18}F) and targeted radionuclide therapy of melanoma (^{131}I).

EXPERIMENTAL SECTION

Materials for Chemical Syntheses. All reagents and solvents were obtained from the following commercial suppliers: Aldrich, Acros Organics, or Carlo Erba. Column chromatography was performed with Merck neutral aluminum oxide 90 standardized (63−200 μm) or silica gel A normal phase (35−70 μm). Thin layer chromatography (TLC) was performed on Merck neutral aluminum oxide 60F_{254} plates or Merck silica gel 60F_{254} plates. The plates were visualized with UV light (254 nm) and/or by development with iodine, nynhydrin, or potassium permanganate. Melting points were determined on an electrothermal IA9300 (capillary) or a Reichert−Jung−Koffler apparatus and were not corrected. NMR spectra (400 or 200 MHz) for ^{1}H or (100 or 50 MHz) for ^{13}C were recorded on a Bruker Avance 400 or Bruker Avance 200 instruments using $CDCl_3$ or DMSO-d_6 as solvent. ^{19}F NMR spectra (470 MHz) were recorded on a Bruker DRX 500 with tetrafluorotoluene (−63 ppm) as internal reference. Infrared spectra were recorded in KBr pellets or in CCl_4 on a FTIR Nicolet Impact 410 or an FT Vector 22 instrument (ν expressed in cm^{-1}). Mass spectra (MS) were obtained in electron impact mode on 5989A instruments (Agilent Technologies). Electrospray ionization mass spectra (ESI-MS) were obtained on a TSQ 7000 ThermoQuest Finnigam (Les Ulis, France). The samples were analyzed in CH_3OH/H_2O (1/1, v/v, containing 1% HCOOH) or CH_3CN/H_2O (1/1, v/v, containing 1% HCOOH) in positive mode or in CH_3OH/H_2O (1/1, v/v, containing 1% NH_4OH) in negative mode, at a final concentration of 8−12 pmol/μL. Each ESI-MS spectrum was recorded by averaging of 10 spectra. Microanalyses were performed by Analytical Laboratory of the CNRS (Vernaison, France) for the elements mentioned and were within 0.4% of the theoretical values unless indicated. All air-sensitive reactions were run under argon atmosphere. All solvents were dried using common techniques.[36]

Materials for Radiolabeling with ^{125}I and ^{131}I. [^{125}I]NaI (3.7 GBq/mL, 643.8 MBq/mg) and [^{131}I]NaI (66.2 GBq/mL, 712.8 GBq/mg) were purchased from PerkinElmer Life and Analytical Sciences (331 Treble Cove Road, Billerica, MA 01862, USA) as a no-carrier-added solution in reductant free 1.0 × 10^{-5} M aqueous sodium hydroxide solution (pH 8−11) for [^{125}I]NaI and as a no-carrier-added solution in reductant free 0.1 M aqueous sodium hydroxide solution (pH 12−14) for [^{131}I]NaI. Extrelut and citrate buffer solution (pH = 4) were purchased from Merck (Darmstadt, Germany). The radio TLC strips (Merck neutral aluminum oxide 60F_{254} plates) were developed with CH_2Cl_2/EtOH (97/3, v/v) and measured on an AMBIS 400 (Scanalytics, CSPI, San Diego, CA, USA). Analytical HPLC measurements were performed on a system consisting of a HP1100 (Hewlett-Packard, Les Ulis, France) and a Flow One A_{500} Radiomatic detector (Packard, Canberra, Australia). The separation was carried out on a C-18 column (Purospher RP_{18} e, 150−4.6, 5 μm) using the following conditions: gradient time = 10 min, flow rate = 0.5 mL/min, H_2O/MeOH/(50:50 → 0:100) (NH_4OH 0.2%), λ = 254 nm. HPLC purification was performed on a system including a Shimadzu LC 6A

pump, SLC 6B controller, a CR5A integrator, a SPD 6AV UV detector and a flow-trough γ Raytest Steffi detector. The separation was carried out on a C-18 column (ZORBAX 80 Å, 4.6 mm × 150 mm) using the following conditions: gradient time = 20 min, flow rate =1 mL/min, H_2O/MeOH (50:50 → 0:100) (NH_4OH 0.2%), λ = 254 nm. All radiolabeled compounds were compared by TLC or analytical HPLC to the authentic nonradioactive material and to be free of significant chemical and radiochemical impurities.

Materials for Radiolabeling with ^{18}F. No-carrier-added [^{18}F]F^- was produced via the [^{18}O(p, n)^{18}F] nuclear reaction by irradiation of a 2 mL [^{18}O]water target (>97%-enriched, Rotem CortecNet, Paris, France) on an IBA Cyclone-18/9 cyclotron (18 MeV proton beam) (IBA, Louvain-la-Neuve, Belgium), and the aqueous radioactive solution was then transferred to the appropriate hot cell. *Target hardware*: commercial, 2 mL, two-port, stainless steel target holder equipped with a domed-end niobium cylinder insert. *Target to hot cell liquid-transfer system*: 60 m PTFE line (0.8 mm internal diameter; 1/16 in. external diameter), 2.0 bar helium drive pressure, transfer time 3−6 min. Typical production of [^{18}F]fluoride at the end of bombardment for a 20 μA, 30 min (10 μA.h) irradiation: 27.7−29.6 GBq (750−800 mCi). ^{18}F-labeled compounds were HPLC purified using the following equipment and conditions. System: a Waters 600 pump and a Waters 600 controller, a Shimadzu SPD10-AVP UV-multiwavelength detector and a miniature ionization chamber probe. Column: semipreparative Symmetry C-18, Waters (300 mm × 7.8 mm); porosity 7 μm; eluent H_2O/CH_3CN/TFA 75/25/0.1 (v/v/v); flow rate 5−7 mL/min; temperature room temperature; absorbance detection at λ = 254 nm. Chemical and radiochemical purities of all HPLC-purified and formulated compounds for in vivo imaging were determined by analytical HPLC analyses using the following equipment and conditions. System: a Waters Alliance 2690 (or a Waters binary HPLC pump 1525) equipped with a UV spectrophotometer (photodiode array detector, Waters 996) and a Berthold LB509 radioactivity detector. Column: analytical Symmetry-M C-18, Waters (50 mm × 4.6 mm); porosity 5.0 μm. Conditions: isocratic elution with solvent A/solvent B: 55/45 (v/v) [solvent A, H_2O containing Low-UV PIC B7 reagent (20 mL for 1000 mL); solvent B, H_2O/CH_3CN: 30:70 (v/v) containing Low-UV PIC B7 reagent (20 mL for 1000 mL)]; flow rate 2.0 mL/min; temperature rt; absorbance detection at λ = 254 nm.

Materials for Pharmacological Experiments. Male C57Bl6 mice were obtained from Charles River, (l'Arbresle, France) and the B16F0 or syngenic melanoma from ATCC (no. CRL-6322). Mice were maintained at 21 °C with a 12 h/12 h light/dark cycle. They were fed with a breeding diet (diet A04 from Safe, Villemoisson, France) and received water ad libitum. Scintigraphic imaging of radioiodinated compounds in mice was performed using a gamma camera dedicated for small animal imaging (γIMAGER, Biospace Mesures, Paris, France). This camera consists of a R 3292 Hamamatsu position-sensitive photomultiplier having a continuous 4 mm thick ×120 mm diameter CsI(Na) crystal leading to a 10 cm field of view. For [^{125}I] imaging, the camera was equipped with parallel-hole collimator 1.8/0.2/20 (hole diameter/septum thickness/height in mm). All the acquisitions were performed with a 15% window centered on the 35 keV peak of ^{125}I. For whole-body autoradiography study, the radioactivity contained in the animal slices was analyzed using an AMBIS 4000 detector (Scanalytics, CSPI, San Diego, CA, USA), which is a computer-controlled multiwire proportional counter previously described and validated for the evaluation of iodinated agents in mice.[22a]

Models and Protocols for in Vivo Experiments. Animals were handled and cared in accordance with the guidelines for the Care and Use of Laboratory Animals (National Research Council, 1996) and European Directive 86/609/EEC. The animal model used was 6−8 week old C57Bl6 male mice bearing B16F0 murine melanoma. Stock B16F0 melanoma cell cultures were maintained as monolayers in Dulbecco's Modified Eagle's Medium (DMEM)/Glutamax (Invitrogen, Cergy Pontoise, France) supplemented with 10% fetal calf serum (Sigma, Saint Quentin Fallavier, France), 1% vitamins (Invitrogen, Cergy-Pontoise, France), 1 mM sodium pyruvate (Invitrogen), 1% nonessential amino acids (Invitrogen), and 4 μg/mL of gentamycin base (Invitrogen) and passaged by trypsination. The cells were grown at 37 °C in a humidified incubator containing 5% CO_2. Early passages were frozen and stored in liquid nitrogen. For transplantation, cells in exponential growth phase were trypsinized, washed with phosphate buffer saline (PBS), and resuspended in PBS. C57Bl6 mice anesthetized by isoflurane inhalation were inoculated with 3 × 10^5 melanoma B16F0 cells in 0.1 mL by subcutaneous injection on the left flank. Ten days later, the tumors became palpable with a percentage of tumor take of 98−100%.

*2-Fluoroisonicotinic Acid (**5**).* This compound was synthesized according to the procedure developed by Ashimori et al. for preparation of the 2-fluoronicotinic acid.[27] Briefly, to a stirred solution of 2-fluoro-4-picoline[27] (**4**) (16.0 g, 0.14 mol) in water (500 mL) was added, at room temperature, potassium permanganate (53.0 g, 0.34 mol). The mixture was refluxed for 1 h. After cooling to room temperature, potassium permanganate (25.0 g, 0.16 mol) was added to the mixture, which was then refluxed for 12 h. The black precipitate formed was removed by filtration, washed with hot water (100 mL), and the filtrate volume was reduced to 150 mL under vacuum. A concentrated hydrochloric acid solution was added dropwise at 0 °C (pH = 1). After 3 h at 0 °C, the white precipitate was filtered and dried to give acid **5** (7.96 g, 56.4 mmol) as a white solid. Yield 43%; mp 197−199 °C (lit.[37] 195−197 °C). IR (KBr) ν 1211, 1292, 1394, 1475, 1723, 2300−3100 cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.53 (m, 1H), 7.76 (ddd, 1H, $^5J_{H-F}$ = 1.9 Hz, J = 1.3, 5.1 Hz), 8.43 (d, 1H, J = 5.1 Hz), 13.98 (brs, 1H).

*Ethyl 2-Fluoroisonicotinate (**6**).* To a stirred solution of 2-fluoroisonicotinic acid (**5**) (1.00 g, 7.09 mmol) in anhydrous dichloromethane (30 mL) was added at 0 °C, under argon, triethylamine (991 μL, 7.09 mmol). After 5 min, ethyl chloroformate (681 μL, 7.09 mmol) was added and the mixture was stirred at 0 °C for 5 min before addition of *N*, *N*-dimethyl-4-aminopyridine (DMAP, 86 mg, 0.71 mmol). The mixture was stirred at 0 °C for 4 h. After return back to room temperature, the solvent was evaporated under vacuum and the residue was chromatographed (Al_2O_3, CH_2Cl_2) to give ester **6**[38] (0.98 g, 5.79 mmol) as a yellow liquid. Yield 82%; R_f (Al_2O_3, CH_2Cl_2) 0.90. IR (CCl_4) ν 1096, 1210, 1299, 1409, 1572, 1735 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.1 Hz), 4.40 (q, 2H, J = 7.1 Hz), 7.47 (ddd, 1H, $^3J_{H-F}$ = 2.4 Hz, J = 0.8, 1.3 Hz), 7.72 (ddd, 1H, $^5J_{H-F}$ = 1.8 Hz, J = 1.3, 5.1 Hz), 8.33 (td, 1H, $^4J_{H-F}$ = 0.8 Hz, J = 0.8, 5.1 Hz).

*2-Fluoro-4-hydroxymethylpyridine (**7**).* To a stirred solution of 1.0 M lithium aluminum hydride in anhydrous tetrahydrofuran (7.00 mL, 7.00 mmol) was added at −50 °C under argon, a solution of ester **6** (1.19 g, 7.00 mmol) in anhydrous tetrahydrofuran (10 mL). After 15 min, water (7 mL), a 3.0 N aqueous sodium hydroxide solution (7 mL) and water (7 mL) were added successively to the mixture until no more gas evolution was observed. After return back to room temperature, a brine solution (10 mL) was added. The mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed (Al_2O_3, CH_2Cl_2) to give alcohol 7 (0.45 g, 3.50 mmol) as a beige solid. Yield 51%; R_f (Al_2O_3, CH_2Cl_2) 0.32; mp 61−63 °C (lit.[28] 59−60 °C). IR (KBr) ν 1074, 1273, 1420, 1618, 3100−3400 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.81 (brs, 1H), 4.75 (s, 2H), 6.96 (s, 1H), 7.14 (m, 1H), 8.07 (d, 1H, J = 5.1 Hz).

*2-(2-Fluoropyridin-4-yl)methyl Methylsulfonate (**8**).* The title compound was obtained according to the procedure developed by Pesti et al.[28] Briefly, to a solution of alcohol 7 (0.26 g, 2.01 mmol) in anhydrous ethyl acetate (12 mL) were added successively at 0 °C under argon, triethylamine (409 μL, 2.00 mmol), and dropwise methanesulfonyl chloride (196 μL, 2.00 mmol). The mixture was stirred at 0 °C for 15 min before addition of water (10 mL). After return back to room temperature, the mixture was decanted and the organic layer was washed

with water (15 mL), dried on magnesium sulfate, filtered, and evaporated under vacuum. The oily residue was suspended in heptane (5 mL) and left 1 h at 0 °C. The precipitate formed was filtered and dried to give mesylate **8** (0.40 g, 1.95 mmol) as a beige solid. Yield 98%; mp 55−57 °C (lit.[28] 58−59 °C). IR (KBr) ν 1179, 1358, 1414, 1621 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 3.09 (s, 3H), 5.26 (s, 2H), 6.97 (m, 1H), 7.19 (m, 1H), 8.26 (d, 1H, J = 5.2 Hz).

*2-Fluoro-4-(2-hydroxyethyl)pyridine (**9**).* To a solution of anhydrous diisopropylamine (18.5 mL, 0.13 mol) in anhydrous tetrahydrofuran (160 mL) was added dropwise at −80 °C, under argon, a 1.3 M *n*-butyllithium solution in hexane (100 mL, 0.13 mol). The mixture was stirred at −80 °C for 1 h before addition dropwise of a solution of 2-fluoro-4-picoline[27] (**4**) (9.00 g, 6.30 mmol) in anhydrous tetrahydrofuran (60 mL). The mixture was stirred at −80 °C for 1 h once again before addition dropwise of a *p*-formaldehyde (16.6 g, 0.55 mol) suspension in anhydrous tetrahydrofuran (100 mL). The mixture was stirred at −80 °C for 1 h and then at room temperature for 12 h. A saturated aqueous ammonium chloride solution (450 mL) was added to the mixture. The solution was decanted, and the aqueous layer was extracted with dichloromethane (4 × 200 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue obtained was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give alcohol **9** (7.25 g, 51.4 mmol) as an orange-colored oil. Yield 57%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.32. IR (CCl_4) ν 1046, 1149, 1278, 1413, 1613, 2800−3000, 3100−3600, 3634 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 2.87 (t, 2H, J = 6.3 Hz), 3.87 (t, 2H, J = 6.3 Hz), 6.82 (s, 1H), 7.07 (d, 1H, J = 5.2 Hz), 8.01 (d, 1H, J = 5.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 37.7 (d, $^4J_{C-F}$ = 3 Hz), 60.9, 109.5 (d, $^2J_{C-F}$ = 36 Hz), 122.0 (d, $^4J_{C-F}$ = 4 Hz), 146.2 (d, $^3J_{C-F}$ = 14 Hz), 155.0 (d, $^3J_{C-F}$ = 8 Hz), 163.2 (d, $^1J_{C-F}$ = 239 Hz). ^{19}F NMR ($CDCl_3$) δ −70.49. MS m/z 141 (M^+, 23), 111 (100), 91 (34), 83 (14), 64 (10), 57 (12).

*2-(2-Fluoropyridin-4-yl)ethyl Methylsulfonate (**10**).* The title compound was synthesized according to the procedure described for compound **8**, starting from alcohol **9** (6.50 g, 46.1 mmol). Reaction time at 0 °C: 10 min to give mesylate **10** (7.56 g, 34.5 mmol) as a yellow oil. Yield 75%; R_f (Al_2O_3, CH_2Cl_2) 0.52. IR (CCl_4) ν 1179, 1352, 1414, 1613 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 2.95 (s, 3H), 3.09 (t, 2H, J = 6.4 Hz), 4.45 (t, 2H, J = 6.4 Hz), 6.82 (s, 1H), 7.07 (d, 1H, J = 5.1 Hz), 8.15 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.8, 37.7, 68.0, 110.0 (d, $^2J_{C-F}$ = 37 Hz), 122.0 (d, $^4J_{C-F}$ = 4 Hz), 147.9 (d, $^3J_{C-F}$ = 15 Hz), 151.6 (d, $^3J_{C-F}$ = 8 Hz), 164.1 (d, $^1J_{C-F}$ = 239 Hz). ^{19}F NMR ($CDCl_3$) δ −68.10. MS m/z 219 (M^+, 3), 123 (100), 111 (30), 83 (14), 79 (53), 57 (11).

*2-Fluoro-4-(3-hydroxypropyl)pyridine (**11**).* The title compound was synthesized according to the procedure developed for 2-fluoro-4-(2-hydroxyethyl)pyridine (**9**), starting from picoline[27] **4** (7.00 g, 6.30 mmol) and ethylene oxide (4.20 mL, 84.1 mmol) in place of *p*-formaldehyde. The purification was performed using column chromatography (SiO_2, EtOAc/pentane, 6/4, v/v) to give alcohol **11** (5.16 g, 33.3 mmol) as a yellow oil. Yield 53%; R_f (SiO_2, EtOAc/pentane 6/4, v/v) 0.52. IR (CCl_4) ν 1412, 1613, 2939, 3639 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.84 (m, 2H), 2.72 (t, 2H, J = 7.4 Hz), 3.14 (brs, 1H), 3.62 (t, 2H, J = 6.2 Hz), 6.72 (s, 1H), 6.98 (m, 1H), 8.01 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 31.3 (d, $^4J_{C-F}$ = 2 Hz), 32.8, 61.1, 109.2 (d, $^2J_{C-F}$ = 36 Hz), 121.8 (d, $^4J_{C-F}$ = 3 Hz), 147.0 (d, $^3J_{C-F}$ = 15 Hz), 157.5 (d, $^3J_{C-F}$ = 8 Hz), 164.0 (d, $^1J_{C-F}$ = 237 Hz). ^{19}F NMR ($CDCl_3$) δ −69.61. MS m/z 155 (M^+, 6), 137 (25), 124 (12), 111 (100), 91 (21), 77 (14), 57 (14), 51 (16).

*3-(2-Fluoropyridin-4-yl)propyl Methylsulfonate (**12**).* The title compound was synthesized according to the procedure developed for compound **8**, starting from alcohol **11** (5.00 g, 32.2 mmol). Reaction time at 0 °C, 30 min; the purification was performed using column chromatography (SiO_2, EtOAc/pentane, 8/2, v/v) to give compound **12** (4.82 g, 20.7 mmol) as a yellow oil. Yield 69%; R_f (SiO_2, EtOAc/pentane, 8/2, v/v) 0.58. IR (CCl_4) ν 1179, 1351, 1371, 1413, 1613 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 2.12 (m, 2H), 2.83 (t, 2H, J = 7.3 Hz), 3.05 (s, 3H), 4.28 (t, 2H, J = 6.1 Hz), 6.80 (s, 1H), 7.07 (m, 1H), 8.15 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.5, 30.8 (d, $^4J_{C-F}$ = 3 Hz), 37.4, 68.4, 109.2 (d, $^2J_{C-F}$ = 37 Hz), 121.6 (d, $^4J_{C-F}$ = 4 Hz), 147.7 (d, $^3J_{C-F}$ = 15 Hz), 155.5 (d, $^3J_{C-F}$ = 8 Hz), 164.1 (d, $^1J_{C-F}$ = 238 Hz). ^{19}F NMR ($CDCl_3$) δ −68.70. ESI-MS m/z $[M + H]^+_{theoretical}$ = 234.06; $[M + H]^+_{experimental}$ = 231.96.

*N-[2-(Ethylamino)ethyl]phthalimide (**14**).* A suspension of *N*-[2-(ethylamino)ethyl]phthalimide hydrochloride salt (**13**)[29] (2.00 g, 7.86 mmol) in dichloromethane (100 mL) was washed with a 5% aqueous sodium carbonate solution (5 mL). The mixture was extracted with dichloromethane (40 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum to give compound **14** (1.41 g, 6.47 mmol) as a yellow solid. Yield 82%; mp 111−113 °C (dec). IR (KBr) ν 1292, 1395, 1430, 1541, 1637, 1712, 2929, 3200−3600 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, 3H, J = 7.1 Hz), 2.68 (q, 2H, J = 7.1 Hz), 2.92 (t, 2H, J = 6.5 Hz), 3.81 (t, 2H, J = 6.5 Hz), 7.68 (m, 2H), 7.82 (m, 2H).

*N-[2-[[N-Ethyl-N-(2-fluoropyridin-4-yl)methyl]amino]ethyl]phthalimide (**15**).* To a stirred solution of phthalimide **14** (320 mg, 1.46 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) were added successively, under argon, triethylamine (208 μL, 1.50 mmol) and mesylate **8** (300 mg, 1.46 mmol). The mixture was heated at 90 °C for 3 h. After cooling to room temperature, the solvent was evaporated under vacuum and the residue was chromatographed (SiO_2, EtOAc/cyclohexane, 6/4, v/v) to give compound **15** (309 mg, 0.94 mmol) as a beige solid. Yield 64%; R_f (SiO_2, EtOAc/cyclohexane, 6/4, v/v) 0.78; mp 68−70 °C. IR (KBr) ν 1413, 1611, 1708 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.94 (t, 3H, J = 7.1 Hz), 2.51 (q, 2H, J = 7.1 Hz), 2.68 (t, 2H, J = 6.2 Hz), 3.57 (s, 2H), 3.72 (t, 2H, J = 6.2 Hz), 6.70 (brs, 1H), 6.95 (m, 1H), 7.68 (m, 2H), 7.76 (m, 2H), 7.85 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.5, 35.4, 47.3, 50.7, 56.2 (d, $^4J_{C-F}$ = 3 Hz), 108.5 (d, $^2J_{C-F}$ = 37 Hz), 120.9 (d, $^4J_{C-F}$ = 4 Hz), 122.7 (2C), 131.6 (2C), 133.6 (2C), 146.7 (d, $^3J_{C-F}$ = 15 Hz), 155.2 (d, $^3J_{C-F}$ = 8 Hz), 163.6 (d, $^1J_{C-F}$ = 238 Hz), 167.7 (2C). ^{19}F NMR (376 MHz, $CDCl_3$) δ −67.06. MS m/z 327 (M^+, 2), 167 (100), 110 (37), 83 (12), 77 (10), 56 (13).

*N-[2-[[N-Ethyl-N-2-(2-fluoropyridin-4-yl)ethyl]amino]ethyl]phthalimide (**16**).* To a stirred solution of sulfonate **10** (4.00 g, 18.2 mmol) in anhydrous ethanol (105 mL) were added, under argon, phthalimide **14** (8.00 g, 36.7 mmol) and triethylamine (5.10 mL, 36.5 mmol). The mixture was heated at 70 °C for 24 h. After cooling to room temperature, water (90 mL), a 1.0 N aqueous sodium hydroxide solution (10 mL), and a brine solution (20 mL) were added successively. The mixture was decanted, and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (SiO_2, EtOAc/cyclohexane, 7/3, v/v) to give in order of elution: 2-fluoro-4-vinylpyridine[39] (0.79 g, 6.42 mmol) as an orange-colored oil. Yield 35%; R_f (SiO_2, EtOAc/cyclohexane, 7/3, v/v) 0.80. IR (CCl_4) ν 1152, 1290, 1392, 1414, 1551, 1608 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 5.52 (d, 1H, J = 10.8 Hz), 5.95 (d, 1H, J = 17.6 Hz), 6.64 (dd, 1H, J = 10.8, 17.6 Hz), 6.85 (s, 1H), 7.14 (dt, 1H, $^5J_{H-F}$ = 1.5 Hz, J = 1.5, 5.2 Hz), 8.12 (d, 1H, J = 5.2 Hz). Compound **16** (0.98 g, 2.87 mmol) as a yellow oil. Yield 16%; R_f (SiO_2, EtOAc/cyclohexane, 7/3, v/v) 0.54. IR (CCl_4) ν 1397, 1613, 1715 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.95 (t, 3H, J = 7.1 Hz), 2.59 (q, 2H, J = 7.1 Hz), 2.72 (m, 6H), 3.71 (t, 2H, J = 6.6 Hz), 6.68 (s, 1H), 6.93 (dt, 1H, $^5J_{H-F}$ = 1.5 Hz, J = 1.5, 5.2 Hz), 7.69 (m, 2H), 7.77 (m, 2H), 7.91 (d, 1H, J = 5.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.8, 33.1 (d, $^4J_{C-F}$ = 3 Hz), 36.0, 47.5, 50.6, 53.5, 109.4 (d, $^2J_{C-F}$ = 37 Hz), 121.9 (d, $^4J_{C-F}$ = 4 Hz), 123.2 (2C), 132.1 (2C), 134.0 (2C), 147.1 (d, $^3J_{C-F}$ = 15 Hz), 155.7 (d, $^3J_{C-F}$ = 8 Hz), 163.9 (d, $^1J_{C-F}$ = 236 Hz), 168.4 (2C). ^{19}F NMR ($CDCl_3$) δ −69.47. ESI-MS m/z 341.9 $[M + H]^+$.

*N-[2-[[N-Ethyl-N-3-(2-fluoropyridin-4-yl)propyl]amino]ethyl]phthalimide (**17**).* The title compound was synthesized according to the procedure developed for compound **16**, starting from sulfonate **12** (7.00 g, 30.0 mmol), phthalimide **14** (19.65 g, 90.1 mmol), and triethylamine (12.5 mL, 89.9 mmol), compound **17** (3.70 g, 10.4 mmol) was isolated as a yellow oil. Yield 35%; R_f (SiO_2, EtOAc/cyclohexane, 7/3, v/v) 0.38. IR (CCl_4) ν 1396, 1411, 1613, 1716, 1774, 2809, 2948, 2970 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.97 (t, 3H, J = 7.1 Hz), 1.69 (m, 2H), 2.54 (m, 6H), 2.72 (t, 2H, J = 6.6 Hz), 3.77 (t, 2H, J = 6.6 Hz), 6.61 (m, 1H), 6.90 (m, 1H), 7.71 (m, 2H), 7.81 (m, 2H), 8.04 (d, 1H, J = 5.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.7, 27.7, 32.4 (d, $^4J_{C-F}$ = 3 Hz), 36.0, 47.4, 50.7, 52.5, 108.7 (d, $^2J_{C-F}$ = 37 Hz), 121.3 (d, $^4J_{C-F}$ = 4 Hz), 122.9 (2C), 131.9 (2C), 133.7 (2C), 146.9 (d, $^3J_{C-F}$ = 15 Hz), 157.4 (d, $^3J_{C-F}$ = 8 Hz), 163.8 (d, $^1J_{C-F}$ = 236 Hz), 168.1 (2C). ^{19}F NMR ($CDCl_3$) δ −69.62. ESI-MS m/z 356.0 $[M + H]^+$.

*N-[2-[N-Ethyl-N-(6-fluoropyridin-2-yl)]amino)ethyl]phthalimide (**18**).* To a solution of phthalimide **14** (5.01 g, 23.0 mmol) in anhydrous dimethylformamide (70 mL) were added, under argon, triethylamine (6.45 mL, 46.0 mmol) and commercial 2,6-difluoropyridine (4.20 mL, 46.0 mmol). The mixture was stirred at 130 °C for 16 h. After cooling to room temperature, water (700 mL) was added and the mixture was extracted with dichloromethane (3 × 100 mL). The organic layers were combined, washed with a brine solution (2 × 100 mL), dried on magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed (Al_2O_3, CH_2Cl_2), and the product obtained was washed with cyclohexane (5 mL) to give compound **18** (2.87 g, 9.20 mmol) as a yellow solid. Yield 40%; R_f (Al_2O_3, CH_2Cl_2) 0.86; mp 115−117 °C. IR (KBr) ν 1399, 1438, 1498, 1612, 1706 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.17 (t, 3H, J = 7.1 Hz), 3.49 (q, 2H, J = 7.1 Hz), 3.74 (t, 2H, J = 6.2 Hz), 3.93 (t, 2H, J = 6.2 Hz), 5.97 (dd, 1H, J = 2.7, 8.0 Hz), 6.36 (dd, 1H, J = 2.7, 8.0 Hz), 7.44 (q, 1H, $^4J_{H-F}$ = 8.0 Hz, J = 8.0 Hz), 7.70 (m, 2H), 7.81 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 35.7, 43.5, 45.9, 94.5 (d, $^2J_{C-F}$ = 37 Hz), 101.7 (d, $^4J_{C-F}$ = 4 Hz), 123.3 (2C), 132.1 (2C), 133.9 (2C), 141.7 (d, $^3J_{C-F}$ = 8 Hz), 156.9 (d, $^3J_{C-F}$ = 16 Hz), 162.9 (d, $^1J_{C-F}$ = 234 Hz), 168.3 (2C). ^{19}F NMR ($CDCl_3$) δ −68.97 (d, $^4J_{H-F}$ = 8.2 Hz). MS m/z 313 (M^+, 8), 153 (100), 125 (50), 96 (22), 76 (15).

*p-Nitrophenyl 2-fluoroisonicotinate (**19**).* To a stirred suspension of acid **5** (1.00 g, 7.09 mmol) in anhydrous dichloromethane (80 mL) were added, under argon, anhydrous dimethylformamide (4 drops) and thionyl chloride (2.07 mL, 28.5 mmol). The mixture was refluxed for 3.5 h. After cooling to room temperature, the solvent was evaporated under vacuum. The residue obtained was suspended in anhydrous toluene (50 mL), and the solvent was evaporated under reduced pressure. The resulting precipitate was suspended in anhydrous tetrahydrofuran (50 mL) before addition, under argon, of a solution of *p*-nitrophenol (0.99 g, 7.09 mmol) and triethylamine (988 μL, 7.09 mmol) in anhydrous tetrahydrofuran (50 mL). The mixture was stirred at 50 °C for 12 h. After cooling to room temperature, dichloromethane (100 mL) and a 5% aqueous sodium carbonate solution (80 mL) were added successively. The mixture was decanted, and the aqueous layer was extracted with dichloromethane (3 × 60 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum. The precipitate obtained was washed with ether (5 mL) to give ester **19** (1.14 g, 4.35 mmol) as a white solid. Yield 61%; mp 148−150 °C. IR (KBr) ν 1220, 1283, 1406, 1525, 1742 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 7.44 (d, 2H, J = 9.3 Hz), 7.66 (m, 1H), 7.91 (td, 1H, $^5J_{H-F}$ = 1.5 Hz, J = 1.5, 5.1 Hz), 8.36 (d, 2H, J = 9.3 Hz), 8.50 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 110.5 (d, $^2J_{C-F}$ = 40 Hz), 121.2 (d, $^4J_{C-F}$ = 5 Hz), 122.5 (2C), 125.6 (2C), 141.3 (d, $^3J_{C-F}$ = 9 Hz), 146.0, 149.4 (d, $^3J_{C-F}$ = 14 Hz), 154.9, 161.7, 164.4 (d, $^1J_{C-F}$ = 241 Hz). ^{19}F NMR ($CDCl_3$) δ −65.31. MS m/z 262 (M^+, 4), 124 (100), 96 (59), 76 (18), 69 (16), 63 (12), 51 (10).

*N-[2-(N-Ethylamino)ethyl]-2-fluoroisonicotinamide (**20**).* To a solution of ester **19** (4.60 g, 17.5 mmol) in anhydrous tetrahydrofuran (185 mL) was added, under argon, *N*-ethylethylenediamine (1.80 mL, 17.5 mmol). The mixture was stirred at room temperature for 24 h. The solvent was evaporated under vacuum, and the residue was suspended in dichloromethane (95 mL). A 1.0 N aqueous sodium hydroxide solution (140 mL) was added to the mixture. The solution was decanted, and the aqueous layer was extracted with dichloromethane (6 × 120 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 98/2, v/v) to give compound **20** (3.37 g, 15.9 mmol) as a yellow solid. Yield 91%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 98/2, v/v) 0.16; mp 51−52 °C. IR (KBr) ν 1300, 1416, 1554, 1675, 2700−3050, 3100−3600 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, 3H, J = 7.1 Hz), 2.63 (q, 2H, J = 7.1 Hz), 2.76 (brs, 1H), 2.83 (t, 2H, J = 5.8 Hz), 3.50 (m, 2H), 7.33 (s, 1H), 7.54 (m, 1H), 7.74 (brs, 1H), 8.24 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.0, 39.7, 43.7, 48.1, 107.9 (d, $^2J_{C-F}$ = 38 Hz), 119.0 (d, $^4J_{C-F}$ = 4 Hz), 147.3 (d, $^3J_{C-F}$ = 8 Hz), 148.3 (d, $^3J_{C-F}$ = 15 Hz), 164.1 (d, $^1J_{C-F}$ = 240 Hz), 164.3 (d, $^4J_{C-F}$ = 15 Hz). ^{19}F NMR ($CDCl_3$) δ −66.56. MS m/z 212 ($M + H^+$, 1), 124 (8), 96 (15), 71 (19), 58 (100).

*N-[2-[N-Ethyl-N-[2-(1,3-dioxo-1,3-dihydroindol-2-yl)ethyl]]amino]-ethyl]-2-fluoroisonicotinamide (**21**).* To a stirred solution of compound **20** (100 mg, 0.47 mmol) in anhydrous acetonitrile (7 mL) were added successively, under argon, triethylamine (197 μL, 1.42 mmol) and *N*-(2-bromoethyl)phthalimide (361 mg, 1.42 mmol). The mixture was refluxed for 63 h. After cooling to room temperature, the solvent was evaporated under vacuum. The brown oily residue was chromatographed (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) to give compound **21** (99 mg, 0.26 mmol) as a yellow solid. Yield 55%; R_f (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) 0.14; mp 74−76 °C. IR (KBr) ν 1400, 1526, 1654, 1703, 3321 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7.1 Hz), 2.55 (q, 2H, J = 7.1 Hz), 2.79 (m, 4H), 3.52 (q, 2H, J = 4.8 Hz), 3.78 (m, 2H), 7.41 (m, 1H), 7.48 (brs, 1H), 7.60 (td, 1H, $^5J_{H-F}$ = 1.4 Hz, J = 1.4, 5.2 Hz), 7.66 (m, 2H), 7.72 (m, 2H), 8.29 (d, 1H, J = 5.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.5, 36.7, 37.7, 47.4, 51.6, 52.4, 107.9 (d, $^2J_{C-F}$ = 39 Hz), 119.3 (d, $^4J_{C-F}$ = 4 Hz), 123.1 (2C), 131.7 (2C), 134.1 (2C), 147.3 (d, $^3J_{C-F}$ = 7 Hz), 148.1 (d, $^3J_{C-F}$ = 15 Hz), 164.0 (d, $^1J_{C-F}$ = 240 Hz), 164.2 (d, $^4J_{C-F}$ = 4 Hz), 168.7 (2C). ^{19}F NMR ($CDCl_3$) δ −66.99. MS m/z 384 (M^+, 1), 231 (100), 224 (18), 174 (67), 167 (41), 147 (16), 124 (46), 96 (29), 58 (45).

*N-[2-[N-Ethyl-N-(2-hydroxyethyl)amino]ethyl]phthalimide (**22**).* To a solution of phthalimide **14** (15.0 g, 68.7 mmol) in anhydrous ethanol (300 mL) were successively added, under argon, 2-bromoethanol (14.8 mL, 0.21 mol) and triethylamine (29.0 mL, 0.21 mol). The mixture was stirred under reflux for 60 h. After cooling to room temperature, the solvent was evaporated under vacuum and the residue was chromatographed (SiO_2, EtOAc/EtOH, 99/1, v/v) to give compound **22** (13.0 g, 49.6 mmol) as a white solid. Yield 72%; R_f (SiO_2, EtOAc/EtOH, 99/1, v/v) 0.49; mp 56−58 °C. IR (KBr) ν 1018, 1387, 1405, 1702, 2825, 2959, 3400−3600 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.93 (t, 3H, J = 7.1 Hz), 2.58 (q, 2H, J = 7.1 Hz), 2.68 (t, 2H, J = 5.3 Hz), 2.76 (t, 2H, J = 6.2 Hz), 3.11 (brs, 1H), 3.53 (t, 2H, J = 5.3 Hz), 3.77 (t, 2H, J = 6.2 Hz), 7.71 (m, 2H), 7.82 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.1, 36.9, 48.0, 51.9, 56.3, 59.0, 123.4 (2C), 132.1 (2C), 134.1 (2C), 168.7 (2C). MS m/z 262 (M^+, 1), 231 (11), 174 (21), 130 (8), 102 (100), 76 (10), 58 (29).

*N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-phthalimide (**23**).* To a solution of phthalimide **22** (1.65 g, 6.29 mmol) in anhydrous tetrahydrofuran (80 mL) were successively added, under argon, 2-fluoro-3-hydroxypyridine[30] (0.72 g, 6.37 mmol), triphenylphosphine (1.65 g, 6.29 mmol), and dropwise diisopropyl azodicarboxylate (1.71 mL, 8.68 mmol). The mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was chromatographed (Al_2O_3, EtOAc) to give compound **23** (1.77 g, 4.95 mmol) as a yellow solid. Yield 79%;

R_f (Al_2O_3, EtOAc) 0.84; mp 58−60 °C. IR (KBr) ν 1192, 1240, 1290, 1395, 1452, 1712, 2847, 2943 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.29 (t, 3H, J = 7.1 Hz), 2.97 (q, 2H, J = 7.1 Hz), 3.14 (t, 2H, J = 6.5 Hz), 3.24 (t, 2H, J = 5.9 Hz), 4.08 (t, 2H, J = 6.5 Hz), 4.31 (t, 2H, J = 5.9 Hz), 7.35 (ddd, 1H, $^5J_{H-F}$ = 0.9 Hz, J = 4.8, 7.8 Hz), 7.52 (ddd, 1H, $^4J_{H-F}$ = 10.0 Hz, J = 1.6, 7.8 Hz), 7.97 (m, 3H), 8.07 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 36.2, 48.5, 51.4, 52.1, 67.9, 121.7 (d, $^4J_{C-F}$ = 5 Hz), 122.6 (d, $^3J_{C-F}$ = 4 Hz), 122.8 (2C), 132.1 (2C), 132.9 (2C), 137.2 (d, $^3J_{C-F}$ = 13 Hz), 142.2 (d, $^2J_{C-F}$ = 26 Hz), 153.7 (d, $^1J_{C-F}$ = 238 Hz), 168.4 (2C). ^{19}F NMR ($CDCl_3$) δ −84.07 (d, $^4J_{H-F}$ = 9.9 Hz). MS m/z 357 (M^+, 1), 231 (15), 197 (100), 174 (23), 130 (10), 85 (17), 76 (10), 57 (45).

*N-(2-Aminoethyl)-N-ethyl-N-[(2-fluoropyridin-4-yl)methyl]amine (**24**).* To a stirred solution of **15** (0.30 g, 0.91 mmol) in anhydrous ethanol (15 mL) was added, under argon, hydrazine monohydrate (130 μL, 2.73 mmol). The mixture was refluxed for 6 h. After cooling to room temperature and then to 0 °C, the white precipitate was filtered and washed with ethanol (5 mL). The filtrate was evaporated under vacuum, and the residue was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 98/2, v/v) to give amine **24** (129 mg, 0.66 mmol) as a yellow oil. Yield: 72%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 98/2, v/v) 0.29. IR (CCl_4) ν 1278, 1410, 1569, 1613, 2816, 2969, 3300−3400 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (t, 3H, J = 7.1 Hz), 2.53 (q, 2H, J = 7.1 Hz), 2.56 (t, 2H, J = 6.2 Hz), 2.63 (brs, 2H), 2.78 (t, 2H, J = 6.2 Hz), 3.61 (s, 2H), 6.96 (s, 1H), 7.16 (d, 1H, J = 5.1 Hz), 8.11 (d, 1H, J = 5.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 39.5, 48.0, 55.9, 57.2, 108.9 (d, $^2J_{C-F}$ = 38 Hz), 121.3 (d, $^4J_{C-F}$ = 3 Hz), 147.5 (d, $^3J_{C-F}$ = 15 Hz), 156.0 (d, $^3J_{C-F}$ = 8 Hz), 164.3 (d, $^1J_{C-F}$ = 237 Hz). ^{19}F NMR ($CDCl_3$) δ −69.19. ESI-MS m/z 197.9 $[M + H]^+$.

*N-(2-Aminoethyl)-N-ethyl-N-[2-(2-fluoropyridin-4-yl)ethyl]amine (**25**).* The title compound was synthesized according to the procedure developed for amine **24**, starting from compound **16** (600 mg, 1.76 mmol). Reaction time under reflux: 12 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH/NH_4OH, 97/2.5/0.5, v/v/v) to give compound **25** (361 mg, 1.71 mmol) as a brown oil. Yield 97%; R_f (Al_2O_3, CH_2Cl_2/EtOH/NH_4OH, 97/2.5/0.5, v/v/v) 0.34. IR (CCl_4) ν 1149, 1264, 1412, 1555, 1613, 2814, 2970 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.72 (t, 3H, J = 7.1 Hz), 1.16 (brs, 2H), 2.27 (m, 4H), 2.44 (m, 4H), 6.50 (s, 1H), 6.76 (m, 1H), 7.79 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.4, 32.6 (d, $^4J_{C-F}$ = 3 Hz), 39.3, 46.9, 53.5, 55.9, 108.9 (d, $^2J_{C-F}$ = 37 Hz), 121.6 (d, $^4J_{C-F}$ = 4 Hz), 146.7 (d, $^3J_{C-F}$ = 15 Hz), 155.7 (d, $^3J_{C-F}$ = 8 Hz), 163.5 (d, $^1J_{C-F}$ = 236 Hz). ^{19}F NMR ($CDCl_3$) δ −69.42. ESI-MS m/z $[(M - NH_2\text{-}CH{=}CH_2) + H]^+$ = 169.08.

*N-(2-Aminoethyl)-N-ethyl-N-[3-(2-fluoropyridin-4-yl)propyl]amine (**26**).* The title compound was synthesized according to the procedure developed for amine **24**, starting from compound **17** (1.00 g, 2.81 mmol). Reaction time under reflux: 12 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH, 97/3, v/v) to give compound **26** (0.55 g, 2.42 mmol) as a brown oil. Yield 86%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 97/3, v/v) 0.27. IR (CCl_4) ν 1412, 1613, 2336, 2810, 2850−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 7.1 Hz), 1.31 (brs, 2H), 1.70 (quint, 2H, J = 7.5 Hz), 2.39 (m, 6H), 2.60 (m, 4H), 6.66 (brs, 1H), 6.92 (m, 1H), 7.99 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.8, 27.9, 32.8 (d, $^4J_{C-F}$ = 3 Hz), 39.8, 47.5, 52.9, 56.6, 109.0 (d, $^2J_{C-F}$ = 37 Hz), 121.6 (d, $^4J_{C-F}$ = 4 Hz), 147.2 (d, $^3J_{C-F}$ = 16 Hz), 157.5 (d, $^3J_{C-F}$ = 7 Hz), 164.1 (d, $^1J_{C-F}$ = 238 Hz). ^{19}F NMR ($CDCl_3$) δ −69.50. ESI-MS m/z 225.9 $[M + H]^+$.

*N-(2-Aminoethyl)-N-ethyl-N-(6-fluoropyridin-2-yl)amine (**27**).* To a solution of compound **18** (0.60 g, 1.92 mmol) in ethanol (70 mL) was added hydrazine monohydrate (0.93 mL, 19.2 mmol). The mixture was stirred at room temperature for 24 h. The white precipitate was filtered and washed with ethanol (5 mL). The filtrate was then evaporated under vacuum, and the residue was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 9/1, v/v) to give amine **27** (0.28 g, 1.52 mmol) as a brown oil. Yield 79%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 9/1, v/v) 0.35. IR (CCl_4) ν 1240, 1440, 1501, 1617, 2800−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.16 (t, 3H, J = 7.0 Hz), 1.59 (brs, 2H), 2.92 (t, 2H, J = 6.6 Hz), 3.49 (m, 4H), 6.04 (dd, 1H, J = 2.6, 8.0 Hz), 6.28 (dd, 1H, J = 2.6, 8.0 Hz), 7.43 (q, 1H, $^4J_{H-F}$ = 8.0 Hz, J = 8.0 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.2, 40.1, 43.5, 51.0, 93.8 (d, $^2J_{C-F}$ = 37 Hz), 101.6 (d, $^4J_{C-F}$ = 4 Hz), 141.3 (d, $^3J_{C-F}$ = 8 Hz), 157.2 (d, $^3J_{C-F}$ = 17 Hz), 162.9 (d, $^1J_{C-F}$ = 234 Hz). ^{19}F NMR ($CDCl_3$) δ −69.38 (d, $^4J_{H-F}$ = 8.1 Hz). ESI-MS m/z 183.8 $[M + H]^+$.

*N-(2-Aminoethyl)-N-ethyl-N-[2-[[(2-fluoropyridin-4-yl)carbonyl]-amino]ethyl]amine (**28**).* The title compound was synthesized according to the procedure developed for amine **24**, from compound **21** (1.00 g, 2.60 mmol). Reaction time under reflux: 4.5 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH, 93/7, v/v) to give compound **28** (0.65 g, 2.56 mmol) as a yellow oil. Yield 98%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 93/7, v/v) 0.41. IR (CCl_4) ν 1304, 1397, 1567, 1673, 2750−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.0 Hz), 2.64 (m, 4H), 2.75 (t, 2H, J = 5.7 Hz), 2.86 (t, 2H, J = 5.7 Hz), 2.91 (brs, 2H), 3.56 (m, 2H), 7.50 (s, 1H), 7.72 (d, 1H, J = 4.6 Hz), 8.34 (d, 1H, J = 5.1 Hz), 8.75 (brs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.9, 38.8, 39.5, 48.2, 51.7, 54.7, 108.1 (d, $^2J_{C-F}$ = 39 Hz), 119.3 (d, $^4J_{C-F}$ = 4 Hz), 147.8 (d, $^3J_{C-F}$ = 7 Hz), 148.4 (d, $^3J_{C-F}$ = 15 Hz), 164.2 (d, $^1J_{C-F}$ = 240 Hz), 164.4 (d, $^4J_{C-F}$ = 3 Hz). ^{19}F NMR ($CDCl_3$) δ −67.52. ESI-MS m/z 255.0 $[M + H]^+$.

*N-(2-Aminoethyl)-N-ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]-amine (**29**).* The title compound was synthesized according to the procedure developed for amine **27**, starting from compound **23** (1.50 g, 4.20 mmol). Reaction time at room temperature: 14 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH, 9/1, v/v) to give compound **29** (949 mg, 4.18 mmol) as a yellow oil. Yield 99%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 9/1, v/v) 0.36. IR (CCl_4) ν 1120, 1190, 1250, 1283, 1453, 1466, 2700−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.1 Hz), 1.54 (brs, 2H), 2.67 (m, 6H), 2.94 (t, 2H, J = 6.0 Hz), 4.12 (t, 2H, J = 6.0 Hz), 7.13 (ddd, 1H, $^5J_{H-F}$ = 0.9 Hz, J = 4.8, 7.9 Hz), 7.32 (ddd, 1H, $^4J_{H-F}$ = 10.1 Hz, J = 1.6, 7.9 Hz), 7.76 (td, 1H, $^4J_{H-F}$ = 1.6 Hz, J = 1.6, 4.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.6, 39.3, 48.2, 51.8, 56.4, 67.7, 121.4 (d, $^4J_{C-F}$ = 4 Hz), 122.4 (d, $^3J_{C-F}$ = 4 Hz), 136.7 (d, $^3J_{C-F}$ = 13 Hz), 141.8 (d, $^2J_{C-F}$ = 26 Hz), 153.3 (d, $^1J_{C-F}$ = 239 Hz). ^{19}F NMR ($CDCl_3$) δ −84.13 (d, $^4J_{H-F}$ = 9.7 Hz). ESI-MS m/z 227.9 $[M + H]^+$.

*N-[2-[(N-Cyanomethyl-N-ethyl)amino]ethyl]phthalimide (**30**).* To a solution of *N*-[2-(ethylamino)ethyl]phthalimide hydrochloride salt (**13**)[29] (10.0 g, 39.3 mmol) in anhydrous acetonitrile (160 mL) were added successively, under argon, potassium carbonate (5.43 g, 39.3 mmol) and bromoacetonitrile (2.72 mL, 39.3 mmol). The mixture was stirred at room temperature for 48 h. The precipitate was filtered, and the filtrate was evaporated under vacuum. The residue was chromatographed (Al_2O_3, CH_2Cl_2) to give compound **30** (8.24 g, 32.0 mmol) as a beige solid. Yield 82%; R_f (Al_2O_3, CH_2Cl_2) 0.94; mp 80−82 °C. IR (KBr) ν 1100, 1322, 1356, 1398, 1420, 1708, 1767, 2235, 2846, 2979 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 7.1 Hz), 2.55 (q, 2H, J = 7.1 Hz), 2.79 (t, 2H, J = 6.1 Hz), 3.65 (s, 2H), 3.75 (t, 2H, J = 6.1 Hz), 7.67 (m, 2H), 7.78 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.6, 35.2, 41.0, 48.2, 51.0, 114.9, 123.2 (2C), 131.9 (2C), 134.0 (2C), 168.3 (2C). MS m/z 257 (M^+, 2), 97 (100), 76 (12), 69 (23).

*[N-(2-Aminoethyl)-N-ethyl]aminoacetonitrile (**31**).* The title compound was synthesized according to the procedure developed for compound **27**, starting from compound **30** (2.78 g, 10.8 mmol). Reaction time at room temperature: 14 h to give amine **31** (1.17 g, 9.20 mmol) as a yellow oil. Yield 85%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 9/1, v/v) 0.25. IR (CCl_4) ν 1321, 1427, 1459, 1664, 2240, 2829, 2940, 2975 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.2 Hz), 1.58 (brs, 2H), 2.50 (m, 4H), 2.67 (t, 2H, J = 5.5 Hz), 3.50 (s, 2H). ^{13}C NMR

(50 MHz, $CDCl_3$) δ 12.6, 39.0, 41.3, 48.0, 56.3, 115.0. ESI-MS m/z 128.5 $[M + H]^+$.

*N-Ethyl-N-[2-[(6-fluoropyridin-2-yl)amino]ethyl]aminoacetonitrile (**32**).* To a stirred solution of amine **31** (1.90 g, 14.9 mmol) in anhydrous dimethylsulfoxide (40 mL) were successively added under argon, 2,6-difluoropyridine (2.05 mL, 22.4 mmol) and triethylamine (3.15 mL, 22.4 mmol). The mixture was heated at 80 °C for 8 h. After cooling to room temperature, water (400 mL) was added. The mixture was decanted, and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The organic layers were combined, washed with a brine solution (3 × 150 mL), dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (Al_2O_3, EtOAc/cyclohexane, 1/1, v/v) to give compound **32** (1.29 g, 5.82 mmol) as a brown oil. Yield 39%; R_f (Al_2O_3, EtOAc/cyclohexane, 1/1, v/v) 0.53. IR (CCl_4) ν 1228, 1423, 1457, 1500, 1577, 1620, 2750−3000, 3422 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (t, 3H, J = 7.1 Hz), 2.67 (q, 2H, J = 7.1 Hz), 2.83 (t, 2H, J = 5.9 Hz), 3.38 (t, 2H, J = 5.9 Hz), 3.64 (s, 2H), 5.00 (brs, 1H), 6.14 (dd, 1H, J = 2.4, 7.9 Hz), 6.21 (dd, 1H, J = 2.4, 7.9 Hz), 7.47 (q, 1H, $^4J_{H-F}$ = 7.9 Hz, J = 7.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 38.8, 41.0, 48.2, 52.5, 96.0 (d, $^2J_{C-F}$ = 37 Hz), 103.2, 114.7, 141.8 (d, $^3J_{C-F}$ = 8 Hz), 157.8 (d, $^3J_{C-F}$ = 16 Hz), 163.4 (d, $^1J_{C-F}$ = 235 Hz). ^{19}F NMR ($CDCl_3$) δ −70.06 (d, $^4J_{H-F}$ = 7.6 Hz). MS m/z 222 (M^+, 5), 125 (31), 97 (100), 69 (22).

*N-(2-Aminoethyl)-N-ethyl-N-[2-[N-(6-fluoropyridin-2-yl)amino]-ethyl]amine (**33**).* To a solution of lithium aluminum hydride (288 mg, 7.58 mmol) in anhydrous tetrahydrofuran (20 mL) was added under argon, at 0 °C, a solution of nitrile **32** (1.12 g, 5.04 mmol) in anhydrous tetrahydrofuran (10 mL). The mixture was stirred at 0 °C for 2 h, then water (388 μL), a 3.0 N aqueous sodium hydroxide solution (388 μL), and water (388 μL) were successively added to the mixture until no more gas evolution was observed. The precipitate was filtered, and the filtrate was dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (Al_2O_3, CH_2Cl_2/EtOH/NH_4OH, 80/19/1, v/v/v) to give compound **33** (0.55 g, 2.43 mmol) as a brown oil. Yield 48%; R_f (Al_2O_3, CH_2Cl_2/EtOH/NH_4OH, 80/19/1, v/v/v) 0.61. IR (CCl_4) ν 1227, 1456, 1501, 1621, 2750−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.97 (t, 3H, J = 7.0 Hz), 2.08 (brs, 2H), 2.59 (m, 8H), 3.25 (q, 2H, J = 5.5 Hz), 5.39 (brs, 1H), 6.05 (ddd, 1H, $^3J_{H-F}$ = 0.6 Hz, J = 2.4, 7.8 Hz), 6.17 (ddd, 1H, $^5J_{H-F}$ = 0.4 Hz, J = 2.4, 7.8 Hz), 7.39 (q, 1H, $^4J_{H-F}$ = 7.8 Hz, J = 7.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.6, 39.7 (2C), 47.6, 52.3, 56.0, 95.1 (d, $^2J_{C-F}$ = 37 Hz), 103.1 (d, $^4J_{C-F}$ = 4 Hz), 141.5 (d, $^3J_{C-F}$ = 9 Hz), 158.3 (d, $^3J_{C-F}$ = 17 Hz), 163.4 (d, $^1J_{C-F}$ = 236 Hz). ^{19}F NMR ($CDCl_3$) δ −71.02. MS m/z 227 (M + 1, 1), 196 (15), 139 (60), 114 (17), 101 (61), 96 (17), 72 (27), 58 (100).

*N,N-Diethyl-2-fluoroisonicotinamide (**34**).* To a stirred suspension of acid **5** (6.00 g, 42.5 mmol) in anhydrous dichloromethane (200 mL) were successively added, under argon, anhydrous *N,N*-dimethylformamide (2 mL) and thionyl chloride (12.4 mL, 0.17 mol). The mixture was refluxed for 3 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and the residue was dissolved in anhydrous toluene (40 mL). The solvent was evaporated under vacuum, and the residue was dissolved in anhydrous tetrahydrofuran (40 mL). A solution of *N,N*-diethylamine (8.79 mL, 85.0 mmol) in anhydrous tetrahydrofuran (10 mL) was added dropwise, at 0 °C. The mixture was then stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (80 mL) before addition of a 5% aqueous sodium carbonate solution (10 mL). The mixture was extracted with dichloromethane (4 × 80 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (Al_2O_3, EtOAc) to give compound **34** (5.66 g, 28.8 mmol) as a yellow liquid. Yield 68%; R_f (Al_2O_3, EtOAc) 0.78. IR (CCl_4) ν 1298, 1401, 1433, 1562, 1612, 1647, 2936, 2978 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, 3H, J = 7.1 Hz), 1.12 (t, 3H, J = 7.1 Hz), 3.09 (q, 2H, J = 7.1 Hz), 3.42 (q, 2H, J = 7.1 Hz), 6.80 (m, 1H), 7.05 (ddd, 1H, $^5J_{H-F}$ = 1.9 Hz, J = 1.3, 5.1 Hz), 8.15 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.3, 13.7, 39.0, 42.8, 106.6 (d, $^2J_{C-F}$ = 39 Hz), 118.3 (d, $^4J_{C-F}$ = 4 Hz), 148.0 (d, $^3J_{C-F}$ = 15 Hz), 149.9 (d, $^3J_{C-F}$ = 7 Hz), 163.2 (d, $^1J_{C-F}$ = 240 Hz), 166.8 (d, $^4J_{C-F}$ = 3 Hz). ^{19}F NMR ($CDCl_3$) δ −66.03. MS m/z 196 (M^+, 22), 195 (26), 124 (100), 96 (36), 76 (11), 69 (12).

*N,N-Diethyl-N-[(2-fluoropyridin-4-yl)methyl]amine (**35**).* To a solution of compound **34** (0.50 g, 2.55 mmol) in anhydrous tetrahydrofuran (6 mL) was added under argon at 0 °C, a 1.0 M borane solution in anhydrous tetrahydrofuran (7.65 mL, 7.65 mmol). The mixture was heated at 50 °C for 1 h. After cooling to room temperature, methanol (5 mL) and a 1.0 M aqueous sodium hydroxide solution (5 mL) were added dropwise successively at 0 °C. The mixture was heated at 50 °C for 5 h. After cooling to room temperature, the mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed (Al_2O_3, CH_2Cl_2) to give compound **35** (0.42 g, 2.30 mmol) as a colorless liquid. Yield 91%; R_f (Al_2O_3, CH_2Cl_2) 0.51. IR (CCl_4) ν 1167, 1278, 1410, 1571, 1612, 2300, 2805, 2973 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.27 (t, 6H, J = 7.1 Hz), 2.76 (q, 4H, J = 7.1 Hz), 3.82 (s, 2H), 7.21 (s, 1H), 7.41 (m, 1H), 8.35 (d, 1H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.1 (2C), 47.4 (2C), 56.6 (d, $^4J_{C-F}$ = 3 Hz), 108.9 (d, $^2J_{C-F}$ = 37 Hz), 121.3 (d, $^4J_{C-F}$ = 4 Hz), 147.3 (d, $^3J_{C-F}$ = 15 Hz), 156.6 (d, $^3J_{C-F}$ = 8 Hz), 164.4 (d, $^1J_{C-F}$ = 238 Hz). ^{19}F NMR ($CDCl_3$) δ −69.56. MS m/z 182 (M^+, 12), 167 (100), 110 (46), 86 (12), 83 (12), 56 (15).

*4-(N,N-Diethylaminomethyl)-2-fluoropyridine-3-carbaldehyde (**36**).* To a solution of anhydrous diisopropylamine (3.39 mL, 24.0 mmol) in anhydrous tetrahydrofuran (130 mL) was added dropwise under argon and, at −78 °C, a 2.5 M *n*-butyllithium solution in hexane (9.60 mL, 24.0 mmol). The mixture was stirred at −78 °C for 1 h before a dropwise addition of a solution of compound **35** (4.00 g, 21.9 mmol) in anhydrous tetrahydrofuran (65 mL). The mixture was stirred at −78 °C for 7 h before addition of anhydrous *N,N*-dimethylformamide (1.86 mL, 24.0 mmol). The mixture was stirred 5 min at −78 °C before rapid return back to a temperature composed between 5 and 10 °C. A saturated aqueous ammonium chloride solution (300 mL) was added, the solution was decanted, and the aqueous layer was extracted with dichloromethane (4 × 100 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (Al_2O_3, EtOAc/cyclohexane, 6/4, v/v) to give compound **36** (2.83 g, 13.5 mmol) as a colorless liquid. Yield 61%; R_f (Al_2O_3, EtOAc/cyclohexane, 6/4, v/v) 0.56. IR (CCl_4) ν 1269, 1393, 1465, 1553, 1604, 1705, 1741, 2700−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.01 (t, 6H, J = 7.1 Hz), 2.54 (q, 4H, J = 7.1 Hz), 3.96 (s, 2H), 7.71 (d, 1H, J = 5.1 Hz), 8.29 (d, 1H, J = 5.1 Hz), 10.41 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.0 (2C), 47.6 (2C), 54.8 (d, $^4J_{C-F}$ = 4 Hz), 116.7 (d, $^2J_{C-F}$ = 21 Hz), 122.3 (d, $^4J_{C-F}$ = 4 Hz), 151.4 (d, $^3J_{C-F}$ = 18 Hz), 159.4 (d, $^3J_{C-F}$ = 2 Hz), 164.7 (d, $^2J_{C-F}$ = 247 Hz), 188.2 (d, $^3J_{C-F}$ = 2 Hz). ^{19}F NMR ($CDCl_3$) δ −74.73; MS m/z 210 (M^+, 24), 195 (13), 181 (49), 167 (38), 153 (46), 138 (100), 133 (28), 110 (37), 83 (27), 72 (79), 58 (34).

*4-(N,N-Diethylaminomethyl)-2-fluoropyridine-3-carbaldehyde oxime (**37**).* To a solution of compound **36** (150 mg, 0.71 mmol) in methanol (2 mL) was added a solution of hydroxylamine hydrochloride (74 mg, 1.07 mmol) and potassium acetate (105 mg, 1.07 mmol) in water (7 mL). The mixture was stirred at room temperature for 4 h, and methanol was then evaporated under reduced pressure. A saturated aqueous sodium chloride solution (10 mL) was added, and the mixture was extracted with dichloromethane (4 × 20 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (Al_2O_3, EtOAc → EtOAc/EtOH, 85/15, v/v) to

give oxime **37** (131 mg, 0.58 mmol) as a white solid. Yield 81%; R_f (Al_2O_3, EtOAc/EtOH, 85/15, v/v) 0.89; mp 97−99 °C. IR (KBr) ν 1264, 1331, 1392, 1605, 2750−2980, 3100, 3191 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.05 (t, 6H, J = 7.1 Hz), 2.57 (q, 4H, J = 7.1 Hz), 3.83 (s, 2H), 7.56 (d, 1H, J = 5.1 Hz), 8.13 (d, 1H, J = 5.1 Hz), 8.43 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.8 (2C), 47.7 (2C), 55.4 (d, $^4J_{C-F}$ = 4 Hz), 113.6, 122.4 (d, $^4J_{C-F}$ = 4 Hz), 143.6 (d, $^3J_{C-F}$ = 2 Hz), 147.0 (d, $^3J_{C-F}$ = 16 Hz), 154.4, 161.7 (d, $^1J_{C-F}$ = 242 Hz). ^{19}F NMR ($CDCl_3$) δ −71.50. ESI-MS m/z 225.8 $[M + H]^+$.

*N,N-Diethyl-N-[(3-aminomethyl-2-fluoropyridin-4-yl)methyl]amine (**38**).* To a stirred solution of compound **37** (1.30 g, 5.77 mmol) in acetic acid (34 mL), protected against light exposure, was added dropwise during 24 h at room temperature, zinc dust (1.63 g, 24.9 mmol). The mixture was then cooled to 0 °C before addition of a 3.0 N aqueous sodium hydroxide solution (130 mL). After return back to room temperature, the mixture was extracted with dichloromethane (3 × 200 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed (Al_2O_3, CH_2Cl_2/EtOH, 97/3, v/v) to give amine **38** (778 mg, 3.68 mmol) as a yellow oil. Yield 64%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 97/3, v/v) 0.54. IR (CCl_4) ν 1264, 1413, 1608, 2972 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, 6H, J = 7.3 Hz), 2.44 (q, 6H, J = 7.1 Hz), 3.55 (s, 2H), 3.81 (s, 2H), 7.05 (d, 1H, J = 4.9 Hz), 7.95 (d, 1H, J = 4.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.5 (2C), 36.6, 46.7 (2C), 55.4, 123.6 (d, $^4J_{C-F}$ = 4 Hz), 124.2 (d, $^2J_{C-F}$ = 31 Hz), 145.4 (d, $^3J_{C-F}$ = 16 Hz), 152.5 (d, $^3J_{C-F}$ = 5 Hz), 162.6 (d, $^1J_{C-F}$ = 237 Hz). ^{19}F NMR ($CDCl_3$) δ −76.30.

General Procedure for the Synthesis of Amides 39−51 and 73. To a solution of the appropriate amine (1.13 mmol) in anhydrous dichloromethane (15 mL) was added under argon at 0 °C, a 2.0 M trimethylaluminium solution in heptane (0.56 mL, 1.13 mmol). After 5 min, the appropriate ester (0.80 mmol) in anhydrous dichloromethane solution (5 mL) was added and the mixture was refluxed for 6−24 h. After cooling to room temperature, water (50 mL) was added. The mixture was extracted with dichloromethane (5 × 50 mL). The organic layers were combined, dried on magnesium sulfate, filtered, and evaporated under reduced pressure. The oily residue was chromatographed (Al_2O_3) to give the appropriate amide.

*N-[2-[[N-Ethyl-N-(2-fluoropyridin-4-yl)methyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**39**).* Starting from amine **24** (125 mg, 0.63 mmol) and ethyl 6-iodoquinoxaline-2-carboxylate[22a] (160 mg, 0.49 mmol). Reaction time under reflux: 6 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give compound **39** (120 mg, 0.24 mmol) as a brown oil. Yield 38%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.49. IR (CCl_4) ν 1394, 1413, 1613, 1720 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.09 (t, 3H, J = 7.1 Hz), 2.65 (q, 2H, J = 7.1 Hz), 2.75 (t, 2H, J = 5.8 Hz), 3.58 (q, 2H, J = 5.8 Hz), 3.69 (s, 2H), 7.06 (s, 1H), 7.19 (m, 1H), 7.81 (d, 1H, J = 8.9 Hz), 8.04 (m, 2H), 8.33 (brs, 1H), 8.55 (d, 1H, J = 1.8 Hz), 9.57 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.7, 36.9, 47.8, 52.2, 56.8 (d, $^4J_{C-F}$ = 3 Hz), 98.3, 108.9 (d, $^2J_{C-F}$ = 38 Hz), 121.3 (d, $^4J_{C-F}$ = 4 Hz), 130.7, 138.6, 139.5, 139.9, 143.7, 144.5 (2C), 147.6 (d, $^3J_{C-F}$ = 15 Hz), 155.4 (d, $^3J_{C-F}$ = 10 Hz), 162.9, 164.3 (d, $^1J_{C-F}$ = 239 Hz). ^{19}F NMR ($CDCl_3$) δ −68.62; MS m/z 479 (M^+, 3), 167 (100), 128 (9), 110 (36), 101 (10), 56 (11). Amides **40−51** and **73** were obtained using the procedure described above (see Supporting Information).

General Procedure for the Synthesis of Dihydrochloride Salts 52−63, 68. *N-[2-[[N-Ethyl-N-(2-fluoropyridin-4-yl)methyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide Dihydrochloride Salt (**52**).* To a stirred solution of compound **39** (117 mg, 0.24 mmol) in anhydrous dichloromethane (5 mL) was added under argon a 2.0 N hydrochloric acid solution in anhydrous diethylether (10 mL). After 10 min, the solvent was evaporated under vacuum. The residue was suspended in anhydrous diethylether (10 mL), and the mixture was stirred at room temperature for 12 h. The precipitate was then filtered and dried under reduced pressure to give compound **52** (83 mg, 0.15 mmol) as a very hygroscopic beige solid. Yield 64%; mp 139−141 °C. IR (KBr) ν 1164, 1415, 1533, 1616, 1670, 2250−2660, 2945, 3100−3600 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 1.34 (t, 3H, J = 6.9 Hz), 3.26 (m, 4H), 3.60 (m, 2H), 3.77 (m, 2H), 4.52 (m, 2H), 7.60 (s, 1H), 7.70 (m, 1H), 7.91 (d, 1H, J = 8.8 Hz), 8.26 (m, 2H), 8.63 (d, 1H, J = 1.8 Hz), 9.38 (m, 1H), 9.42 (s, 1H), 11.36 (brs, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 11.5, 36.8, 47.3, 51.8, 55.9, 99.3, 108.6 (d, $^2J_{C-F}$ = 38 Hz), 121.8 (d, $^4J_{C-F}$ = 4 Hz), 130.7, 137.5, 139.0, 139.8, 143.6, 144.3, 144.5, 147.1 (d, $^3J_{C-F}$ = 15 Hz), 158.6 (d, $^3J_{C-F}$ = 8 Hz), 162.6, 163.4 (d, $^1J_{C-F}$ = 235 Hz). ^{19}F NMR (DMSO-d_6) δ −69.71. ESI-MS m/z 480.1 $[M + H]^+$. Anal. ($C_{19}H_{19}OIN_5F$, 2HCl) H, N, C; calcd, 41.33; found, 42.19. Dihydrochloride salts **53−63** and **68** were obtained using the procedure described above (see Supporting Information).

*N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-7-bromophenazine-1-carboxamide (**65**).* A solution of 7-bromophenazine-1-carboxylic acid[22b] (**64**) (333 mg, 1.10 mmol) in thionyl chloride (8 mL) was refluxed under argon for 1 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and the residue was dissolved in anhydrous toluene (5 mL). The solvent was evaporated under vacuum, and the residue was dissolved in anhydrous dichloromethane (11 mL). A solution of compound **29** (300 mg, 1.32 mmol) in anhydrous dichloromethane (5 mL) was added at 0 °C, and the mixture was then stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was chromatographed (Al_2O_3, EtOAc) to give compound **65** (537 mg, 1.05 mmol) as a yellow oil. Yield 95%; R_f (Al_2O_3, EtOAc) 0.69. IR (CCl_4) ν 1190, 1249, 1283, 1466, 1517, 1663, 2800−3000, 3250−3350 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.09 (t, 3H, J = 7.1 Hz), 2.80 (q, 2H, J = 7.1 Hz), 2.86 (t, 2H, J = 6.0 Hz), 3.00 (t, 2H, J = 5.5 Hz), 3.70 (q, 2H, J = 6.0 Hz), 4.04 (t, 2H, J = 5.5 Hz), 6.75 (ddd, 1H, $^5J_{H-F}$ = 0.8 Hz, J = 4.8, 7.9 Hz), 6.93 (ddd, 1H, $^4J_{H-F}$ = 10.1 Hz, J = 1.5, 7.9 Hz), 7.45 (td, 1H, $^4J_{H-F}$ = 1.5 Hz, J = 1.5, 4.8 Hz), 7.71 (dd, 1H, J = 2.0, 9.2 Hz), 7.82 (dd, 1H, J = 7.1, 8.7 Hz), 7.89 (d, 1H, J = 9.2 Hz), 8.15 (dd, 1H, J = 1.5, 8.7 Hz), 8.23 (d, 1H, J = 2.0 Hz), 8.84 (dd, 1H, J = 1.5, 7.1 Hz), 10.79 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.0, 38.2, 48.9, 52.1, 53.3, 68.4, 121.3 (d, $^4J_{C-F}$ = 4 Hz), 122.2 (d, $^3J_{C-F}$ = 4 Hz), 125.3, 129.4, 129.9, 130.6, 131.4, 133.3, 135.0, 135.5, 137.0 (d, $^3J_{C-F}$ = 13 Hz), 139.7, 140.5, 141.9 (d, $^2J_{C-F}$ = 26 Hz), 142.8, 143.4, 153.4 (d, $^1J_{C-F}$ = 238 Hz), 164.3. ^{19}F NMR ($CDCl_3$) δ −83.93. ESI-MS m/z 512.0 $[M + H]^+$.

*N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-7-(tributylstannyl)phenazine-1-carboxamide (**66**).* To a solution of compound **65** (0.60 g, 1.17 mmol) in anhydrous toluene (20 mL), beforehand degassed under argon, were successively added hexabutylditin (587 μL, 1.56 mmol) and freshly prepared tetrakis(triphenylphosphine)palladium(0)[32] (20 mg). The resulting solution was refluxed for 17 h under argon. After cooling to room temperature, the mixture was filtered through Celite 521, washed with toluene (3 × 100 mL), and the filtrate was evaporated under vacuum. The residue obtained was then chromatographed (Al_2O_3, EtOAc/cyclohexane, 7/3, v/v) to give compound **66** (381 mg, 0.53 mmol) as a yellow oil. Yield 45%; R_f (Al_2O_3, EtOAc/cyclohexane, 7/3, v/v) 0.72. IR (CCl_4) ν 1377, 1465, 1661, 2870, 2873, 2927, 2959 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.81 (t, 9H, J = 7.2 Hz), 1.11 (m, 9H), 1.29 (m, 6H), 1.50 (m, 6H), 2.82 (m, 4H), 2.99 (t, 2H, J = 5.7 Hz), 3.71 (q, 2H, J = 5.6 Hz), 4.05 (t, 2H, J = 5.7 Hz), 6.69 (dd, 1H, J = 4.8, 7.8 Hz), 6.89 (m, 1H), 7.44 (td, 1H, $^4J_{H-F}$ = 1.6 Hz, J = 1.6, 4.7 Hz), 7.80 (m, 2H), 8.04 (d, 1H, J = 8.5 Hz), 8.25 (dd, 1H, J = 1.5, 8.7 Hz), 8.29 (s, 1H), 8.86 (dd, 1H, J = 1.4, 7.2 Hz), 11.13 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.9 (3C, $^1J_{^{119}Sn-C}$ = 344 Hz, $^1J_{^{117}Sn-C}$ = 328 Hz), 12.1, 13.6 (3C), 27.0 (3C, $^2J_{^{119}Sn/^{117}Sn-C}$ = 35 Hz), 27.6 (3C, $^3J_{^{119}Sn/^{117}Sn-C}$ = 20 Hz), 38.2, 49.0, 52.2, 53.4, 68.6, 121.2 (d, $^4J_{C-F}$ = 4 Hz), 122.1 (d, $^3J_{C-F}$ = 4 Hz), 127.1, 129.2, 129.7, 133.5, 134.9, 136.9 (d, $^3J_{C-F}$ = 13 Hz), 138.1, 138.3, 140.7, 141.4, 142.0 (d, $^2J_{C-F}$ = 26 Hz),

142.1, 143.2, 148.5, 153.5 (d, $^1J_{C-F}$ = 239 Hz), 164.7. ^{19}F NMR ($CDCl_3$) δ −83.97. ESI-MS m/z 724.4 $[M + H]^+$.

*(N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-7-iodophenazine-1-carboxamide (**67**).* To a stirred solution of stannane **66** (270 mg, 0.37 mmol) in chloroform (7 mL) was added dropwise, during 4 h, a solution of diiodine (192 mg, 0.76 mmol) in chloroform (14 mL). The mixture was then stirred 14 h at room temperature before addition of a saturated aqueous sodium carbonate solution (40 mL). The solution was decanted, and the organic layer was washed with a 5% aqueous sodium hydrogenosulfite solution (2 × 15 mL), dried on magnesium sulfate, filtered, and evaporated under vacuum. The residue was chromatographed (Al_2O_3, EtOAc/cyclohexane, 7/3, v/v) to give compound **67** (112 mg, 0.20 mmol) as a yellow oil. Yield 54%; R_f (Al_2O_3, EtOAc/cyclohexane, 7/3, v/v) 0.68. IR (CCl_4) ν 1119, 1189, 1246, 1286, 1458, 1649, 2800−3000, 3246 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.14 (t, 3H, J = 7.1 Hz), 2.86 (m, 4H), 3.05 (t, 2H, J = 5.5 Hz), 3.76 (q, 2H, J = 5.5 Hz), 4.10 (t, 2H, J = 5.5 Hz), 6.80 (dd, 1H, J = 4.9, 7.9 Hz), 6.97 (m, 1H), 7.53 (m, 1H), 7.83 (dd, 1H, J = 1.4, 9.2 Hz), 7.93 (m, 2H), 8.25 (dd, 1H, J = 1.5, 8.7 Hz), 8.60 (brs, 1H), 8.92 (dd, 1H, J = 1.5, 7.2 Hz), 10.92 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.1, 38.3, 49.0, 52.2, 53.4, 68.5, 97.7, 121.5 (d, $^4J_{C-F}$ = 4 Hz), 122.3 (d, $^3J_{C-F}$ = 4 Hz), 129.4, 129.8, 130.7, 133.6, 135.7, 137.3 (d, $^3J_{C-F}$ = 13 Hz), 138.6, -140.1, 140.2, 140.9, 142.0 (d, $^2J_{C-F}$ = 26 Hz), 143.2, 143.4, 153.6 (d, $^1J_{C-F}$ = 239 Hz), 164.6. ^{19}F NMR ($CDCl_3$) δ −83.95. ESI-MS m/z 560.0 $[M + H]^+$.

*N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-6-(tributylstannyl)imidazo[1,2-a]pyridine-2-carboxamide (**69**).* To a stirred solution of compound **50** (200 mg, 0.40 mmol) in anhydrous toluene (9 mL), beforehand degassed under argon, were successively added hexabutylditin (638 μL, 1.20 mmol) and freshly prepared tetrakis(triphenylphosphine)palladium(0)[32] (20 mg). The resulting solution was refluxed for 12 h under argon. After cooling to room temperature, the mixture was filtered through Celite 545, washed with toluene (2 × 10 mL), and the filtrate was evaporated under vacuum. The residue obtained was then chromatographed (Al_2O_3, EtOAc/cyclohexane, 8/2, v/v) to give compound **69** (246 mg, 0.37 mmol) as a yellow oil. Yield 93%; R_f (Al_2O_3, EtOAc/cyclohexane, 8/2, v/v) 0.40. IR (CCl_4) ν 1250, 1340, 1378, 1465, 1507, 1565, 1670, 2854, 2873, 2927, 2959 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85 (t, 9H, J = 7.1 Hz), 1.03 (m, 6H), 1.26 (m, 9H), 1.51 (m, 6H), 2.66 (q, 2H, J = 7.1 Hz), 2.75 (t, 2H, J = 6.2 Hz), 2.92 (t, 2H, J = 6.1 Hz), 3.50 (q, 2H, J = 6.2 Hz), 4.06 (t, 2H, J = 6.1 Hz), 6.87 (ddd, 1H, $^5J_{H-F}$ = 0.7 Hz, J = 4.8, 7.9 Hz), 7.17 (m, 2H), 7.40 (d, 1H, J = 8.9 Hz), 7.58 (td, 1H, $^4J_{H-F}$ = 1.6 Hz, J = 1.6, 4.8 Hz), 7.71 (m, 1H), 7.92 (s, 1H), 8.01 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.8 (3C, $^1J_{^{119}Sn-C}$ = 349 Hz, $^1J_{^{117}Sn-C}$ = 334 Hz), 11.9, 13.6 (3C), 27.1 (3C, $^2J_{^{119}Sn/^{117}Sn-C}$ = 64 Hz), 28.9 (3C, $^3J_{^{119}Sn/^{117}Sn-C}$ = 21 Hz), 37.2, 48.7, 52.3, 53.4, 68.4, 113.1, 117.5 ($^3J_{^{119}Sn/^{117}Sn-C}$ = 34 Hz), 121.4 (d, $^4J_{C-F}$ = 4 Hz), 122.8 (d, $^3J_{C-F}$ = 4 Hz), 123.3, 130.7 ($^2J_{^{119}Sn/^{117}Sn-C}$ = 51 Hz), 132.2 ($^3J_{^{119}Sn/^{117}Sn-C}$ = 24 Hz), 137.0 (d, $^3J_{C-F}$ = 13 Hz), 139.4, 142.2 (d, $^2J_{C-F}$ = 25 Hz), 144.4, 153.8 (d, $^1J_{C-F}$ = 239 Hz), 162.9. ^{19}F NMR ($CDCl_3$) δ −86.21. ESI-MS m/z 662.3 $[M + H]^+$.

*N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-7-(tributylstannyl)acridone-4-carboxamide (**70**).* The title compound was synthesized according to the procedure described for stannane **69**, starting from compound **51** (0.40 g, 0.70 mmol). Reaction time under reflux: 12 h. The purification was performed using column chromatography (Al_2O_3, EtOAc/cyclohexane, 8/2, v/v) to give compound **70** (208 mg, 0.28 mmol) as a yellow oil. Yield 40%; R_f (Al_2O_3, EtOAc/cyclohexane, 8/2, v/v) 0.43. IR (CCl_4) ν 1465, 1510, 1608, 1652, 2220, 2854, 2873, 2927, 2959 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (m, 12H), 1.09 (m, 6H), 1.32 (sex, 6H, J = 7.2 Hz), 1.55 (m, 6H), 2.70 (q, 2H, J = 7.1 Hz), 2.84 (t, 2H, J = 5.9 Hz), 2.96 (t, 2H, J = 5.2 Hz), 3.57 (q, 2H, J = 5.9 Hz), 4.08 (t, 2H, J = 5.2 Hz), 7.00 (m, 1H), 7.03 (t, 1H, J = 7.7 Hz), 7.18 (ddd, 1H, $^4J_{H-F}$ = 10.1 Hz, J = 1.6, 7.9 Hz), 7.34 (d, 1H, J = 8.1 Hz), 7.43 (m, 1H), 7.69 (td, 1H, $^4J_{H-F}$ = 1.7 Hz, J = 1.7, 4.9 Hz), 7.72 (dd, 1H, J = 1.2, 8.1 Hz), 7.82 (dd, 1H, J = 1.4, 7.7 Hz), 8.52 (s, 1H), 8.58 (dd, 1H, J = 1.3, 8.0 Hz), 12.33 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.8 (3C, $^1J_{^{119}Sn-C}$ = 342 Hz, $^1J_{^{117}Sn-C}$ = 327 Hz), 12.0, 13.7 (3C), 27.2 (3C, $^2J_{^{119}Sn/^{117}Sn-C}$ = 42 Hz), 29.2 (3C, $^3J_{^{119}Sn/^{117}Sn-C}$ = 20 Hz), 37.5, 48.2, 52.2, 52.4, 68.2, 117.2, 117.5, 119.5, 121.0, 121.8 (d, $^4J_{C-F}$ = 4 Hz), 123.0 (d, $^3J_{C-F}$ = 4 Hz), 123.1, 131.4, 131.7, 134.7, 135.0, 137.8 (d, $^3J_{C-F}$ = 13 Hz), 140.3, 141.0, 141.4 ($^2J_{^{119}Sn/^{117}Sn-C}$ = 35 Hz), 142.0 (d, $^2J_{C-F}$ = 25 Hz), 153.8 (d, $^1J_{C-F}$ = 238 Hz), 168.5, 178.1. ^{19}F NMR ($CDCl_3$) δ −83.85. ESI-MS m/z 739.3 $[M + H]^+$.

*N-[2-[N-Ethyl-N-[2-(2-nitropyridin-3-yloxy)ethyl]amino]ethyl]-phthalimide (**71**).* To a solution of compound **22** (11.3 g, 43.1 mmol) in anhydrous tetrahydrofuran (400 mL) were added successively, under argon, triphenylphosphine (11.3 g, 43.1 mmol), commercial 2-nitro-3-hydroxypyridine (6.02 g, 43.0 mmol), and dropwise diisopropyl azodicarboxylate (8.70 mL, 44.2 mmol). The mixture was stirred at room temperature for 60 h, and the solvent was evaporated under vacuum. Unreacted 2-nitro-3-hydroxypyridine was removed by chromatography (Al_2O_3, EtOAc/cyclohexane, 9/1, v/v). After evaporation, the residue containing the desired product was dissolved in dichloromethane (200 mL) and pentane (600 mL) was added. The mixture was stirred at room temperature for 5 min, and the precipitate was filtered and dried under vacuum to give compound **71** (12.7 g, 33.0 mmol) as a yellow solid. Yield 77%; R_f (Al_2O_3, EtOAc/cyclohexane, 9/1, v/v) 0.76; mp 124−126 °C. IR (KBr) ν 1274, 1397, 1536, 1702, 1767, 2830 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (t, 3H, J = 7.1 Hz), 2.63 (q, 2H, J = 7.1 Hz), 2.81 (t, 2H, J = 6.4 Hz), 2.94 (t, 2H, J = 5.8 Hz), 3.76 (t, 2H, J = 6.4 Hz), 4.10 (t, 2H, J = 5.8 Hz), 7.50 (m, 2H), 7.60 (m, 2H), 7.75 (m, 2H), 8.05 (dd, 1H, J = 2.5, 3.4 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.0, 36.3, 48.5, 51.4, 51.8, 68.8, 122.7 (2C), 123.6, 128.6, 131.9 (2C), 133.8 (2C), 138.8, 147.1, 148.6, 168.3 (2C). ESI-MS m/z 385.0 $[M + H]^+$.

*N-(2-Aminoethyl)-N-ethyl-N-[2-(2-nitropyridin-3-yloxy)ethyl]amine (**72**).* The title compound was synthesized according to the procedure described for amine **24**, starting from compound **71** (2.00 g, 5.20 mmol). Reaction time under reflux: 12 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH, 95/5, v/v) to give compound **72** (1.01 g, 3.97 mmol) as a yellow oil. Yield 76%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 95/5, v/v) 0.36. IR (CCl_4) ν 1116, 1289, 1505, 1548, 1608, 2874, 2934, 2970 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.11 (t, 3H, J = 7.1 Hz), 2.72 (m, 8H), 2.99 (t, 2H, J = 5.6 Hz), 4.28 (t, 2H, J = 5.6 Hz), 7.63 (dd, 1H, J = 4.3, 8.4 Hz), 7.71 (dd, 1H, J = 1.5, 8.4 Hz), 8.13 (dd, 1H, J = 1.5, 4.3 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.0, 39.6, 48.5, 52.1, 56.6, 68.9, 123.7, 128.6, 139.1, 147.2, 150.3. ESI-MS m/z 255.0 $[M + H]^+$.

*N-[2-[N-Ethyl-N-[2-(2-nitropyridin-3-yloxy)ethyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**73**).* The title compound was synthesized starting from amine **72** (600 mg, 2.36 mmol) and ethyl 6-iodoquinoxaline-2-carboxylate[22a] (774 mg, 2.36 mmol) according to the general procedure for the synthesis of amides described above. Reaction time under reflux: 12 h. The purification was performed using column chromatography (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give compound **73** (902 mg, 1.68 mmol) as a light-sensitive brown oil. Yield 71%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.46. IR (CCl_4) ν 1117, 1289, 1526, 1549, 1683, 2800−3000, 3410 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.1 Hz), 2.70 (q, 2H, J = 7.1 Hz), 2.83 (t, 2H, J = 6.1 Hz), 2.99 (t, 2H, J = 5.3 Hz), 3.57 (q, 2H, J = 6.1 Hz), 4.17 (t, 2H, J = 5.3 Hz), 7.36 (dd, 1H, J = 4.2, 8.4 Hz), 7.43 (dd, 1H, J = 1.7, 8.4 Hz), 7.61 (d, 1H, J = 8.8 Hz), 7.94 (dd, 1H, J = 1.7, 4.2 Hz), 7.99 (dd, 1H, J = 1.8, 8.8 Hz), 8.29 (m, 1H), 8.53 (d, 1H, J = 1.8 Hz), 9.57 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.4, 37.8, 48.9, 52.3, 53.3, 69.4, 98.1, 123.3, 128.6, 130.9, 138.6, 139.2, 139.5, 139.8, 144.1, 144.5, 144.7, 147.3, 149.0, 163.1. ESI-MS m/z 536.9 $[M + H]^+$.

*tert-Butyl N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]carbamate (**74**).* To a solution of amine **29** (500 mg, 2.20

mmol) in anhydrous dichloromethane (15 mL) were successively added triethylamine (306 μL, 2.20 mmol) and di-*tert*-butyl dicarbonate (480 mg, 2.20 mmol). The mixture was stirred at room temperature for 20 h. A 1.0 N aqueous hydrochloric acid solution (10 mL) was added. The mixture was decanted, and the organic layer was washed successively with a saturated aqueous sodium hydrogenocarbonate solution (2 × 5 mL) and water (2 × 5 mL). The organic layer was then dried on magnesium sulfate, filtered, and evaporated under reduced pressure to give compound **74** (506 mg, 1.55 mmol) as an orange-colored oil. Yield 70%. IR (CCl_4) ν 1172, 1250, 1453, 1466, 1498, 1580, 1716, 2978 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.01 (t, 3H, J = 7.1 Hz), 1.38 (s, 9H), 2.61 (m, 4H), 2.88 (t, 2H, J = 5.7 Hz), 3.16 (q, 2H, J = 5.9 Hz), 4.04 (t, 2H, J = 5.7 Hz), 5.05 (m, 1H, NH), 7.07 (ddd, 1H, $^5J_{H-F}$ = 0.8 Hz, J = 4.8, 7.8 Hz), 7.27 (m, 1H), 7.70 (td, 1H, $^4J_{H-F}$ = 1.6 Hz, J = 1.6, 4.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.9, 28.4 (3C), 38.5, 48.4, 52.1, 53.3, 68.1, 121.7 (d, $^4J_{C-F}$ = 4 Hz), 123.0 (d, $^3J_{C-F}$ = 4 Hz), 137.4 (d, $^3J_{C-F}$ = 13 Hz), 142.2 (d, $^2J_{C-F}$ = 26 Hz), 153.4 (d, $^1J_{C-F}$ = 239 Hz), 156.2. ^{19}F NMR ($CDCl_3$) δ −83.92 (d, J_{H-F} = 9.1 Hz). MS m/z 327 (M^+, 1), 254 (5), 197 (100), 85 (13), 72 (7), 57 (29).

*tert-Butyl N-[2-[N-Ethyl-N-[2-(2-nitropyridin-3-yloxy)ethyl]amino]-ethyl]carbamate (**75**).* The title compound was synthesized according to the procedure described for **74**, starting from compound **72** (500 mg, 1.97 mmol). Reaction time at room temperature: 18 h to give compound **75** (444 mg, 1.25 mmol) as a yellow oil. Yield 64%. IR (CCl_4) ν 1172, 1289, 1367, 1500, 1549, 1716, 2977 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, 3H, J = 7.1 Hz), 1.36 (s, 9H), 2.60 (m, 4H), 2.89 (t, 2H, J = 5.6 Hz), 3.13 (q, 2H, J = 5.9 Hz), 4.13 (t, 2H, J = 5.6 Hz), 7.51 (m, 2H), 8.05 (dd, 1H, J = 2.4, 3.4 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.0, 28.5 (3C), 38.6, 48.5, 52.0, 53.5, 68.9, 79.1, 123.7, 139.3, 147.3, 149.0, 156.2. ESI-MS m/z 355.1 $[M + H]^+$.

*N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-6-tributylstannylquinoxaline-2-carboxamide (**76**).* The title compound was synthesized according to the procedure described for stannane **66**, starting from compound **44** (0.40 g, 0.79 mmol). Reaction time under reflux: 4 h. The purification was performed using column chromatography (Al_2O_3, EtOAc/cyclohexane, 6/4, v/v) to give compound **76** (318 mg, 0.47 mmol) as a red oil. Yield 60%; R_f (Al_2O_3, EtOAc/cyclohexane, 6/4, v/v) 0.58. IR (CCl_4) ν 1120, 1190, 1250, 1283, 1466, 1521, 1682, 2800−3000 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.86 (t, 9H, J = 7.2 Hz), 1.12 (m, 9H), 1.32 (sex, 6H, J = 7.2 Hz), 1.57 (m, 6H), 2.71 (q, 2H, J = 7.2 Hz), 2.84 (t, 2H, J = 6.0 Hz), 2.98 (t, 2H, J = 5.6 Hz), 3.61 (q, 2H, J = 6.0 Hz), 4.11 (t, 2H, J = 5.6 Hz), 6.91 (dd, 1H, J = 4.8, 7.7 Hz), 7.20 (m, 1H), 7.59 (td, 1H, $^4J_{H-F}$ = 1.5 Hz, J = 1.5, 4.8 Hz), 7.86 (m, 2H), 8.27 (s, 1H, $^3J_{^{119}Sn/^{117}Sn-H}$ = 41.0 Hz), 8.46 (te, 1H), 9.61 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.9 (3C, $^1J_{^{119}Sn-C}$ = 343 Hz, $^1J_{^{117}Sn-C}$ = 327 Hz), 12.2, 13.6 (3C), 27.3 (3C, $^2J_{^{119}Sn/^{117}Sn-C}$ = 56 Hz), 29.0 (3C, $^3J_{^{119}Sn/^{117}Sn-C}$ = 21 Hz), 37.5, 48.7, 52.4, 53.2, 68.5, 121.5 (d, $^4J_{C-F}$ = 4 Hz), 122.7 (d, $^3J_{C-F}$ = 4 Hz), 128.0, 137.2 (d, $^3J_{C-F}$ = 13 Hz), 137.7, 137.9, 140.2, 142.2 (d, $^2J_{C-F}$ = 26 Hz), 142.9, 143.4, 143.6, 148.8, 153.7 (d, $^1J_{C-F}$ = 239 Hz), 163.4. ^{19}F NMR (470 MHz, $CDCl_3$) δ − 83.82. ESI-MS m/z 674.3 $[M + H]^+$.

General Procedures for Preparation of Radioiodinated Compounds via Nucleophilic Isotopic Exchange. *Method A.* To a solution of the appropriate compound (2−3 mg) in citrate buffer pH = 4 (500 μL) were added, in a closed vial, an aqueous copper sulfate solution (0.5 mg, 100 μL) used as catalyst and [^{125}I]NaI (26−110 μL, 62−246 MBq). The reaction mixture was heated at 130−150 °C for 20−60 min. After cooling to room temperature, the residue was taken up in water (500 μL) and a 1.0 N aqueous NaOH solution (100 μL) was added. The vial cap and septum were removed. The resulting suspension was passed through an Extrelut column and eluted, after 10 min, with dichloromethane (5 × 3 mL). The collected organic extracts were evaporated under reduced pressure, taken up with methanol (200 μL), and purified by HPLC at a flow rate of 1 mL/min. The fractions containing the product were collected, evaporated to dryness, redissolved in dichloromethane (2 mL), and treated with a 2.0 N hydrochloric acid solution in anhydrous ether (5 mL). The resulting hydrochloride solution was evaporated under reduced pressure, and the dry residue was suspended in anhydrous ether (5 mL). The solvent was then evaporated under vacuum for 30 min to give the radiolabeled compound.

Method B. According to the same procedure developed in method A except for the HPLC purification.

The experimental conditions and characterization of radioiodinated final compounds are summarized in Table 2.

*[^{125}I]N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-6-iodoimidazo[1,2-a]pyridine-2-carboxamide Dihydrochloride Salt ([^{125}**I**]**62**).* In a closed vial containing compound **69** in EtOH (30 μL, 5.1 mg.mL^{-1}) were added in the following order: a citrate buffer solution pH = 4 (100 μL), [^{125}I]NaI (90 μL, 193.8 MBq), and an aqueous solution of chloramine T monohydrate (100 μL, 0.5 mg·mL^{-1}). The resulting solution was vortexed at room temperature for 30 min. The reaction was quenched with an aqueous 3.0 N sodium hydroxide solution (150 μL). The mixture was vortexed for 5 min, and the vial cap and septum were removed. The reaction mixture was transferred to an Extrelut column, and the vial was rinsed with a solution of H_2O/EtOH (1/1, v/v, 2 × 100 μL). After 10 min, the column was eluted with dichloromethane (5 × 2 mL). The organic extracts were collected and evaporated under reduced pressure. The residue was taken up with methanol (200 μL) and purified by HPLC (R_t = 11.2 min for [^{125}I]**50**, R_t = 22.5 min for **69**). The fractions containing the product were collected and evaporated to dryness, redissolved in dichloromethane (2 mL), and treated with a 2.0 N hydrochloric acid solution in anhydrous ether (5 mL). The resulting hydrochloride solution was evaporated under reduced pressure, and the dry residue was suspended in anhydrous ether (5 mL). The solvent was then evaporated under vacuum for 30 min to yield the expected compound [^{125}I]**62** (34 MBq). Radiochemical yield, 18%; specific activity, 96.5 GBq/μmol; radiochemical purity, 96.4%.

*[^{125}I]N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-7-iodoacridone-4-carboxamide Hydrochloride Salt ([^{125}**I**]**63**).* In a closed vial containing compound **70** (0.14 mg, 0.19 μmol) in EtOH (30 μL) were added in the following order: a solution of acetic acid 1% in ethanol (30 μL), [^{125}I]NaI (90 μL, 197.6 MBq), and an aqueous solution of chloramine T monohydrate (15 μL, 0.4 mg·mL^{-1}). The resulting solution was vortexed at room temperature for 10 min. The reaction was quenched with an aqueous 0.1 N sodium hydroxide solution (20 μL). The mixture was vortexed for 5 min, and the vial cap and septum were removed. The reaction mixture was transferred to an Extrelut column, and the vial was rinsed with a solution of H_2O/EtOH (1/1, v/v, 2 × 100 μL). After 10 min, the column was eluted with dichloromethane (5 × 2 mL). The organic extracts were collected and evaporated under reduced pressure. The residue was taken up with methanol (200 μL) and purified by HPLC (R_t = 15.4 min for [^{125}I]**51**, R_t = 22.4 min for **70**). The fractions containing the product were collected and evaporated to dryness, redissolved in dichloromethane (2 mL), and treated with a 2.0 N hydrochloric acid solution in anhydrous ether (5 mL). The resulting hydrochloride solution was evaporated under reduced pressure, and the dry residue was suspended in anhydrous ether (5 mL). The solvent was then evaporated under vacuum for 30 min to yield the expected compound [^{125}I]**63** (94.7 MBq). Radiochemical yield, 48%; specific activity, 96.5 GBq/μmol; radiochemical purity, 97.6%.

In Vivo [^{125}I] γ Imaging of Melanoma Bearing Mice. Protocols were performed under the authorization of the French Direction des Services Vétérinaires (authorization no. C63-113-10, CE18-09) and conducted under the supervision of authorized investigators in accordance with the institution's recommendations for the use

of laboratory animals. On day 14 after tumor implantation (tumor weight 0.25 ± 0.10 g), each [^{125}I]-labeled compound was administered intravenously via a tail vein (range 1.26−5.29 MBq/animal) in three mice for each compound. Radiotracers [^{125}I]**52**−**61** and [^{125}I]**68** were injected in solution in physiological serum while [^{125}I]**42** was injected in a solution of dimethylsulfoxide 10% in physiological serum. Biodistribution of radioiodinated compounds in B16F0 melanoma-bearing C57Bl6 mice was followed by serial scintigraphic imaging using the γ IMAGER. The injected activity to each mouse was also determined from scintigraphic imaging of the syringe before and after the injection. At different times after administration (1 h, 3 h, 6 h, 24 h, 72 h, 5 d, 7 d, 10 d, and 14 d), mice were anaesthetized by ip administration (200 μL/mouse) of a ketamine−xylazine mixture in saline:ketamine (100 mg/kg, Imalgene, Rhône Mérieux, Lyon, France) and xylazine (10 mg/kg, Rompun, Bayer, France). A 10 min duration image was acquired on anesthetized mice placed in an anterior position over the collimator. Reproducibility in animal positioning for the serial images was achieved using a graduated reference grid. Quantitative analysis of scintigraphic scans was performed using the GAMMAVISION+ software (Biospace Mesures, Paris, France). Regions of interest (ROIs) were delineated on the images: whole body area, tumor, and the areas with a significant tracer concentration. In the various ROIs, the activity (cpm) was quantified according to the validated methodology.[40] These values were normalized to the injected dose. For the tumor, the activity was also normalized to the tumor weight.

Whole-Body Autoradiography on Animal Slices. For an accurate evaluation in all organs, [^{125}I]**56** was administered intravenously via a tail vein (0.1 μmol, 2 MBq/animal) in 10 animals. Two injected mice were sacrificed by CO_2 inhalation, quickly frozen in liquid nitrogen, and embedded in carboxymethylcellulose (CMC) at different times after administration (1, 3, 6, 24, or 72 h). The frozen animals were cryosectioned in 40 μm thick slices using a Reichert−Jung cryopolycut (Leica Instruments, Rueil Malmaison, France) at −22 °C and dehydrated for 48 h in the cryochamber.[41] Eight slices per mouse and per time point were selected for the analysis corresponding to distant slices and the section of the organs of interest. Measurements were performed on an AMBIS 4000 detector (Scanalytics, CSPI, San Diego, CA, USA) following an acquisition time of 1000 min. The radioactivity of different organs was quantified after contouring suitable zones on the two-dimensional image of the slice displayed on the computer screen for analysis. Surfacic activity (net cpm/mm^2) was converted into radioactive concentration (kBq/g) and expressed as percentage of injected dose/g of tissue (% ID/g). For two animals, urine and feces were collected up to 72 h and counted to determine the cumulative urinary and fecal excretions.

Dosimetry Parameters. From the biodistribution values, the biological tumor half-life of [^{125}I]**56** was calculated using an adaptation of the MIRD program to the mouse. With a view to using [^{131}I]**56** analogue for targeted radionuclide therapy of melanoma and taking into account its physical properties, the effective half-life of [^{131}I]**56** and the tumor delivered doses (Gy/injected MBq) were calculated.

^{18}F Radiochemistry. Radiosyntheses using ^{18}F, including the HPLC purifications, were performed in a 7.5 cm lead-shielded cell using a computer-assisted Zymate robot system (Zymark Corporation, USA).

*Preparation of N-[2-[N-Ethyl-N-[2-(2-[^{18}F]fluoropyridin-3-yloxy)ethyl] amino]ethyl]-6-iodoquinoxaline-2-carboxamide ([^{18}F]**44**).* Acetonitrile (0.8 mL) containing *tert*-butyl *N*-[2-[*N*-ethyl-*N*-[2-(2-nitropyridin-3-yloxy)ethyl]amino]ethyl]carbamate (**75**) (4.0−6.0 mg, 11.3−16.9 μmol) was added to the Vacutainer tube containing the dried K[^{18}F]F−K_{222} complex (37 GBq batches, prepared as previously reported).[42] The tube (not sealed) was then thoroughly vortexed (15 s) and placed in a heating block (at 145 °C, for 2 min) without stirring the contents. After evaporation of acetonitrile, DMSO (0.6 mL) was added to the tube and was further heated for 6 min. The reaction vessel was then cooled using an ice−water bath and the contents diluted with water (1 mL) and transferred to a reservoir on top of a C-18 cartridge (PrepSep R-C18, Fisher Scientific, activated with EtOH (2 mL) and then rinsed with water (10 mL)). The reaction vessel was rinsed twice with water (1 mL), which was also transferred and added to the diluted reaction mixture on the reservoir. After addition of another 2 mL of water, the whole solution was passed through the C-18 cartridge. The cartridge was washed with water (1 mL) and partially dried for 0.5 min by applying a nitrogen stream. The intermediate *tert*-butyl *N*-[2-[*N*-ethyl-*N*-[2-(2-[^{18}F]fluoropyridin-3-yloxy)ethyl]amino]ethyl]carbamate ([^{18}F]**74**) was eluted from the cartridge with CH_2Cl_2 (3 mL) into a 5 mL reaction vial containing TFA (0.1 mL). Two 1 mL quantities of CH_2Cl_2 were used to wash the cartridge and to completely transfer the fluorine-18-labeled ester. The resulting CH_2Cl_2/TFA solution (50/1, v/v) was concentrated to dryness (at 65−75 °C under a gentle nitrogen stream for 4−6 min), giving the corresponding amine *N*-(2-aminoethyl)-*N*-ethyl-*N*-[2-(2-fluoropyridin-3-yloxy)ethyl]-amine ([^{18}F]**29**). This residue was first redissolved in CH_2Cl_2 (2 mL) and concentrated again to dryness to minimize TFA presence (at 65−75 °C under a gentle nitrogen stream for another 2−3 min), then redissolved in a CH_2Cl_2/NEt_3 mixture (1/1 v/v, 0.4 mL,). A solution of CH_2Cl_2 (0.4 mL) containing 6-iodoquinoxaline-2-carbonyl chloride[35] (4 mg, 12.5 μmol) was then added to the solution, and the vessel was gently vortexed for 10 min at room temperature. Dichloromethane was evaporated (at 65−75 °C under a gentle nitrogen stream for 2−4 min), and the crude was successively redissolved in acetonitrile (0.4 mL) and then water (0.6 mL) before injection into HPLC for purification (see Materials for Radiolabeling with ^{18}F section for a complete description of the HPLC equipment and conditions).

*Formulation of [^{18}F]**44**.* Formulation of the labeled product for iv injection was effected as follows: The HPLC-collected fraction containing the radiotracer was diluted with water (30 mL). The resulting solution was passed through a Sep-pak Plus C-18 cartridge (Waters, washed with ethanol (2 mL) and then rinsed with water (10 mL) prior to use). The cartridge was washed with water (10 mL) and partially dried by applying a nitrogen stream for 10 s. The radiotracer was eluted with EtOH (2 mL) followed by physiological saline (8 mL) and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to lower the ethanol concentration below 10%. This whole process was performed using a remote-controlled dedicated homemade device based on a literature procedure.[43]

*Quality Control of [^{18}F]**44**.* The radiotracer preparation was visually inspected for clarity, absence of color, and particles. An aliquot of the preparation was removed for determination of pH using standard pH paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC, using a sample of authentic **44** (R_t: 2.03 min) (see Materials for Radiolabeling with ^{18}F section for a complete description of the HPLC equipment and conditions). Specific radioactivity of the radiotracer was calculated from three consecutive HPLC analyses (average) and determined as follows: the area of the UV absorbance peak corresponding to the radiolabeled product was measured (integrated) on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance.

PET Imaging Assay with [^{18}F]44. Mice bearing subcateneous grafts of B16F0 melanoma cells on their right flank and B16F10 melanoma cells on their left flank were anaesthetized with isoflurane (3% for induction and 2 ± 0.5% for maintaining in oxygen flux), injected intravenously with 7.4 MBq of the radiolabeled compound, and placed immediately in the field of view of a microPET FOCUS 200 camera (Siemens, Knoxville, TN, USA). Dynamic whole body PET sequences were acquired in 3-D mode during the first 60 min following injection. Two other 30 min scans were acquired at 2 and 4 h after injection. Image analysis and quantification were performed using Anatomist software. All values of radioactivity concentrations were normalized by the

injected dose and expressed as a percentage of the injected dose per volume of tissue (% ID/cc).

^{131}I Radiochemistry. *[^{131}I]N-[2-[N-Ethyl-N-[2-(2-fluoropyridin-3-yloxy)ethyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide Dihydrochloride Salt [^{131}I]**56***. In a closed vial containing compound **76** (0.52 mg, 0.77 μmol) in ethanol (500 μL) were added, in the following order: a citrate buffer solution pH = 4 (150 μL), [^{131}I]NaI (117 μL, 2.42 GBq), and an aqueous solution of chloramine-T monohydrate (400 μL, 0.5 mg.mL^{-1}). The resulting solution was vortexed at room temperature for 30 min. The reaction was quenched successively with an aqueous sodium metabisulfite solution (400 μL, 0.2 g.mL^{-1}) and an aqueous 3 N sodium hydroxide solution (500 μL). The mixture was vortexed for 5 min, and the vial cap and septum were removed. The reaction mixture was transferred to an Extrelut column, and the vial was rinsed with a solution of H_2O/EtOH (1/1, v/v, 2 × 100 μL). After 10 min, the column was eluted with dichloromethane (5 × 2 mL). The organic extracts were collected, evaporated under reduced pressure, taken up with methanol (200 μL), and purified by HPLC (retention time: 18.0 min). The fractions containing the product were collected, evaporated to dryness, redissolved in dichloromethane (2 mL), and treated with a 2.0 N hydrochloric acid solution in anhydrous ether (5 mL). The resulting hydrochloride solution was evaporated under reduced pressure, and the dry residue was suspended in anhydrous ether (5 mL). The solvent was then evaporated under vacuum for 30 min to yield the expected compound [^{131}I]**56** (1.14 GBq). Radiochemical yield, 47%; 106.9 GBq/μmol; radiochemical purity, 98.5%.

Therapy Experiment on C57Bl6 Mice Bearing B16F0 Tumors. Targeted radionuclide therapy experiment was performed as previously described with [^{131}I]**3**.[23a] [^{131}I]**56** treatment was administered intravenously at days 6 and 10 (2 × 18.5 MBq) into a group of 10 mice. In the same conditions, an untreated group (10 mice) was studied. Pretreatment with Lugol's iodine solution was added in all group mice drinking water to block thyroid. To monitor tumoral growth, tumor volume in mm^3 was calculated twice a week from the measurement of two perpendicular diameters using a calliper according to the formula $L \times S^2/2$ where L and S are the largest and smallest diameters in mm respectively.[44]

■ ASSOCIATED CONTENT

Supporting Information. Table of elemental analysis for target compounds **42**, **52**−**63**, and **68**, biodistribution data of [^{125}I]**56** and chemical synthesis of hydrochloride salts **53**−**63** and **68**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

SPECT, single photon emission computed tomography; PET, positron emission tomography; CT, computed tomography; MRI, magnetic resonance imaging; FDG, fluorodeoxyglucose; α-MSH, α-melanocyte stimulating hormone; MC1R, melanocortin-1 receptors; SAR, structure−activity relationship; BZA, *N*-(2-diethylaminoethyl)-4-iodobenzamide; DMAP, *N*,*N*-dimethylaminopyridine; LAH, lithium aluminum hydride; LDA, lithium diisopropylamide; DIAD, diisopropyl azodicarboxylate; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DMSO, methylsulfoxide; TFA, trifluoroacetic acid; DMEM, Dulbecco's Modified Eagle's Medium; PBS, phosphate buffered saline; CMC, carboxymethylcellulose; MIRD, medical internal radiation dose; K_{222}, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane; CAT, chloramine-T

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