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Design, synthesis and preliminary biological evaluations of novel amphiphilic drug carriers

Sandrine Périno,[a] Christiane Contino-Pépin,[a,*] Sylvain Jasseron,[a] Maryse Rapp,[b] Jean-Claude Maurizis[b] and Bernard Pucci[a,*]

[a]*Laboratoire de Chimie Bioorganique et des Systèmes Moléculaires Vectoriels, Faculté des Sciences, 33, rue Louis Pasteur, 84000 Avignon, France*
[b]*INSERM U484, rue Montalembert, BP 184, 63005 Clermont-Ferrand Cedex, France*

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Abstract—The synthesis of a new fluorocarbon amphiphilic drug carrier is described. A polyfunctional amino acid endowed with a fluorocarbon chain and a sugar moiety providing the amphiphilic character constitutes the central element of this structure. A ^{14}C-radiolabelled acetyl group was grafted onto the third function and the bioavailability of this molecule was specified in mice after IV administration. This amphiphilic drug carrier exhibits a rapid and homogeneous distribution to the whole tissues and slow elimination half-lives (higher than one day) through a biliary excretion without any toxicity (no measured DL 50 for concentrations up to 500 mg/kg).

The preparation of drug bearing macromolecular conjugates has been widely explored since the last decade.[1–5] These systems exhibited therapeutic molecules linked to a polymeric backbone by means of biodegradable covalent bonds. In this field, we have developed researches on the biomedical potentialities of a new class of oligomeric carriers called ''Telomers''.[6] Preliminary biological studies have shown that these multifunctional macromolecules exhibit good biocompatibility making them suitable for drug delivery systems.[7,8] Therefore, the grafting of cytotoxic agents and possibly different targeting devices to the polymeric backbone of telomers resulted in macromolecular prodrugs with an increased bioavailability and a better therapeutic index than the parent drugs.[6,9] Nevertheless, despite the numerous therapeutic potentialities exhibited by our so-called ''telomers'', the several difficulties in predicting and controlling the drug-telomer conjugate architecture prompted us to develop a new class of multivalent carriers, having the main potentialities and advantages of telomers but with a well-defined structure.

To this purpose, we focused our investigations on the design of amphiphilic multifunctional molecular carriers with a modular structure including an amino acid scaffold upon which are anchored several architectural components:

- A perfluoroalkyl tail as a hydrophobic part.
- A glycosidic or peptidic hydrophilic head providing the solubility in water and/or a cell targeting.
- A labelling domain for the in vivo follow-up and/or a drug moiety.

The general structure of the investigated molecules is shown in Figure 1. We can expect that, compared to telomers, the combination of these structural domains on a molecular carrier should provide less complicate data interpretation with more wide therapeutic exploitation. As regards the hydrophobic tail, it has to be specified

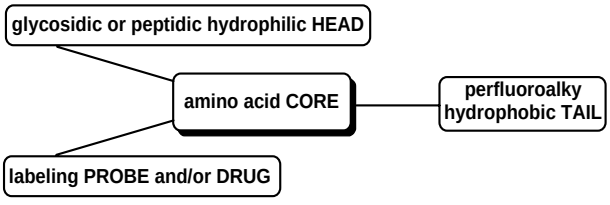

Figure 1. General structure of new amphiphilic molecular carriers.

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* Corresponding authors. Tel.: +33490144442; fax: +33490144449; e-mail addresses: christine.contino@univ-avignon.fr; bernard.pucci@univ-avignon.fr

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that a fluoroalkyl chain was chosen instead of a classical hydrocarbon one to minimize the cell toxicity of such amphiphilic molecules. Indeed, it is well known that hydrocarbon and fluorocarbon chains exhibit poor miscibility, thus fluorocarbon surfactants and phospholipids of biological membranes do not mix very well.[10] In those conditions, fluorocarbon amphiphiles do not destabilize cell membranes at a concentration higher than their critical micellar concentration (CMC) and are less toxic than hydrocarbon analogues.[11]

In this context, we preliminary investigated the in vivo behaviour and biocompatibility of the single carrier through the follow-up of a radiolabelled derivative. We describe herein the synthesis and biodistribution in mouse of amphiphilic carrier **4**, labelled with ^{14}C.

The synthesis of the radiolabelled molecule **4** was performed easily as summarized in Figure 2.

After reduction of the azide derivative **1**, previously obtained from the corresponding commercial iodide, a suitable lysinoyl protected moiety was introduced on the molecular architecture using conventional methods of peptidic synthesis. The consecutive removal of the Boc *N*-protecting group of lysine was followed by coupling lactobionolactone achieved from dehydration of lactobionic acid. The acylation of the sugar hydroxyl groups, performed by using acetic anhydride in pyridine, gave compound **3** in satisfactory yield.[12] Then, after hydrogenolysis of the benzyloxycarbonyl protective function of the lysine ε-amino group, we have acylated the resulting amine moiety and introduced a ^{14}C atom by using ^{14}C-labelled acetic anhydride in pyridine.

Finally, a deacetylation of the saccharidic moiety by transesterification in methanol with catalytic amount of sodium methylate was performed to provide compound **4**. Its nonradiolabelled analogue **4a** was synthesized likewise from acetic anhydride. This molecule **4a** was fully characterized by NMR spectroscopy and mass spectra.[13] The specific radioactivity of the ^{14}C-labelled derivative **4** was 1.34 μCi/mg.

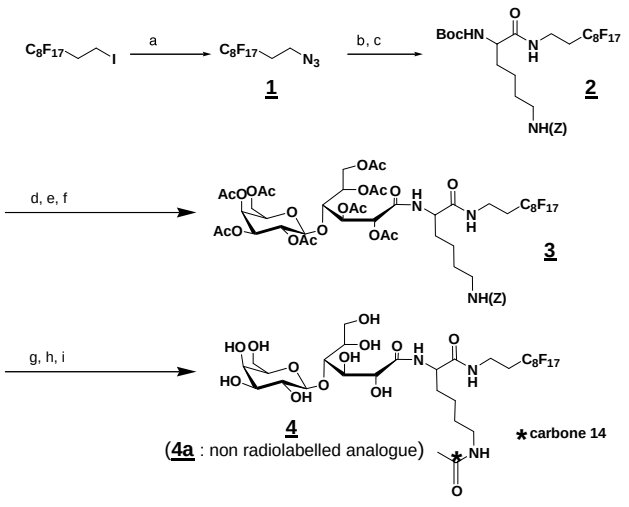

Figure 2. Synthesis of the ^{14}C-labelled carrier. Reagents: (a) NaN_3, DMF (85%); (b) H_2/Pd/C (100%); (c) BocLys(Z)OH, DCC, HOBT (79%); (d) TFA/CH_2Cl_2 (100%); (e) lactobionic acid, TEA, MeOH; (f) $(AcO)_2O$, Py (65%); (g) H_2/Pd/C (100%); (h) ^{14}C-labelled acetic anhydride, Py (46%); (i) MeO^-Na^+/MeOH (84%).

According to the chemical structure of the compound **4**, we predicted that it would exhibit amphiphilic behaviour in biological fluids. For this purpose, we focussed our attention on the determination of its essential tensioactive properties. All these physicochemical studies were performed on the nonradiolabelled analogue **4a**.

The surface tension vs concentration of its aqueous solution was measured at 20 °C by using a KRUSS R12 tensiometer, following the Wilhelmy plate method. The critical aggregation concentration (CAC) value of this compound was 1.61×10^{-2} mM. Such a low value could be ascribed to the presence of the fluorocarbon chain which generally induces a dramatic decrease of the CMC (or CAC) by comparison with hydrocarbon analogues.[14]

Moreover, the tensiometric curve led us to specify the polar head interfacial cross-section of compound **4a** by applying the Gibbs absorption isotherm: $a = 56$ Å^2 (25 °C). This value is in good agreement with measurements already performed on various hydrocarbon or fluorocarbon surfactants derived from lactobionamide ($a = 40$–50 Å^2).[15]

The particles size that compound **4a** formed in water at a concentration higher than its CAC was observed at 25 °C by light scattering with a Zetasizer Nano-S model ZEN 1600 granulometer.

This measurement led us to specify the mean hydrodynamic diameter of these particles: 26 nm (average size measured with six different batches) whatever the concentration used between 0.05 and 0.5 mM (Fig. 3). Such a value was markedly higher than that usually measured for micelles and could be ascribed to aggregates. Thus, amphiphilic compound **4a**, which displays a very good solubility in water, self-organizes at low concentration as supramolecular aggregates different from classic micellar systems whose average size is usually lower than 5 nm. Such a behaviour is frequently observed with fluorocarbon surfactants which have a strong tendency to form cylindrical or rod-like micelles.[16,17]

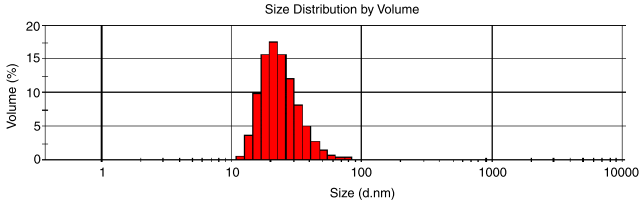

Figure 3. Histogram aggregate size distribution of compound **4a** in aqueous solution determined as hydrodynamic diameter versus volume percentage of populations by DLS.

Table 1. Distribution of radioactivity in tissues after iv administration of 13.4 μCi of labelled carrier

	15 min	1 h	6 h	24 h	$T_{1/2}$ (h)
Blood	31.97 ± 2.97	22.85 ± 1.78	9.62 ± 1.33	9.47 ± 0.88	32.5
Kidney	13.49 ± 3.02	15.29 ± 0.94	9.04 ± 1.01	8.94 ± 1.22	48.2
Liver	15.84 ± 5.76	16.32 ± 1.82	31.77 ± 3.27	25.97 ± 2.08	138.4
Heart	12.73 ± 2.50	11.22 ± 2.42	5.42 ± 1.11	4.73 ± 0.49	38.2
Lung	27.05 ± 4.09	22.64 ± 1.95	9.97 ± 1.72	13.21 ± 2.27	ND
Spleen	9.21 ± 1.92	10.92 ± 3.25	7.63 ± 0.67	6.44 ± 1.00	53.7
Skin	10.95 ± 1.95	10.82 ± 2.36	8.94 ± 2.78	6.53 ± 1.38	42.7
Brain	1.03 ± 0.2	0.77 ± 0.17	0.49 ± 0.07	0.38 ± 0.04	72.6
Tumour	5.08 ± 1.31	13.31 ± 0.58	11.49 ± 1.19	6.81 ± 1.45	36.8

Results, given in percentage of administered dose per g of tissue, are means of five measures ± SD.

Male C57B16 mice were grafted by subcutaneous injection of 10^6 syngenic B16 melanoma cells. Animals were used when the mean diameter of the tumours reached 0.5 cm. 10 mg of the labelled compound **4** (13.4 μCi) dissolved in 200 μl of physiological serum was injected in the caudal vein. Animals were sacrificed by CO_2 inhalation 15 min, 1, 6 and 24 h after administration. Mice were immediately frozen in liquid nitrogen and sliced (40 μm) using a cryomicrotome. Tissue radioactive concentration was determined with a computer-controlled multiwire proportional counter (Ambis 4000) allowing direct quantitation of regions of interest.[18] Elimination half-lives were determined using the Kinetica 2000 software (Innaphase).

The radioactivity follow-up performed on C57B16 mice bearing grafted B16 syngeneic melanoma tumours showed a rapid and relatively homogeneous distribution of the radiolabelled molecule to the whole tissues, except brain (Table 1). Maximal tissue concentrations were observed 1 h after administration. Following this distribution phase, slow elimination half-lives were measured. In the blood, this one was 32 h, while in the other tissues such as tumour or spleen, longer half-lives were determined (37 h for the tumour, 54 h for the spleen).

High radioactive concentrations in the liver after 6 h indicate an important biliary excretion. The elimination half-life was the highest (138 h) in this tissue. Two hypotheses can be proposed to explain such a behaviour. We might assume that the size of the particles formed in water by the carrier **4** induces its partial accumulation within the liver as it is usually observed for various liposomes or nanoparticles.[19,20]

More probably, considering the small size (26 nm) of the aggregates produced by compound **4**, these accumulation and retention times could be the consequence of a chemical interaction between the galactopyranosyl moiety of compound **4** and specific receptors present in the liver. Indeed, β-galactose residues are known to be recognized by asialoglycoproteins receptors (ASGP-R) present on the surface of hepatocytes.[21] Also, several lactose-endowed macromolecules devoted to promoting an efficient entry in liver cells are described in the literature.[22,23]

These results warrant several remarks and comments on the potentialities of such an amphiphilic carrier:

- First, this amphiphilic fluorinated carrier exhibits a half-life time higher than one day in all tissues and in particular in blood. This result suggests that the binding of an active principle to such a carrier could permit to extend the presence of the drug into the whole organism, including tumoural tissues, and to increase its pharmacological effect. Moreover, several biological studies showed that compound **4** did not exhibit any lethal toxicity up to a concentration of 500 mg/kg after IV administration in mice (data not shown). Thus, these preliminary biological findings appear to substantiate the possible use of these new amphiphilic carriers for delayed delivery of drugs.
- Second, the high concentration of radioactivity measured within the lungs can be reasonably ascribed to the ^{14}C-CO_2 arising from the catabolism of the ε-acetyl group of lysine. Such a result has to be favourably considered, indeed it suggests that a drug linked to this amphiphilic carrier through an amide group may be released over a long period within the organism.
- However, the ubiquitous distribution of the carrier and above all its great affinity for the liver could possibly induce an increase of the whole toxicity of the drug. Thus, to overcome such a drawback, it would be attractive to endow this molecular amphiphilic carrier with specific moieties providing a selective affinity for tumoural sites. In this field, we previously directed chemotherapeutic agents to highly vascularized zones surrounding tumours by grafting RGD sequences onto the polymeric backbone of our THAM-derived telomers.[9] The application of such a cell targeting concept to our amphiphilic multifunctional molecular carriers is currently undertaken. In those compounds, a peptidic homing device will constitute the hydrophilic head of the carrier.

In conclusion, all the physicochemical and biological studies carried out with the fluorinated amphiphilic compound **4** seem to validate its therapeutic potentialities as a promising vector for targeted drug delivery to tumours as well for molecular imaging of neoangiogenesis.

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12. Compound **3**: white powder (51%), mp 65 °C (dec); $[\alpha_D]$: + 3 (C, 1, $CHCl_3$); MS (ESI) *m*/*z*: 1403 $(M+H)^+$; 1425 $(M+Na)^+$; ^1H NMR (250 MHz, DMSO-d_6, δ): 8.07 (m, 2H), 7.34 (s, 5H), 7.01 (m, 1H), 5.47 (d, 1H), 5.30–5.10 (m, 2H), 5.02–4.79 (m, 5H), 4.51–3.90 (m, 8H), 3.35 (m, 2H), 2.96 (m, 2H), 2.30 (m, 2H), 2.14, 2.09, 2.04, 2.01, 1.99, 1.96, 1.92 (s, 27H), 1.59–1.23 (m, 6H). ^{19}F NMR (250 MHz, DMSO-d_6, δ) : −80.22 (s, 3F), −113 (s, 2F), −121.4 (s, 6F), −122.2 (s, 2F), −123 (s, 2F), −125.4 (s, 2F). ^{13}C NMR (250 MHz, $CDCl_3$, δ): 171.3; 170.5; 170.1; 170; 169.7; 169.2; 167.9; 156.7; 136.7; 128.4; 128; 127.9; 101.6; 77.9; 72.7; 71.1; 70.9; 70; 69.4; 69; 66.9; 66.5; 61.6; 61; 52.6; 40.4; 31.9; 31.2; 30.5; 29.1; 22.2; 20.6; 20.5; 20.4; 20.3.
13. Compound **4a**: white powder (20%), mp 145.6–148.9 °C (dec); $[\alpha_D]$: + 6 (C, 1, MeOH); MS (ESI) *m*/*z*: 974.5 $(M+H)^+$; 996.5 $(M+Na)^+$; ^1H NMR (250 MHz, DMSO-d_6, δ): 8.12 (t, 1H), 7.71 (t, 1H), 7.22 (t, 1H), 5.47 (d, 1H), 5.19 (d, 1H), 4.82 (m, 2H), 4.64–4.51 (m, 3H), 4.36–4.18 (m, 3H), 3.73–3.35 (m, 5H), 2.96 (m, 2H), 2.47–2.39 (m, 2H), 1.99 (s, 3H), 1.59–1.23 (m, 6H). ^{19}F NMR (250 MHz, DMSO-d_6, δ): −80.22 (s, 3F), −113.45 (s, 2F), −121.7 (s, 6F), −122.5 (s, 2F), −123.2 (s, 2F), −125.76 (s, 2F). ^{13}C NMR (250 MHz, DMSO-d_6, δ): 172.9; 170.0; 169.1; 105.3; 83.9; 76.3; 73.7; 72.5; 71.8; 71.6; 68.6; 62.7; 61.0; 52.6; 48.0; 44.6; 42.9; 41.0; 32.0; 27.6; 23.1; 21.7.
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