Supporting Information for

Lipid Based Nanovectors Containing Ruthenium Complexes: A Potential Route in Cancer Therapy

Mauro Vaccaro[a,b,c], Raffaella Del Litto[a], Gaetano Mangiapia[a,b], Anna M. Carnerup[c], Gerardino D'Errico[a,b], Francesco Ruffo[a], and Luigi Paduano*[a,b]

[a)] Department of Chemistry, Università degli Studi di Napoli "Federico II", Via Cinthia, Naples, Italy.
[b)] CSGI – Consorzio Interuniversitario per lo sviluppo dei Sistemi a Grande Interfase
[c)] Physical Chemistry I , Center for Chemistry and Chemical Engineering
Lund University, Getingevägen 60, 221 00 Lund, Sweden
* To whom the correspondence should be addressed. Fax: +390 81 674090; Tel: +390 81 674250; E-mail: luigi.paduano@unina.it

Materials

NMR Spectra. NMR spectra were recorded with a Varian Gemini-200 spectrometer. The following abbreviations were used for describing NMR multiplicities: s, singlet; d, doublet; t, triplet; app, apparent; m, multiplet. The coupling costants are reported in Hz. DMF was dried using standard procedures. ^{1}H NMR spectrum of **1**[1,2] ($CDCl_3$, 298 K): δ 8.02 (s, 1H, NH), 7.53 (d, 1H, H6-uracyl, $^3J_{H-H}$= 8.2 Hz), 6.03 (d, 1H, H1', $^3J_{H-H}$= 5.6 Hz), 5.79 (d, 1H, H5-uracyl), 5.36 (m, 6H, H2', H3' and 2 CH=CH), 4.42 (app d, 1H, H4'), 4.34 (m, 2H, H5'), 4.27 (s, 2H, PEG-OCH_2CO), 3.75-3.50 (m, 28 H, CH_2-PEG), 3.35 (s, 3H, OMe), 2.30 (m, 4H, 2 CH_2CO-), 1.95 (m, 8H, -CH_2CH=), 1.58 (m, 4H, -CH_2CH_2CO-), 1.45-1.10 (m, 40H, CH_2), 0.86 (t, 6H, Me).

Synthesis of DOPU. To a suspension of **1** (0.10 g, 0.087 mmol) in dry DMF (2 ml) was added solid K_2CO_3 (0.027 g, 0.20 mmol) followed by 4-bromomethylpyridine hydrobromide (0.033 g, 0.13 mmol). After 2 hours of stirring at 333 K the solvent is removed in vacuo from the resulting greenish solution. The residue was dissolved in dichloromethane and the solution was filtered through a thin layer of Celite. The product was purified by chromatography on Silica Gel (dichloromethane:methanol 9:1) and was isolated as a yellow waxy solid in 90% yield. ^{1}H NMR spectrum ($CDCl_3$, 298 K): δ 8.50 (d, 2H, H2-py and H6-py, $^3J_{H-H}$= 5.8 Hz), 7.54 (d, 1H, H6-uracyl, $^3J_{H-H}$= 8.2 Hz), 7.24 (d, 2H, H3-py and H5-py), 6.05 (d, 1H, H1', $^3J_{H-H}$= 5.4 Hz), 5.87 (d, 1H, H5-uracyl), 5.33 (m, 6H, H2', H3' and 2 CH=CH), 5.05 (app d, 2H, NCH_2, J_{gem}= 3.8 Hz), 4.50-4.10 (m, 5H, H4', H5', PEG-OCH_2CO), 3.75-3.50 (m, 28 H, CH_2-PEG), 3.35 (s, 3H, OMe), 2.33 (m, 4H, 2 CH_2CO-), 1.98 (m, 8H, -CH_2CH=), 1.50 (m, 4H, -CH_2CH_2CO-), 1.45-1.10 (m, 40H, CH_2), 0.86 (t, 6H, Me). ^{13}C NMR spectrum ($CDCl_3$, 298 K): δ 149.9 (C2-py and C6-py), 138.2 (C6-uracyl), 130.0 (CH=), 129.6 (CH=), 123.1 (C3-py and C5-py), 102.8 (C5-uracyl), 88.1 (C1'), 80.0 (C2'), 72.4 (C3' and C4'), 71.9-70.5 (CH_2-PEG), 68.7

(C5'), 63.4 (PEG-OCH_2CO), 59.0 (OMe), 43.3 (NCH_2), 33.7 (CH_2CO-), 31.9 (-CH_2CH=), 29.7-22.6 (CH_2), 14.1 (Me).

Synthesis of DOPURu. A solution of **DOPU** (0.095 g, 0.076 mmol) and $[RuCl_4(DMSO)_2][H(DMSO)_2]$ (0.020 g, 0.038 mmol) in a mixture acetone-d_6:DMSO-d_6 2:1 was heated at 323 K overnight. Removal of the solvents afforded **DOPURu** as an orange oil. Selected ^{1}H NMR data (acetone-d_6, 298 K, broad signals): δ 9.0 (1H, NH^+), 8.3 (3H, H2-pyH^+, H6-pyH^+, H6-uracyl in DOPUH^+), 7.9 (2H, H3-pyH^+ and H5-pyH^+), 7.3 (1H, H6-uracyl), 6.2 (br, 2H, H1'), 5.9 (br, 2H, H5-uracyl), 5.5 (m, 12H, H2', H3' and 2 CH=CH), 5.1 (m, 2H, NCH_2 in DOPUH^+), 4.45 (br, 2H, H4'), 4.3 (br, 4H, H5'), 4.25 (br, 4H, PEG-OCH_2CO), 3.8-3.3 (m, 56 H, CH_2-PEG), 3.3 (br, 6H, OMe), 2.3 (br, 8H, CH_2CO-), 2.1 (m, 16H, -CH_2CH=), 1.5 (m, 8H, -CH_2CH_2CO-), 1.4-1.0 (m, 80H, CH_2), 0.8 (m, 12H, Me), -0.6 (2H of py-Ru).

Light Scattering Measurements. The setup for the dynamic light scattering measurement was a CGS3 based compact goniometer system from ALV-GmbH, Langen, Germany. The light source was constituted by a 22mW He-Ne laser, operating at 6328 Å. The detection system was comprised of a near mono-modal optical fiber and two matched photo-multipliers put in a pseudo-cross geometry. A more detailed description about the instrumentation can be found elsewhere[1].

Analysis of the DLS data was performed by fitting directly to the experimentally measured time correlation function of the scattered intensity $G^{(2)}(t)$ often presented as the normalized function, $g^{(2)}(t)$-1 [2]. The models used in the fitting procedures are expressed with respect to the normalized time correlation function of the electric field, $g^{(1)}(t)$, which is related to $g^{(2)}(t)$, by Siegert relation [3, 4]

$$g^{(2)}(t) - 1 = \beta \left| g^{(1)}(t) \right|^2 \qquad (1)$$

where β (≤ 1) is the coherence factor, which accounts for deviation from ideal correlation and depends on the experimental geometry.

$g^{(1)}(t)$ can either be a single-exponential or multi-exponential decay with corresponding relaxation times, τ, depending on the system investigated. A distribution of relaxation times, $A(\tau)$, can be obtained by an inverse Laplace transformation of a multi-exponential

$$g^{(1)}(t) = \int_{-\infty}^{+\infty} \tau A(\tau) \exp\left(-\frac{t}{\tau}\right) \mathrm{d}\ln\tau \qquad (2)$$

where $\tau = \Gamma^{-1}$, while Γ is the relaxation rate that is used to calculate the diffusion coefficient D.

The relaxation time distribution $\tau A(\tau)$ is obtained by regularized inverse Laplace transformation (RILT) of the measured intensity correlation function using calculation algorithms provided by DynaLS (Alango Software). The relaxation rate Γ is obtained from the first moment of the relaxation time distribution, and from its value is estimated the apparent translational diffusion coefficient D, by this relation

$$D = \lim_{q \to 0} \frac{\Gamma}{q^2} \tag{3}$$

where q is the absolute value of the scattering vector ($q = 4\pi n_0 / \lambda \sin(\theta/2)$), with n_0 the refractive index of the solvent, λ the incident wavelength and θ is the scattering angle. Thus D is obtained from the slope of Γ as a function of q^2, where Γ is measured at different scattering angles.

Small Angle Neutron Scattering Measurements.
Small angle neutron scattering measurements were performed at the KWS2 instrument located at the FRM-2 reactor of the Heinz Meier Leibnitz of Garching bei München. Neutrons with an average wavelength λ of 7Å and a wavelength spread $\Delta\lambda/\lambda \leq 0.2$ were used. A two-dimensional array detector at three different sample-to-detector distances (2m, 8m and 20m) detected neutrons scattered from the samples. These configurations allowed collecting the scattering cross section in an interval of transferred moment q ranged between $0.002\,\text{Å}^{-1}$ and $0.18\,\text{Å}^{-1}$. The measurements time was ranged between 15min and 60min per sample. The obtained raw data were then corrected for electronic background and empty cell scattering. Detector efficiency corrections and transformation to absolute scattering cross sections $d\Sigma/d\Omega$ were made by using a Plexiglass sample, according to the standard procedure[5].

Cryo-TEM Micrography Measurements.
Cryogenic-transmission electron microscopy (Cryo-TEM) images were carried out at the Center for Chemistry and Chemical Engineering in Lund, Sweden, on a Philips CM120 BioTWIN Cryo electron microscope operating at 120 kV. A small drop of the sample solution was applied on a copper EM grid with a holey carbon film, and excess solution was blotted with a filter paper, leaving thus a thin sample film spanning the holes in the carbon film. Sample preparation was carried out in a controlled environment vetrification (CEVS) system to avoid water evaporation and to ensure cryo-fixation of the specimen at a controlled temperature (25°C)[6, 7].

EPR Measurements.
EPR spectra were recorded at 25 °C with a Bruker Elexys E500 X-band spectrometer.
The spectrum of DOPURu in chloroform was registered in the range 2500-4500 G, with the instrument parameters set as follows: modulation amplitude, 9 G; resolution, 1024 points; time constant, 20.48 ms; modulation frequency, 100 KHz; incident power, 10 mW. This spectrum shows the typical signal of the paramagnetic Ru^{3+} ion with a pseudo-octahedral coordination with an axial symmetry.[8]
Liposomes formed by DOPURu in water are EPR silent because of the strong spin exchange between the paramagnetic centres situated on the aggregate surface very close one another. Consequently, the DOPURu structuring in the system has been investigating by including in the system a small amount (1% by mole on the DOPURu content) of the spin-probe 1-acyl-2-[5-(4,4-dimethyloxazolidine-*N*-oxyl)]stearoyl-*sn*-glycero-3-phosphocholine (5-PCSL) purchased from Avanti Polar Lipids. In this case the spectrum was registered in the range 3440-3560 G with the instrument parameters set as follows:

modulation amplitude, 1 G; resolution, 1024 points; time constant, 20.48 ms; modulation frequency, 100 KHz; modulation amplitude, 1.0 G; incident power, 6.37 mW. The spectrum of 5-PCSL in DOPURu liposomes present a clearly defined axially anisotropic lineshape, as expected for a spin-probe almost rigidly inserted in a bilayer structure.[9] Values of the outer hyperfine splitting, $2A_{max}$, were determined by measuring the difference between the low-field maximum and the high-field minimum, through a home-made, MATLAB-based software routine. In general, $2A_{max}$ is dependent on both the amplitude (i.e., order) and rate of chain rotational motion, and is therefore a useful parameter for characterising chain dynamics in phospholipid membranes.[10]

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