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Original article

Synthesis, radioiodination and *in vivo* screening of novel potent iodinated and fluorinated radiotracers as melanoma imaging and therapeutic probes

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ABSTRACT

In order to develop new iodinated and fluorinated matched-pair radiotracers for Single-Photon Emission Computed Tomography (SPECT)/Positron Emission Tomography (PET) imaging and targeted radionuclide therapy of melanoma, we successfully synthesized and radiolabelled with iodine-125 seven new derivatives, starting from our previously described lead structure **3**. The relevance of these radiotracers for gamma scintigraphic imaging of melanoma in rodent was assessed. The tumoural radioactivity uptake was most often high and specific even at early time points (12.1–18.3% ID/g at 3 h p.i. for [^{125}I]**39**–**42**) and a fast clearance from the non-target organs was observed. Also, calculated effective doses that could be delivered to tumours when using corresponding [^{131}I]-labelled analogues were generally higher than 100 cGy/MBq injected (98.9–150.5 cGy/MBq for [^{131}I]**39**–**42**). These results make compounds **39**–**42** suitable candidates for (i) PET imaging of melanoma after labelling with fluorine-18 and (ii) targeted radionuclide therapy of disseminated melanoma after labelling with iodine-131.

1. Introduction

In contrast to many other malignancies, the incidence of melanoma, the most lethal form of skin cancer, is steadily increasing worldwide [1–4]. In the United States the estimated lifetime risk of developing cutaneous melanoma is currently 1 in 58 overall against 1 in 250 in 1980 [5,6]. Survival largely depends on the stage of detection [7,8]. At the earliest invasive stages, melanoma is usually cured by surgical excision and the estimated 5-year overall survival rate is 97% [6]. Unfortunately, this cancer can rapidly spread to almost all the organs, and for patients with advanced-stage disease, the survival rate decreases dramatically (12% at 5 years) [9]. This poor prognosis is due to the ineffectiveness of existing chemo- or immunotherapies [6,9,10]. In addition, although promising results in terms of responses to treatment are obtained with new targeted therapy approaches, median progression-free survival is still disappointingly low [11–13]. Powerful tools for both early detection and new efficient treatment strategies of melanoma are therefore urgently needed.

An interesting area of melanoma research is the development of radiopharmaceuticals specific to this pathology. Among all the

Abbreviations: DAFBA, *N*-(2-diethylaminoethyl)-4-fluorobenzamide; DAST, (diethylamino)sulphur trifluoride; DME, dimethoxyethane; ESI-MS, electrospray ionization mass spectra; ID, injected dose; i.v., intravenous injection; PET, positron emission tomography; p.i., post injection; RCP, radiochemical purity; RCY, radiochemical yield; ROI, region of interest; RP-HPLC, reverse-phase high performance liquid chromatography; SA, specific activity; SPECT, single-photon emission computed tomography; TBDMSCl, *tert*-butyldimethylsilyl chloride; TBAF, tetrabutylammonium fluoride.

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approaches investigated, melanins are relevant molecular targets due to the capacity of these amorphous, irregular natural polymers to bind with many drugs, and especially those with coplanar fused aromatic rings [14]. Based on the work done on the benzamide family [15–20], recent studies using radiohalogenated heteroaromatic analogues have revealed that this class of compounds is suitable for positron emission tomography (PET) imaging (e.g. [^{18}F]**1**, [^{18}F]DAFBA [21–26]) or targeted radionuclide therapy (e.g. [^{131}I]**2**, [^{131}I]MIP-1145) of melanoma (Fig. 1) [27–30]. These findings prompted us to investigate a new approach consisting in using iodinated and fluorinated matched-pair radiotracers targeting melanins and offering potential for both diagnosis *via* single-photon emission computed tomography (SPECT, iodine-123) or PET imaging (iodine-124 or fluorine-18), and therapy (iodine-131), depending on the type of radionuclide introduced on the same chemical scaffold [31].

Fourteen iodinated compounds bearing a 2- or 6-fluoropyridine moiety on the *N*,*N*-diethylethylenediamine side chain were recently synthesized, radiolabelled with iodine-125 and screened by gamma scintigraphic imaging to evaluate their *in vivo* biodistribution profiles in B16F0 primary melanoma-bearing mice [31,32]. This screening allowed the selection of the tracer **3** (Fig. 1) with high, specific and long-lasting tumoural uptake. This compound was then radiolabelled with fluorine-18 in a three-step procedure. A first PET imaging experiment using the same murine model confirmed the promising results obtained by gamma scintigraphic imaging, and the utility of combining such tracer specificity with the performance of PET technology. Compound **3** was finally radiolabelled with iodine-131 and evaluated in B16F0 primary melanoma-bearing mice for a first targeted radionuclide therapy assay. This treatment induced a significant tumoural growth inhibition. Promising properties of **3** radiolabelled with either fluorine-18 or iodine-131 gave a first validation of our concept and underscored the potential of this class of iodinated and fluorinated matched-pair radiotracers for both diagnosis and targeted radionuclide therapy of melanoma [32]. To further improve pharmacokinetic profiles of the tracers, and facilitate radiofluorination processes, we planned to extend this strategy to fluoroaliphatic derivatives of the lead compound **3** as depicted in Fig. 2. Here we describe the synthesis, and iodine-125 radiolabelling of these new analogues and their preliminary *in vivo* screening in a melanoma-bearing mice model.

2. Results and discussion

2.1. Chemistry

The synthesis of seven new derivatives showing structural similarity to **3** was undertaken, based on the replacement of the (2-fluoropyridin-3-yloxy)ethyl moiety by various fluoroaliphatic groups (Fig. 2). As a first approach, we designed compounds bearing saturated fluoroalkyl chains. In parallel, and keeping in mind that haloalkylamines can undergo intramolecular cyclisation [33,34], we synthesized more constrained derivatives containing fluoroallyl and fluoropropargyl synthons, together with pegylated analogues enlarging the distance between the fluorine atom and the tertiary amine. Finally, we explored the Huisgen 1,3-dipolar cycloaddition between azides and alkynes, known as "click" chemistry, which is recognized as a powerful synthetic methodology allowing rapid and convenient radiofluorinations [35].

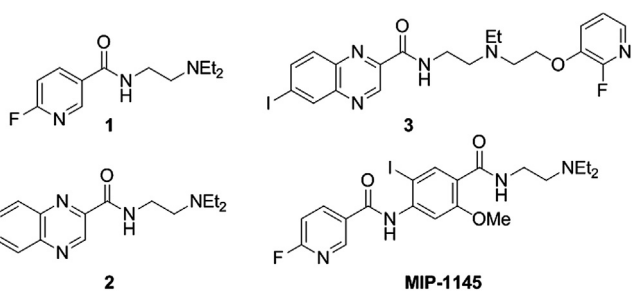


Fig. 1. Several benzamide derivatives developed for imaging and/or targeted radionuclide therapy of melanoma.

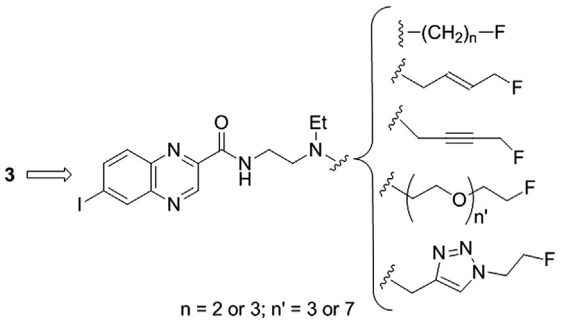
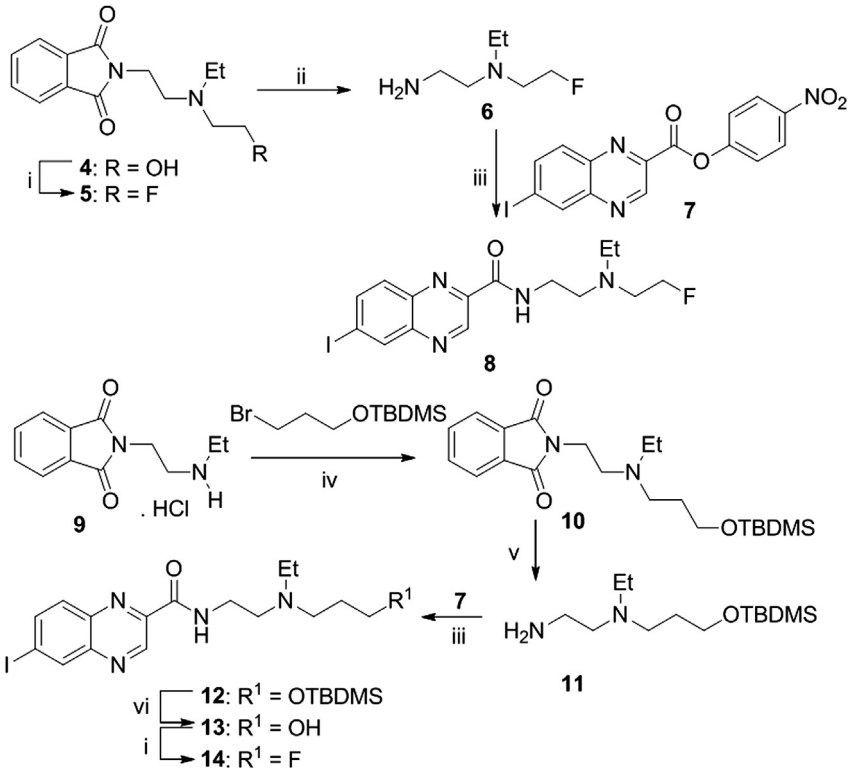


Fig. 2. Pharmacomodulation of the fluoro side chain of **3**.

The synthesis of compounds **8** and **14**, bearing fluoroethyl or fluoropropyl side chains respectively, is illustrated in Scheme 1. First, alcohol **4** [32] was converted into the fluorinated compound **5** using (diethylamino)sulphur trifluoride (DAST) at −50 °C. Deprotection of the phthalimide **5** was achieved by the action of hydrazine monohydrate to give unstable primary amine **6**, which was rapidly condensed with *p*-nitrophenyl 6-iodoquinoxaline-2-carboxylate (**7**) [36] to provide the desired amide **8**. A modified synthetic pathway was designed to produce the fluoropropyl derivative **14** in order to introduce the fluorine atom as late as possible in the synthesis. Phthalimide **9** [37] was alkylated with commercially available (3-bromopropoxy)-*tert*-butyldimethylsilane to afford compound **10**. In a similar manner as for **6**, phthalimide **10** was then deprotected to give primary amine **11** in quantitative yield and coupled to the activated ester **7** to provide amide **12**. Desilylation of **12** using tetrabutylammonium fluoride (TBAF) afforded alcohol **13**, which was finally converted into the desired fluorinated compound **14** following the protocol optimized for **5**. We note that due to its instability, tracer **14** has to be synthesized and purified just before use.

To synthesize amides **17** and **20** (Scheme 2) we investigated a direct alkylation of *N*-[2-(ethylamino)ethyl]-6-iodoquinoxaline (**15**) [36] using either (*E*)-4-fluoro-but-2-enyl toluene-4-sulfonate (**16**) [38] or 4-fluoro-but-2-ynyl toluene-4-sulfonate (**19**). The latter was produced *via* a two-step reaction sequence according to the procedure developed for compound **16** involving ditosylation [39] of commercially available 1,4-butynediol, and subsequent monofluorination [38] of **18** with TBAF in refluxing tetrahydrofuran. This strategy afforded amides **17** and **20** with moderate yields of 47% and 38% respectively. The (*E*)-configuration of final alkene **17** was extrapolated from the vicinal spin–spin coupling constant of 15.8 Hz between the two olefinic protons of the corresponding dihydrochloride salt **39**.

Pegylated derivatives **33** and **34** were synthesized by a common strategy outlined in Scheme 3. Commercially available tetra- and octaethylene glycols were first monoprotected using *tert*-butyldimethylsilyl chloride (TBDMSCl) according to a slightly modified protocol developed by Kan and co-workers [40]. Iodination of the remaining free alcohol function of **21** and **22**, in the presence of triphenylphosphine and imidazole, afforded derivatives **23** and **24**. Subsequent nucleophilic substitution using phthalimide **9** [37] provided intermediates **25** and **26**. Under similar reaction conditions (temperature and reaction time), we observed that two equivalents of potassium carbonate were required to obtain **26** in



Scheme 1. Syntheses of compounds **8** and **14**. Reagents and conditions: (i) DAST, DME, −50 °C, 15 min, then rt, 1.5 h; (ii) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 1 h; (iii) THF, rt, overnight; (iv) K_2CO_3, CH_3CN, 50 °C, 36 h; (v) $NH_2NH_2{\cdot}H_2O$, EtOH, rt, 18 h; (vi) TBAF, THF, rt, 1.5 h.

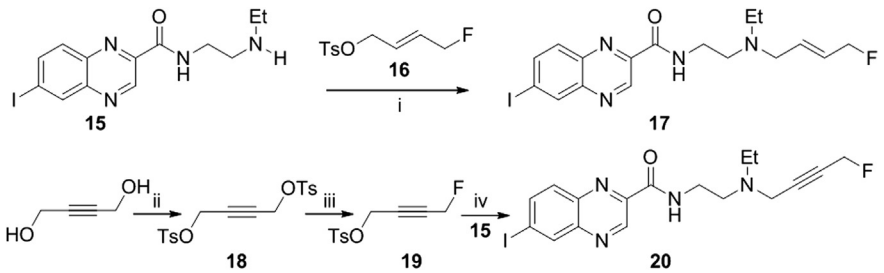
65% yield, whereas only one equivalent was needed to produce **25** (72%). Deprotection of phthalimides **25** and **26** using hydrazine monohydrate in ethanol immediately followed by peptidic coupling with activated ester **7** [36] provided silylated derivatives **29** and **30**, respectively. After desilylation with TBAF, the resulting alcohols **31** and **32** were converted into the corresponding alkyl fluorides **33** and **34** using DAST as fluorinating reagent.

The sequence depicted in Scheme 4 illustrates the synthesis of amide **37**, obtained by coupling alkyne **35** and azide **36** using copper(I) generated *in situ* as catalyst. Alkyne **35** was synthesized through alkylation of compound **15** with propargyl bromide. Unstable 1-azido-2-fluoroethane (**36**) was produced *in situ* by the action of freshly dried cesium fluoride on 2-azidoethyl *p*-toluenesulfonate [41] according to the procedure described by Bejot et al. [42].

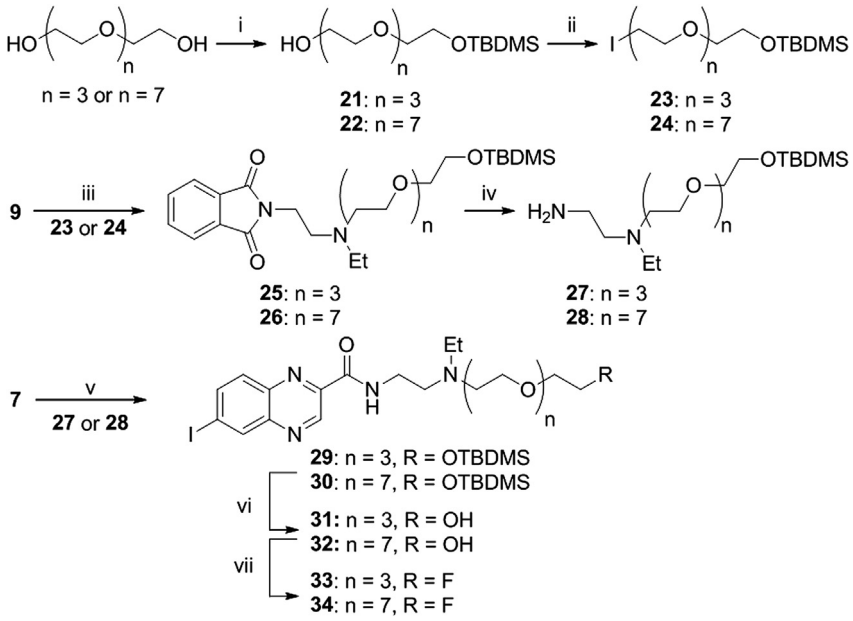
To improve the solubility of final compounds in water, hydrochloride salts **38**–**42** were prepared in 78–89% yields by treatment of corresponding amides **8**, **17**, **20**, **33** and **37** respectively, with an anhydrous 2 N HCl/ether solution (Table 1). However, in the case of derivatives **14** and **34**, the conversion into hydrochloride salts failed due to unexpected chlorine-for-fluorine nucleophilic substitution.

2.2. Radiochemistry

Derivatives **14**, **34**, **38**–**42** were radioiodinated at low specific activity using a nucleophilic aromatic isotopic exchange reaction with no-carrier-added [^{125}I]NaI in the presence of copper sulphate and under acidic conditions (Table 2). The radiolabelling process for each radiotracer was optimized with respect to the following reaction parameters: temperature, solvent and reaction time. The highest radiolabelling efficiencies were usually achieved after 25–45 min heating at 130 °C in either acetic acid solution (method A) or citrate buffer (pH = 4, method B). According to radio-thin layer chromatography (radio-TLC) and radio-reversed phase-high pressure liquid chromatography (radio-RP-HPLC) analyses of the reaction mixtures, only a limited number of by-products were produced when using these optimized reaction parameters. After purification on an Extrelut® column and then semi-preparative RP-HPLC, expected radiotracers [^{125}I]**14**, [^{125}I]**34** and [^{125}I]**38**–**42** were produced with moderate radiochemical yields (RCY, 8–50%), high radiochemical purities (RCP, >96%) and with a specific activity (SA) range of 4.0–39.6 MBq/μmol (Table 2).



Scheme 2. Syntheses of compounds **17** and **20**. Reagents and conditions: (i) CH_3CN, rt, 60 h; (ii) *p*-toluenesulfonyl chloride, KOH, Et_2O, −15 °C then 0 °C, 2 h; (iii) TBAF, THF, reflux, 2 h; (iv) CH_3CN, rt, 72 h.



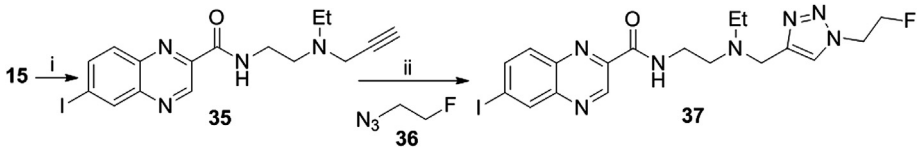
Scheme 3. Syntheses of pegylated derivatives **33** and **34**. Reagents and conditions: (i) (a) NaH, THF, 0 °C, 5 min; (b) TBDMSCl, rt, 1 or 4 h; (ii) imidazole, Ph_3P, I_2, CH_2Cl_2, 0 °C, 30 min and then rt, 1 h or 48 h; (iii) K_2CO_3, CH_3CN, 50 °C, 6 or 7 d; (iv) $NH_2NH_2{\cdot}H_2O$, EtOH, rt, 20 h; (v) THF, rt, 21 h; (vi) TBAF, THF, rt, 1.5 h or 2 h; (vii) DAST, DME, −50 °C, 15 min then rt, 1.5 h.

2.3. *In vivo* screening in melanoma-bearing mice

The pharmacological evaluation of all final radiotracers was performed according to the standard protocol established by Miot-Noirault et al. [43]. The *in vivo* gamma imaging screening of the [^{125}I]-labelled radiotracers was carried out in a B16F0-bearing mouse model and high tumoural uptake was globally observed (Table 3). For all the radiotracers tested, tumour accumulation was clearly evidenced from the first gamma scintigrams obtained at 1 h post injection (p.i.) (Fig. 3). Also, the tumours remained visible for at least 10 days after injection of the radioactive compounds. At early time points, radioactivity was mainly localized in the abdominal region, consistent with the previously observed elimination of this class of compounds *via* the urinary and hepatobiliary systems [28]. Tumours were visualized with a high contrast due to a rapid clearance of the radioactivity from the non-target organs (e.g. Fig. 3, 24 h p.i. scintigram).

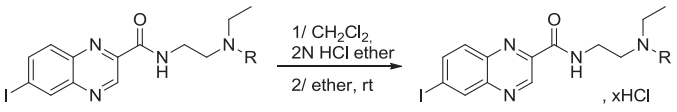
Compared with our reference radiotracer [^{125}I]**3**, a significantly higher uptake of radioactivity in the melanoma tumour was observed with [^{125}I]**38** (Table 3, entry 4: 21.4 ± 4.9% injected dose/gram (ID/g) *vs.* entry 1: 12.7 ± 2.3% ID/g for [^{125}I]**3** at 1 h p.i.). Unfortunately, [^{125}I]**38** administered by the intravenous (i.v.) route proved highly toxic, with only one mouse out of six surviving 2 days after injection. By contrast, compound [^{125}I]**34** exhibited a lower tumoural uptake than [^{125}I]**3** (Table 3, entry 3). With regard to radiotracers [^{125}I]**14** and [^{125}I]**39**–**42**, specific accumulation of the radioactivity in B16F0 melanoma tumours, at least comparable or higher than [^{125}I]**3** was evidenced. Interestingly, similar uptake values were observed at early time points after injection of [^{125}I]**40** or [^{125}I]**3**, but from 24 h p.i. a significantly higher retention of radioactivity in the tumour was noted for [^{125}I]**40** (11.6 ± 1.7% ID/g for [^{125}I]**40** *vs.* 5.2 ± 1.2% ID/g for [^{125}I]**3** at 5 days p.i.). Biodistribution profiles of [^{125}I]**14** and [^{125}I]**39**–**42** are promising and consistent for PET imaging purposes. However, due to its chemical instability, derivative **14** bearing a 3-fluoropropyl side chain appeared less attractive than compounds **39**–**42**. According to us, and in regard to melanoma uptake, [^{125}I]**39**–**42** were the most promising and non toxic radiotracers. This was confirmed by the high tumour to muscle ratios determined for these compounds as early as 1 h p.i. (in the range of 5.76 ± 1.22–7.84 ± 2.83).

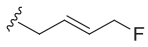
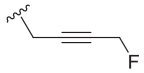
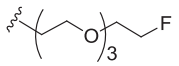
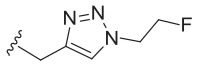
To provide further data on the potential of this new class of compounds for targeted radionuclide therapy of melanoma, we also estimated the dosimetry parameters of the corresponding [^{131}I]-labelled analogues. Based on the radioactive concentration values calculated from planar scintigraphic images, effective radiation doses that could be delivered to melanoma tumours were calculated using an adaptation of the MIRD software to the mouse and expressed as cGy/injected MBq (Table 4). Compared with [^{131}I]**3** (106.4 cGy/injected MBq), the estimated effective doses were slightly lower for compounds [^{131}I]**14**, [^{131}I]**34** and [^{131}I]**39** (93.0, 58.9 and 98.9 cGy/injected MBq respectively). However, promising results were obtained with tracers [^{131}I]**38**, [^{131}I]**40** and [^{131}I]**42** (164.8, 140.1 and 150.5 cGy/injected MBq respectively). The absorbed doses would be respectively 1.55, 1.32 and 1.41 times higher than when using [^{131}I]**3**, our previous lead tracer.



Scheme 4. Synthesis of compound **37**. Reagents and conditions: (i) propargyl bromide, NEt_3, EtOH, 72 h, rt. (ii) (a) CsF, 2-azidoethyl *p*-toluenesulfonate [41], *tert*-amyl alcohol, CH_3CN, 100 °C, 2 h; (b) 1.0 M aq. $CuSO_4$. $5H_2O$, 1.0 M aq. ascorbic acid, rt, 12 h.

Table 1
Syntheses of hydrochloride salts **38**–**42**.

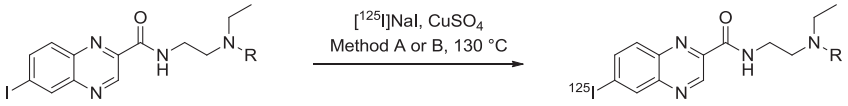


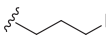
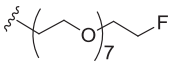
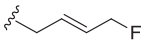
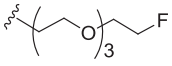
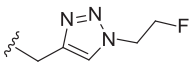
Entry	Starting matérial	R	Reaction time at rt	Product	*x*	Yield (%)
1	**8**		14 h	**38**	2	78
2	**17**		12 h	**39**	2	89
3	**20**		12 h	**40**	2	81
4	**33**		5 min	**41**	2	83
5	**37**		12 h	**42**	3	86

3. Conclusion

In the present manuscript, we report on the synthesis, radiolabelling with iodine-125 and *in vivo* screening using gamma scintigraphic imaging of seven new iodinated and fluorinated matched-pair radiotracers for SPECT/PET imaging and targeted radionuclide therapy of melanoma. This investigation revealed that the replacement of the (2-fluoropyridin-3-yloxy)ethyl side chain of our lead structure **3** by different fluoroaliphatic moieties preserved the affinity of this class of compounds for melanoma *in vivo* and even enhanced the uptake of radioactivity in the tumour in some cases. We consider that tracers **39**–**42** hold great potential for PET imaging of melanoma due to their high and specific accumulation in the murine B16F0 tumours even at early time points after injection (12.1–18.3% ID/g at 3 h p.i. and 12.1–23.3% ID/g at 6 h p.i. for [^{125}I]**39**–**42**). With this end in view, radiolabelling of these compounds with fluorine-18 and their preclinical evaluation as PET imaging probes are currently under way in our laboratory. In

Table 2
Labelling conditions and radiochemical data for [^{125}I]**14**, [^{125}I]**34**, and [^{125}I]**38**–**42**.



Entry	Starting matérial	R	Product	Method	Reaction time (min)	HPLC R_t (min)[a]	RCY (%)[b]	RCP (%)[c]	SA (MBq/μmol)[d]
1	**14**		[^{125}I]**14**	A[e]	30	17.4	16	>99.0	4.0
2	**34**		[^{125}I]**34**	B[e]	25	13.5	22	>99.0	15.6
3	**38**		[^{125}I]**38**	A	30	10.9	33	97.2	39.6
4	**39**		[^{125}I]**39**	B	30	14.2	34	98.3	22.9
5	**40**		[^{125}I]**40**	B	40	15.5	8	96.3	5.2
6	**41**		[^{125}I]**41**	B	25	15.6	50	>99.0	19.3
7	**42**		[^{125}I]**42**	B	45	11.6	35	98.8	34.6

[a] HPLC R_t: HPLC retention time.
[b] Radiochemical yields (RCY) were calculated by dividing the radioactivity of the final product by the initial amount of radioactive sodium iodide introduced.
[c] Radiochemical purities (RCP) were assessed by RP-HPLC analyses.
[d] SA: specific activity.
[e] The final conversion step for the synthesis of corresponding hydrochloride salt has not been performed.

Table 3
Uptake of radioactivity in melanoma B16F0 tumours at different time points after i.v. injection of [^{125}I]**14**, [^{125}I]**34**, and [^{125}I]**38**–**42** in C57BL/6J mice. Data for [^{125}I]**3** were already published [32] and included for comparison. Radioactive concentration values were calculated from planar scintigraphic images [43].

% ID/g ± SD in B16F0 tumour										
Entry	Cpd	1 h	3 h	6 h	24 h	72 h	5 d	7 d	10 d	14 d
1	[^{125}I]**3**	12.7 ± 2.3[b]	20.2 ± 4.2[b]	18.8 ± 1.6[b]	18.2 ± 0.6[b]	10.3 ± 1.7[b]	5.2 ± 1.2[b]	3.2 ± 0.4[b]	1.7 ± 0.4[b]	0.6 ± 0.3[b]
2	[^{125}I]**14**	10.0 ± 2.8[c]	13.5 ± 0.4[c]	15.3 ± 0.7[c]	11.4 ± 3.0[b]	8.5 ± 1.6[a]	5.4 ± 0.5[a]	3.0 ± 0.1[a]	1.4 ± 0.2[a]	0.8 ± 0.1[a]
3	[^{125}I]**34**	6.8 ± 1.8[c]	7.8 ± 1.5[c]	7.2 ± 1.1[c]	6.8 ± 1.7[c]	3.8 ± 0.7[c]	2.7 ± 0.3[c]	1.5 ± 0.2[b]	0.8 ± 0.3[b]	0.3 ± 0.01[a]
4	[^{125}I]**38**	21.4 ± 4.9[d]	22.5 ± 3.6[d]	25.0 ± 8.0[d]	24.8 ± 6.5[d]	20.0	8.4	n.d.	1.6	0.6
5	[^{125}I]**39**	10.8 ± 2.3[b]	12.3 ± 2.8[b]	13.9 ± 1.3[b]	15.0 ± 0.5[b]	10.0 ± 0.9[b]	5.6±1[b]	3.3 ± 0.6[b]	1.9 ± 0.3[b]	1.3 ± 0.6[b]
6	[^{125}I]**40**	12.3 ± 4.9[b]	18.3 ± 1.5[b]	23.3 ± 3.5[b]	23.5 ± 1.4[b]	14.9 ± 1.8[b]	11.6 ± 1.7[b]	8.0 ± 0.2[b]	4.2 ± 0.2[b]	2.2 ± 0.2[b]
7	[^{125}I]**41**	11.7 ± 2.2[f]	12.1 ± 1.7[e]	12.1 ± 1.9[e]	12.4 ± 2.3[e]	8.6 ± 3.6[e]	5.8 ± 2.4[e]	2.7 ± 0.8[e]	1.3 ± 0.4[e]	0.6 ± 0.3[e]
8	[^{125}I]**42**	13.0 ± 1.5[b]	14.5 ± 0.1[b]	15.9 ± 1.7[b]	15.6 ± 3.4[b]	11.0 ± 0.7[b]	8.6 ± 0.4[b]	5.3 ± 0.8[b]	3.7 ± 0.3[b]	1.8 ± 0.4[b]

n.d. non determined.
[a] $n = 2$.
[b] $n = 3$.
[c] $n = 4$.
[d] $n = 6$.
[e] $n = 7$.
[f] $n = 8$.

addition, with a fast clearance from non-target organs and very favourable dosimetry parameters, they are also new attractive candidates for efficient targeted radionuclide therapy of the disseminated disease and will be evaluated for this application.

4. Experimental section

4.1. Materials and methods

4.1.1. Chemistry

All reagents and solvents were purchased in the following commercial suppliers: Sigma Aldrich, Acros Organics, Carlo Erba and SDS. All solvents were dried using common techniques [44]. Unless otherwise noted, moisture sensitive reactions were conducted under dry argon. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates or neutral aluminium oxide 60 F_{254} plates (Merck or SDS) and visualized with UV light and/or developed with iodine, nynhydrin or potassium permanganate. Column chromatography was performed on silica gel 60A normal phase, 35–70 μm (Merck or SDS) or neutral aluminium oxide 90 standardized, 63–200 μm (Merck, column chromatographic adsorption analysis acc. to Brockmann). Uncorrected melting points (mp) were recorded on an electrothermal IA9300 (capillary) or a Reichert-Jung-Koffler apparatus. NMR spectra (400 or 200 MHz for 1H and 100 or 50 MHz for 13C) were recorded on a Bruker Avance 400 or 200 instrument; 19F NMR spectra (470 MHz) were recorded on a Bruker DRX 500 apparatus using tetrafluorotoluene as internal reference (−63 ppm). δ were expressed in ppm. Infrared spectra

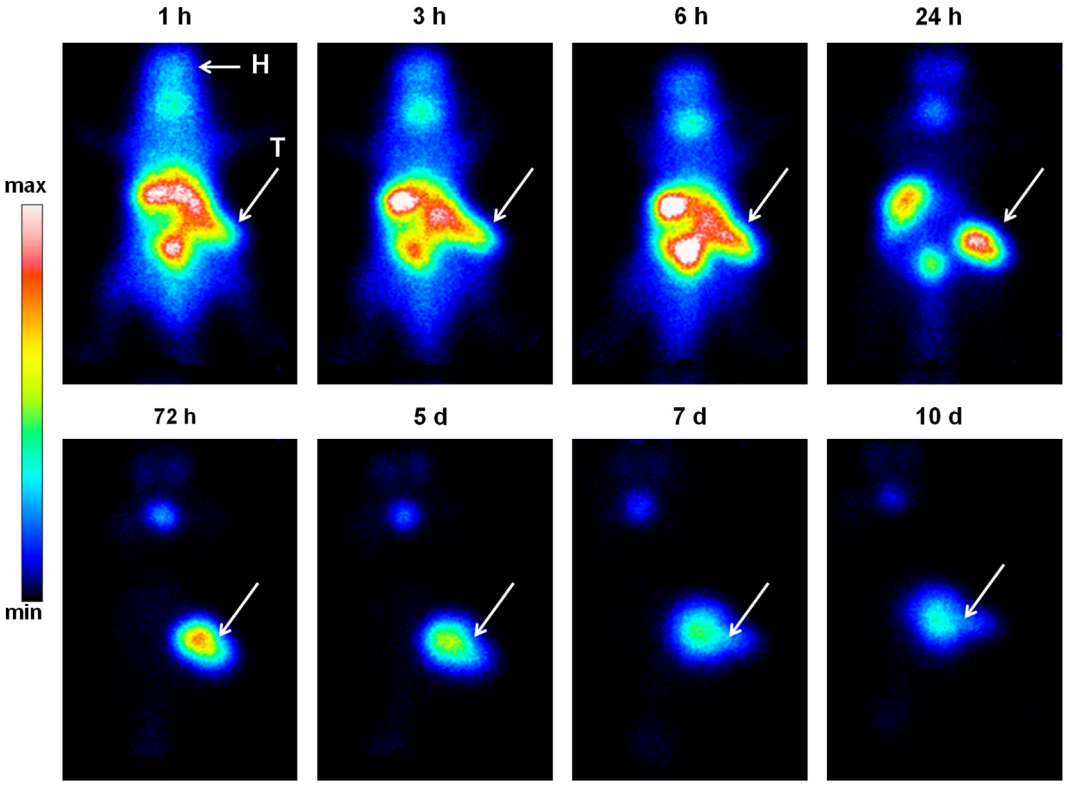


Fig. 3. Representative planar gamma scintigraphic images of a C57BL/6J mouse bearing a B16F0 melanoma tumour after i.v. injection of [^{125}I]**40** (3.7 MBq); T: tumour; H: head.

Table 4
Tumoural dosimetry parameters calculated from the MIRD software for corresponding radiotracers labelled with iodine-131.

Delivered dose		
Entry	Cpd	cGy/injected MBq
1	[^{131}I]**3**	106.4
2	[^{131}I]**14**	93.0
3	[^{131}I]**34**	58.9
4	[^{131}I]**38**	164.8
5	[^{131}I]**39**	98.9
6	[^{131}I]**40**	140.1
7	[^{131}I]**41**	103.5
8	[^{131}I]**42**	150.5

(IR) were recorded on a FTIR Nicolet Impact 410, a FT Vector 22 or a Nicolet IS10 with attenuated total reflectance (ATR) accessory. Electron impact mode mass spectra (MS) were obtained on a 5989A instrument (Agilent Technologies) or HP5890 series II chromatograph coupled to HP5985B mass spectrometer. The analysis of samples was performed in CH_3CN at a final concentration of 1 pmol/μL. Electrospray ionization mass spectra (ESI-MS) were recorded on TSQ 7000 ThermoQuest Finnigam (Les Ulis, France) or Esquire-LC (Bruker Daltonics, Wissenbourg, France) spectrometers. The analysis of samples was performed in CH_3CN at a final concentration of 1 pmol/μL, 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 and 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 indicated.

4.1.2. Radiochemistry

[^{125}I]NaI (3.7 GBq/mL, 643.8 MBq/mg) was purchased from PerkinElmer Life and Analytical Sciences (331 Treble Cove Road, Billerica, MA 01862, US) as a no-carrier-added solution in reductant free 1.0×10^{-5} M aqueous sodium hydroxide solution (pH 8–11). Extrelut® and citrate buffer solution (pH = 4) were purchased from Merck (Darmstadt, Germany). The radio TLC plates (Merck neutral aluminium oxide 60 F_{254} or Fluka, aluminium oxide on TLC-PET foils, 60Å, 0.2 mm) were developed with CH_2Cl_2/EtOH (98/2, v/v) and measured on an AMBIS 400 (Scanalytics, CSPI, San Diego, CA, USA). Analytical RP-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 mm, 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. For radiotracers [^{125}I]**38**, [^{125}I]**39**, [^{125}I]**40** and [^{125}I]**42**, semi-preparative RP-HPLC purifications were performed on a system including a Shimadzu LC 6A pump, SLC 6B controller, a CR5A integrator, a SPD 6AV UV detector and a flow-through gamma Raytest Steffi detector. The separation was carried out on a C_{18} column (ZORBAX 80Å, 4.6 × 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. For radiotracers [^{125}I]**14**, [^{125}I]**34** and [^{125}I]**41** semi-preparative RP-HPLC purification was performed on a Perkin Elmer system consisting of a Flexar LC autosampler and PDA detector, a Series 200 pump, a Peltier column oven and vacuum degasser, and a GabiStar Raytest detector. In these cases, the separation was carried out on a C_{18} column (SymmetryPrep C_{18}, 7.8 × 300 mm, 7 μm, Waters) using the following conditions: gradient time = 20 min, flow rate = 2.5 mL/min, H_2O/MeOH (30:70 → 0:100) (NH_4OH 0.2%), λ = 254 nm for [^{125}I]**14** or isocratic elution: flow rate = 3 mL/min, H_2O/MeOH (30:70) (NH_4OH 0.2%), λ = 254 nm for [^{125}I]**41** or flow rate = 2.5 mL/min, H_2O/MeOH (25:75) (NH_4OH 0.2%), λ = 254 nm for [^{125}I]**34**. All radiotracers were shown by radio-TLC and radio-RP-HPLC to be identical to the authentic non-radioactive material and to be free of significant chemical and radiochemical impurities.

4.1.3. Primary murine B16F0 melanoma model

Protocols were performed under the authorization of the French "Direction des Services Vétérinaires" (authorization no. C63-113-10) and conducted under the supervision of authorized investigators in conformity with the institution's recommendations for the use of laboratory animals. 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.

C57BL/6J male mice (6–8 weeks old) were obtained from Charles River (l'Arbresle, France) and the B16F0 syngenic melanoma from ATCC (no. CRL-6322). Primary melanoma model was induced by subcutaneous injection of B16F0 murine melanoma cells in C57BL/6J mice. B16F0 melanoma cells were cultured as described previously [32]. Briefly, B16F0 melanoma cell cultures were maintained as monolayers in Dulbecco's Modified Eagle's Medium (DMEM)/Glutamax (Invitrogen, Cergy Pontoise, France) supplemented with 10% foetal calf serum (Sigma, Saint Quentin Fallavier, France), 1% vitamins (Invitrogen, Cergy-Pontoise, France), 1 mM sodium pyruvate (Invitrogen), 1% non essential amino acids (Invitrogen), and 4 μg/mL of gentamycin base (Invitrogen). Cells were grown at 37 °C in a humidified incubator containing 5% CO_2. For transplantation, cells in exponential growth phase were trypsinized, washed with phosphate buffer saline (PBS), and resuspended in PBS. C57BL/6J mice anesthetized by isoflurane (2%) inhalation were inoculated with 3×10^5 melanoma B16F0 cells in 0.1 mL of PBS by subcutaneous injection on the right flank.

4.2. Chemistry

*4.2.1. N-[2-[N-Ethyl-N-(2-fluoroethyl)amino]ethyl]phthalimide (**5**)*

To a solution of alcohol **4** [32] (100 mg, 0.38 mmol) in anhydrous dimethoxyethane (DME, 7 mL) was added at −50 °C, (diethylamino)sulphur trifluoride (DAST, 100 μL, 0.76 mmol) under argon. The mixture was stirred at −50 °C for 15 min and then at room temperature for 1.5 h. Dichloromethane (5 mL) and a saturated aqueous sodium carbonate solution (5 mL) were successively added. The organic layer was dried on magnesium sulphate, filtered and evaporated under *vacuum*. The residue was purified by chromatography (SiO_2, ethyl acetate (EtOAc)/pentane, 1/1, v/v) to give compound **5** (51 mg, 0.19 mmol) as a white solid. Yield 50%; R_f (SiO_2, EtOAc/pentane, 1/1, v/v) 0.53; mp 57–59 °C; IR (KBr) ν 1023, 1403, 1715, 1769, 2700–3000 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (t, 3H, J = 7.1 Hz, C<u>H</u>$_3$), 2.68 (q, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH$_3$), 2.86 (m, 4H, (CO)$_2$NCH$_2$C<u>H</u>$_2$N, NC<u>H</u>$_2$CH$_2$F), 3.78 (t, 2H, J = 6.6 Hz, (CO)$_2$NC<u>H</u>$_2$CH$_2$N), 4.47 (td, 2H, $^2J_{H-F}$ = 47.5 Hz, J = 5.1 Hz, C<u>H</u>$_2$F), 7.70 (m, 2H, H-5, H-6), 7.83 (m, 2H, H-4, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.8, 35.8, 48.5, 51.3, 53.3 (d, $^2J_{C-F}$ = 21 Hz), 82.5 (d, $^1J_{C-F}$ = 168 Hz), 123.3 (2C), 132.2 (2C), 134.0 (2C), 168.5 (2C); ^{19}F NMR ($CDCl_3$) δ −220.20 (tt, $^3J_{H-F}$ = 25.0 Hz, $^2J_{H-F}$ = 47.5 Hz); MS *m*/*z* 264 (M^+, 1), 104 (100), 76 (38), 56 (18).

*4.2.2. N-(2-Aminoethyl)-N-ethyl-N-(2-fluoroethyl)amine (**6**)*

To a stirred solution of **5** (752 mg, 2.85 mmol) in absolute ethanol (30 mL) was added hydrazine monohydrate (324 μL, 6.68 mmol). The mixture was refluxed for 1 h. After cooling to room temperature, the white precipitate was filtered, washed with ethanol (10 mL) and the filtrate was evaporated under *vacuum*. The residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH/

NH_4OH, 80/19/1, v/v/v) to give compound **6** (213 mg, 1.59 mmol) as an orange-coloured oil. Yield 56%; R_f (Al_2O_3, CH_2Cl_2/EtOH/NH_4OH, 80/19/1, v/v/) 0.41; IR (CCl_4) ν 1034, 1453, 1684, 2700–3000 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.01 (t, 3H, J = 7.1 Hz, CH_3), 2.59 (m, 4H, CH_2CH_3, $H_2NCH_2CH_2N$), 2.74 (td, 2H, $^3J_{H-F}$ = 26.5 Hz, J = 5.3 Hz, CH_2CH_2F), 2.78 (t, 2H, J = 5.3 Hz, $H_2NCH_2CH_2N$), 4.30 (br s, 2H, NH_2), 4.46 (td, 2H, $^2J_{H-F}$ = 47.5 Hz, J = 5.0 Hz, CH_2F); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.7, 38.8, 48.3, 53.4 (d, $^2J_{C-F}$ = 20 Hz), 54.9, 82.7 (d, $^1J_{C-F}$ = 167 Hz); ^{19}F NMR ($CDCl_3$) δ −218.92; ESI-MS m/z 135.0 $[M + H]^+$.

4.2.3. N-[2-[[N-Ethyl-N-(2-fluoroethyl)]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**8**)

To a stirred solution of amine **6** (110 mg, 0.80 mmol) in anhydrous tetrahydrofuran (THF, 15 mL) was added *p*-nitrophenyl 6-iodoquinoxaline-2-carboxylate (**7**) [36] (200 mg, 0.48 mmol) under argon. The mixture was stirred at room temperature overnight. The solvent was evaporated under *vacuum* and the residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH, 98/2, v/v) to give compound **8** (190 mg, 0.46 mmol) as an orange-coloured oil. Yield 96%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 98/2, v/v) 0.72; IR (CCl_4) ν 1474, 1522, 1685, 2855, 2927 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, 3H, J = 7.1 Hz, CH_3), 2.75 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.87 (t, 1H, J = 6.0 Hz, $(CO)NHCH_2CH_2N$), 2.92 (td, 2H, $^3J_{H-F}$ = 26.8 Hz, J = 5.0 Hz, CH_2CH_2F), 3.62 (q, 2H, J = 6.0 Hz, $(CO)NHCH_2CH_2N$), 4.58 (td, 2H, $^2J_{H-F}$ = 47.7 Hz, J = 5.0 Hz, CH_2F), 7.81 (d, 1H, J = 8.8 Hz, H-8), 8.05 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.45 (br s, 1H, NH), 8.58 (d, 1H, J = 1.8 Hz, H-5), 9.62 (s, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 37.3, 48.5, 52.9, 53.6 (d, $^2J_{C-F}$ = 20 Hz), 82.5 (d, $^1J_{C-F}$ = 167 Hz), 98.0, 130.9, 138.6, 139.6, 139.7, 144.0, 144.4, 144.6, 163.1; ^{19}F NMR ($CDCl_3$) δ −220.22 (tt, $^3J_{H-F}$ = 26.4 Hz, $^2J_{H-F}$ = 47.4 Hz); MS m/z 416 (M^+, 1), 104 (100), 76 (12), 56 (8).

4.2.4. N-[2-[N-[3-(tert-Butyldimethylsilyloxy)propyl]-N-ethylamino]ethyl]phthalimide (**10**)

To a solution of phthalimide **9** [37] (1.00 g, 3.95 mmol) in anhydrous acetonitrile (30 mL) were successively added, under argon, potassium carbonate (1.09 g, 7.90 mmol) and commercially available (3-bromopropoxy)-*tert*-butyldimethylsilane (1.00 g, 3.95 mmol). The mixture was then stirred at 50 °C for 36 h. After cooling to room temperature, a saturated aqueous sodium carbonate solution (90 mL) was added and the aqueous layer was then extracted with dichloromethane (3 × 60 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The obtained liquid residue was purified by chromatography (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) to give compound **10** (600 mg, 1.54 mmol) as a colourless liquid. Yield 39%; R_f (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) 0.25; IR (ATR diamond accessory) ν 777, 838, 1101, 1256, 1397, 1435, 1470, 1718, 2857, 2930, 2955 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ −0.02 (s, 6H, $(CH_3)_2Si$), 0.84 (s, 9H, $(CH_3)_3C$), 0.97 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.57 (quint., 2H, J = 6.8 Hz, $NCH_2CH_2CH_2O$), 2.53 (m, 4H, CH_2CH_3, $NCH_2CH_2CH_2O$), 2.68 (t, 2H, J = 6.9 Hz, $(CO)_2NCH_2CH_2N$), 3.53 (t, 2H, J = 6.3 Hz, $NCH_2CH_2CH_2O$), 3.75 (t, 2H, J = 6.9 Hz, $(CO)_2NCH_2CH_2N$), 7.69 (m, 2H, H-5, H-6), 7.82 (m, 2H, H-4, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.3 (2C), 12.0, 18.3, 26.0 (3C), 30.5, 36.2, 47.6, 50.2, 50.9, 61.2, 123.2 (2C), 132.3 (2C), 133.9 (2C), 168.4 (2C); ESI-MS m/z 391.3 $[M + H]^+$.

4.2.5. N-[3-(tert-Butyldimethylsilyloxy)propyl]-N-ethyl-1,2-ethylenediamine (**11**)

To a solution of phthalimide **10** (400 mg, 1.02 mmol) in absolute ethanol (12 mL) was added hydrazine monohydrate (500 µL, 10.24 mmol). The mixture was stirred at room temperature for 18 h and cooled to 0 °C for 2 h. The resulting white precipitate was filtered, washed successively with ice-cold ethanol (10 mL) and dichloromethane (10 mL) and the filtrate was evaporated under *vacuum*. The residue was suspended in dichloromethane (10 mL) and the remaining precipitate was filtered again and washed with dichloromethane (10 mL). The last steps were repeated until no more white solid appeared after evaporation of the filtrate. Amine **11** (266 mg, 1.02 mmol) was obtained as a colourless oil and used without further purification. Yield quant.; R_f (SiO_2, CH_2Cl_2/EtOH, 9/1, v/v) 0.15; IR (NaCl) ν 776, 836, 1099, 1255, 1472, 1575, 2857, 2955, 3200–3400 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.88 (s, 9H, $(CH_3)_3C$), 1.00 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.62 (quint., 2H, J = 6.8 Hz, $NCH_2CH_2CH_2O$), 2.49 (m, 6H, CH_2CH_3, $NCH_2CH_2CH_2O$, $(CO)_2NCH_2CH_2N$), 2.72 (t, 2H, J = 6.1 Hz, $(CO)_2NCH_2CH_2N$), 3.63 (t, 2H, J = 6.3 Hz, $NCH_2CH_2CH_2O$); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.3 (2C), 11.9, 18.4, 26.0 (3C), 30.4, 39.9, 47.8, 50.1, 56.8, 61.4.

4.2.6. N-[2-[N-Ethyl-N-[3-(tert-butyldimethylsilyloxy)propyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**12**)

To a suspension of activated ester **7** [36] (295 mg, 0.70 mmol) in anhydrous THF (4 mL) was added, a solution of amine **11** (283 mg, 1.09 mmol) in anhydrous THF (5 mL) under argon. The mixture was then stirred at room temperature overnight, poured into a 1 N aqueous sodium hydroxide solution (30 mL) and extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The crude product was then purified by chromatography (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) to give compound **12** (368 mg, 0.68 mmol) as a yellow solid. Yield 97%; R_f (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) 0.20; mp 60–62 °C; IR (ATR diamond accessory) ν 775, 836, 1099, 1255, 1357, 1387, 1473, 1519, 1594, 1660, 1684, 2855, 2930, 2955, 3376 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(CH_3)_2Si$), 0.84 (s, 9H, $(CH_3)_3C$), 1.07 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.69 (quint., 2H, J = 6.7 Hz, $NCH_2CH_2CH_2O$), 2.62 (m, 4H, CH_2CH_3, $NCH_2CH_2CH_2O$), 2.70 (t, 2H, J = 6.0 Hz, $(CO)_2NCH_2CH_2N$), 3.56 (q, 2H, J = 5.8 Hz, $(CO)_2NCH_2CH_2N$), 3.70 (t, 2H, J = 6.2 Hz, $NCH_2CH_2CH_2O$), 7.82 (d, 1H, J = 8.8 Hz, H-8), 8.06 (dd, 1H, J = 1.9, 8.8 Hz, H-7), 8.41 (br s, 1H, NH), 8.60 (d, 1H, J = 1.9 Hz, H-5), 9.64 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 12.1, 18.3, 26.0 (3C), 30.6, 37.3, 47.6, 49.9, 52.3, 61.1, 97.9, 130.9, 138.6, 139.6 (2C), 144.2, 144.4, 144.7, 162.9; ESI-MS m/z 543.2 $[M + H]^+$.

4.2.7. N-[2-[N-Ethyl-N-(3-hydroxypropyl)amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**13**)

To a stirred solution of compound **12** (428 mg, 0.79 mmol) in THF (13 mL) was added a solution of tetrabutylammonium fluoride (TBAF) 1 M in THF (1.18 mL, 1.18 mmol). The mixture was stirred at room temperature for 1.5 h and the reaction was stopped by addition of a saturated aqueous sodium hydrogencarbonate solution (100 mL), followed by distilled water (50 mL) and then ethyl acetate (50 mL). After decantation, the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with brine (50 mL), dried on magnesium sulphate, filtered and evaporated under *vacuum*. The residue was then purified by chromatography (SiO_2, CH_2Cl_2/EtOH, 85/15 and then 80/20, v/v) to give compound **13** (249 mg, 0.58 mmol) as a yellow solid. Yield 74%; R_f (SiO_2, CH_2Cl_2/EtOH, 9/1, v/v) 0.24; mp 78–80 °C; IR (ATR diamond accessory) ν 1064, 1351, 1476, 1527, 1593, 1679, 2835, 2940, 3326, 3462 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.04 (t, 3H, J = 7.1 Hz, CH_3), 1.73 (quint., 2H, J = 5.7 Hz, $NCH_2CH_2CH_2O$), 2.64 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.74 (m, 4H, $CONHCH_2CH_2N$, $NCH_2CH_2CH_2O$), 3.64 (q, 2H, J = 6.1 Hz, $CONHCH_2CH_2N$), 3.82 (t, 2H, J = 5.4 Hz, $NCH_2CH_2CH_2O$), 7.81 (d, 1H, J = 8.8 Hz, H-8), 8.04 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.32 (br s, 1H, NH), 8.57 (d, 1H, J = 1.8 Hz, H-5), 9.60 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.6, 28.4, 37.5, 47.7, 52.8, 53.5, 63.6, 98.2, 130.9, 138.5, 139.5, 139.8, 143.8, 144.4, 144.5, 163.2; ESI-MS m/z 429.1 $[M + H]^+$.

4.2.8. N-[2-[N-Ethyl-N-(3-fluoropropyl)amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**14**)

To a solution of alcohol **13** (20 mg, 46.7 μmol) in anhydrous DME (0.8 mL) was added under argon and at −50 °C, DAST (12.5 μL, 93.4 μmol). The reaction media was stirred at −50 °C for 15 min, then at room temperature for 1.5 h, and poured into a saturated aqueous sodium hydrogencarbonate solution (10 mL). The mixture was extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The residue was purified by chromatography (SiO_2, EtOAc) to give compound **14** (7 mg, 16.3 μmol) as a yellow oil. Yield 35%; R_f (SiO_2, EtOAc) 0.36; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.10 (t, 3H, J = 7.1 Hz, C$\underline{H}_3$), 1.82 (quint., 2H, J = 6.5 Hz, NCH$_2$C$\underline{H}_2$CH$_2$F), 2.64 (m, 6H, C$\underline{H}_2$CH$_3$, CONHCH$_2$C$\underline{H}_2$N, NC$\underline{H}_2$CH$_2$CH$_2$F), 3.57 (q, 2H, J = 5.9 Hz, CONHC$\underline{H}_2$CH$_2$N), 3.89 (m, 2H, NCH$_2$CH$_2$C$\underline{H}_2$F), 7.87 (d, 1H, J = 8.8 Hz, H-8), 8.08 (dd, 1H, J = 1.9, 8.8 Hz, H-7), 8.41 (br s, 1H, NH), 8.60 (d, 1H, J = 1.9 Hz, H-5), 9.63 (s, 1H, H-3); ^{19}F NMR (CD_3CN) δ −220.47; ESI-MS m/z 431.1 $[M + H]^+$.

4.2.9. N-[2-[N-Ethyl-N-[(E)-4-fluorobut-2-enyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**17**)

To a solution of (*E*)-4-fluoro-but-2-enyl toluene-4-sulfonate (**16**) [38] (380 mg, 1.56 mmol) in anhydrous acetonitrile (16 mL) was added dropwise and under argon a solution of *N*-(2-ethylaminoethyl)-6-iodoquinoxaline-2-carboxamide (**15**) [36] (464 mg, 1.13 mmol) in anhydrous acetonitrile (17 mL). The mixture was stirred at room temperature for 60 h. The solvent was then evaporated under *vacuum* and the residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give compound **17** (235 mg, 0.53 mmol) as a yellow solid. Yield 47%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.36; mp 50–52 °C (dec.); IR (CCl_4) ν 1160, 1353, 1475, 1520, 1682, 2817, 2850–3000, 3409 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.08 (t, 3H, J = 7.1 Hz, C$\underline{H}_3$), 2.63 (q, 2H, J = 7.1 Hz, C$\underline{H}_2$CH$_3$), 2.72 (t, 2H, J = 6.2 Hz, (CO)NHCH$_2$C$\underline{H}_2$N), 3.21 (m, 2H, NC$\underline{H}_2$CH=CHCH$_2$F), 3.57 (q, 2H, J = 5.9 Hz, (CO)NHC$\underline{H}_2$CH$_2$N), 4.81 (dd, 2H, $^2J_{H-F}$ = 47.0 Hz, J = 4.3 Hz, C$\underline{H}_2$F), 5.90 (m, 2H, NCH$_2$C$\underline{H}$=C$\underline{H}$CH$_2$F), 7.81 (d, 1H, J = 8.8 Hz, H-8), 8.07 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.32 (m, 1H, NH), 8.59 (d, 1H, J = 1.8 Hz, H-5), 9.63 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.2, 37.4, 47.8, 51.9, 55.2 (d, $^4J_{C-F}$ = 1 Hz), 83.0 (d, $^1J_{C-F}$ = 162 Hz), 98.1, 127.4 (d, $^2J_{C-F}$ = 17 Hz), 130.9, 133.2 (d, $^3J_{C-F}$ = 12 Hz), 138.7, 139.7, 139.8, 144.1, 144.5, 144.7, 163.0; ^{19}F NMR ($CDCl_3$) δ −211.13 (t, $^2J_{H-F}$ = 48.2 Hz); ESI-MS m/z 442.9 $[M + H]^+$.

4.2.10. 1,4-bis(p-Toluenesulfonyloxy)but-2-yne (**18**)

This compound was synthesized according to the procedure described by Shine et al. for the tosylation of hex-3-yne-2,4-diol [39]. Briefly, to a stirred solution of *p*-toluenesulfonyl chloride (28.2 g, 0.15 mol) in diethylether (340 mL) was added commercially available 1,4-butynediol (5.00 g, 58.1 mmol). The mixture was cooled to −15 °C and potassium hydroxide (23.7 g, 0.42 mmol) was added dropwise. The resulting solution was stirred at 0 °C for 2 h and poured into cold water (200 mL). After return back to room temperature, the solution was decanted, the organic layer was collected while the aqueous layer was extracted with dichloromethane (3 × 200 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum* to give compound **18** (21.8 g, 55.3 mmol) as a beige solid. Yield 95%; mp 94–96 °C (Lit. [45]. : 94–95 °C); IR (KBr) ν 934, 1178, 1350, 1374, 2991 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.45 (s, 6H, 2C$\underline{H}_3$), 4.57 (s, 4H, 2C$\underline{H}_2$OTos), 7.35 (d, 4H, J = 8.3 Hz, 4H$_{ar}$), 7.76 (d, 4H, J = 8.3 Hz, 4H$_{ar}$).

4.2.11. 4-Fluoro-but-2-ynyl toluene-4-sulfonate (**19**)

To a stirred solution of ditosyl **18** (503 mg, 1.28 mmol) in anhydrous THF (20 mL) was added, a solution of TBAF 1.0 M in THF (1.57 mL, 1.57 mmol) under argon. The mixture was refluxed for 2 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and the residue was purified by chromatography (SiO_2, CH_2Cl_2) to give compound **19** (174 mg, 0.72 mmol) as an orange-coloured oil. Yield 56%; R_f (SiO_2, CH_2Cl_2) 0.71; IR (CCl_4) ν 1179, 1181, 1381, 2950 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.46 (s, 3H, C$\underline{H}_3$), 4.74 (td, 2H, $^5J_{H-F}$ = 6.7 Hz, J = 1.7 Hz, C$\underline{H}_2$OTos), 4.87 (td, 2H, $^2J_{H-F}$ = 33.7 Hz, J = 1.7 Hz, C$\underline{H}_2$F), 7.36 (d, 2H, J = 8.3 Hz, 2H$_{ar}$), 7.82 (d, 2H, J = 8.3 Hz, 2H$_{ar}$); ^{13}C NMR (50 MHz, acetone-d_6) δ 21.5, 58.5 (d, $^4J_{C-F}$ = 3 Hz), 70.9 (d, $^1J_{C-F}$ = 164 Hz), 82.8 (d, $^3J_{C-F}$ = 12 Hz), 83.8 (d, $^2J_{C-F}$ = 22 Hz), 128.9 (2C), 130.9 (2C), 134.1, 146.3; ^{19}F NMR ($CDCl_3$) δ −217.56.

4.2.12. N-[2-[[N-Ethyl-N-(4-fluorobut-2-ynyl)]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**20**)

To a stirred solution of compound **19** (491 mg, 2.03 mmol) in anhydrous acetonitrile (17 mL) was added dropwise amine **15** [36] (0.50 g, 1.35 mmol). After 72 h at room temperature, water (5 mL) and a 2.0 M aqueous sodium carbonate solution (5 mL) were added. The resulting solution was extracted with dichloromethane (3 × 10 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give compound **20** (226 mg, 0.51 mmol) as a yellow oil. Yield 38%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.86; IR (CCl_4) ν 1475, 1522, 1685, 2928 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 1.11 (t, 3H, J = 7.2 Hz, C$\underline{H}_3$), 2.64 (q, 2H, J = 7.2 Hz, C$\underline{H}_2$CH$_3$), 2.80 (t, 2H, J = 6.1 Hz, (CO)NHCH$_2$C$\underline{H}_2$N), 3.59 (m, 4H, (CO)NHC$\underline{H}_2$CH$_2$N, NC$\underline{H}_2$C≡C), 4.97 (td, 2H, $^2J_{H-F}$ = 47.5 Hz, J = 1.7 Hz, C$\underline{H}_2$F), 7.80 (d, 1H, J = 8.8 Hz, H-8), 8.05 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.25 (br s, 1H, NH), 8.58 (d, 1H, J = 1.8 Hz, H-5), 9.62 (s, 1H, H-3); ^{13}C NMR (50 MHz, acetone-d_6) δ 12.9, 37.7, 42.2 (d, $^4J_{C-F}$ = 3 Hz), 48.3, 52.9, 71.5 (d, $^1J_{C-F}$ = 161 Hz), 80.3 (d, $^2J_{C-F}$ = 22 Hz), 85.5 (d, $^3J_{C-F}$ = 12 Hz), 98.3, 131.8, 139.1, 140.2, 140.5, 145.0, 145.3, 145.5, 163.5; ^{19}F NMR ($CDCl_3$) δ −213.79; ESI-MS m/z 441.1 $[M + H]^+$.

4.2.13. 2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (**21**)

A solution of sodium hydride (60% in mineral oil, 2.94 g, 73.5 mmol) in anhydrous THF (135 mL) was stirred at room temperature for 5 min under argon. Dried tetraethylene glycol (8.80 mL, 51.0 mmol) was added dropwise at 0 °C and the reaction mixture was stirred for 5 more min before addition of *tert*-butyldimethylsilyl chloride (TBDMSCl, 12.00 g, 79.6 mmol). The resulting mixture was stirred at room temperature for 1 h and the reaction was stopped by addition of water (150 mL). The mixture was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined, washed successively with a saturated aqueous ammonium chloride solution (150 mL) and brine (150 mL), dried on magnesium sulphate, filtered and evaporated under *vacuum*. The crude product was purified by chromatography (SiO_2 pad, EtOAc/pentane, 75/25, v/v then 50/50, v/v and finally CH_2Cl_2/EtOH, 98/2, v/v) to yield compound **21** (6.42 g, 20.8 mmol) as a yellow oil. Yield 41%; R_f (SiO_2, EtOAc/pentane, 75/25, v/v) 0.16; IR (ATR diamond accessory) ν 778, 837, 1109, 1255, 1472, 2859, 2930, 3300–3600 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.05 (s, 6H, (C$\underline{H}_3$)$_2$Si), 0.88 (s, 9H, (C$\underline{H}_3$)$_3$C), 2.63 (br s, 1H, O$\underline{H}$), 3.65 (m, 16H, 8C$\underline{H}_2$O); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 18.4, 26.0 (3C), 61.8, 62.8, 70.4–70.7 (4C), 72.6, 72.7; MS m/z 251 (M-57, 9), 163 (26), 119 (46), 103 (55), 89 (100), 75 (93).

4.2.14. 2,2,3,3-Tetramethyl-4,7,10,13,16,19,22,25-octaoxa-3-silaheptacosan-27-ol (**22**)

This compound was synthesized according to the procedure described for **21**, starting from octaethylene glycol (6.01 g,

16.2 mmol), sodium hydride (60% in mineral oil, 0.91 g, 22.7 mmol) and TBDMSCl (3.67 g, 24.3 mmol). Reaction time: 4 h at room temperature; the purification was performed using column chromatography (SiO_2, EtOAc/EtOH, 95/5, v/v then 8/2, v/v) to give compound **22** (5.32 g, 11.0 mmol) as a yellow oil. Yield 68%; R_f (SiO_2, EtOAc/EtOH, 95/5, v/v) 0.21; IR (NaCl) ν 778, 836, 1106, 1254, 2873, 2927, 3100–3600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.00 (s, 6H, $(C\underline{H}_3)_2Si$), 0.83 (s, 9H, $(C\underline{H}_3)_3C$), 3.58 (m, 32H, $16C\underline{H}_2O$); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 18.4, 26.0 (3C), 61.7, 62.7, 70.4–70.6 (12C), 72.6, 72.7; MS *m*/*z* 251 (13), 233 (6), 207 (53), 189 (12), 163 (34), 145 (15), 119 (49), 115 (27), 89 (100), 75 (46).

4.2.15. 15-Iodo-2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecane (**23**)

To a stirred solution of alcohol **21** (5.00 g, 16.2 mmol) in anhydrous dichloromethane (150 mL) were successively added imidazole (1.44 g, 21.1 mmol), triphenylphosphine (5.53 g, 21.1 mmol) and iodine (5.35 g, 21.1 mmol) at 0 °C under argon. The mixture was stirred at 0 °C for 30 min, then at room temperature for 1 h. A 10% aqueous sodium bisulfite solution (170 mL) was added and the mixture was forcefully stirred for 2 min (until fading). After decantation the organic layer was collected and the aqueous layer was extracted with dichloromethane (3 × 80 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The obtained solid was suspended in *n*-pentane (150 mL) and the mixture was stirred at room temperature for 45 min before filtration. The precipitate was washed with *n*-pentane (30 mL). The filtrate was evaporated under *vacuum* and the oily residue was purified by chromatography (SiO_2 pad, CH_2Cl_2/EtOH, 98/2, v/v) to give compound **23** as a pale yellow oil (5.62 g, 13.4 mmol). Yield 83%; R_f (SiO_2, CH_2Cl_2/EtOH, 98/2, v/v) 0.25; IR (ATR diamond accessory) ν 778, 837, 1109, 1256, 1472, 2858, 2928 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (s, 6H, $(C\underline{H}_3)_2Si$), 0.87 (s, 9H, $(C\underline{H}_3)_3C$), 3.24 (t, 2H, J = 7.0 Hz, $C\underline{H}_2I$), 3.53 (t, 2H, J = 5.4 Hz, $OC\underline{H}_2CH_2OSi$), 3.64 (m, 8H, $4C\underline{H}_2O$), 3.73 (m, 4H, $ICH_2C\underline{H}_2$, $C\underline{H}_2OSi$); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 3.0, 18.4, 26.0 (3C), 62.8, 70.3–70.8 (4C), 72.1, 72.7; MS *m*/*z* 361 (M-57, 3), 317 (14), 273 (4), 229 (5), 199 (13), 155 (100).

4.2.16. 27-Iodo-2,2,3,3-tetramethyl-4,7,10,13,16,19,22,25-octaoxa-3-silaheptacosane (**24**)

This compound was synthesized according to the procedure described for **23**, starting from compound **22** (2.00 g, 4.13 mmol), imidazole (365 mg, 5.36 mmol), triphenylphosphine (1.41 g, 5.36 mmol) and iodine (1.36 g, 5.36 mmol). Reaction time: 2 d at room temperature; the purification was performed using column chromatography (SiO_2, EtOAc) to give compound **24** as a pale yellow oil (1.89 g, 3.18 mmol). Yield 77%; R_f (SiO_2, EtOAc) 0.30; IR (NaCl) ν 778, 835, 1108, 1253, 2859, 2927 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.06 (s, 6H, $(C\underline{H}_3)_2Si$), 0.89 (s, 9H, $(C\underline{H}_3)_3C$), 3.26 (t, 2H, J = 7.0 Hz, $C\underline{H}_2I$), 3.55 (t, 2H, J = 5.5 Hz, $OC\underline{H}_2CH_2OSi$), 3.65 (m, 24H, $12C\underline{H}_2O$), 3.70 (m, 4H, $C\underline{H}_2CH_2I$, $OCH_2C\underline{H}_2OSi$); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 3.0, 18.4, 26.0 (3C), 62.8, 70.3–70.7 (12C), 72.1, 72.7; MS *m*/*z* 361 (2), 331 (1), 287 (6), 243 (8), 233 (4), 199 (28), 189 (8), 159 (16), 155 (100), 145 (9), 115 (15).

4.2.17. N-(16-Ethyl-2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-16-aza-3-silaoctadecan-18-yl)phthalimide (**25**)

To a stirred solution of phthalimide **9** [37] (3.04 g, 12.0 mmol) in anhydrous acetonitrile (80 mL) were successively added potassium carbonate (1.65 g, 12.0 mmol) and compound **23** (5.00 g, 12.0 mmol) under argon. The mixture was heated at 50 °C for 6 d. After cooling to room temperature, a saturated aqueous sodium carbonate solution was added (200 mL) and the resulting solution was extracted with dichloromethane (3 × 150 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The oily residue was purified by chromatography (SiO_2, EtOAc) to afford compound **25** (4.38 g, 8.61 mmol) as a pale yellow oil. Yield 72%; R_f (SiO_2, EtOAc) 0.25; IR (NaCl) ν 837, 1105, 1254, 1396, 1468, 1592, 1713, 2858, 2900 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (s, 6H, $(C\underline{H}_3)_2Si$), 0.87 (s, 9H, $(C\underline{H}_3)_3C$), 0.97 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 2.61 (q, 2H, J = 7.1 Hz, $C\underline{H}_2CH_3$), 2.74 (m, 4H, $(CO)_2NCH_2C\underline{H}_2N$, $NC\underline{H}_2CH_2O$), 3.57 (m, 12H, $6C\underline{H}_2O$), 3.76 (m, 4H, $C\underline{H}_2OSi$, $(CO)_2NC\underline{H}_2CH_2N$), 7.70 (m, 2H, H-5, H-6), 7.80 (m, 2H, H-4, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 12.0, 18.4, 26.0 (3C), 36.2, 48.4, 51.5, 53.0, 62.8, 69.8, 70.5, 70.6, 70.7, 70.8, 72.7, 123.2 (2C), 132.3 (2C), 133.9 (2C), 168.4 (2C); ESI-MS *m*/*z* 509.4 $[M + H]^+$.

4.2.18. N-(28-Ethyl-2,2,3,3-tetramethyl-4,7,10,13,16,19,22,25-octaoxa-28-aza-3-silaheptacosan-30-yl)phthalimide (**26**)

To a stirred solution of phthalimide **9** [37] (489 mg, 1.92 mmol) in anhydrous acetonitrile (15 mL) was added potassium carbonate (530 mg, 3.84 mmol) under argon. The reaction mixture was stirred at room temperature for 1 h before addition of compound **24** (1.14 g, 1.92 mmol). The mixture was heated at 50 °C for 7 d, cooled to room temperature and partitioned between a saturated aqueous sodium carbonate solution (50 mL) and dichloromethane (30 mL). After decantation, the organic layer was collected while the aqueous layer was extracted with dichloromethane (3 × 30 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The oily residue was purified by chromatography (SiO_2, CH_2Cl_2/EtOH, 95/5, v/v) to afford compound **26** (854 mg, 1.25 mmol) as a pale yellow oil. Yield 65%; R_f (SiO_2, CH_2Cl_2/EtOH, 95/05, v/v) 0.32; IR (ATR diamond accessory) ν 838, 1112, 1255, 1397, 1436, 1469, 1717, 2862, 2929 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 (s, 6H, $(C\underline{H}_3)_2Si$), 0.88 (s, 9H, $(C\underline{H}_3)_3C$), 0.99 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 2.66 (q, 2H, J = 7.5 Hz, $C\underline{H}_2CH_3$), 2.73 (m, 4H, $(CO)_2NCH_2C\underline{H}_2N$, $NC\underline{H}_2CH_2O$), 3.56 (m, 4H, $NC\underline{H}_2CH_2O$, $OC\underline{H}_2CH_2OSi$), 3.63 (m, 24H, $12C\underline{H}_2O$), 3.76 (m, 4H, $(CO)_2NC\underline{H}_2CH_2N$, $C\underline{H}_2OSi$), 7.70 (m, 2H, H-5, H-6), 7.83 (m, 2H, H-4, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 12.0, 18.4, 26.0 (3C), 36.1, 48.5, 51.4, 53.0, 62.8, 70.6 (13C), 72.7, 123.2 (2C), 132.3 (2C), 133.9 (2C), 168.4 (2C); ESI-MS *m*/*z* 685.5 $[M + H]^+$.

4.2.19. 16-Ethyl-2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-16-aza-3-silaoctadecan-18-amine (**27**)

To a stirred solution of **25** (1.50 g, 2.95 mmol) in ethanol (150 mL) was added hydrazine monohydrate (1.43 mL, 29.5 mmol). The mixture was stirred at room temperature for 20 h and then cooled at 0 °C for 3 h. The white precipitate was filtered and washed with ice-cold ethanol (50 mL) and dichloromethane (50 mL) and the filtrate was evaporated under *vacuum*. The residue was suspended in dichloromethane (20 mL) and the remaining precipitate was filtered again and washed with dichloromethane (20 mL). The last steps were repeated until no more white solid appeared after evaporation of the filtrate. Amine **27** (1.12 g, 2.95 mmol) was obtained as a pale yellow oil and used without further purification. Yield quant.; R_f (Al_2O_3, CH_2Cl_2/EtOH, 8/2, v/v) 0.32; IR (ATR diamond accessory) ν 834, 1107, 1252, 1472, 1574, 2857, 2929, 3100–3300 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 (s, 6H, $(C\underline{H}_3)_2Si$), 0.88 (s, 9H, $(C\underline{H}_3)_3C$), 1.01 (t, 3H, J = 7.1 Hz, $CH_2C\underline{H}_3$), 2.68 (m, 8H, $C\underline{H}_2CH_3$, $H_2NC\underline{H}_2CH_2N$, $H_2NCH_2C\underline{H}_2N$, $NC\underline{H}_2CH_2O$), 3.55 (m, 12H, $6C\underline{H}_2O$), 3.72 (m, 2H, $C\underline{H}_2OSi$); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 11.9, 18.4, 26.0 (3C), 39.7, 48.8, 52.7, 56.4, 62.8, 70.5 (5C), 72.7.

4.2.20. 28-Ethyl-2,2,3,3-tetramethyl-4,7,10,13,16,19,22,25-octaoxa-28-aza-3-silaheptacosan-30-amine (**28**)

This compound was synthesized according to the procedure described for **27**, starting from compound **26** (607 mg, 0.89 mmol)

and hydrazine monohydrate (430 μL, 8.86 mmol). Reaction time: 20 h at room temperature to give amine **28** (492 mg, 0.89 mmol) as a pale yellow oil which was used without further purification. Yield quant.; R_f (Al_2O_3, CH_2Cl_2/EtOH, 9/1, v/v) 0.23; IR (NaCl) ν 837, 1108, 1252, 1299, 1472, 1648, 2859, 2929, 3100–3600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.87 (s, 9H, $(CH_3)_3C$), 1.00 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.61 (m, 8H, CH_2CH_3, $H_2NCH_2CH_2N$, $H_2NCH_2CH_2N$, NCH_2CH_2O), 3.51 (t, 2H, J = 6.3 Hz, NCH_2CH_2O), 3.53 (t, 2H, J = 5.4 Hz, OCH_2CH_2OSi), 3.63 (m, 24H, $12CH_2O$), 3.74 (t, 2H, J = 5.4 Hz, CH_2OSi); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 12.0, 18.4, 26.0 (3C), 39.9, 48.7, 53.0, 56.9, 62.8, 70.1–70.6 (13C), 72.7.

4.2.21. N-(16-Ethyl-2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-16-aza-3-silaoctadecan-18-yl)-6-iodoquinoxaline-2-carboxamide (**29**)

To a stirred suspension of activated ester **7** [36] (305 mg, 0.72 mmol) in anhydrous THF (7 mL) was added under argon a solution of **27** (430 mg, 1.14 mmol) in anhydrous THF (8 mL). The mixture was stirred at room temperature for 21 h and the solvent was then evaporated under *vacuum*. The obtained residue was dissolved in dichloromethane (15 mL) and a 1 N aqueous sodium hydroxide solution was added (45 mL). After decantation, the organic layer was collected while the aqueous layer was extracted with dichloromethane (3 × 15 mL). The organic layers were combined, washed with a 5% aqueous sodium carbonate solution (2 × 45 mL), dried on magnesium sulphate, filtered and evaporated under *vacuum*. The oily residue was purified by chromatography (SiO_2, CH_2Cl_2/EtOH, 9/1, v/v) to provide compound **29** (459 mg, 0.70 mmol) as a pale yellow oil. Yield 96%; R_f (SiO_2, CH_2Cl_2/EtOH, 9/1, v/v) 0.30; IR (NaCl) ν 834, 1108, 1253, 1353, 1387, 1472, 1523, 1592, 1676, 1714, 2857, 2928, 3025, 3300–3400 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (s, 6H, $(CH_3)_2Si$), 0.87 (s, 9H, $(CH_3)_3C$), 1.08 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.78 (m, 6H, CH_2CH_3, NCH_2CH_2O, $CONHCH_2CH_2N$), 3.60 (m, 16H, $CONHCH_2CH_2N$, $7CH_2O$), 7.82 (d, 1H, J = 8.8 Hz, H-8), 8.07 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.46 (br s, 1H, NH), 8.60 (d, 1H, J = 1.8 Hz, H-5), 9.63 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 12.1, 18.4, 26.0 (3C), 37.4, 48.7, 52.9, 53.0, 62.7, 70.7 (5C), 72.7, 98.0, 130.9, 138.6, 139.7 (2C), 144.2, 144.4, 144.7, 163.0; ESI-MS m/z 661.3 $[M + H]^+$.

4.2.22. N-(28-Ethyl-2,2,3,3-tetramethyl-4,7,10,13,16,19,22,25-octaoxa-28-aza-3-silaheptacosan-30-yl)-6-iodoquinoxaline-2-carboxamide (**30**)

This compound was synthesized according to the procedure described for **29**, starting from compound **28** (450 mg, 0.81 mmol) and activated ester **7** (220 mg, 0.52 mmol). Reaction time: 21 h at room temperature; the purification was performed using column chromatography (SiO_2, CH_2Cl_2/EtOH, 95/5, v/v then 9/1, v/v) to provide compound **30** (435 mg, 0.52 mmol) as a pale yellow oil. Yield 99%; R_f (SiO_2, CH_2Cl_2/EtOH, 9/1, v/v) 0.28; IR (NaCl) ν 835, 1107, 1252, 1352, 1473, 1527, 1592, 1677, 2860, 2927, 3300–3400 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.03 (s, 6H, $(CH_3)_2Si$), 0.85 (s, 9H, $(CH_3)_3C$), 1.04 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.65 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.74 (m, 4H, NCH_2CH_2O, $CONHCH_2CH_2N$), 3.61 (m, 32H, $CONHCH_2CH_2N$, $15CH_2O$), 7.80 (d, 1H, J = 8.8 Hz, H-8), 8.05 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.41 (br s, 1H, NH), 8.57 (d, 1H, J = 1.8 Hz, H-5), 9.61 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ −5.2 (2C), 12.2, 18.4, 26.0 (3C), 37.6, 48.7, 52.9, 53.0, 62.8, 70.2–70.6 (13C), 72.7, 97.9, 130.9, 138.6, 139.7 (2C), 144.2, 144.4, 144.7, 163.0; ESI-MS m/z 837.4 $[M + H]^+$.

4.2.23. N-(12-Ethyl-1-hydroxy-3,6,9-trioxa-12-azatetradecan-14-yl)-6-iodoquinoxaline-2-carboxamide (**31**)

To a stirred solution of compound **29** (173 mg, 0.26 mmol) in THF (8 mL) was added a solution of TBAF 1 M in THF (393 μL, 0.39 mmol). The mixture was then stirred at room temperature for 2 h. The reaction was stopped by addition of a saturated aqueous sodium hydrogencarbonate solution (80 mL) followed by distilled water (40 mL) and then ethyl acetate (40 mL). After decantation, the aqueous layer was extracted with ethyl acetate (3 × 40 mL). The organic layers were combined, washed with brine (40 mL), dried on magnesium sulphate, filtered and evaporated under *vacuum*. The obtained residue was purified by chromatography (SiO_2, CH_2Cl_2/EtOH, 95/5 to 80/20, v/v) to give compound **31** (132 mg, 0.24 mmol) as a pale yellow oil. Yield 93%; R_f (SiO_2, CH_2Cl_2/EtOH, 80/20, v/v) 0.26; IR (NaCl) ν 1125, 1353, 1473, 1527, 1592, 1671, 2871, 3300–3400 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.10 (t, 3H, J = 7.1 Hz, CH_3), 2.82 (m, 6H, CH_2CH_3, NCH_2CH_2O, $CONHCH_2CH_2N$), 3.60 (m, 16H, $CONHCH_2CH_2N$, $7CH_2O$), 7.83 (d, 1H, J = 8.8 Hz, H-8), 8.06 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.57 (br s, 1H, NH), 8.59 (d, 1H, J = 1.8 Hz, H-5), 9.62 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.7, 37.3, 48.9, 52.9, 53.1, 61.7, 69.7, 70.3–70.6 (4C), 72.7, 98.0, 130.9, 138.6, 139.7 (2C), 144.2, 144.4, 144.7, 163.2; ESI-MS m/z 547.2 $[M + H]^+$.

4.2.24. N-(24-Ethyl-1-hydroxy-3,6,9,12,15,18,21-heptaoxa-24-azahexacosan-26-yl)-6-iodoquinoxaline-2-carboxamide (**32**)

This compound was synthesized according to the procedure described for **31**, starting from compound **30** (425 mg, 0.51 mmol) and TBAF 1 M in THF (762 μL, 0.76 mmol). Reaction time: 1.5 h at room temperature; the purification was performed using column chromatography (SiO_2, CH_2Cl_2/EtOH, 90/10 and then 80/20, v/v) to afford compound **32** as a yellow oil (273 mg, 0.38 mmol). Yield 75%; R_f (SiO_2, CH_2Cl_2/EtOH, 80/20, v/v) 0.36; IR (ATR diamond accessory) ν 1127, 1353, 1474, 1529, 1593, 1675, 2800–3000, 3100–3600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.06 (t, 3H, J = 7.1 Hz, CH_3), 2.66 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.76 (m, 4H, NCH_2CH_2O, $CONHCH_2CH_2N$), 2.91 (m, 1H, OH), 3.60 (m, 32H, $CONHCH_2CH_2N$, $15CH_2O$), 7.81 (d, 1H, J = 8.8 Hz, H-8), 8.05 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.44 (br s, 1H, NH), 8.58 (d, 1H, J = 1.8 Hz, H-5), 9.61 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.1, 37.5, 48.7, 52.9, 53.0, 61.7, 70.0–70.6 (13C), 72.7, 98.0, 130.9, 138.6, 139.7 (2C), 144.2, 144.4, 144.7, 163.0; ESI-MS m/z 723.3 $[M + H]^+$.

4.2.25. N-(12-Ethyl-1-fluoro-3,6,9-trioxa-12-azatetradecan-14-yl)-6-iodoquinoxaline-2-carboxamide (**33**)

This compound was synthesized according to the procedure described for **14**, starting from compound **31** (100 mg, 0.18 mmol) and DAST (49 μL, 0.37 mmol). Reaction time: 15 min at −50 °C and 1.5 h at room temperature; the purification was performed using column chromatography (SiO_2, EtOAc/EtOH, 85/15, v/v, 0.5% NH_4OH) to yield compound **33** as a yellow oil (68 mg, 0.12 mmol). Yield 68%; R_f (SiO_2, EtOAc/EtOH, 85/15, v/v) 0.30; IR (ATR diamond accessory) ν 1046, 1107, 1352, 1473, 1523, 1592, 1669, 2868, 2916, 3300–3400 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, 3H, J = 7.1 Hz, CH_3), 2.65 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.77 (m, 4H, NCH_2CH_2O, $CONHCH_2CH_2N$), 3.61 (m, 12H, $CONHCH_2CH_2N$, $5CH_2O$), 3.69 (m, 2H, CH_2CH_2F), 4.53 (td, 2H, $^2J_{H-F}$ = 47.7 Hz, J = 4.1 Hz, CH_2F), 7.81 (d, 1H, J = 8.8 Hz, H-8), 8.07 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.42 (br s, 1H, NH), 8.60 (d, 1H, J = 1.8 Hz, H-5), 9.63 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.5, 37.1, 48.9, 52.8 (2C), 70.8 (6C), 83.2 ($^1J_{C-F}$ = 169 Hz), 98.1, 131.0, 138.6, 139.5, 139.7, 144.1, 144.4, 144.7, 163.3; ^{19}F NMR ($CDCl_3$) δ −223.1; ESI-MS m/z 549.2 $[M + H]^+$.

4.2.26. N-(24-Ethyl-1-fluoro-3,6,9,12,15,18,21-heptaoxa-24-azahexacosan-26-yl)-6-iodoquinoxaline-2-carboxamide (**34**)

This compound was synthesized according to the procedure described for **14**, starting from compound **32** (94 mg, 0.13 mmol) and DAST (34.4 μL, 0.26 mmol). Reaction time: 15 min at −50 °C and 1.5 h at room temperature; the purification was performed using column chromatography (SiO_2, EtOAc/EtOH, 80/20, v/v, 0.5% NH_4OH) to yield compound **34** as a yellow oil (63 mg, 86.9 μmol).

Yield 67%; R_f (SiO_2, EtOAc/EtOH, 5/5, v/v, 0.5% NH_4OH) 0.32; IR (ATR diamond accessory) ν 1129, 1353, 1474, 1527, 1593, 1675, 2800–3000, 3300–3400 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, 3H, J = 7.1 Hz, C<u>H</u>$_3$), 2.67 (q, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH$_3$), 2.79 (m, 4H, NC<u>H</u>$_2$CH$_2$O, CONHCH$_2$C<u>H</u>$_2$N), 3.61 (m, 30H, CONHC<u>H</u>$_2$CH$_2$N, 14C<u>H</u>$_2$O), 4.55 (td, 2H, $^2J_{H-F}$ = 47.8 Hz, J = 4.1 Hz, C<u>H</u>$_2$F), 7.83 (d, 1H, J = 8.8 Hz, H-8), 8.08 (dd, 1H, J = 1.6, 8.8 Hz, H-7), 8.46 (br s, 1H, NH), 8.61 (d, 1H, J = 1.6 Hz, H-5), 9.64 (s, 1H, H-3); ^{19}F NMR ($CDCl_3$) δ −223.2; ESI-MS m/z 725.2 $[M + H]^+$; Anal. ($C_{29}H_{46}O_8IN_4F$, 3H_2O) calculated: C: 44.73; H: 6.73; N: 7.20; found: C: 44.84; H: 7.16, N: 5.82.

4.2.27. N-[2-[(N-Ethyl)-(N-prop-2-ynyl)amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**35**)

To a solution of compound **15** [36] (500 mg, 1.35 mmol) in anhydrous ethanol (10 mL) were successively added propargyl bromide (122 μL, 1.62 mmol) and triethylamine (187 μL, 1.35 mmol). The mixture was stirred at room temperature for 72 h before addition of water (33 mL). The resulting solution was then extracted with dichloromethane (3 × 33 mL). The organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The obtained residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give compound **35** (522 mg, 1.28 mmol) as a beige solid. Yield 95%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.48; mp 135–137 °C; IR (KBr) ν 1047, 1176, 1355, 1474, 1534, 1670, 2967, 3000–3600 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.12 (t, 3H, J = 7.1 Hz, C<u>H</u>$_3$), 2.23 (t, 1H, J = 2.3 Hz, C≡C<u>H</u>), 2.67 (q, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH$_3$), 2.84 (t, 2H, J = 6.0 Hz, (CO)NHCH$_2$C<u>H</u>$_2$N), 3.52 (d, 2H, J = 2.3 Hz, C<u>H</u>$_2$C≡CH), 3.62 (q, 2H, J = 6.0 Hz, (CO)NHC<u>H</u>$_2$CH$_2$N), 7.78 (d, 1H, J = 8.8 Hz, H-8), 8.02 (dd, 1H, J = 1.9, 8.8 Hz, H-7), 8.32 (m, 1H, NH), 8.54 (d, 1H, J = 1.9 Hz, H-5), 9.59 (s, 1H, H-3); ^{13}C NMR (50 MHz, acetone-d_6) δ 13.2, 37.8, 41.9, 48.1, 52.8, 74.5, 79.3, 98.3, 131.9, 139.2, 140.3, 140.6, 145.2, 145.4, 145.6, 163.5; ESI-MS m/z 409.1 $[M + H]^+$.

4.2.28. N-[2-[(N-Ethyl)-[[1-(2-fluoroethyl)-1H-(1,2,3)triazol-4-yl]methyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide (**37**)

Cesium fluoride (336 mg, 2.21 mmol) was dried by azeotropic distillation with anhydrous acetonitrile (4 × 7 mL) at 90 °C under *vacuum*. After cooling to room temperature, a solution 2-azidoethyl of *p*-toluenesulfonate (176 mg, 0.73 mmol), synthesized according to the procedure described by Demko et al. [41], in a mixture of anhydrous *tert*-amyl alcohol and acetonitrile (7.4 mL, 1/1, v/v) was added under argon. The mixture was stirred at 100 °C for 2 h to yield 2-fluoro-1-azidobutane (**36**). Back to room temperature, a 1.0 M aqueous pentahydrate copper (II) sulphate solution (370 μL), a 1.0 M aqueous ascorbic acid solution (370 μL) and compound **35** (300 mg, 0.73 mmol) were successively added. The mixture was stirred at room temperature for 12 h before addition of water (5 mL) and a 2.0 M aqueous sodium carbonate solution (5 mL). The solution was extracted with dichloromethane (4 × 10 mL) and the organic layers were combined, dried on magnesium sulphate, filtered and evaporated under *vacuum*. The obtained residue was purified by chromatography (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) to give compound **37** (311 mg, 0.63 mmol) as a brown oil. Yield 86%; R_f (Al_2O_3, CH_2Cl_2/EtOH, 99/1, v/v) 0.41; IR (CCl_4) ν 1145, 1354, 1388, 1437, 1473, 1522, 1659, 1673, 2837, 2971, 3246, 3386 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.12 (t, 3H, J = 7.1 Hz, C<u>H</u>$_3$), 2.64 (q, 2H, J = 7.1 Hz, C<u>H</u>$_2$CH$_3$), 2.76 (t, 2H, J = 6.1 Hz, (CO)NHCH$_2$C<u>H</u>$_2$N), 3.59 (q, 2H, J = 6.1 Hz, (CO)NHC<u>H</u>$_2$CH$_2$N), 3.88 (s, 2H, NC<u>H</u>$_2$C), 4.57 (td, 2H, $^3J_{H-F}$ = 27.5 Hz, J = 4.6 Hz, C<u>H</u>$_2$CH$_2$F), 4.71 (td, 2H, $^2J_{H-F}$ = 51.0 Hz, J = 4.6 Hz, C<u>H</u>$_2$F), 7.65 (s, 1H, C=C<u>H</u>CH$_2$CH$_2$F), 7.83 (d, 1H, J = 8.8 Hz, H-8), 8.05 (dd, 1H, J = 1.8, 8.8 Hz, H-7), 8.40 (m, 1H, NH), 8.57 (d, 1H, J = 1.8 Hz, H-5), 9.60 (s, 1H, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.1, 37.2, 47.6, 48.1, 50.4 (d, $^2J_{C-F}$ = 20 Hz), 51.5, 81.5 (d, $^1J_{C-F}$ = 172 Hz), 98.0, 123.5 (d, $^3J_{C-F}$ = 14 Hz), 130.7, 138.4, 139.4, 139.6, 144.0, 144.2, 144.5, 145.3, 162.9; ^{19}F NMR ($CDCl_3$) δ −223.21; ESI-MS m/z 498.0 $[M + H]^+$.

4.2.29. N-[2-[[N-Ethyl-N-(2-fluoroethyl)]amino]ethyl]-6-iodoquinoxaline-2-carboxamide dihydrochloride salt (**38**)

To a stirred solution of compound **8** (180 mg, 0.43 mmol) in anhydrous dichloromethane (5 mL) was added under argon an anhydrous 2 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 under argon at room temperature for 14 h. The precipitate was then filtered and dried under reduce pressure to give dihydrochloride salt **38** (166 mg, 0.34 mmol) as a yellow solid. Yield 78%; mp 184–186 °C; IR (KBr) ν 1178, 1351, 1466, 1516, 1676, 2200–2800, 2946, 3049, 3200–3400 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.27 (t, 3H, J = 7.1 Hz, C<u>H</u>$_3$), 3.33 (m, 4H, (CO)NHCH$_2$C<u>H</u>$_2$N, C<u>H</u>$_2$CH$_3$), 3.61 (qd, 2H, $^3J_{H-F}$ = 28.6 Hz, J = 4.2 Hz, C<u>H</u>$_2$CH$_2$F), 3.78 (q, 2H, J = 6.0 Hz, (CO)NHC<u>H</u>$_2$CH$_2$N), 4.69 (br s, 5H), 4.92 (td, 2H, $^2J_{H-F}$ = 47.5 Hz, J = 4.0 Hz, C<u>H</u>$_2$F), 7.93 (d, 1H, J = 8.8 Hz, H-8), 8.26 (dd, 1H, J = 1.9, 8.8 Hz, H-7), 8.64 (d, 1H, J = 1.9 Hz, H-5), 9.40 (t, 1H, J = 6.0 Hz, NH), 9.46 (s, 1H, H-3), 10.89 (br s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 8.3, 33.9, 48.1, 50.7, 51.8 (d, $^2J_{C-F}$ = 20 Hz), 78.7 (d, $^1J_{C-F}$ = 164 Hz), 99.6, 130.7, 137.5, 138.9, 139.9, 143.6, 144.3, 144.4, 163.6; ^{19}F NMR (DMSO-d_6) δ −220.98 (tt, $^2J_{H-F}$ = 47.3 Hz, $^3J_{H-F}$ = 28.6 Hz); ESI-MS m/z 417.0 $[M + H]^+$. Anal. ($C_{15}H_{18}OIN_4F$, 2HCl) calculated: C: 36.83; H: 4.12; N: 11.45; found: C: 36.59; H: 4.29; N: 11.07.

4.2.30. N-[2-[N-Ethyl-N-((E)-4-fluorobut-2-enyl)]amino]ethyl]-6-iodoquinoxaline-2-carboxamide dihydrochloride salt (**39**)

This compound was synthesized according to the procedure described for **38**, starting from compound **17** (200 mg, 0.45 mmol). Reaction time: 12 h at room temperature to give compound **39** (206 mg, 0.40 mmol) as a very hygroscopic beige solid. Yield 89%; mp 139–141 °C (dec.); IR (KBr) ν 1166, 1522, 1676, 2300–2600, 3200–3500 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.27 (t, 3H, J = 7.1 Hz, C<u>H</u>$_3$), 3.24 (m, 4H, (CO)NHCH$_2$C<u>H</u>$_2$N, C<u>H</u>$_2$CH$_3$), 3.75 (q, 2H, J = 5.9 Hz, (CO)NHC<u>H</u>$_2$CH$_2$N), 3.88 (m, 2H, NC<u>H</u>$_2$CH=CHCH$_2$F), 4.94 (m, 1H, NH), 4.95 (dd, 2H, $^2J_{H-F}$ = 46.6 Hz, J = 4.6 Hz, C<u>H</u>$_2$F), 5.96 (m, 1H, NCH$_2$C<u>H</u>=CHCH$_2$F), 6.23 (tt, 1H, $^4J_{H-F}$ = 15.8 Hz, J = 4.6, 15.8 Hz, NCH$_2$CH=C<u>H</u>CH$_2$F), 7.93 (d, 1H, J = 8.8 Hz, H-8), 8.26 (dd, 1H, J = 1.5, 8.8 Hz, H-7), 8.64 (d, 1H, J = 1.5 Hz, H-5), 9.39 (t, 1H, J = 5.6 Hz, NH), 9.46 (s, 1H, H-3), 10.66 (br s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 8.3, 33.9, 47.0, 49.7, 52.5, 81.9 (d, $^1J_{C-F}$ = 162 Hz), 99.5, 121.4 (d, $^2J_{C-F}$ = 14 Hz), 130.7, 135.0 (d, $^3J_{C-F}$ = 17 Hz), 137.5, 138.9, 139.9, 143.5, 144.2, 144.4, 163.5; ^{19}F NMR (DMSO-d_6) δ −218.25 (tdd, $^2J_{H-F}$ = 46.4 Hz, $^3J_{H-F}$ = 16.5 Hz, $^4J_{H-F}$ = 2.5 Hz); ESI-MS m/z 442.9 $[M + H]^+$. Anal. ($C_{17}H_{20}OIN_4F$, 2HCl) calculated: C: 39.63; H: 4.30; N: 10.88; found: C: 41.33; H: 4.56; N: 11.30.

4.2.31. N-[2-[[N-Ethyl-N-(4-fluorobut-2-ynyl)]amino]ethyl]-6-iodoquinoxaline-2-carboxamide dihydrochloride salt (**40**)

This compound was synthesized according to the procedure described for **38**, starting from compound **20** (100 mg, 0.23 mmol). Reaction time: 12 h at room temperature to give compound **40** (95 mg, 0.19 mmol) as a very hygroscopic yellow solid. Yield 81%; mp 105–107 °C; IR (KBr) ν 1165, 1356, 1474, 1534, 1672, 2400–2800, 2900–3650 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.29 (t, 3H, J = 7.0 Hz, C<u>H</u>$_3$), 3.34 (m, 4H, (CO)NHCH$_2$C<u>H</u>$_2$N, C<u>H</u>$_2$CH$_3$), 3.80 (m, 2H, (CO)NHC<u>H</u>$_2$CH$_2$N), 4.38 (m, 2H, NC<u>H</u>$_2$C≡C), 5.17 (d, 2H, $^2J_{H-F}$ = 46.9 Hz, C<u>H</u>$_2$F), 7.63 (br s, 2H, NH), 7.91 (d, 1H, J = 8.8 Hz, H-8), 8.23 (dd, 1H, J = 1.5, 8.8 Hz, H-7), 8.60 (d, 1H, J = 1.5 Hz, H-5), 9.44 (m, 2H, H-3, NH), 11.58 (br s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 8.7, 34.1, 41.1, 48.0, 50.6, 70.7 (d, $^1J_{C-F}$ = 162 Hz), 79.0 (d, $^3J_{C-F}$ = 12 Hz), 84.1 (d, $^2J_{C-F}$ = 22 Hz), 99.6, 130.7, 137.5, 138.9, 139.8,

143.5, 144.4, 144.5, 163.6; ^{19}F NMR (DMSO-d_6) δ −218.61; ESI-MS m/z 441.1 $[M + H]^+$. Anal. ($C_{17}H_{18}OIN_4F$, 2HCl) calculated: C: 39.79; H: 3.93; N: 10.92; found: C: 39.94; H: 4.04; N: 10.93.

4.2.32. N-(12-Ethyl-1-fluoro-3,6,9-trioxa-12-azatetradecan-14-yl)-6-iodoquinoxaline-2-carboxamide dihydrochloride salt (**41**)

This experiment was carried out in a glove compartment, under argon atmosphere. To a solution of compound **33** (10 mg, 18.2 μmol) in anhydrous dichloromethane (1 mL) was added a 2.0 N hydrochloric acid solution in anhydrous diethylether (2 mL) under argon. After 5 min, the solvent was evaporated under a flux of argon. The residue was suspended in anhydrous diethylether (2 mL) and dried under a flux of argon to give dihydrochloride salt **41** (9.4 mg, 15.1 μmol) as a very hygroscopic yellow solid. Yield 83%; IR (ATR diamond accessory) ν 409, 1048, 1130, 1355, 1475, 1538, 1592, 1674, 2953, 3300–3400 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, 3H, J = 7.1 Hz, C$\underline{H}_3$), 3.37 (m, 6H, C$\underline{H}_2$CH$_3$, NC$\underline{H}_2$CH$_2$O, CONHCH$_2$C$\underline{H}_2$N), 3.65 (m, 10H, 5C$\underline{H}_2$O), 3.77 (m, 2H, C$\underline{H}_2$CH$_2$F), 4.02 (q, 2H, J = 6.2 Hz, CONHC$\underline{H}_2$CH$_2$N), 4.54 (td, 2H, $^2J_{H-F}$ = 47.7 Hz, J = 4.2 Hz, C$\underline{H}_2$F), 7.96 (d, 1H, J = 8.9 Hz, H-8), 8.08 (dd, 1H, J = 1.9, 8.9 Hz, H-7), 8.59 (d, 1H, J = 1.6 Hz, H-5), 9.28 (br s, 1H, NH), 9.56 (s, 1H, H-3); ^{19}F NMR ($CDCl_3$) δ −222.76; ESI-MS m/z 549.1 $[M + H]^+$; Anal. ($C_{21}H_{30}FIN_4O_4$, 2HCl, 5H$_2$O) calculated: C: 35.46; H: 5.95; N: 7.88; found: C: 35.81; H: 6.23; N: 8.04.

4.2.33. N-[2-[(N-Ethyl)-[[1-(2-fluoroethyl)-1H-(1,2,3)triazol-4-yl] methyl]amino]ethyl]-6-iodoquinoxaline-2-carboxamide trihydrochloride salt (**42**)

This compound was synthesized according to the procedure described for **38**, starting from **37** (200 mg, 0.40 mmol). Reaction time: 12 h at room temperature to give compound **42** (209 mg, 0.34 mmol) as a very hygroscopic yellow solid. Yield 86%; mp 98–100 °C; IR (KBr) ν 1040, 1169, 1356, 1474, 1534, 1670, 2600–3600 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.34 (t, 3H, J = 7.0 Hz, C$\underline{H}_3$), 3.15 (m, 2H, CH$_2$CH$_3$), 3.30 (m, 2H, (CO)NHCH$_2$C$\underline{H}_2$N), 3.84 (m, 2H, (CO)NHC$\underline{H}_2$CH$_2$N), 4.55 (m, 2H, C$\underline{H}_2$F), 4.70 (s, 2H, NC$\underline{H}_2$C), 4.89 (td, 2H, $^3J_{H-F}$ = 20.9 Hz, J = 4.4 Hz, C$\underline{H}_2$F), 5.36 (m, 2H, NH), 7.92 (d, 1H, J = 8.8 Hz, H-8), 8.24 (dd, 1H, J = 1.6, 8.8 Hz, H-7), 8.47 (s, 1H, C=C$\underline{H}$CH$_2$CH$_2$F), 8.62 (d, 1H, J = 1.6 Hz, H-5), 9.44 (m, 2H, H-3, NH), 11.08 (br s, 1H, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 8.6, 34.1, 45.4, 47.3, 50.2 (d, $^2J_{C-F}$ = 19 Hz), 50.3, 81.8 (d, $^1J_{C-F}$ = 167 Hz), 99.6, 127.6 (d, $^3J_{C-F}$ = 12 Hz), 130.7, 136.1, 137.5, 138.9, 139.8, 143.6, 144.3, 144.4, 163.6; ^{19}F NMR (DMSO-d_6) δ −223.95; ESI-MS m/z 498.1 $[M + H]^+$. Anal. ($C_{18}H_{21}OIN_7F$, 3HCl, H_2O) calculated: C: 34.61; H: 4.20; N: 15.70; found: C: 37.74; H: 4.61; N: 15.49.

4.3. Radiochemistry

4.3.1. Method A

To a solution of the appropriate compound (2–4 mg) in acetic acid (600 μL) were added, in a sealed vial equipped with a needle (25 G, 0.5 × 16 mm), a copper sulphate solution in acetic acid (100 μL, 1 mg mL^{-1}) used as catalyst and [^{125}I]NaI (119–333 MBq). The reaction mixture was heated at 130 °C for 25–45 min. Back to room temperature, the residue was taken up in water (500 μL) and a 1.0 N aqueous sodium hydroxide solution (100 μL) was added.

4.3.2. Method B

To a solution of the appropriate compound (2–4 mg) in citrate buffer pH = 4 (500 μL) were added, in a sealed vial equipped with a needle (25 G, 0.5 × 16 mm), an aqueous copper sulphate solution (0.5 mg, 100 μL) and [^{125}I]NaI (181–363 MBq). The reaction mixture was heated at 130 °C for 25–30 min. After cooling to room temperature, the residue was taken up in water (500 μL) and a 1.0 N aqueous sodium hydroxide solution (100 μL) was added.

4.3.3. General method for purification

The vial cap and septum were removed. The resulting suspension was deposited on an extrelut® column. After 10 min, the column was eluted with dichloromethane (5 × 3 mL). The collected organic extracts were evaporated under reduced pressure, taken up with methanol (2 × 100 μL), and purified by RP-HPLC. Fractions containing the expected radiolabelled 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 reduce 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 radiolabelled tracer as corresponding hydrochloride salt.

The labelling conditions and characterization data for radiotracers [^{125}I]**14**, [^{125}I]**34**, and [^{125}I]**38**–**42** are given in Table 2.

For subsequent injection in mice, radiotracers [^{125}I]**38**, [^{125}I]**39**, [^{125}I]**40**, [^{125}I]**41** and [^{125}I]**42** were prepared in solution in physiological serum while [^{125}I]**14** and [^{125}I]**34**, which were not converted into corresponding hydrochloride salts, were dissolved in a solution of ethanol 5% in physiological serum.

4.4. In vivo scintigraphic imaging

In vivo scintigraphic imaging protocol was submitted and approved by the french regional ethical animal use committee (CEMEA, Auvergne, authorization no. CE18-09, 2010). Scintigraphic imaging of radioiodinated compounds in mice was performed using a gamma camera dedicated to 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 centred on the 35 keV peak of iodine-125.

On day 14 after tumour implant, each [^{125}I]-labelled compound was administered intravenously *via* the tail vein with 3.7 MBq/mouse (range 3–8 animals/compound). At different time points after radiotracer administration (1 h, 3 h, 6 h, 24 h, 72 h, 5 d, 7 d, 10 d and 14 d), mice were anaesthetized by intraperitoneal (i.p.) 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 prone position over the collimator of the camera. Reproducibility in animal positioning for the serial images was achieved using a graduated reference grid.

The injected activity to each mouse was also determined from scintigraphic imaging of the syringe before and after the injection of the radiotracer. Quantitative analysis of scintigraphic scans was performed using the GAMMAVISION+® software (Biospace Mesures, Paris, France). For each scintigram, regions of interest (ROIs) were manually drawn around the tumour, the whole body area, and the controlateral muscle which was chosen as background. For each ROI, total activity, min and max pixel values, ROI size (in mm^2), average activity per mm^2 and standard deviation were obtained. Activities were normalized to the injected activity and corrected for the radioactive decay. For the tumour, the activity was also normalized to the tumour weight as previously described [43] and expressed as percentage of injected dose per gram of tumour (%ID/g).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.11.047. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] V. Gray-Schopfer, C. Wellbrock, R. Marais, Nature 445 (2007) 851–857.
[2] S.N. Markovic, L.A. Erickson, R.D. Rao, R.H. Weenig, B.A. Pockaj, A. Bardia, C.M. Vachon, S.E. Schild, R.R. McWilliams, J.L. Hand, S.D. Laman, L.A. Kottschade, W.J. Maples, M.R. Pittelkow, J.S. Pulido, J.D. Cameron, E.T. Creagan, Mayo Clin. Proc. 82 (2007) 490–513.
[3] S.N. Markovic, L.A. Erickson, R.D. Rao, R.H. Weenig, B.A. Pockaj, A. Bardia, C.M. Vachon, S.E. Schild, R.R. McWilliams, J.L. Hand, S.D. Laman, L.A. Kottschade, W.J. Maples, M.R. Pittelkow, J.S. Pulido, J.D. Cameron, E.T. Creagan, Mayo Clin. Proc. 82 (2007) 364–380.
[4] R.M. MacKie, A. Hauschild, A.M. Eggermont, Ann. Oncol. 20 (Suppl. 6) (2009) vi1–7.
[5] D.S. Rigel, J. Russak, R. Friedman, CA Cancer J. Clin. 60 (2010) 301–316.
[6] J.E. Gershenwald, M.I. Ross, N. Engl. J. Med. 364 (2011) 1738–1745.
[7] M.I. Ross, Semin. Cutan. Med. Surg. 29 (2010) 238–248.
[8] S.Q. Wang, P. Hashemi, Semin. Cutan. Med. Surg. 29 (2010) 174–184.
[9] J. Lutzky, Semin. Cutan. Med. Surg. 29 (2010) 249–257.
[10] C. Garbe, T.K. Eigentler, U. Keilholz, A. Hauschild, J.M. Kirkwood, Oncologist 16 (2011) 5–24.
[11] S. Dalle, N. Poulalhon, L. Thomas, N. Engl. J. Med. 365 (2011) 1448–1449.
[12] J.A. Sosman, K.B. Kim, L. Schuchter, R. Gonzalez, A.C. Pavlick, J.S. Weber, G.A. McArthur, T.E. Hutson, S.J. Moschos, K.T. Flaherty, P. Hersey, R. Kefford, D. Lawrence, I. Puzanov, K.D. Lewis, R.K. Amaravadi, B. Chmielowski, H.J. Lawrence, Y. Shyr, F. Ye, J. Li, K.B. Nolop, R.J. Lee, A.K. Joe, A. Ribas, N. Engl. J. Med. 366 (2012) 707–714.
[13] R.N. Amaria, K.D. Lewis, A. Jimeno, Drugs Today (Barc) 48 (2012) 109–118.
[14] R.M. Ings, Drug Metabol. Rev. 15 (1984) 1183–1212.
[15] D. Oltmanns, M. Eisenhut, W. Mier, U. Haberkorn, Curr. Med. Chem. 16 (2009) 2086–2094.
[16] M. Eisenhut, W.E. Hull, A. Mohammed, W. Mier, D. Lay, W. Just, K. Gorgas, W.D. Lehmann, U. Haberkorn, J. Med. Chem. 43 (2000) 3913–3922.
[17] X. Liu, T.Q. Pham, P. Berghofer, J. Chapman, I. Greguric, P. Mitchell, F. Mattner, C. Loc'h, A. Katsifis, Nucl. Med. Biol. 35 (2008) 769–781.
[18] J.M. Michelot, M.F. Moreau, A.J. Veyre, J.F. Bonafous, F.J. Bacin, J.C. Madelmont, F. Bussiere, P.A. Souteyrand, L.P. Mauclaire, F.M. Chossat, et al., J. Nucl. Med. 34 (1993) 1260–1266.
[19] N. Moins, M. D'Incan, J. Bonafous, F. Bacin, P. Labarre, M.F. Moreau, D. Mestas, E. Noirault, F. Chossat, E. Berthommier, J. Papon, M. Bayle, P. Souteyrand, J.C. Madelmont, A. Veyre, Eur. J. Nucl. Med. Mol. Imaging 29 (2002) 1478–1484.
[20] T.Q. Pham, I. Greguric, X. Liu, P. Berghofer, P. Ballantyne, J. Chapman, F. Mattner, B. Dikic, T. Jackson, C. Loc'h, A. Katsifis, J. Med. Chem. 50 (2007) 3561–3572.
[21] D. Denoyer, I. Greguric, P. Roselt, O.C. Neels, N. Aide, S.R. Taylor, A. Katsifis, D.S. Dorow, R.J. Hicks, J. Nucl. Med. 51 (2010) 441–447.
[22] D. Denoyer, T. Potdevin, P. Roselt, O.C. Neels, L. Kirby, I. Greguric, A. Katsifis, D.S. Dorow, R.J. Hicks, J. Nucl. Med. 52 (2011) 115–122.
[23] I. Greguric, S.R. Taylor, D. Denoyer, P. Ballantyne, P. Berghofer, P. Roselt, T.Q. Pham, F. Mattner, T. Bourdier, O.C. Neels, D.S. Dorow, C. Loc'h, R.J. Hicks, A. Katsifis, J. Med. Chem. 52 (2009) 5299–5302.
[24] S. Garg, K. Kothari, S.R. Thopate, A.K. Doke, P.K. Garg, Bioconjug. Chem. 20 (2009) 583–590.
[25] G. Ren, Z. Miao, H. Liu, L. Jiang, N. Limpa-Amara, A. Mahmood, S.S. Gambhir, Z. Cheng, J. Nucl. Med. 50 (2009) 1692–1699.
[26] L. Rbah-Vidal, A. Vidal, S. Besse, F. Cachin, M. Bonnet, L. Audin, S. Askienazy, F. Dollé, F. Degoul, E. Miot-Noirault, N. Moins, P. Auzeloux, J.M. Chezal, Eur. J. Nucl. Med. Mol. Imaging (2012), http://dx.doi.org/10.1007/s00259-012-2168-y.
[27] M. Bonnet-Duquennoy, J. Papon, F. Mishellany, P. Labarre, J.L. Guerquin-Kern, T.D. Wu, M. Gardette, J. Maublant, F. Penault-Llorca, E. Miot-Noirault, A. Cayre, J.C. Madelmont, J.M. Chezal, N. Moins, Int. J. Cancer 125 (2009) 708–716.
[28] J.M. Chezal, J. Papon, P. Labarre, C. Lartigue, M.J. Galmier, C. Decombat, O. Chavignon, J. Maublant, J.C. Teulade, J.C. Madelmont, N. Moins, J. Med. Chem. 51 (2008) 3133–3144.
[29] M. Bonnet, F. Mishellany, J. Papon, A. Cayre, F. Penault-Llorca, J.C. Madelmont, E. Miot-Noirault, J.M. Chezal, N. Moins, Pigm. Cell Melanoma Res. 23 (2010) e1–11.
[30] J.L. Joyal, J.A. Barrett, J.C. Marquis, J. Chen, S.M. Hillier, K.P. Maresca, M. Boyd, K. Gage, S. Nimmagadda, J.F. Kronauge, M. Friebe, L. Dinkelborg, J.B. Stubbs, M.G. Stabin, R. Mairs, M.G. Pomper, J.W. Babich, Cancer Res. 70 (2010) 4045–4053.
[31] J.M. Chezal, F. Dollé, J.C. Madelmont, A. Maisonial, E. Miot-Noirault, N. Moins, J. Papon, B. Kuhnast, B. Tavitian, R. Boisgard, World Patent WO 2009095872, 2009.
[32] A. Maisonial, B. Kuhnast, J. Papon, R. Boisgard, M. Bayle, A. Vidal, P. Auzeloux, L. Rbah, M. Bonnet-Duquennoy, E. Miot-Noirault, M.J. Galmier, M. Borel, S. Askienazy, F. Dolle, B. Tavitian, J.C. Madelmont, N. Moins, J.M. Chezal, J. Med. Chem. 54 (2011) 2745–2766.
[33] B. Cohen, E.R.V. Artsdalen, J. Harris, J. Am. Chem. Soc. 70 (1948) 281–285.
[34] O.C. Dermer, G.E. Ham, Ethylenimine and Other Aziridines, Chemistry and Applications, Academic Press, New York, 1969.
[35] M. Glasser, E.G. Robins, J. Labelled Compd. Radiopharm. 52 (2009) 407–414.
[36] D. Denoyer, P. Labarre, J. Papon, E. Miot-Noirault, M.J. Galmier, J.C. Madelmont, J.M. Chezal, N. Moins, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 875 (2008) 411–418.
[37] J.H. Jones, W.J. Holtz, E.J. Cragoe, J. Med. Chem. 16 (1973) 537–542.
[38] F. Dolle, P. Emond, S. Mavel, S. Demphel, F. Hinnen, Z. Mincheva, W. Saba, H. Valette, S. Chalon, C. Halldin, J. Helfenbein, J. Legaillard, J.C. Madelmont, J.B. Deloye, M. Bottlaender, D. Guilloteau, Bioorg. Med. Chem. 14 (2006) 1115–1125.
[39] H.J. Shine, P. Rangappa, J.N. Marx, D.C. Shelly, T. Ould-Ely, K.H. Whitmire, J. Org. Chem. 70 (2005) 3877–3883.
[40] T. Kan, Y. Kita, Y. Morohashi, Y. Tominari, S. Hosoda, T. Tomita, H. Natsugari, T. Iwatsubo, T. Fukuyama, Org. Lett. 9 (2007) 2055–2058.
[41] Z.P. Demko, K.B. Sharpless, Org. Lett. 3 (2001) 4091–4094.
[42] R. Bejot, T. Fowler, L. Carroll, S. Boldon, J.E. Moore, J. Declerck, V. Gouverneur, Angew. Chem. Int. Ed. Engl. 48 (2009) 586–589.
[43] E. Miot-Noirault, J. Papon, M. Gardette, M. Bonnet-Duquennoy, P. Labarre, A. Maisonial, J.C. Madelmont, J. Maublant, J.M. Chezal, N. Moins, Cancer Biother. Radiopharm. 24 (2009) 629–636.
[44] W.L.F. Armarego, D.D. Perrin, Purification of Laboratory Chemicals, fourth ed., Elsevier, 1997.
[45] G. Eglington, M.C. Whiting, J. Chem. Soc. (1950) 3650–3655.