PEGylation enhances the tumor selectivity of melanoma-targeted conjugates

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID:	OB-ART-08-2014-001751.R2
Article Type:	Paper
Date Submitted by the Author:	24-Oct-2014
Complete List of Authors:	ANDRE, Mathieu; INSERM UMR 990, ; INSERM UMR 990, BESSE, Sophie; INSERM UMR 990, CHEZAL, jean; INSERM UMR 990, MOUNETOU, Emmanuelle; INSERM UMR 990,

SCHOLARONE™
Manuscripts

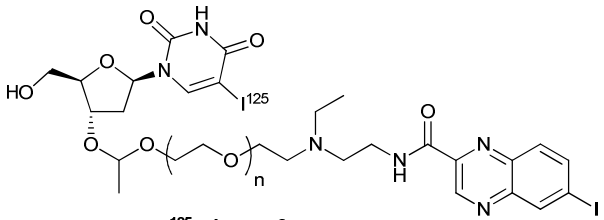
PEGylation enhances the tumor selectivity of melanoma-targeted conjugates

Mathieu André,[a,b] Sophie Besse,[a] Jean-Michel Chezal[a,b] and Emmanuelle Mounetou*[a,b]

[a]*INSERM - Université d'Auvergne UMR 990, IMTV, BP 184, F-63005 Clermont-Ferrand Cedex, France. E-mail: emmanuelle.mounetou@inserm.fr; Phone: (33) 4 73 15 08 00 ; Fax: (33) 4 73 15 08 01.*

[b]*Clermont Université, Université d'Auvergne, IMTV, BP 10448, F-63005 Clermont-Ferrand Cedex, France*

Table of contents entry



[^{125}I] 1a n = 0
[^{125}I] 1b n = 5
[^{125}I] 1c n = 9

Three preselected conjugates of 5-iodo-2'-deoxyuridine (IUdR) to the ICF01012 melanoma-carrier, PEGylated and non- PEGylated, were radiolabelled with iodine-125, and their *in vivo* distribution profile was evaluated for potential intratumoural selective delivery.

Abstract

In the development of our melanoma-selective delivery approach, three preselected conjugates of 5-iodo-2'-deoxyuridine (IUdR) to the ICF01012 melanoma-carrier were radiolabelled with iodine-125, and their *in vivo* distribution profile was determined. A radioiodination method for the conjugate **1a** and its PEGylated derivatives **1b-c** was developed *via* electrophilic iododestannylation in good radiochemical yield with excellent radiochemical purity (>99%). When administered to melanoma-bearing mice, the PEGylated conjugates exhibited an increased tumour uptake with a prolonged residence time. PEGylation also resulted in enhanced tumour selectivity compared with the non-PEGylated parent. These characteristics support further development of this model to achieve maximal concentration of anticancer therapeutics at the local site of action and minimize distribution to non-targeted sites.

Introduction

In anticancer therapy research, the development of efficient tumour-selective approaches remains a challenge. The main limitation of cancer chemotherapy is its low ability to target neoplastic cells versus normal cells, resulting in severe systemic toxicities.[1] Conventional anticancer agents therefore generally possess a very low therapeutic index, and there is an important need for treatments with improved selectivity.[2] This is particularly urgent for melanoma, an extremely aggressive malignancy of melanocytes with high metastatic potential. When disseminated, melanoma is one of the most difficult tumour types to treat.[3] Chemotherapy is mostly ineffective as response rate generally remains under 20%, and patients have a very poor prognosis. The average median survival rate of patients with metastatic melanoma is 6 months.[4] Globally, the low response rates following systemic treatments still pose enormous challenges in melanoma treatment and, the development of more selective melanoma chemotherapy is therefore urgently needed.

Our strategy to achieve melanoma selectivity is based on the design of drug conjugates including a carrier moiety to target the malignant melanocytes, derived from *N,N*-

diethylaminoethyleneheteroarylamide (e.g. ICF01012) (Fig. 1). ICF01012 has demonstrated high long-lasting affinity for the tumour tissues, offering successful applications as a radiopharmaceutical in diagnosis[5] and in targeted radionuclide therapy of melanoma.[6] ICF01012 was also able to carry a wide variety of chemical structures to the pigmented melanoma cells.[7,8] However, the ICF01012 conjugates can be sequestrated in their subcellular target[7], i. e. the melanosome, through a scavenger effect.[9] We initiated a tumour-selective delivery approach based on ICF 01012 drug conjugates with a spacer that incorporates a pre-determined breaking point allowing potential drug release at the target site. We designed a model consisting of 5-iodo-2'-deoxyuridine (IUdR), an anti-metabolite, conjugated to the ICF01012 carrier *via* various cleavable linkers (ester, acetal, carbamate, and carbonate). We demonstrated that the acetal conjugate **1a** and its PEGylated derivatives **1b-c** were of particular interest: they displayed a pH-dependent stability owing to their acid-sensitive spacer (Fig. 1).[10] As the targeted melanosomes are acidic compartments, this profile should thus allow rapid hydrolysis of the conjugate and drug release upon internalization in malignant melanocytes.

Here we report the evaluation of the *in vivo* melanoma targeting properties of the selected conjugate **1a** and its PEGylated derivatives **1b-c**. We specifically studied the effects of PEGylation on tissue distribution to achieve the highest accumulation and longest residence time in the tumour. It is known that PEGylation**,** i.e. the process by which polyethylene glycol (PEG) chains are attached to bioactive substances, may confer resistance to enzyme degradation, and also improve pharmacokinetic profile.[11] PEGylated conjugates as Certolizumab pegol (Cimzia®) are used in clinical practice and have significantly achieve clinical response or remission of various chronic diseases, including rheumatoid arthritis and Crohn's disease.[12] So far only few general rules can however be drawn, and the behaviour of PEG conjugates can be assessed only through case by case experimentation.[13] As the presence of an iodine-substituted purine ring allowed the introduction of a iodine radioisotope, we synthesized the radiolabelled conjugates **[^{125}I]1a-c**. The appropriate range of energy decay (35 keV) and half-life time ($T_{1/2}$ = 60 d) of iodine-125

(^{125}I) enabled us to carry out a subsequent quantitative and comparative *in vivo* tissue distribution study of these conjugates in B16F0 melanoma-bearing mice.

Results and discussion

Synthesis

It is strongly recommended that the synthesis of radiolabelled compounds be performed via a single, fast, and high-yield step. The current method of choice for the regiospecific incorporation of radioiodine with high specific activity into an unactivated small-molecule drug candidates is electrophilic iododemetallation.[14-16] Among the known demetallation methods, the radioiododestannylation is the best established.[17,18] Good regiochemical control at the site of electrophilic substitution, mild conditions of reaction, high yield and stability of organostannanes precursors have favoured to their frequent use. Moreover, the increased lipophilicity of the tin precursor compared with the iodinated product allows easy purification by reversed-phase HPLC.

Tri-*n*-butylstannane precursor **3a** was successfully prepared by palladium(II)-catalysed Stille coupling recation of the previously synthesized iodo-analog **2a**[10,19] with hexabutylditin in refluxing dioxane (Scheme 2).[18] However, under similar conditions, the reaction of the PEGylated derivatives **2b** and **2c**[10] with hexabutylditin did not give the desired tri-*n*-butylstannyled compounds, but instead led to the formation of the hydrodeiodinated products. In these cases, insertion of the bulky tri-*n*-butylstannanyl group was probably hampered by the steric hindrance due to the highly mobile PEG spacer backbone. To overcome this problem, the synthesis of trimethyltin analogues was considered. Replacing the tri-*n*-butyl by the smaller trimethyl group allowed the stannylation of **2b** and **2c** in 70% and 80% yield respectively (Scheme 2). Although trimethylstannane precursors are less frequently used due to their high toxicity, they sometimes achieve higher iododestannylation yields than tri-*n*-butylstannanes. The primary alcohol function of the stannanes **3a-b** was then converted into a mesyl group to give the

corresponding mesylates **4a-c** in good yields. The targeting moiety was next introduced by nucleophilic substitution of the methane sulfonates **4a-c** by *N*-(2-ethylaminoethyl)-6-iodoquinoxaline-2-carboxamide[20] in the presence of N,N-diisopropylethylamine (DIPEA) in anhydrous acetonitrile (MeCN). Final deprotection of the silyl esters under classical conditions, *i.e.* tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF), yielded the desired conjugates **6a-c**.

As previously stated the iodination of organostannanes proceeds *via* electrophilic iododemetallation. The most convenient procedure to generate an electrophilic form of iodine involves *in situ* oxidation of sodium radioiodide by a mild oxidant.[15] Chloramine-T (CAT) and organic peroxides have been widely used as oxidizing agents to release the electrophilic species from sodium or potassium iodide in these procedures.[21] To optimize the radioiodation of the conjugates **6a-c**, the effect of these two different oxidants on the radiochemical yield were compared. Additional variables such as solvent composition, reaction times, and quenching conditions were also evaluated. In a pilot experiment, tributylstannyl conjugate **6a** was converted at room temperature to the radioiodo compound **[^{125}I]1a** with sodium [^{125}I]-iodide in ethanol and aqueous 25% hydrogen peroxide-acetic acid solution as the oxidant system. However, the conversion rate was low (3-6%). Attempts to improve the iodination yield by varying the solvent (chloroform, ethanol) and the reaction times (1 to 15 min) were unsuccessful. Exploratory radioiodonations were then performed using CAT. The results are summarised in Table 1. Unlike the previous tests, all the radioiodination reactions using chloramine T afforded good radiochemical yields of at least 60%. It has been demonstrated that optimum radiochemical yields in electrophilic radiohalogenations occur at slightly acidic pH.[22] Higher conversion rates were, however, obtained in dilute hydrochloric acid than in citrate buffer (entry 2-4 *vs* 1, Table 1). Increasing CAT quantities did not give better results and confirmed that only catalytic amounts are required (entry 4 *vs* 2, 3, Table 1). Lengthening the reaction time did not improve the radiochemical yield (entry 2 *vs* 3, Table 1). Finally, the optimized conditions (entry 2, Table 1) provided a reproducible procedure for the radioiodination of the PEG derivatives **6b** and **6c** successfully affording the radiolabelled **[^{125}I]1b** and **[^{125}I]1c**. The ^{125}I-labelled

target conjugates **[^{125}I]1a-c** were sufficiently well resolved from their trimethyltin precursors **6a-c**, allowing the preparation of radioiodinated no-carrier-added products, with high specific activities, using a single HPLC purification. The radiochemical purity determined by analytical HPLC with simultaneous UV and radioactivity detections exceeded 99% for all the radiolabelled conjugates **[^{125}I]1a-c**. Their identity was confirmed by co-elution with the independently prepared standards **1a-c**.[10]

Radioiododestannylation in the presence of chloramine-T was the method of choice for the synthesis of ^{125}I-labelled target conjugates **[^{125}I]1a-c**. The advantages of this procedure include rapid reaction times at room temperature, good radiochemical yield and excellent chemical and radiochemical purities. Finally, this high specific activity radioiodination method allowed the intravenous (i.v.) administration of all the three conjugates **[^{125}I]1a-c** at required doses for an accurate quantitative biodistribution study.

Biodistribution

The quantitative biodistribution of the three radiolabelled conjugates **[^{125}I]1a-c** was studied at 1 h, 3 h and 24 h after i.v. administration in mice subcutaneously implanted with B16F0 melanoma, and *ex vivo* γ-counting of the tissues. B16F0 murine melanoma is a spontaneous melanoma derived from C57BL/6 mice and one of the very few pigmented melanoma lines available for use in mice. It is the most widely model used in melanoma investigative chemotherapy because tumour growth is rapid and tumour are non- or low-immunogenic. Table 2 presents the pharmacokinetic data obtained in blood and representative non-target normal organs. Maximal uptake was achieved at 1 h post-injection (p.i.) followed by a time dependent decrease in all the organs considered. While the conjugates were thus rapidly distributed, 60-70% of the initial detected dose at 1 h p.i. was already eliminated at 3 h p.i. Blood kinetics displayed a fairly different profile for **[^{125}I]1a**, with a 2 h delayed maximal radioactivity, and for **[^{125}I]1b** with a slightly slower elimination rate (53% *vs* 60-70%). More precisely, the PEGylated derivatives **[^{125}I]1a** and **b** showed a much lower kidney uptake than **[^{125}I]1a**. This trend was also noted for the liver (e.g., 4.54±1.24%ID/g for **[^{125}I]1a** *vs* 1.69±0.04%ID/g and 0.74±0.03%ID/g for **[^{125}I]1b** and

c respectively at 1 h p.i.). For **[^{125}I]1c**, the quantitative distribution was finally lower compared with **[^{125}I]1a** and **b** in all the non-target tissues considered**.** Hence, PEGylation tends to decrease concentration in normal tissues, in particular preserving accumulation in the organs involved in the metabolic and elimination pathways.

An up to 7-fold higher melanoma tumour uptake was found for the PEGylated derivatives **[^{125}I]1b** and **[^{125}I]1c** compared with the parent **[^{125}I]1a** (Fig. 2). The tumoural concentration reached the maximal value of 4.37±1.32%ID/g at 3 h p.i. for the PEGylated conjugate **[^{125}I]1b**. A relatively high and stable uptake was also observed for the other PEGylated congener **[^{125}I]1c** at all-time points, whereas tumoural radioactivity levels for the parent **[^{125}I]1a** were invariably lower and decreased over time. These tumoural concentrations were in the range of those obtained previously with other ICF01012 conjugates (or heteroarylcarboxamide-derived conjugates) aimed at melanoma targeting.[7,8] Distribution kinetics in the melanoma tumour differentiated the three conjugates and was again in favour of the PEGylated derivatives. For the parent **[^{125}I]1a**, the concentration was already maximal (1.01±0.51 %ID/g) at 1 h p.i. and then fell by 40% at 3 h p.i., although it was still detectable at 24h p.i. For the PEGylated conjugate **[^{125}I]1b**, the highest tumoural uptake was rapidly achieved at 3 h p.i. with a 7-fold increase compared with the non-PEGylated parent **[^{125}I]1a**. In addition, the accumulation in the melanoma tumour showed a high retention, e.g. ~ 40% of the maximal activity persisted in the tumour up to 24 h p.i. The other PEGylated congener **[^{125}I]1c** was also rapidly taken up in the tumour. The 2-fold higher uptake value compared with the parent **[^{125}I]1a** reached at 1h p.i. (2.03±0.49%ID/g) remained constant at this initial significant level throughout the period examined. Finally, the residence time in the tumour was prolonged and presented a 5-fold to 7-fold increase for the PEGylated derivatives **[^{125}I]1b** and **[^{125}I]1c** compared with the parent **[^{125}I]1a**. These data reveal other benefits of PEGylation in terms of enhanced uptake and accumulation in the melanoma tumour.

Tumour to non-target organ ratios dramatically increased at all-time points in the case of the PEGylated derivatives **[^{125}I]1b** and **[^{125}I]1c** compared with the parent **[^{125}I]1a** (Fig. 3-6). By early 3 h p.i., tumour to muscle and tumour to blood ratios ranged from 21.8 to 29.7 and 4.20 to 5.62 respectively

for the PEGylated derivatives **[^{125}I]1b** and **[^{125}I]1c** but were below 4.00 for **[^{125}I]1a** (Fig. 3-4). This trend was confirmed up to 24h with 6-fold to 11-fold increased tumour to non-target organ ratios for the PEGylated conjugates. The longer PEG chain derivative **[^{125}I]1c** always displayed the highest ratio values even at early time points. These data clearly demonstrate the high tumour selectivity of PEGylated derivatives **[^{125}I]1b** and particularly **[^{125}I]1c**.

Considering the biodistribution data presented here, the three conjugates **[^{125}I]1a-c** studied were rapidly distributed in the melanoma tumour and simultaneously cleared from non-target organs. This pharmacokinetic profile was observed particularly for the PEGylated derivatives **[^{125}I]1b** and **[^{125}I]1c**, supporting the PEGylation approach applied to our melanoma-targeted conjugates. Uptake data tend to demonstrate that PEGylation both favoured and prolonged tumoural accumulation. Conversely, on the basis of kinetic data, none of the other major organs considered showed any increase in activity over time. As confirmed by the elevated tumour to non-target organs ratios reached at all-time points for the conjugates **[^{125}I]1b** and **[^{125}I]1c** (Fig. 3-6), PEGylation could thus allow rapid and persistent high tumour selectivity. Introduction of the PEG chain could also greatly reduced the exposure of the organs involved in the metabolism and excretion, limiting risks of both hepatotoxicity and nephrotoxicity in further potential chemotherapeutic applications (Fig. 5-6). These important results are consistent with those of our previous study demonstrating that PEGylation increased the stability and improved the metabolic profile of our melanoma-targeted conjugates.[10] They also illustrate that PEGylation offers various advantages to optimize tissue distribution. The two key properties of the polymer are thus its great flexibility owing to the absence of bulky substituents along the chain, and the high hydration of the polymeric backbone.[23] Finally, increasing the PEG chain length did not yield any significant further improvement and the use of a longer polymer conferred little benefit. This result was in agreement with the literature[24] and confirmed that each conjugate requires a dedicated study to determine and choose the best PEG structure (e. g. the number of monomers to link per molecule).

Conclusion

A high specific activity radioiodination method was developed to synthesize three conjugates aimed at melanoma-selective delivery for their *in vivo* evaluation. **[^{125}I]1a-c** were successfully prepared by radioiododestannylation in a rapid procedure under mild conditions with good radiochemical yields and excellent chemical and radiochemical purities. The biodistribution study in B16F0 melanoma-bearing mice showed that the PEGylated derivatives **[^{125}I]1b** and **[^{125}I]1c** displayed an improved profile compared with the non-PEGylated parent **[^{125}I]1a**. They displayed enhanced and prolonged selective accumulation at the targeted tumour site combined to low uptake and fast clearance from non-target organs suggesting that PEGylation is a successful approach to modulating pharmacokinetics. Considering all these results, the PEGylated derivative **1b** probably offered the best compromise in terms of tumour targeting and accumulation associated with low non-targeted organ uptake. These properties are promising and support further development of melanoma-selective agents with improved therapeutic index for anticancer therapy.

Experimental

General

Chemicals were purchased from Sigma Aldrich, Acros Organics, Carlo Erba and SDS and were used without purification. Tetrahydofuran (THF) was distilled from sodium/benzophenone and dichloromethane (DCM) from calcium hydride before use. All reactions were conducted under a dry argon atmosphere. Sodium [^{125}I]-iodide (3.7 GBq mL^{-1}, 643.8 MBq mg^{-1}) as no carrier- added solution in reductant-free aqueous sodium hydroxide (1.0×10^{-5} M) was purchased from PerkinElmer Life and Analytical Sciences (Billerica, MA 01862, US). Citrate buffer solution (pH = 4) was purchased from Merck (Darmstadt, Germany). Analytical thin layer chromatography (TLC) was conducted on precoated silica gel plates (Merck 60 F_{254}, 0.25 mm thick) with both detections by ultraviolet light at 254 nm and visualization by potassium permanganate solution (1%). Flash chromatography was performed using

silica gel 60 A (Merck , 40-63 μm and, SDS, 35-70 μm). Radiolabelled conjugates purification was accomplished by reverse-phase HPLC with a Perkin Elmer series 200 system equipped with a UV PDA detector and a Raytest GABI Star γ detector (Raytest, Straubenhardt, Germany). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC-200 spectrometer (Bruker, Wissembourg, France) using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in parts per million (ppm) from $CDCl_3$ ($\delta_H = 7.26$, $\delta_C = 77.36$). Analytical HPLC was performed on a Hewlett-Packard HP1100 liquid chromatograph (Hewlett-Packard, Les Ulis, France) equipped with a Packard Flow one A_{500} Radiomatic detector (Packard Instruments SA, Rungis, France). Elemental analyses were performed by the CNRS Service Central d'Analyses (Vernaison, France). Electrospray ionization mass spectrometry (ESI) was performed on a Bruker ESQUIRE–LC spectrometer (Bruker Daltonics, Wissembourg, France).

N-(2-ethylaminoethyl)-6-iodoquinoxaline-2-carboxamide was prepared as previously described.[20]

General procedure for the preparation of organostannes (3a-c)

To a solution of the iodo precursors **2a-c** in anhydrous dioxane (0.03 M) were added the convenient hexaalkylditin (2.2 eq.) and dichlorobis(triphenylphosphine) palladium (0.02 eq.). The mixture was stirred for 5h at reflux, filtered through Celite® 545 washed with DCM and concentrated under reduced pressure.

3'-*O*-[1-2-(hydroxyethoxy)ethyl]-5-tributylstannyl-5'-*O*-triisopropylsilyl-2'-deoxyuridine (3a). Prepared from **2a** (0.500 g, 0.84 mmol) with hexabutylditin. After column chromatography on silica gel (EtOAc-cyclohexane, 50-50, v-v) the pure organostanne **3a** was obtained as a colourless oil (0.304 g, 50%). R_f 0.30 (EtOAc-cyclohexane, 50-50, v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (9H, m, $Sn[CH_2CH_2CH_2CH_3]_3$), 1.03-1.10 (27H, m, 3 x ^{i}Pr, $Sn[CH_2CH_2CH_2CH_3]_3$), 1.22-1.61 (15H, m, $O[CHCH_3]O$, $Sn[CH_2CH_2CH_2CH_3]_3$), 1.91-2.25 (1H, H-2'), 2.44-2.61 (1H, m, H-2'), 3.55-3.93 (6H, m, H-5', OCH_2CH_2OH), 4.05-4.17 (1H, m, H-4'), 4.41-4.52 (1H, m, H-3'), 4.86 (1H, q, J = 5.0 Hz,

O[C*H*CH_3]O), 6.12-6.21 (1H, m, H-1'), 7.12 (1H, s, H-6), 8.09 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$): δ10.0 ($^{1}J_{Sn\text{-}C}$ = 172 Hz, Sn(*C*$H_2CH_2CH_2CH_3)_3$), 12.0 (Si[*C*H(CH_3)$_2$]$_3$), 13.8 (Sn[$CH_2CH_2CH_2$*C*H_3]$_3$), 18.2 (Si[CH(*C*H_3)$_2$]$_3$), 20.1 (O[CH*C*H_3]O), 27.4 ($^{2}J_{Sn\text{-}C}$ = 30 Hz, Sn[CH_2*C*$H_2CH_2CH_3$]$_3$), 29.0 (Sn[CH_2CH_2*C*H_2CH_3]$_3$), 37.9 (C-2'), 62.0 (OCH_2*C*H_2OH), 63.8 (C-5'), 66.1 (O*C*H_2CH_2OH), 75.0 (C-3'), 85.6 (C-4'), 85.9 (C-1'), 99.5 (O[*C*HCH_3]O), 112.9 (C-5), 143.1 (C-6), 151.1 (C-2 *C*=O), 166.3 (C-4 *C*=O); MS (ESI): *m/z* cluster peaks around 761.14 and 759.16 [M - H]$^-$; Found C, 53.55; H, 8.70; N, 3.65. $C_{34}H_{66}N_2O_7SiSn$ requires C, 53.61; H, 8.73; N, 3.68%.

3'-*O*-(19-hydroxy-1-methyl-2,5,8,11,14,17-hexaoxanonadec-1-yl)-5'-*O*-triisopropylsilyl-5-trimethylstannyl-2'-deoxyuridine (3b). Prepared from **2b** (1.235 g, 1.51 mmol) with hexamethylditin. After column chromatography on silica gel (EtOAc-DCM-EtOH, 45-45-10, v-v-v) the pure organostanne **3b** was obtained as a colourless oil (0.902 g, 70%). R_f 0.34 (EtOAc-DCM-EtOH, 45-45-10, v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.27 (9H, s, Sn(C*H*$_3$)$_3$), 0.97-1.18 (21H, m, 3 x ^{i}Pr), 1.33 (3H, d, *J* = 5.0 Hz, O[CHC*H*$_3$]O), 1.93-2.17 (1H, H-2'), 2.41-2.52 (1H, m, H-2'), 2.85 (1H, br s, O*H*), 3.54-3.81 (24H, m, 6 x OC*H*$_2$C*H*$_2$O), 3.84-3.92 (2H, m, H-5'), 4.02-4.15 (1H, m, H-4'), 4.38-4.49 (1H, m, H-3'), 4.81-4.90 (1H, m, O[C*H*CH_3]O), 6.13-6.22 (1H, m, H-1'), 7.20 (1H, s, H-6), 8.42 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$): δ-9.2 ($^{1}J_{Sn\text{-}C}$ = 179 Hz (^{117}Sn), 188 Hz (^{119}Sn), Sn[*C*H$_3$]$_3$), 11.9 (Si[*C*H(CH_3)$_2$]$_3$), 18.1 (Si[CH(*C*H_3)$_2$]$_3$), 20.1 (O[CH*C*H_3]O), 38.1 (C-2'), 62.7 (O[CHCH_3]O*C*H_2CH_2O), 63.9 (C-5'), 70.3-70.6 (O[CHCH_3]OCH_2*C*H_2O, 4 x O*C*H_2*C*H_2O, OCH_2*C*H_2OH), 72.6 (O*C*H_2CH_2OH), 75.2 (C-3'), 85.6 (C-4'), 85.9 (C-1'), 99.4 (O[*C*HCH_3]O), 112.7 (C-5), 143.1 (C-6), 151.0 (C-2 *C*=O), 166.4 (C-4 *C*=O); Found C, 49.38; H, 8.11; N, 3.11. $C_{35}H_{68}N_2O_{13}SiSn$ requires C, 49.13; H, 8.01; N, 3.27%.

3'-*O*-(31-hydroxy-1-methyl-2,5,8,11,14,17,20,23,26,29-decaoxahentriacont-1-yl)-5'-*O*-triisopropylsilyl-5-trimethylstannyl-2'-deoxyuridine (3c). Prepared from **2c** (0.250 g, 0.25 mmol) hexamethylditin. After column chromatography on silica gel (EtOAc-DCM-EtOH, 20-70-10, v-v-v) the pure organostanne **3c** was obtained as a colourless oil (0.206 g, 80%). R_f 0.29 (EtOAc-DCM-EtOH, 20-

70-10, v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.26 (9H, s, Sn(C*H*$_3$)$_3$), 1.00-1.08 (21H, m, 3 x ^{i}Pr), 1.33 (3H, d, J = 5.0 Hz, O[CHC*H*$_3$]O), 1.91-2.15 (1H, H-2'), 2.40-2.53 (1H, m, H-2'), 3.12 (1H, br s, O*H*), 3.50-3.79 (40H, m, 10 x OC*H*$_2$C*H*$_2$O), 3.83-3.91 (2H, m, H-5'), 4.01-4.12 (1H, m, H-4'), 4.37-4.47 (1H, m, H-3'), 4.77-4.87 (1H, m, O[C*H*CH$_3$]O), 6.13-6.22 (1H, m, H-1'), 7.19 (1H, s, H-6), 8.59 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$): δ-9.2 ($^lJ_{Sn-C}$ = 179 Hz (^{117}Sn), 189 Hz (^{119}Sn), Sn[*C*H$_3$]$_3$), 12.0 (Si[*C*H(CH$_3$)$_2$]$_3$), 18.1 (Si[CH(*C*H$_3$)$_2$]$_3$), 20.2 (O[CH*C*H$_3$]O), 38.2 (C-2'), 62.5 (O[CHCH$_3$]O*C*H$_2$CH$_2$O), 63.9 (C-5'), 69.9-70.7 (O[CHCH$_3$]OCH$_2$*C*H$_2$O , 8 x O*C*H$_2$*C*H$_2$O, OCH$_2$*C*H$_2$OH), 72.3 (O*C*H$_2$CH$_2$OH), 75.2 (C-3'), 85.6 (C-4'), 85.7 (C-1'), 99.4 (O[*C*HCH$_3$]O), 112.7 (C-5), 143.1 (C-6), 150.9 (C-2 *C*=O), 166.2 (C-4 *C*=O); Found C, 50.28; H, 8.32; N, 2.49. $C_{43}H_{84}N_2O_{16}SiSn$ requires C, 50.05; H, 8.20; N, 2.71%.

General procedure for the preparation of mesylates (4a-c)

To a solution of the organostannes **3a-c** and DIPEA (2.5 eq.) in anhydrous DCM (0.08 M) was added mesyl chloride (1.2 eq.). The mixture was stirred for 1 h at room temperature and then poured into a mixture of sat. aq. $NaHCO_3$ solution. The aqueous layer was extracted with three times with DCM, the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure.

3'-*O*-{1-[2-(methylsulfonyloxy)ethoxy]ethyl}-5-tributylstannyl-5'-*O*-triisopropylsilyl-2'-deoxyuridine (4a). Prepared from **3a** (0.510 g, 0.67 mmol). After column chromatography on silica gel (EtOAc-cyclohexane, 45-55, v-v) the pure mesylate **4a** was obtained as a colourless oil (0.426 g, 76%). R_f 0.35 (EtOAc-cyclohexane, 45-55, v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.89 (9H, t, J = 7.0 Hz, Sn[CH$_2$CH$_2$CH$_2$C*H*$_3$]$_3$), 0.99-1.13 (27H, m, 3 x ^{i}Pr, Sn[C*H*$_2$CH$_2$CH$_2$CH$_3$]$_3$), 1.22-1.43 (9H, m, O[CHC*H*$_3$]O, Sn[CH$_2$C*H*$_2$CH$_2$CH$_3$]$_3$), 1.46-1.56 (6H, m, Sn[CH$_2$CH$_2$C*H*$_2$CH$_3$]$_3$), 1.94-2.16 (1H, H-2'), 2.39-2.51 (1H, m, H-2'), 3.03 (3H, s, OSO$_2$C*H*$_3$), 3.79-3.94 (4H, m, H-5', OC*H*$_2$CH$_2$OMs), 4.03-4.10 (1H, m, H-4'), 4.35 (2H, t, J = 5.2 Hz, OCH$_2$C*H*$_2$OMs), 4.45 (1H, t, J = 5.8 Hz, H-3'), 4.86 (1H, q, J =

5.0 Hz, O[C*H*CH$_3$]O), 6.12-6.22 (1H, m, H-1'), 7.11 (1H, s, H-6), 7.87 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$): δ10.0 ($^{1}J_{Sn\text{-}C}$ = 171 Hz (^{117}Sn), 179 Hz (^{119}Sn), Sn[*C*H$_2$CH$_2$CH$_2$CH$_3$]$_3$), 12.0 (Si[*C*H(CH$_3$)$_2$]$_3$), 13.8 (Sn[CH$_2$CH$_2$CH$_2$*C*H$_3$]$_3$), 18.1 (Si[CH(*C*H$_3$)$_2$]$_3$), 20.0 (O[CH*C*H$_3$]O), 27.4 ($^{2}J_{Sn\text{-}C}$ = 30 Hz, Sn[CH$_2$*C*H$_2$CH$_2$CH$_3$]$_3$), 29.1 (Sn[CH$_2$CH$_2$*C*H$_2$CH$_3$]$_3$), 37.7 (C-2'), 37.8 (OSO$_2$*C*H$_3$), 62.3 (O*C*H$_2$CH$_2$OMs), 63.8 (C-5'), 69.1 (OCH$_2$*C*H$_2$OMs), 75.8 (C-3'), 85.6 (C-4'), 85.8 (C-1'), 99.2 (O[*C*HCH$_3$]O), 112.9 (C-5), 143.0 (C-6), 151.0 (C-2 *C*=O), 166.1 (C-4 *C*=O).

3'-*O*-(1-methyl-21,21-dioxo-2,5,8,11,14,17,20-heptaoxa-21-thiadocos-1-yl)-5'-*O*-triisopropylsilyl-5-trimethylstannyl-2'-deoxyuridine (4b). Prepared from **3b** (0.902 g, 1.05 mmol). After column chromatography on silica gel (EtOAc-DCM-EtOH, 47.5-47.5-5, v-v-v) the pure mesylate **4b** was obtained as a white foam (0.910 g, 93%). R_f 0.29 (EtOAc-DCM-EtOH, 47.5-47.5-5, v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.27 (9H, s, Sn(C*H*$_3$)$_3$), 1.01-1.22 (21H, m, 3 x ^{i}Pr), 1.34 (3H, m, O[CHC*H*$_3$]O), 1.93-2.17 (1H, H-2'), 2.41-2.50 (1H, m, H-2'), 3.08 (3H, s, OSO$_2$C*H*$_3$), 3.53-3.80 (22H, m, 5 x OC*H*$_2$C*H*$_2$O, OC*H*$_2$CH$_2$OMs), 3.84-3.92 (2H, m, H-5'), 4.06-4.13 (1H, m, H-4'), 4.35-4.45 (3H, m, H-3', OCH$_2$C*H*$_2$OMs), 4.81-4.86 (1H, m, O[C*H*CH$_3$]O), 6.13-6.23 (1H, m, H-1'), 7.19 (1H, s, H-6), 8.25 (1H, br s, N*H*); ^{13}C NMR ($CDCl_3$): δ-9.1 ($^{1}J_{Sn\text{-}C}$ = 176 Hz (^{117}Sn), 185 Hz (^{119}Sn), Sn[*C*H$_3$]$_3$), 12.0 (Si[*C*H(CH$_3$)$_2$]$_3$), 18.1 (Si[CH(*C*H$_3$)$_2$]$_3$), 20.1 (O[CH*C*H$_3$]O), 37.8 (OSO$_2$*C*H$_3$), 38.5 (C-2'), 63.8 (O[CHCH$_3$]O*C*H$_2$CH$_2$O), 64.1 (C-5'), 69.1 (OCH$_2$*C*H$_2$OMs), 69.4 (O*C*H$_2$CH$_2$OMs), 70.6-70.7 (O[CHCH$_3$]OCH$_2$*C*H$_2$O , 4 x O*C*H$_2$*C*H$_2$O), 74.5 (C-3'), 85.6 (C-4'), 85.9 (C-1'), 99.3 (O[*C*HCH$_3$]O), 112.6 (C-5), 143.0 (C-6), 150.9 (C-2 *C*=O), 166.3 (C-4 *C*=O).

3'-*O*-(1-methyl-33,33-dioxo-2,5,8,11,14,17,20,23,26,29,32-undecaoxa-33-thiatetratriacont-1-yl)-5'-*O*-triisopropylsilyl-5-trimethylstannyl-2'-deoxyuridine (4c). Prepared from **3c** (0.186 g, 0.18 mmol). After column chromatography on silica gel (EtOAc-DCM-EtOH, 46-46-8, v-v-v) the pure mesylate **4c** was obtained as a light yellow oil (0.118 g, 60%). R_f 0.32 (EtOAc-DCM-EtOH, 46-46-8, v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.26 (9H, s, Sn(C*H*$_3$)$_3$), 1.00-1.11 (21H, m, 3 x ^{i}Pr), 1.34 (3H, t, *J* = 5.0 Hz, O[CHC*H*$_3$]O), 1.94-2.15 (1H, H-2'), 2.40-2.53 (1H, m, H-2'), 3.07 (3H, s, OSO$_2$C*H*$_3$), 3.59-

3.67 (36H, m, 9 x OCH_2CH_2O), 3.73-3.80 (2H, m, OCH_2CH$_2$OMs), 3.81-3.92 (2H, m, H-5'), 3.96-4.14 (1H, m, H-4'), 4.35-4.39 (2H, m, OCH$_2$CH_2OMs), 4.44-4.47 (1H, m, H-3'), 4.81-4.85 (1H, m, O[C*H*CH$_3$]O), 6.13-6.20 (1H, m, H-1'), 7.18 (1H, s, H-6), 8.41 (1H, br s, N*H*); ^{13}C NMR (CDCl$_3$): δ-9.1 ($^{1}J_{Sn\text{-}C}$ = 179 Hz (^{117}Sn), 188 Hz (^{119}Sn), Sn[*C*H$_3$]$_3$), 12.0 (Si[*C*H(CH$_3$)$_2$]$_3$), 18.1 (Si[CH(*C*H$_3$)$_2$]$_3$), 20.2 (O[CH*C*H$_3$]O), 37.8 (OSO$_2$*C*H$_3$), 38.1 (C-2'), 63.8 (O[CHCH$_3$]O*C*H$_2$CH$_2$O), 64.1 (C-5'), 69.1 (OCH$_2$*C*H$_2$OMs), 69.4 (O*C*H$_2$CH$_2$OMs), 70.5-70.7 (O[CHCH$_3$]OCH$_2$*C*H$_2$O , 8 x OCH$_2$*C*H$_2$O), 74.7 (C-3'), 85.6 (C-4'), 85.9 (C-1'), 99.4 (O[*C*HCH$_3$]O), 112.5 (C-5), 143.0 (C-6), 150.8 (C-2 *C*=O), 166.1 (C-4 *C*=O).

General procedure for the preparation of the conjugates (5a-c)

To a solution of the mesylates **4a-c** and DIPEA (1.5 eq.) in anhydrous MeCN (0.05 M) was added *N*-(2-ethylaminoethyl)-6-iodoquinoxaline-2-carboxamide[19] (1.5 eq.). The mixture was stirred for a different time at a different temperature for each compound then poured into sat. aq. NaHCO$_3$ solution. The aqueous layer was extracted with three times with DCM, the combined organic layers were dried over MgSO$_4$ and concentrated under reduced pressure.

3'-*O*-(1-{2-[ethyl(2-{[(6-iodoquinoxalin-2-yl)carbonyl]amino}ethyl)amino]ethoxy}ethyl)-5-tributylstannyl-5'-*O*-triisopropylsilyl-2'-deoxyuridine (5a). Prepared from **4a** (0.330 g, 0.39 mmol). Time of reaction: 6 d, temperature: 55 °C. After column chromatography on silica gel (EtOAc-EOH-NEt$_3$, 85-15-2, v-v-v) the pure conjugate **5a** was obtained as a brown oil (0.100 g, 23%). R_f 0.35 (EtOAc-EOH-NEt$_3$, 85-15-2, v-v-v); ^{1}H NMR (200 MHz, CDCl$_3$): δ 0.92 (9H, t, J = 7.0 Hz, Sn[CH$_2$CH$_2$CH$_2$C*H$_3$*]$_3$), 0.99-1.11 (30H, m, 3 x ^{i}Pr, Sn[C*H$_2$*CH$_2$CH$_2$CH$_3$]$_3$, NCH$_2$C*H$_3$*), 1.22-1.36 (9H, m, O[CHC*H$_3$*]O, Sn[CH$_2$C*H$_2$*CH$_2$CH$_3$]$_3$), 1.43-1.68 (6H, m, Sn[CH$_2$CH$_2$C*H$_2$*CH$_3$]$_3$), 1.94-2.11 (1H, m, H-2'), 2.34-2.51 (1H, m, H-2'), 2.61-2.80 (6H, m, 3 x NC*H$_2$*), 3.49-3.71 (6H, m, H-5', OC*H$_2$*CH$_2$N, NCH$_2$C*H$_2$*NH), 4.04-4.08 (1H, m, H-4'), 4.37-4.41 (1H, m, H-3'), 4.80 (1H, q, J = 5.0 Hz,

O[C*H*CH_3]O), 6.15 (1H, m, H-1'), 7.12 (1H, s, H-6), 7.82 (1H, d, *J* = 8.8 Hz, Ar*H 'm'* to I), 7.88 (1H, br s, H-3), 8.08 (1H, dd, *J* = 8.8 Hz, 1.9, Ar*H 'o'* to I), 8.38 (1H, br s, ArCON*H*), 8.61 (d, 1H, *J* = 1.9 Hz, Ar*H 'o'* to I), 9.64 (1H, s, Ar*H 'o'* to CONH); ^{13}C NMR ($CDCl_3$): δ 9.9 ($^{1}J_{Sn\text{-}C}$ = 171 Hz (^{117}Sn), 179 Hz (^{119}Sn), Sn[*C*$H_2CH_2CH_2CH_3$]$_3$), 12.0 (Si[*C*H(CH_3)$_2$]$_3$, NCH$_2$*C*H_3), 13.8 (Sn[$CH_2CH_2CH_2$*C*H_3]$_3$), 18.1 (Si[CH(*C*H_3)$_2$]$_3$), 20.2 (O[CH*C*H_3]O), 27.3 ($^{2}J_{Sn\text{-}C}$ = 30 Hz, Sn[CH_2*C*$H_2CH_2CH_3$]$_3$), 29.0 ($^{3}J_{Sn\text{-}C}$ = 10 Hz, Sn[CH_2CH_2*C*H_2CH_3]$_3$), 37.6 (NCH$_2$*C*H_2NH), 38.1 (C-2'), 48.6 (N*C*H_2CH_3), 52.9, 53.2 (OCH$_2$*C*H_2N, N*C*H_2CH_2NH), 63.7 (O*C*H_2CH_2N), 64.0 (C-5'), 75.2 (C-3'), 85.6, 85.7 (C-1', C-4'), 98.0 (Ar*C*I), 99.4 (O[*C*HCH$_3$]O), 112.8 (C-5), 130.8 (Ar*C*H *'m'* to I), 138.6 (Ar*C*H *'β'* to N), 139.6 (Ar*C 'p'* to I), 139.7 (Ar*C*H *'o'* to I), 142.9 (C-6), 144.2, 144.4 (Ar*C*CONH, Ar*C 'm'* to I), 144.8 (Ar*C*H *'o'* to CONH), 151.1 (C-2 C=O), 163.0 (Ar*C*ONH), 166.4 (C-4 C=O).

3'-*O*-[20-ethyl-24-(6-iodoquinoxalin-2-yl)-1-methyl-24-oxo-2,5,8,11,14,17-hexaoxa-20,23-diazatetracos-1-yl]-5'-*O*-triisopropylsilyl-5-trimethylstannyl-2'-deoxyuridine (5b). Prepared from **4b** (0.865 g, 0.93 mmol). Time of reaction: 90 h, temperature: 65 °C. After column chromatography on silica gel (DCM-EOH-NH_4OH, 93-7-1, v-v-v) the pure conjugate **5b** was obtained as a brown gum (0.395 g, 35%). R_f 0.30 (DCM-EOH-NH_4OH, 93-7-1, v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.27 (9H, s, Sn(C*H*$_3$)$_3$), 1.01-1.10 (24H, m, 3 x ^{i}Pr, NCH$_2$C*H*$_3$), 1.33 (3H, m, O[CHC*H*$_3$]O), 1.92-2.16 (1H, m, H-2'), 2.40-2.54 (1H, m, H-2'), 2.66 (2H, q, *J* = 6.8 Hz, NC*H*$_2$CH$_3$), 2.73-2.81 (4H, m, 2 x NC*H*$_2$), 3.55-3.80 (24H, m, 5 x OC*H*$_2$C*H*$_2$O, OC*H*$_2$CH$_2$N, NCH$_2$C*H*$_2$NH), 3.84-3.92 (2H, m, H-5'), 4.12-4.13 (1H, m, H-4'), 4.38-4.48 (1H, m, H-3'), 4.78-4.88 (1H, m, O[C*H*CH_3]O), 6.13-6.22 (1H, m, H-1'), 7.19 (1H, s, H-6), 7.81 (1H, d, *J* 8.8 Hz, Ar*H 'm'* to I), 8.07 (1H, dd, *J* = 8.8, 1.9, Ar*H 'o'* to I), 8.25-8.43 (2H, m, H-3, ArCON*H*), 8.60 (d, 1H, *J* 1.9, Ar*H 'o'* to I), 9.64 (1H, s, Ar*H 'o'* to CONH); ^{13}C NMR ($CDCl_3$): δ -9.1 ($^{1}J_{Sn\text{-}C}$ = 179 Hz (^{117}Sn), 188 Hz (^{119}Sn), Sn(*C*H_3)$_3$), 12.0 (Si[*C*H(CH_3)$_2$]$_3$), 12.2 (NCH$_2$*C*H_3), 18.1 (Si[CH(*C*H_3)$_2$]$_3$), 20.2 (O[CH*C*H_3]O), 37.6 (NCH$_2$*C*H_2NH), 38.1 (C-2'), 48.7 (N*C*H_2CH_3), 52.8, 53.0 (OCH$_2$*C*H_2N, N*C*H_2CH_2NH), 63.8 (C-5'), 63.9 (O*C*H_2CH_2N), 70.2-70.6 (O[CH*C*H_3]OCH$_2$*C*H_2, 4 x O*C*H_2*C*H_2O, O*C*H_2CH_2N), 75.1 (C-3'), 85.6 (C-4'), 85.7 (C-1'), 98.0 (Ar*C*I), 99.4 (O[*C*HCH$_3$]O),

112.7 (C-5), 130.9 (Ar*C*H *'m'* to I), 138.7 (Ar*C*H *'β'* to N), 139.6 (Ar*C 'p'* to I), 139.7 (Ar*C*H *'o'* to I), 143.0 (C-6), 144.3, 144.4 (Ar*C*CONH, Ar*C 'm'* to I), 144.8 (Ar*C*H *'o'* to CONH), 150.9 (C-2 C=O), 163.0 (Ar*C*ONH), 166.2 (C-4 C=O).

3'-*O*-[32-ethyl-36-(6-iodoquinoxalin-2-yl]-1-methyl-36-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,35-diazahexatriacont-1-yl)-5'-*O*-triisopropylsilyl-5-trimethylstannyl-2'-deoxyuridine (5c). Prepared from **4c** (0.100 g, 0.93 mmol). Time of reaction: 72 h, temperature: 70 °C. After column chromatography on silica gel (AcOEt-DCM-EtOH-NH_4OH, 30-60-10-0.5, v-v-v-v) the pure conjugate **5c** was obtained as a brown gum (32 mg, 26%). R_f 0.26 (AcOEt-DCM-EtOH-NH_4OH, 30-60-10-0.5, v-v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.26 (9H, s, Sn(C*H*$_3$)$_3$), 1.02-1.12 (24H, m, 3 x ^{i}Pr, NCH$_2$C*H*$_3$), 1.53 (3H, m, O[CHC*H*$_3$]O), 1.94-2.18 (1H, m, H-2'), 2.39-2.52 (1H, m, H-2'), 2.67 (2H, q, *J* = 6.8, NC*H*$_2$CH$_3$), 2.72-2.83 (4H, m, 2 x NC*H*$_2$), 3.52-3.82 (40H, m, 9 x OC*H*$_2$C*H*$_2$O, OC*H*$_2$CH$_2$N, NCH$_2$C*H*$_2$NH), 3.82-3.90 (2H, m, H-5'), 4.01-4.11 (1H, m, H-4'), 4.37-4.49 (1H, m, H-3'), 4.75-4.84 (1H, m, O[C*H*CH$_3$]O), 6.12-6.23 (1H, m, H-1'), 7.21 (1H, s, H-6), 7.82 (1H, d, *J* = 8.8 Hz, Ar*H 'm'* to I), 8.09 (1H, dd, *J* = 8.8 Hz, 1.9, Ar*H 'o'* to I), 8.37-8.49 (2H, m, H-3, ArCON*H*), 8.61 (d, 1H, *J* = 1.9 Hz, Ar*H 'o'* to I), 9.65 (1H, s, Ar*H 'o'* to CONH); ^{13}C NMR ($CDCl_3$): δ -9.2 ($^1J_{Sn\text{-}C}$ = 178 Hz (^{117}Sn), 188 Hz (^{119}Sn), Sn(*C*H$_3$)$_3$), 12.0 (Si[*C*H(CH$_3$)$_2$]$_3$), 12.2 (NCH$_2$*C*H$_3$), 18.1 (Si[CH(*C*H$_3$)$_2$]$_3$), 20.1 (O[CH*C*H$_3$]O), 37.6 (NCH$_2$*C*H$_2$NH), 38.1 (C-2'), 48.7 (N*C*H$_2$CH$_3$), 52.9, 53.0 (OCH$_2$*C*H$_2$N, N*C*H$_2$CH$_2$NH), 63.8 (C-5'), 63.9 (O*C*H$_2$CH$_2$N), 70.1-70.7 (O[CH*C*H$_3$]OCH$_2$*C*H$_2$, 8 x O*C*H$_2$*C*H$_2$O, O*C*H$_2$CH$_2$N), 75.1 (C-3'), 85.7 (C-4'), 85.8 (C-1'), 98.0 (Ar*C*I), 99.3 (O[*C*HCH$_3$]O), 112.6 (C-5), 130.9 (Ar*C*H *'m'* to I), 138.6 (Ar*C*H *'β'* to N), 139.6 (Ar*C 'p'* to I), 139.7 (Ar*C*H *'o'* to I), 143.0 (C-6), 144.3, 144.5 (Ar*C*CONH, Ar*C 'm'* to I), 144.8 (Ar*C*H *'o'* to CONH), 150.9 (C-2 C=O), 163.1 (Ar*C*ONH), 166.2 (C-4 C=O).

General procedure for the preparation of deprotected conjugates (6a-c)

To a solution of conjugates **5a-c** in anhydrous THF (0.03 M) was added tetrabutylammonium fluoride (1 M in THF, 2 eq.). The mixture was stirred for 4 h at room temperature and poured into sat.aq. $NaHCO_3$ solution. The aqueous layer was extracted with three times with DCM, the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure.

3'-*O*-(1-{2-[ethyl(2-{[(6-iodoquinoxalin-2-yl)carbonyl]amino}ethyl)amino]ethoxy}ethyl)-5-tributylstannyl-2'-deoxyuridine (6a). Prepared from **5a** (0.190 g, 0.17 mmol). After column chromatography on silica gel (EtOAc-EtOH-NEt_3, 90-10-2, v-v-v) the pure deprotected conjugate **6a** was obtained as a light brown foam (0.138 g, 84%). R_f 0.26 (EtOAc-EtOH-Net_3, 90-10-2, v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ0.84 (9H, t, J = 7.0 Hz, Sn[$CH_2CH_2CH_2CH_3$]$_3$), 0.96-1.06 (9H, m, Sn[$CH_2CH_2CH_2CH_3$]$_3$, NCH_2CH_3), 1.20-1.28 (9H, m, O[$CHCH_3$]O, Sn[$CH_2CH_2CH_2CH_3$]$_3$), 1.43-1.52 (6H, m, Sn[$CH_2CH_2CH_2CH_3$]$_3$), 2.27-2.40 (2H, m, H-2'), 2.61-2.80 (6H, m, 3 x NCH_2), 3.47-3.65 (4H, m, OCH_2CH_2N, NCH_2CH_2NH), 3.76-3.82 (H-5'), 3.99-4.08 (1H, m, H-4'), 4.50-4.54 (1H, m, H-3'), 4.81-4.82 (1H, m, O[$CHCH_3$]O), 6.07 (1H, t, J = 6.2 Hz, H-1'), 7.32 (1H, s, H-6), 7.78 (1H, d, J = 8.9 Hz, Ar*H 'm'* to I), 8.05 (1H, dd, J = 8.9 Hz, 1.8, Ar*H 'o'* to I), 8.35-8.41 (2H, m, H-3, ArCON*H*), 8.58 (d, 1H, J = 1.8 Hz, Ar*H 'o'* to I), 9.60 (1H, s, Ar*H 'o'* to CONH); ^{13}C NMR ($CDCl_3$): δ9.9 ($^1J_{Sn\text{-}C}$ = 171 Hz (^{117}Sn), 179 Hz (^{119}Sn), Sn[$CH_2CH_2CH_2CH_3$]$_3$), 11.9 (NCH_2CH_3), 13.8 (Sn[$CH_2CH_2CH_2CH_3$]$_3$), 20.2 (O[$CHCH_3$]O), 27.3 ($^2J_{Sn\text{-}C}$ = 30 Hz, Sn[$CH_2CH_2CH_2CH_3$]$_3$), 29.0 ($^3J_{Sn\text{-}C}$ = 10 Hz, Sn[$CH_2CH_2CH_2CH_3$]$_3$), 37.7 (NCH_2CH_2NH), 38.5 (C-2'), 48.8 (NCH_2CH_3), 53.1, 53.3 (OCH_2CH_2N, NCH_2CH_2NH), 62.4 (C-5'), 63.7 (OCH_2CH_2N), 74.4 (C-3'), 85.6 (C-4'), 87.3 (C-1'), 98.2 (Ar*C*I), 99.4 (O[*C*HCH$_3$]O), 112.9 (C-5), 129.8 (Ar*C*H *'m'* to I), 138.7 (Ar*C*H *'β'* to N), 139.6 (Ar*C 'p'* to I), 139.9 (Ar*C*H *'o'* to I), 144.0, 144.5 (Ar*C*CONH, Ar*C 'm'* to I), 144.7 (Ar*C*H *'o'* to CONH), 144.9 (C-6), 151.1 (C-2 C=O), 163.2 (Ar*C*ONH), 166.4 (C-4 C=O); MS (ESI): *m/z* 959.43 and 957.42 $[M + H]^+$; Found C, 47.88; H, 6.43; N, 8.55. $C_{38}H_{59}IN_6O_7Sn$ requires C, 47.66; H, 6.22; N, 8.77%.

3'-*O*-[20-ethyl-24-(6-iodoquinoxalin-2-yl)-1-methyl-24-oxo-2,5,8,11,14,17-hexaoxa-20,23-diazatetracos-1-yl]-5-trimethylstannyl-2'-deoxyuridine (6b). Prepared from **5b** (0.350 g, 0.29 mmol).

After column chromatography on silica gel (EtOAc-DCM-EtOH-NH_4OH, 70-20-10-0.5, v-v-v-v) the pure deprotected conjugate **6b** was obtained as a yellow gum (0.205 g, 67%). R_f 0.31 (EtOAc-DCM-EtOH-NH_4OH, 70-20-10-0.5, v-v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.27 (9H, s, Sn(CH_3)$_3$), 1.07 (3H, t, J = 5.2 Hz, NCH_2CH_3), 1.31 (3H, d, J = 5.0 Hz, O[$CHCH_3$]O), 2.22-2.41 (2H, m, H-2'), 2.66 (2H, q, J = 6.8 Hz, NCH_2CH_3), 2.72-2.81 (4H, m, 2 x NCH_2), 3.48-3.70 (24H, m, 5 x OCH_2CH_2O, OCH_2CH_2N, NCH_2CH_2NH), 3.73-3.93 (2H, m, H-5'), 4.02-4.13 (1H, m, H-4'), 4.54-4.62 (1H, m, H-3'), 4.83 (1H, q, J = 5.0 Hz, O[$CHCH_3$]O), 6.13-6.21 (1H, m, H-1'), 7.57 (1H, s, H-6), 7.82 (1H, d, J = 8.8 Hz, Ar*H 'm'* to I), 8.08 (1H, dd, J = 8.8 Hz, 1.9 Hz, Ar*H 'o'* to I), 8.15 (1H, br s, H-3), 8.42 (1H, br s, ArCONH), 8.61 (d, 1H, J = 1.9 Hz, Ar*H 'o'* to I), 9.64 (1H, s, Ar*H 'o'* to CONH); ^{13}C NMR ($CDCl_3$): δ -9.2 ($^1J_{Sn\text{-}C}$ = 178 Hz (^{117}Sn), 187 Hz (^{119}Sn), Sn(CH_3)$_3$), 12.2 (NCH_2CH_3), 20.1 (O[$CHCH_3$]O), 37.6 (NCH_2CH_2NH), 38.8 (C-2'), 48.7 (NCH_2CH_3), 52.8, 53.0 (OCH_2CH_2N, NCH_2CH_2NH), 62.0 (C-5'), 63.7 (O[$CHCH_3$]OCH_2CH_2O), 70.1-70.7 (O[$CHCH_3$]OCH_2CH_2, 4 x OCH_2CH_2O, OCH_2CH_2N), 73.6 (C-3'), 85.7 (C-1'), 86.5 (C-4'), 98.0 (ArCI), 98.9 (O[$CHCH_3$]O), 112.7 (C-5), 130.9 (ArCH *'m'* to I), 138.6 (ArCH *'β'* to N), 139.6 (ArC *'p'* to I), 139.7 (ArCH *'o'* to I), 144.3, 144.4 (ArCCONH, ArC *'m'* to I), 144.7 (C-6), 144.8 (ArCH *'o'* to CONH), 151.1 (C-2 C=O), 163.0 (ArCONH), 166.3 (C-4 C=O); MS (ESI): *m/z* 1053.37 and 1051.36 $[M + H]^+$; Found C, 44.79; H, 5.96; N, 7.74. $C_{39}H_{61}IN_6O_{12}Sn$ requires C, 44.54; H, 5.86; N, 7.99%.

3'-*O*-[32-ethyl-36-(6-iodoquinoxalin-2-yl)-1-methyl-36-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,35-diazahexatriacont-1-yl]-5-trimethylstannyl-2'-deoxyuridine (6c). Prepared from **5c** (0.032 g, 0.02 mmol). After column chromatography on silica gel (EtOAc-DCM-EtOH-NH_4OH, 20-68-12-0.5, v-v-v-v) the pure deprotected conjugate **6c** was obtained as a brown gum (8 mg, 30%). R_f 0.28 (EtOAc-DCM-EtOH-NH_4OH, 20-68-12-0.5, v-v-v-v); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.26 (9H, s, Sn(CH_3)$_3$), 1.06 (3H, t, J = 5.2 Hz, NCH_2CH_3), 1.32 (3H, d, J = 5.0 Hz, O[$CHCH_3$]O), 2.14-2.40 (2H, m, H-2'), 2.66 (2H, q, J = 6.8 Hz, NCH_2CH_3), 2.73-2.84 (4H, m, 2 x NCH_2), 3.58-3.74 (40H, m, 9 x OCH_2CH_2O, OCH_2CH_2N, NCH_2CH_2NH), 3.69-3.89 (2H, m, H-5'), 4.03-4.10 (1H, m, H-4'), 4.54-4.64 (1H, m, H-

3'), 4.83 (1H, q, *J* = 5.0 Hz, O[C*H*CH_3]O), 6.15-6.22 (1H, m, H-1'), 7.55 (1H, s, H-6), 7.81 (1H, d, *J* = 8.8 Hz, Ar*H 'm'* to I), 8.07 (1H, dd, *J* = 8.8 Hz, 1.9 Hz, Ar*H 'o'* to I), 8.15 (1H, br s, H-3), 8.49 (1H, br s, ArCON*H*), 8.60 (d, 1H, *J* = 1.9 Hz, Ar*H 'o'* to I), 9.63 (1H, s, Ar*H 'o'* to CONH); ^{13}C NMR ($CDCl_3$): δ -9.2 ($^{1}J_{Sn\text{-}C}$ = 179 Hz (^{117}Sn), 188 Hz (^{119}Sn), Sn(CH_3)$_3$), 12.2 (NCH$_2$*C*H$_3$), 20.1 (O[CH*C*H$_3$]O), 37.5 (NCH$_2$*C*H$_2$NH), 38.8 (C-2'), 48.6 (N*C*H$_2$CH$_3$), 52.8, 53.0 (OCH$_2$*C*H$_2$N, N*C*H$_2$CH$_2$NH), 62.1 (C-5'), 63.6 (O[CHC*H$_3$*]O*C*H$_2$CH$_2$O), 70.1-70.9 (O[CH*C*H$_3$]OCH$_2$*C*H$_2$, 8 x O*C*H$_2$*C*H$_2$O, O*C*H$_2$CH$_2$N), 73.6 (C-3'), 85.7 (C-1'), 86.4 (C-4'), 98.1 (Ar*C*I), 98.9 (O[*C*HCH$_3$]O), 112.6 (C-5), 130.9 (Ar*C*H *'m'* to I), 138.6 (Ar*C*H *'β'* to N), 139.6 (Ar*C 'p'* to I), 139.8 (Ar*C*H *'o'* to I), 144.2, 144.3 (Ar*C*CONH, Ar*C 'm'* to I), 144.7 (C-6), 144.8 (Ar*C*H *'o'* to CONH), 151.1 (C-2 C=O), 163.0 (Ar*C*ONH), 166.3 (C-4 C=O); MS (ESI): *m/z* 1229.40 and 1227.38 $[M + H]^+$; Found C, 46.29; H, 6.47; N, 6.84. $C_{47}H_{77}IN_6O_{16}Sn$ requires C, 45.97; H, 6.33; N, 6.84%.

General procedure for the preparation of radioiodinated conjugates [^{125}I]1a-c

To a hydrochloric acid solution (100 µL, 0,1 M) was added the precursors **6a-c** in EtOH (100 µL, 1 mg mL^{-1}) and sodium [^{125}I]-iodide (55-80 µL, 200-300 MBq) followed by chloramine T (40 µL, 1 mg mL^{-1}). After 10 min at room temperature, the reaction was quenched by the addition of aqueous sodium metabisulfite solution (100 µL, 0.5 mg mL^{-1}) and aqueous sodium hydroxide solution (200 µL, 0,1 M). The radioiodinated conjugates were purified by reverse-phase HPLC using a C_{18} column (WatersSymmetryPrep C_{18}, 7.8 × 300 mm, 7 µm, Waters). Mobile phase A was H_2O/NH_4OH (99.9/0.1, v-v) and mobile phase B was MeOH/NH_4OH (99.9/0.1, v-v). The flow rate was 2.0 mL min^{-1}, and a gradient mobile phase composition was used: 8 min gradient from 60% to 80% B, 2 min isocratic at 80% B, 10 min gradient to 100% B and 5 min isocratic at 100% B. Determination of the chemical and radiochemical purities was carried out by analytical HPLC performed on a C_{18} reverse phase column (Purospher RP$_{18}$ e, 150 × 4.6 mm, 5 µm) with the above mentioned elution conditions, an a flow rate of

0.5 mL min^{-1}. Each radiodinated product **[^{125}I]1a-c** co-migrated with the non-radioactive standard **1a-c**[10] independently prepared and showed chemical and radiochemical purities exceeding 99%.

3'-*O*-(1-{2-[ethyl(2-{[(6-iodoquinoxalin-2-yl)carbonyl]amino}ethyl)amino]ethoxy}ethyl)-5-[^{125}I]iodo-2'-deoxyuridine ([^{125}I]1a). Yield : 81%. Rt = 12.5 min.

3'-*O*-(20-ethyl-24-(6-iodoquinoxalin-2-yl)-1-methyl-24-oxo-2,5,8,11,14,17-hexaoxa-20,23-diazatetracos-1-yl)-5-[^{125}I]iodo-2'-deoxyuridine ([^{125}I]1b). Yield : 40%. Rt = 13.6 min.

3'-*O*-(32-ethyl-36-(6-iodoquinoxalin-2-yl)-1-methyl-36-oxo-2,5,8,11,14,17,20,23,26,29-decaoxa-32,35-diazahexatriacont-1-yl)-5-[^{125}I]iodo-2'-deoxyuridine ([^{125}I]1c). Yield : 25%. Rt = 14.4 min.

Biodistribution

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 cell line from ATCC (no. CRL-6322). Primary melanoma model was induced by subcutaneous injection of B16F0 murine melanoma cells in C57BL/6J mice as previously described.[6] On day 14 after tumour implant, each [^{125}I]-labelled conjugate was administered intravenously *via* the tail vein (1.5-3.3 MBq / mouse, n = 4 animals / conjugate). Animals were sacrificed by CO_2 inhalation at various time intervals after injection and selected organs were promptly excised, harvested and weighted. Radioactivity was measured for each organ using a Perkin Elmer Wallac 1480 Wizard 3'' automatic gamma counter (Perkin Elmer, Courtabeuf, France). The results were corrected for radioactive decay and expressed as percentage of injected dose per gram of tissue (%ID/g).

References

1 C. Garbe, T.K. Eigentler, U. Keilhotz, A. Hauschild, J.M. Kirkwood, *The Oncologist*, 2011, **16**, 5-24.

2 (*a*) M.J. Akhtar, M. Ahamed, H.A. Alhadlaq, S.A. Alrokayan and S. Kumar, *Clin. Chim. Acta*, 2014, **436**, 78-92; (*b*) M.S. Kim, *Biomol. Ther*., 2011, **19**,371-389; (*c*) Y. Singh, M. Palombo and P.J. Sinko, *Curr. Med. Chem*., 2008, **15**, 1802-1826.

3 A.P. Algazi, C.S Soon, and A.I. Daud, *Cancer Manag. Res*., 2012, **2**, 197-211.

4 (*a*) B. Homet and A. Ribas, *J. Pathol*., 2014, **232**, 134-141; (*b*) L. Finn, S.N. Markovic and R.W. Joseph, *BMC Med*., 2012, 10-23.

5 J.M. Chezal, J. Papon, P. Labarre, C. Lartigue, M.J. Galmier, C. Decombat, O. Chavignon, J. Maublant, J.C. Teulade, J.C. Madelmont and N. Moins, *J. Med. Chem.*, 2008, **51,** 3133–3144.

6 (*a*) 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, and N. Moins, *Int. J. Cancer,* 2009, **125**, 708– 716 ; (*b*) F. Degoul, M. Borel, N. Jacquemot, S. Besse, Y. Communal, F. Mishellany, J. Papon, F.Penault-Llorca, D. Donnarieix, M. Doly, L. Maigne, E. Miot-Noirault, A. Cayre, J. Cluzel, N. Moins, J.-M. Chezal and M Bonnet, *Int. J. Cancer*, 2013, **13,** 1042– 1053.

7 (*a*) M. Rapp, J.C.Maurizis, J.Papon, P.Labarre, T.Wu, A.Croisy, J.L. Guerquin-Kern, J.C.Madelmont and E. Mounetou, *J Pharmacol. Exper.Ther.,* 2008, **326**, 171-177; (*b*) M. Gardette, J. Papon, M. Bonnet, N. Desbois, P. Labarre, T. D. Wu, E. Miot-Noirault, J. C.Madelmont, J. L. Guerquin-Kern, J. M. Chezal and N. Moins, *Invest. New Drugs,* 2011, **29**, 1253– 1263.

8 (*a*) M. Vivier, M. Rapp M, J. Papon, P. Labarre , M.J. Galmier, J. Sauzière and J.C. Madelmont. *J Med Chem.*, 2008, **51**, 1043-1047; (*b*) J. Morlieras, J.-M. Chezal, E. Miot-Noirault, A. Roux, L. Heinrich-Balard, R. Cohen, S. Tarrit, C. Truillet, A. Mignot, R. Hachani, D. Kryza, R. Antoine, P. Dugourd, P. Perriat, M. Janier, L. Sancey, F. Lux and O.Tillement, *Nanoscale*, 2013, **5**, 1603– 1615.

9 K. G. Chen, J. C. Valencia, B. Lai, G. Zhang, J. K. Paterson, F. Rouzaud, W. Berens, S. M. Wincovitch, S. H. Garfield, R. D. Leapman, V. J. Hearing and M. M. Gottesman, *Proc. Natl. Acad. Sci. U.S.A*. 2006, **123**, 9903– 9907.

10 M. André, S. Tarrit, M.J. Couret, M.J. Galmier, E. Débiton, J.M. Chezal, and E. Mounetou, *Org. Biomol. Chem.*, 2013, **11**, 6372-6384.

11 (*a*) J.M. Harris and R.B. Chess, *Nat. Rev. Drug Discov.,* 2003, **2**, 214-221; (*b*) W. Mier, S. Krämer, S. Zitzmann, A. Altmann, K. Leotta, U. Schierbaum, M. Schnölzer, M. Eisenhut and U. Haberkorn, *Org. Biomol. Chem.*, 2013, **11**, 2706-2711; (*c*) P. Bailon and C.Y.Won, *Expert. Opin. Drug Deliv.*, 2009, **6**, 1-16.

12 (*a*) W. Da., L. Zhu,. L. Wang and Y. Lu, *Adv. Ther*., 2013, **30**, 541-553 ; (*b*) E.D. Deeks, *Drugs*, 2013, **73**, 75-97.

13 P. Caliceti and F.M. Veronese, *Adv. Drug Deliv. Rev*., 2003, **55**, 1261-1277.

14 R.H. Seevers, and R.E.Counsell, *Chem.Rev.*, 1982, **82**, 575-590.

15 G.W. Kabalka, and R.S. Varma, *Tetrahedron*, 1989, **45**, 6601-6621.

16 G.W. Kabalka, and A.R. Mereddy, *Nucl. Med. Biol*., 2004, **31**, 935-938.

17 (*a*) R. Bolton , *J. Labelled Compd. Radiopharm*. 2002, **45**, 485-528; (*b*) W.A. Wolkert, and T.J. Hoffman, *Chem.Rev.*, 1999, **99**, 2269-2292.

18 H Azizian, C. Eaborn, and A.J.Pidcock, *J. Organomet. Chem.*, 1981, **215**, 49-58.

19 M. André, B. Letribot, M. Bayle, E. Mounetou and J.M. Chezal *Tet. Lett.* 2012, **53**, 6107-6110.

20 D. Denoyer, P. Labarre, J. Papon, E. Miot-Noirault, M.J. Galmier, J.C. Madelmont, J.M. Chezal and N. Moins, *J. Chromato. B*, 2008, **875**, 411-418.

21 A.A. Hussain, J.A. Jona, A. Yamada, and L.W. Ditert, *Anal. Biochem*., 1995, **224**, 221-226.

22 G. Petzold, and H.H. Coenen, *J. Labelled Compd. Radiopharm*., 1981, **18**, 1319-1336.

23 G. Pasut and F.M. Veronese, *J. Control Release.* 2012, **161**, 461-472.

24 G. Pasut and F.M. Veronese, *Adv. Drug Deliv. Rev*., 2009, **61**, 1177-1188.

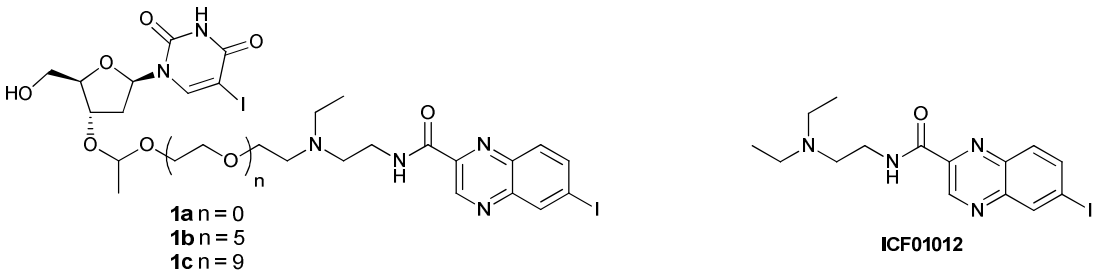


Fig. 1 Chemical structure of the conjugate **1a**, PEGylated derivatives **1b**, **1c** and melanoma tracer ICF01012.

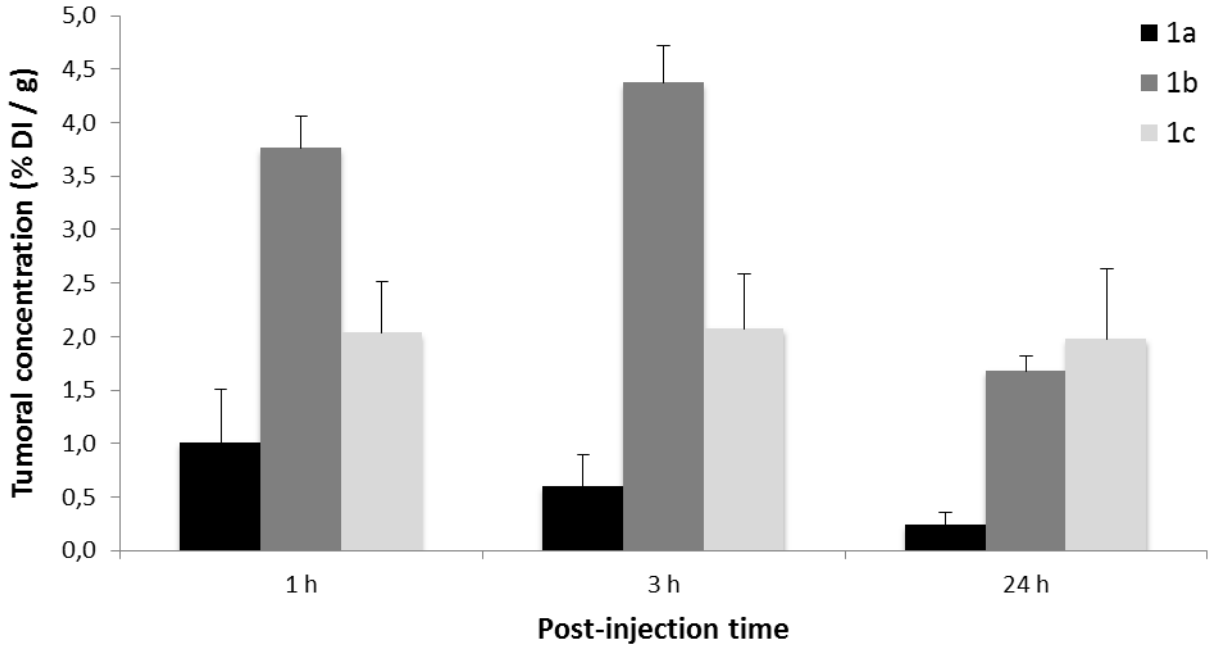


Fig. 2 Comparative tumoural accumulation in C57BL/6J mice bearing B16F0 melanoma xenografts at 1 h, 3 h and 24 h after i.v. injection of the radioconjugates [^{125}I]**1a-c**. Values are expressed as the percentage of the injected dose per gram of tumour (%DI/g) and correspond to the mean ± standard deviation for four animals for each point.

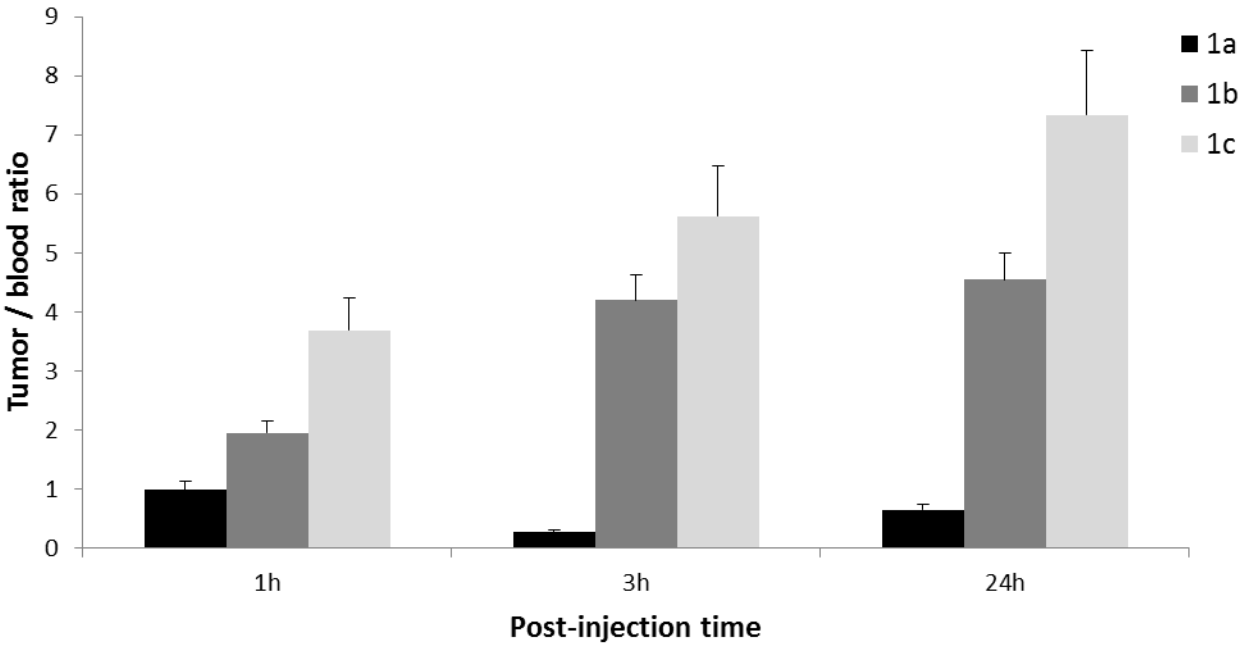


Fig. 3 Comparative tumour/blood uptake ratios for C57BL/6J mice bearing B16F0 melanoma xenografts at 1 h, 3 h and 24 h after i.v. injection of the radioconjugates [^{125}I]**1a-c**. Values are expressed as the percentage of the injected dose per gram of tumour (%DI/g) and correspond to the mean ± standard deviation for four animals for each point.

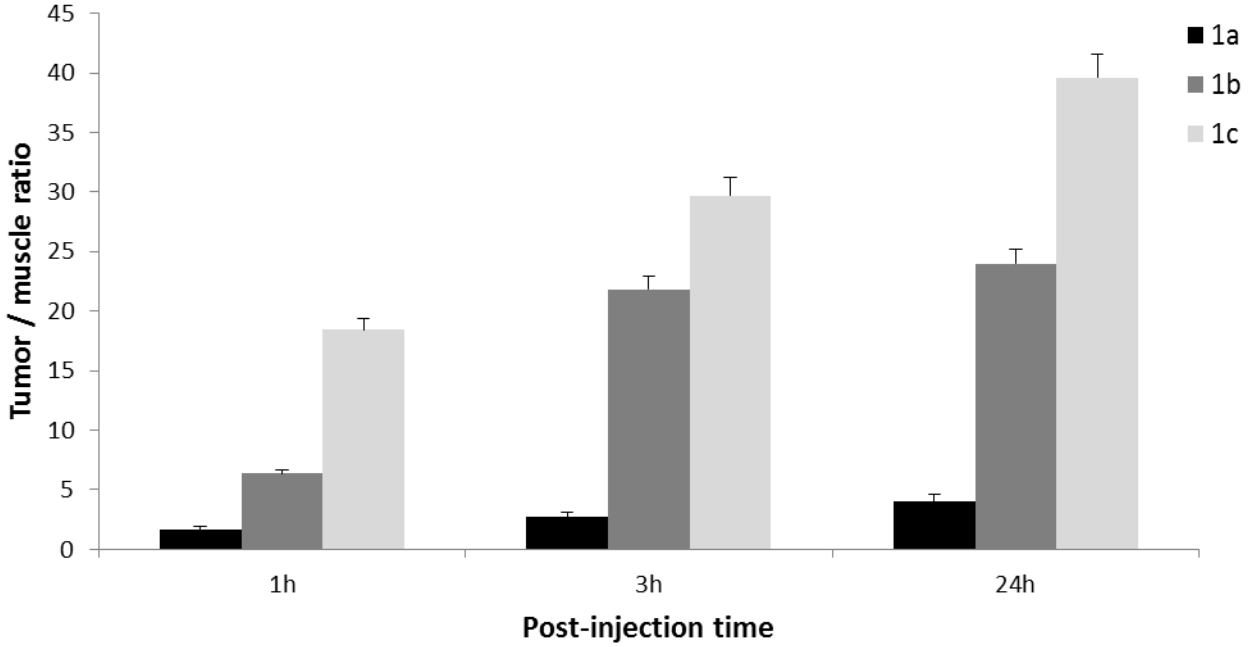


Fig. 4 Comparative tumour/muscle uptake ratios for C57BL/6J mice bearing B16F0 melanoma xenografts at 1 h, 3 h and 24 h after i.v. injection of the radioconjugates [^{125}I]**1a-c**. Values are expressed as the percentage of the injected dose per gram of tumour (%DI/g) and correspond to the mean ± standard deviation for four animals for each point.

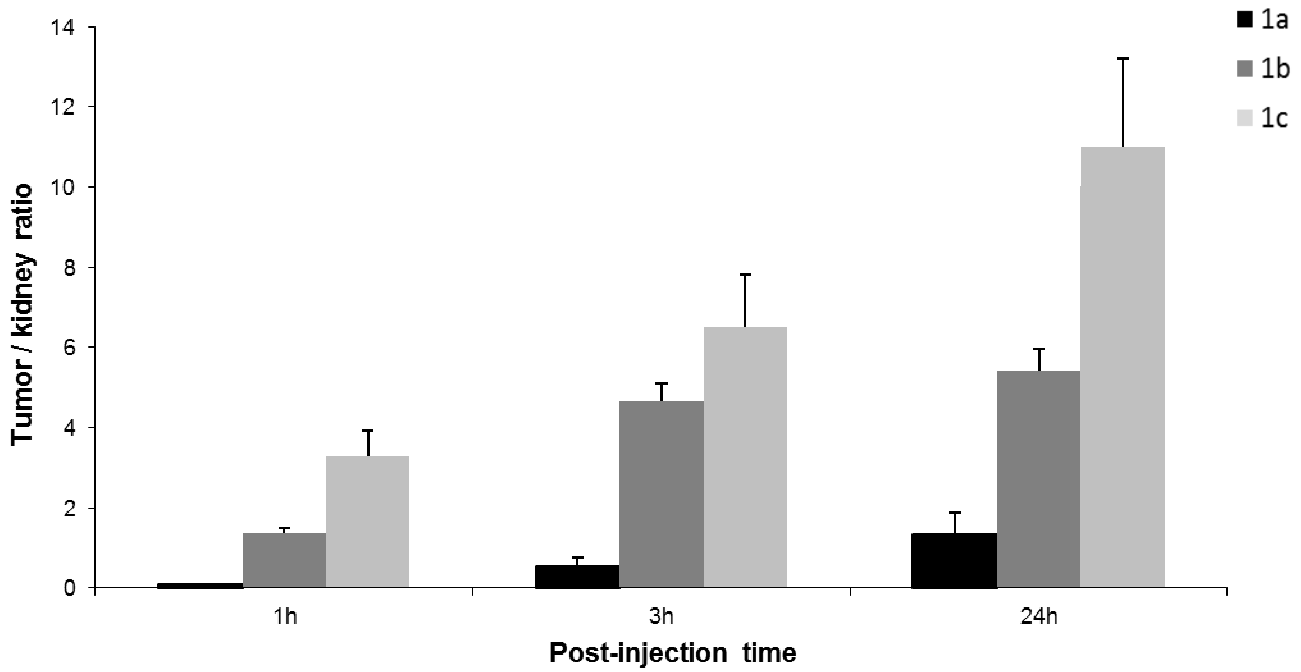


Fig. 5 Comparative tumour/kidney uptake ratios for C57BL/6J mice bearing B16F0 melanoma xenografts at 1 h, 3 h and 24 h after i.v. injection of the radioconjugates [^{125}I]**1a-c**. Values are expressed as the percentage of the injected dose per gram of tumour (%DI/g) and correspond to the mean ± standard deviation for four animals for each point.

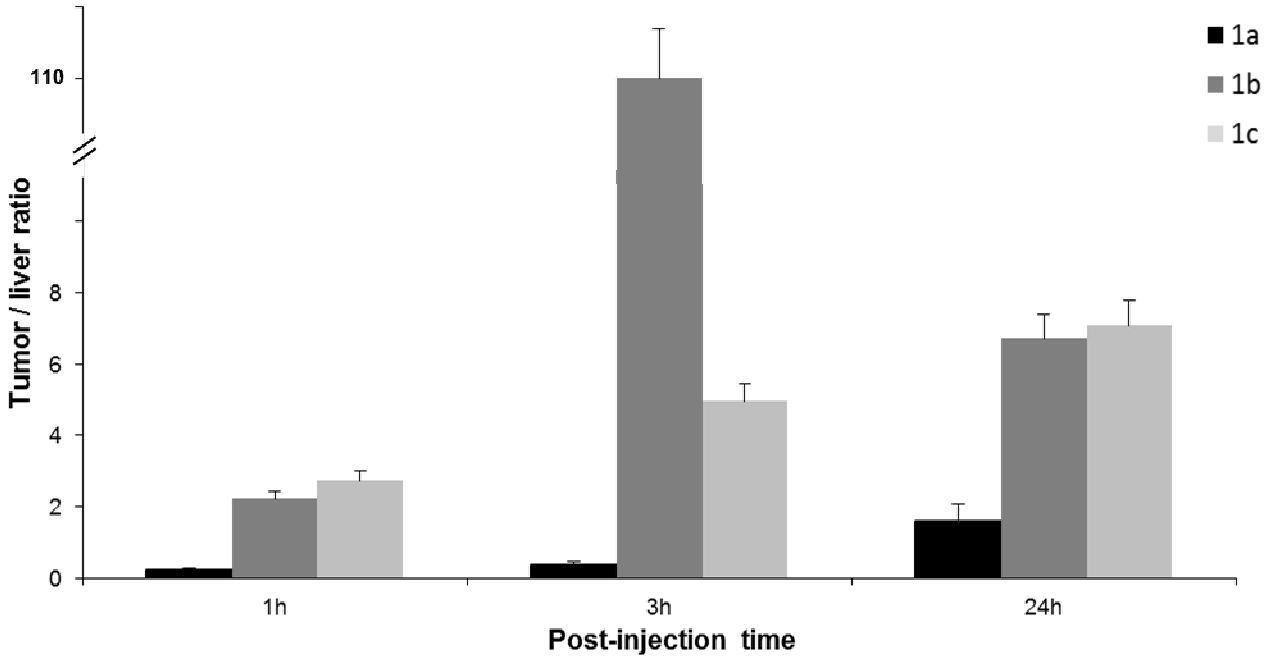
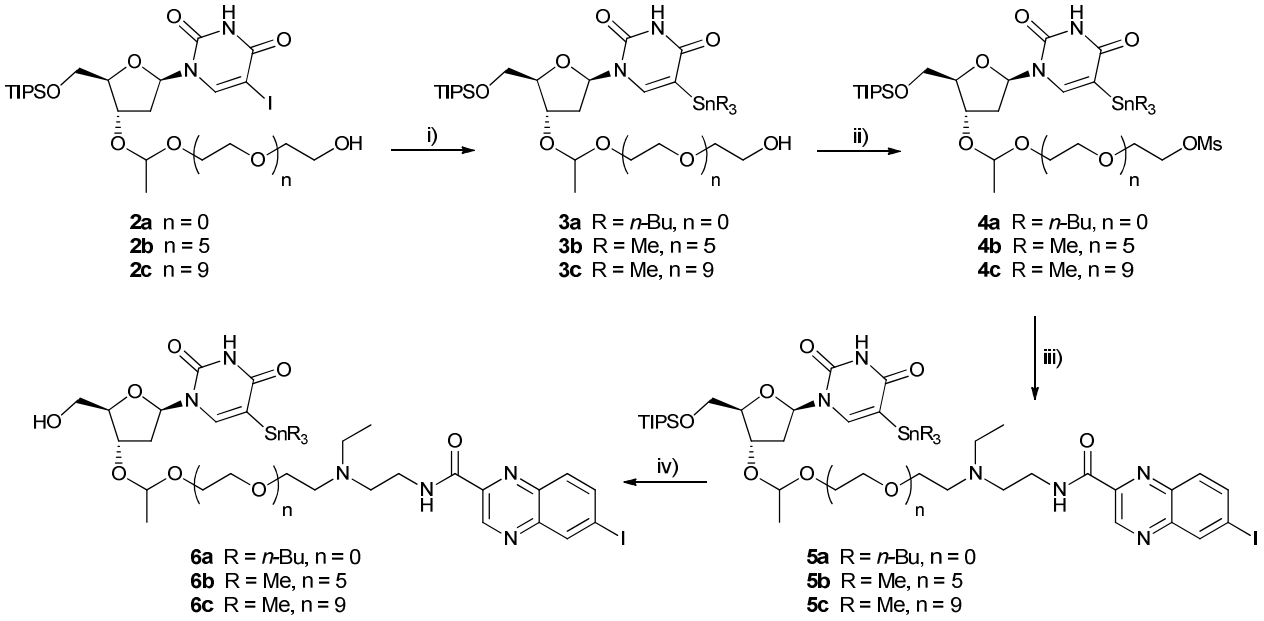
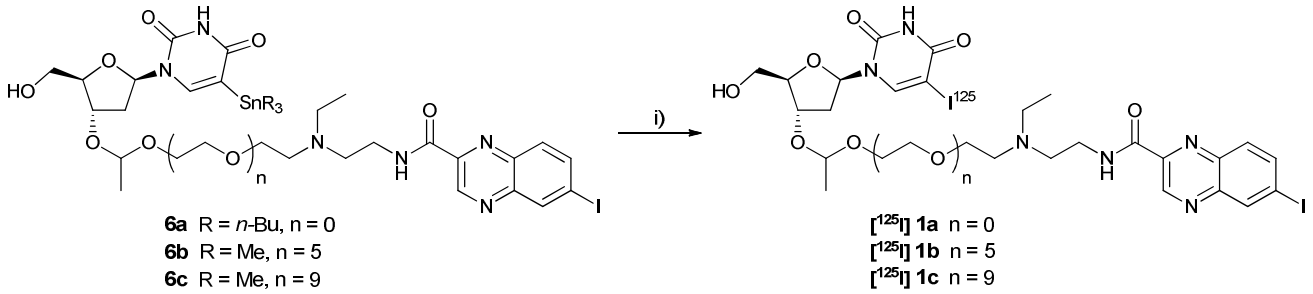


Fig. 6 Comparative tumour/liver uptake ratios for C57BL/6J mice bearing B16F0 melanoma xenografts at 1 h, 3 h and 24 h after i.v. injection of the radioconjugates [^{125}I]**1a-c**. Values are expressed as the percentage of the injected dose per gram of tumour (%DI/g) and correspond to the mean ± standard deviation for four animals for each point.



Scheme 2 Synthesis of the organostanne precursors **6a-c**. (i) Sn_2R_6, $Pd(PPh_3)_2Cl_2$, dioxane, reflux, 5 h, **3a**: 50%, **3b**: 70%, **3c**: 80%; (ii) MsCl, DIPEA, DCM rt, 1 h, **4a**: 76%, **4b**: 93%, **4c**: 60%; (iii) *N*-(2-diethylaminoethyl)-6-iodoquinoxaline-2-carbamide, DIPEA, MeCN, **5a**: 55°C, 6d, 23%, **5b**: 65°C, 90 h, 35%; **5c**: 70°C, 72 h, 26%; (iv) TBAF, THF, rt, 4 h, **6a**: 84%, **6b**: 67%, **6c**: 50%.



Scheme 3 Radiosynthesis of the conjugates **[^{125}I]1a-c**. (i) (a) $Na^{125}I$, chloramine-T, $[H^+]$/EtOH, rt; (b) aq.$Na_2S_2O_5$, NaOH 0.1 N; (c) RP-HPLC purification, **[^{125}I]1a**: 81%, **[^{125}I]1b**: 79%, **[^{125}I]1c**: 45%.

Table 1 Optimization of chloramine-T (CAT) catalysed radioiodination for conjugate **[^{125}I]1a** [a]

Entry	Medium[b]	Oxidant / vol (µL) [c]	Time (min)	Quencher / vol (µL) [d]	Conversion (%)[e]
1	citrate buffer	CAT / 40	20	$Na_2S_2O_5$ / 100	60
2	HCl 0.1 M	CAT / 40	10	$Na_2S_2O_5$ / 100	80
3	HCl 0.1 M	CAT / 40	20	$Na_2S_2O_5$ / 100	81
4	HCl 0.1 M	CAT / 100	10	$Na_2S_2O_5$ / 100	81

[a] General conditions: 100 µL of **6a** in EtOH (1 mg mL^{-1}), 1.3 µL of Na[^{125}I]I (100 µCi), 100 µL of acidic medium at room temperature. [b] Volume: 100 µL. [c] Aqueous solution (1 mg mL^{-1}). [d] Aqueous solution (0.5 mg mL^{-1}). [e] Determined by radio-HPLC analysis.

Table 2 Biodistribution data of [^{125}I]**1a** in C57BL/6J mice bearing B16F0 melanoma xenografts [a]

Conjugate	Time (h)	Organ [b]			
		Blood	Muscle	Liver	Kidney
[^{125}I]1a	1	1.03±0.20	0.62±0.31	4.54±1.24	13.9±2.50
	3	2.27±0.29	0.22±0.03	1.66±0.50	1.13±0.44
	24	0.37±0.11	0.06±0.02	0.15±0.10	0.18±0.05
[^{125}I]1b	1	1.93±0.73	0.59±0.03	1.69±0.04	2.76±0.08
	3	1.04±0.49	0.20±0.02	0.04±0.02	0.94±0.30
	24	0.37±0.05	0.07±0.02	0.25±0.03	0.31±0.04
[^{125}I]1c	1	0.55±0.02	0.11±0.02	0.74±0.03	0.62±0.02
	3	0.37±0.07	0.07±0.02	0.42±0.03	0.32±0.03
	24	0.27±0.08	0.05±0.02	0.28±0.13	0.18±0.05

[a] mice were injected by the i.v. route. [b] Values are expressed as the percentage of the injected dose per gram of tissue (%DI/g) and correspond to the mean ± standard deviation for four animals for each point.