## SUPPLEMENTARY MATERIALS

### 1 Synthesis of DSPE-PEG$_{3400}$-AL

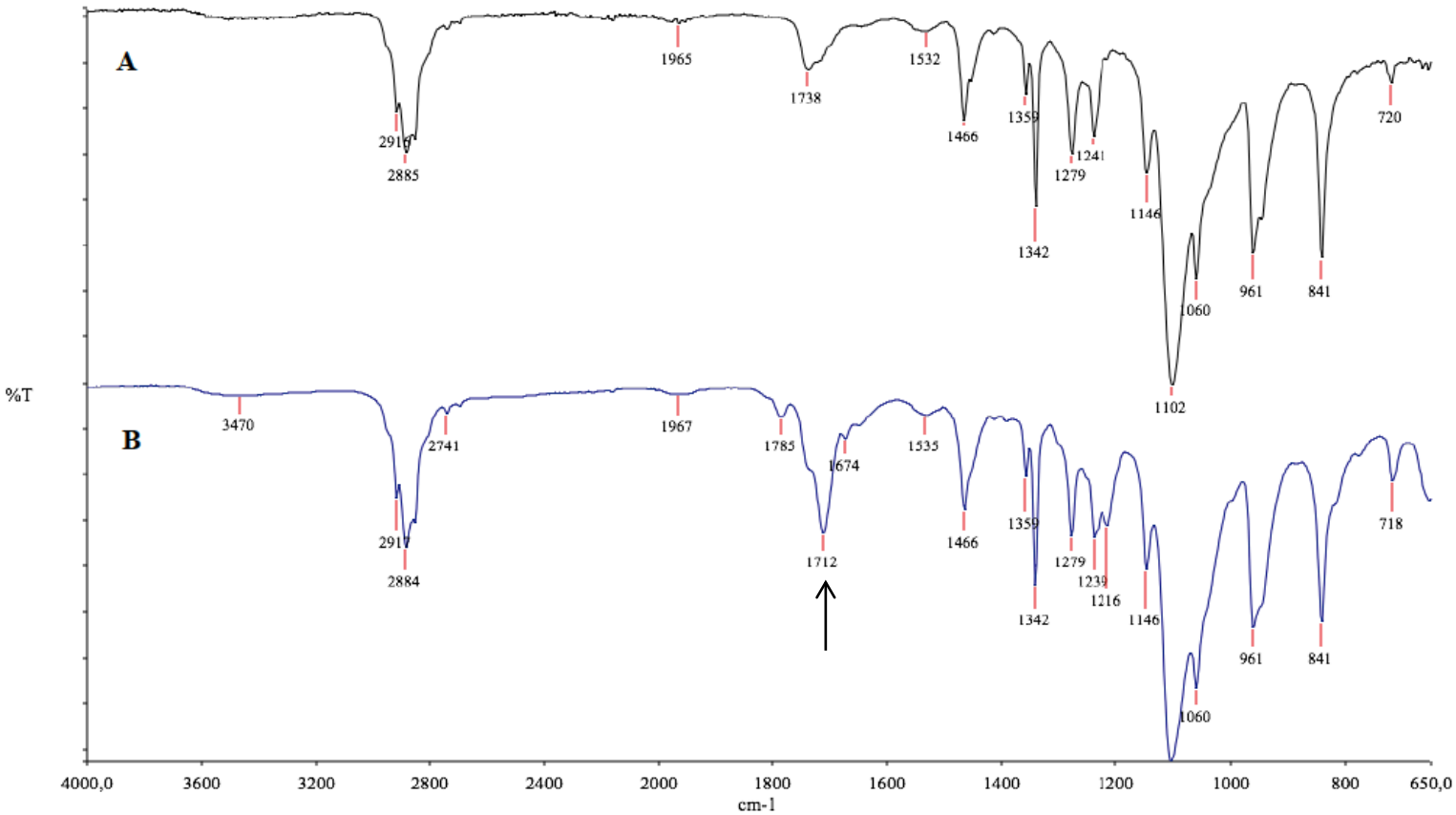


**Figure 1** – FT-IR spectra of DSPE-PEG3400-AL (A) and DSPE-PEG3400-NHS (B). Intensity of peak at 1712 cm$^{-1}$, correspondent to C=O stretch of NHS groups, is strongly reduced in the product, which is consistent with nucleophilic substitution of NHS for alendronate.

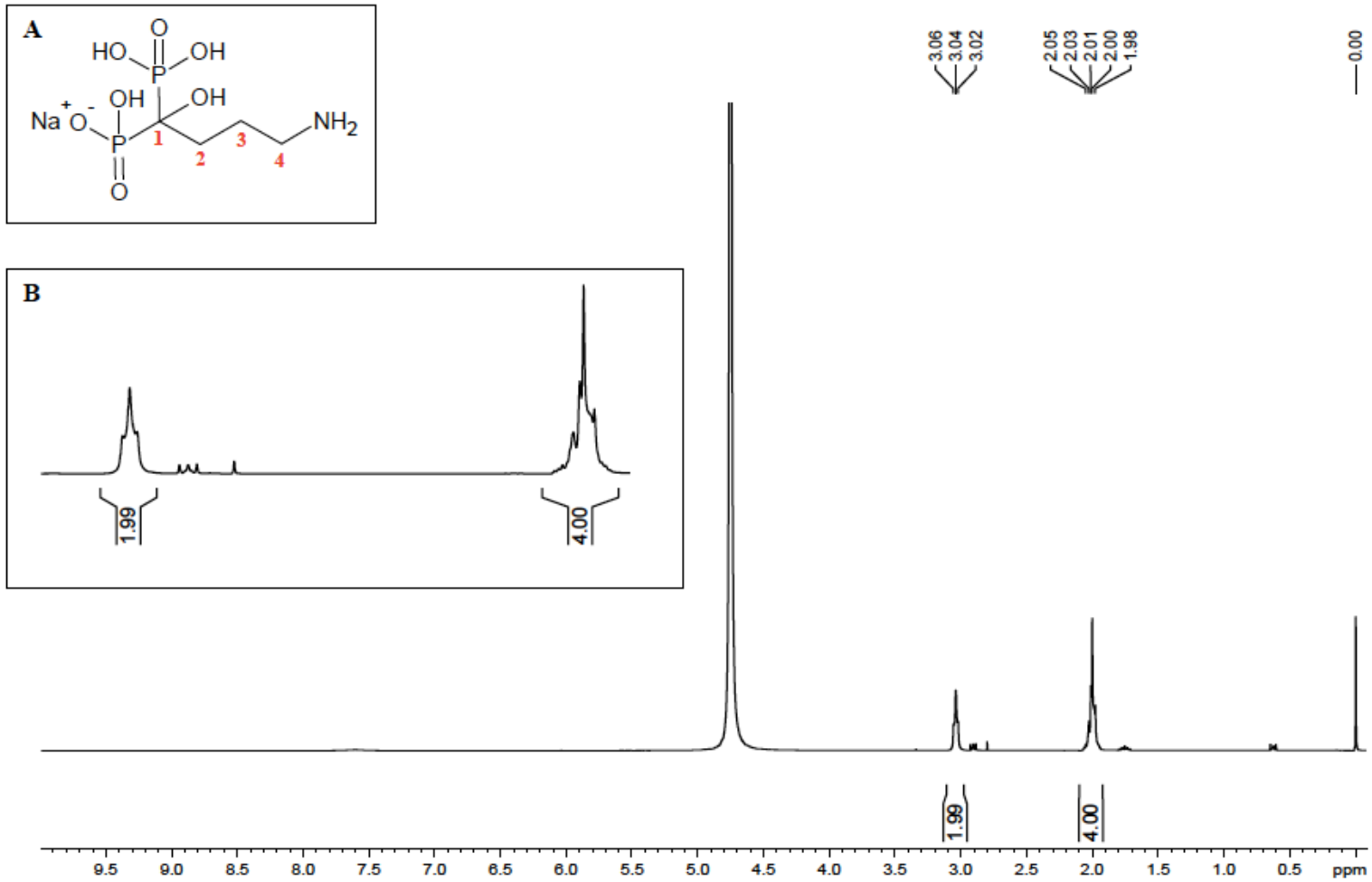


**Figure 2 -** $^{1}H$ NMR of sodium alendronate in $D_2O$ at 400 MHz. Chemical structure of sodium alendronate is represented in A. In B, it is observed the range between 1.5 and 3.5 ppm, evidencing the multiplicity of the signals.

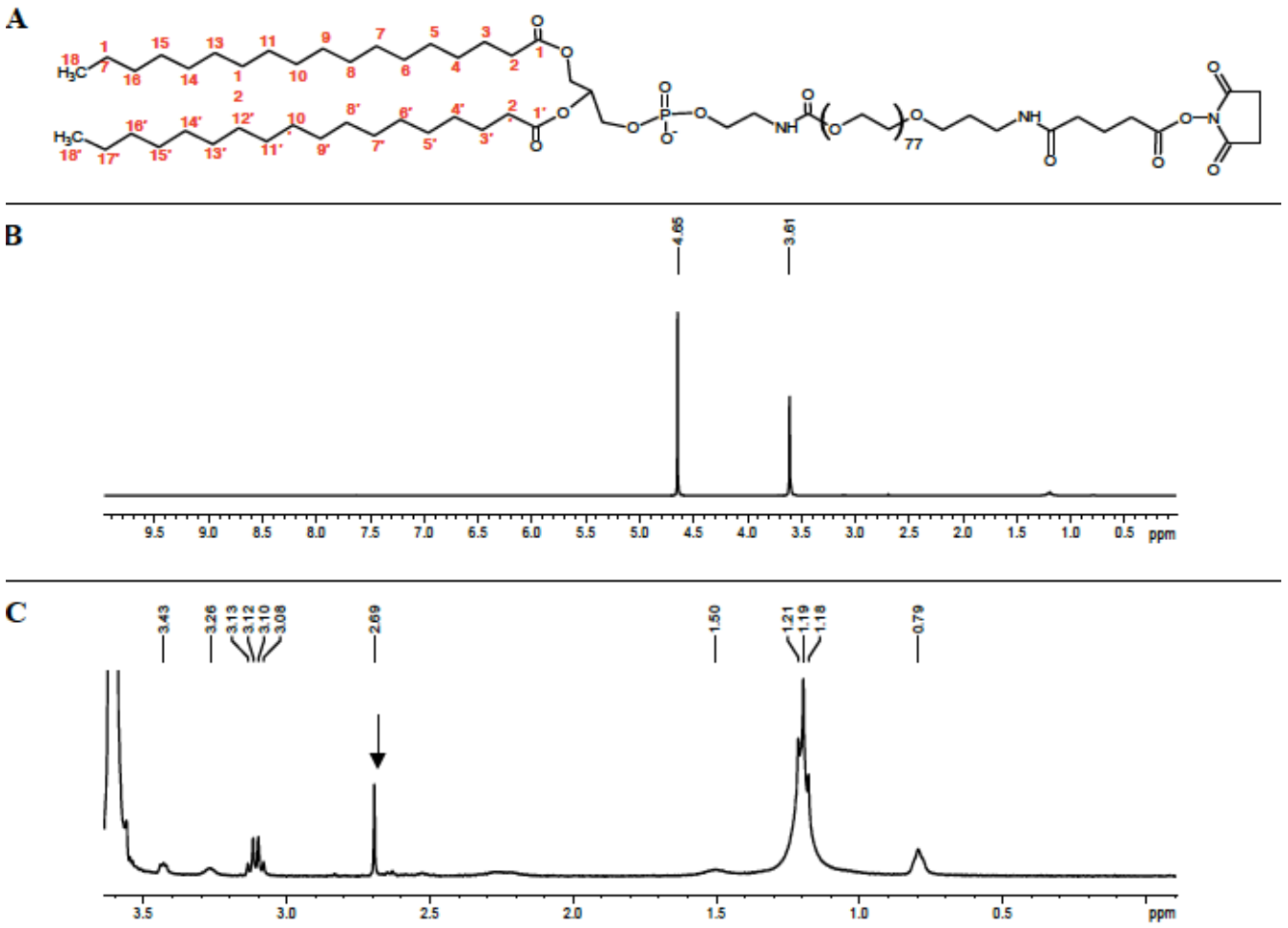


**Figure 3 -** $^{1}H$ NMR of DSPE-PEG$_{3400}$-NHS in $D_2O$ at 400 MHz. Chemical structure of DSPE-PEG$_{3400}$-NHS is represented in A. In B, it is observed the range between 0 and 10 ppm, evidencing the main peak of PEG chain (3.61 ppm). C represents the range between 0 and 3.6 ppm, evidencing the carbon lateral chains (1.19 ppm), terminal methyl groups (0.79 ppm) and NHS (2.69 ppm, arrow).

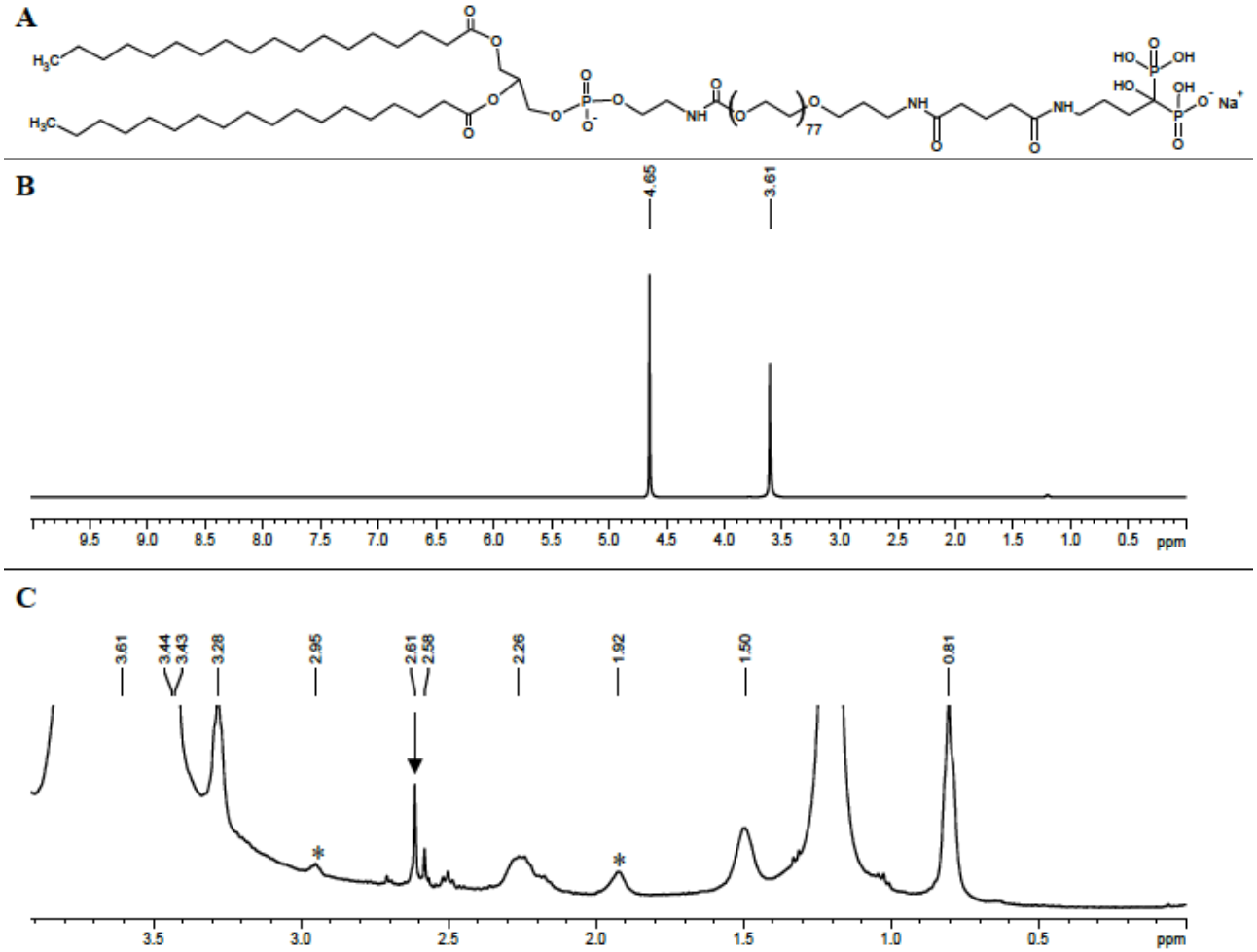


**Figure 4 -** $^{1}H$ NMR of DSPE-$PEG_{3400}$-AL in $D_2O$ at 400 MHz. Chemical structure of DSPE-$PEG_{3400}$-AL is represented in A. In B, it is observed the range between 0 and 10 ppm, evidencing the main peak of PEG chain (3.61 ppm). C represents the range between 0 and 4 ppm, the reduction of signal referent to NHS groups (2.61 ppm, arrow), and appearance of peaks referent to alendronate (1.92 and 2.95 ppm, *).

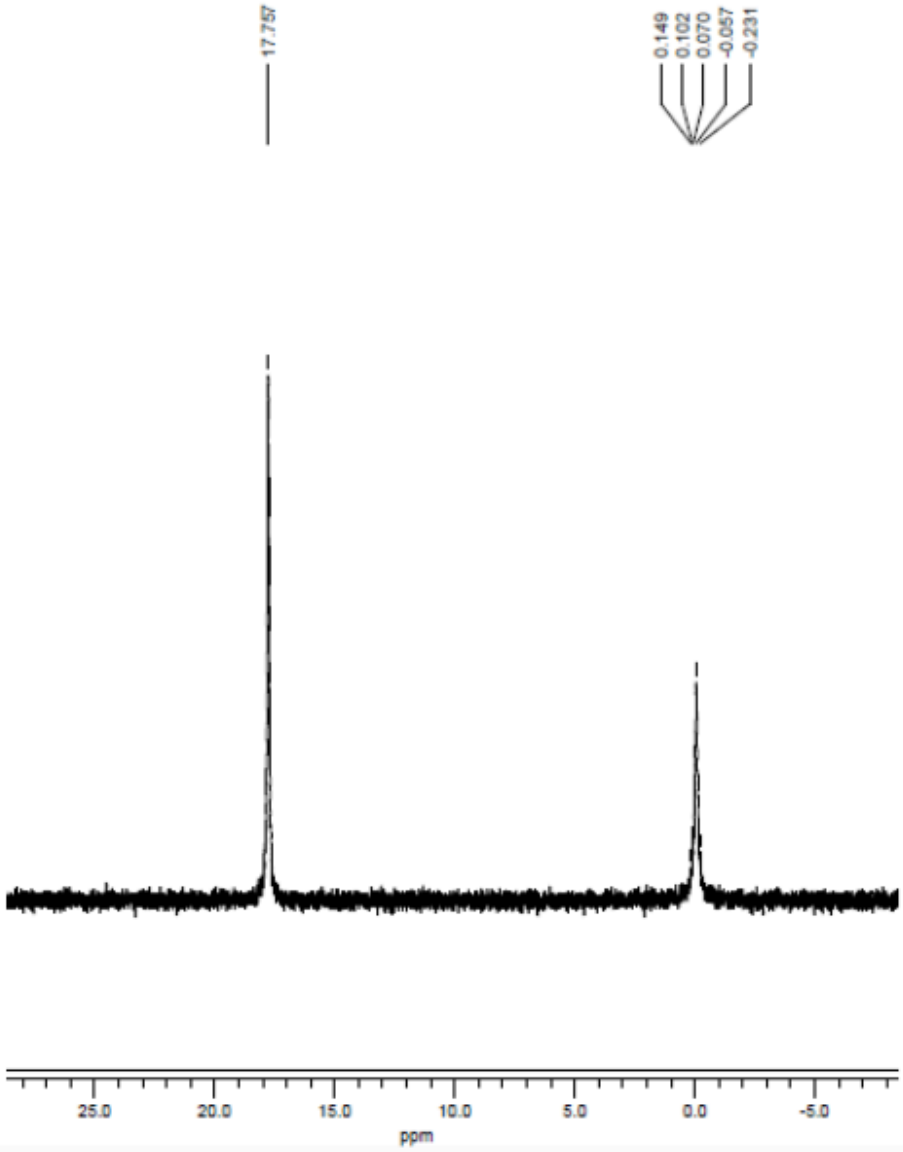


**Figure 5 -** $^{31}P$ NMR of DSPE-$PEG_{3400}$-AL in $D_2O$ at 400 MHz. It is observed two signals in 17.76 and 0.07 ppm, respectively. These signals are related to the groups phosphonate and phosphate, respectively.

## 2 HPLC parameters for DOX quantification

HPLC analyses were performed in a Waters chromatographer (Waters Instruments, 1200 series, Milford, USA). Separations were performed using a 250 x 4.6 mm, 5 µm column (Merck, ACE® 250-4.6 C8, Aberdeen, Scotland). The eluent system consisted of methanol:phosphate buffer pH 3 (in a ratio of 65:35, respectively), and the flow rate was of 1.0 $mL.min^{-1}$. Samples (20 µL) were injected, and the eluate was monitored at excitation/emission wavelengths of 477/555 nm.

## 3 Differential Scanning Analyses (DSC)

A mixture of liposome constituents (Table 1) was introduced in pre-weighed DSC aluminum pans and hydrated over a saturated solution of $MgCl_2$, at 4°C (39 % relative humidity), until constant weight. At equilibrium, the pans were weighed, sealed and analyzed by DSC. The water fraction for the different samples ranged between 0.10 and 0.12. Calorimetric analyses were performed at a heating rate of 5°C.min-1. Indium was used for temperature calibration. Samples were scanned from −50 to 80°C. Data acquisition and analysis were performed on a microcomputer using the software TA Universal Analysis provided by TA Instruments. The transition temperatures were defined by the position of the peak maximum.

**Table 1** – Composition of the samples submitted to DSC analyses

| Sample | Mass (mg) | | | | |
|---|---|---|---|---|---|
| | **DOPE** | **CHEMS** | **DSPE-$PEG_{3400}$-AL** | **DOX** | **HBS** |
| **DOPE:HBS** | 4.2 | - | - | - | 14.0 |
| **DOPE:CHEMS:HBS** | 4.2 | 1.8 | - | - | 14.0 |
| **DOPE:CHEMS:DSPE-$PEG_{3400}$-AL:HBS** | 4.2 | 1.8 | 2.3 | - | 14.0 |
| **DOPE:CHEMS:DSPE-$PEG_{3400}$-AL:DOX:HBS** | 4.2 | 1.8 | 2.3 | 2.0 | 14.0 |

**Abbreviations:** DOPE, 1,2-dioleoyl-glycero-3-phosphatidylethanolamine; HBS, HEPES-buffered saline; CHEMS, cholesteryl hemisuccinate; DOX, doxorubicin.

## 4 Small Angle X-Ray Scattering Analyses (SAXS)

SAXS measurements were carried out at D1B-SAXS1 beamline of the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, Brazil), at a fixed X-ray wavelength of

1.488 nm. SAXS patterns were detected using a 300 k Pilatus detector providing a scattering vector ($q$) range of 0.15 - 4.78 $nm^{-1}$. Temperature was controlled through a Peltier TCCS-3 Hecus. The samples were inserted into a metal ring with 1 mm diameter and 20 μm wall thickness, sealed with a polyimide tape (Kapton™). The SAXS curves were plotted as a function of $q$, where $q = 4\pi\sin(\theta)/\lambda$, being θ the scattering angle and λ the wavelength of the incident radiation. The lattice parameters ($a$) were calculated using the formula $a = 2\pi/q$, which is valid for the first-order peaks of all packing structure types analyzed in this work. The system scattering vector was calibrated by measuring an empty Kapton sealed ring.

## 5 DOX biodistribution analyses

### 5.1 Standard samples preparation

Appropriate amount of DOX were dissolved in 10% DMSO in methanol to prepare a stock solution of 1.0 mg/mL. Then stock solutions were diluted with 10% DMSO in methanol to the concentration of 50 μg/mL as working standard solutions. All solutions were kept at 4 °C before use.

Plasma calibration standards were prepared by adding blank plasma with the appropriate amount of working standard solutions to reach eight concentrations ranging from 12.5 ng/mL to 2000 ng/mL. Promptly after preparation, all solutions were transferred into amber colored volumetric flasks and kept at 4 °C. Standards calibration samples were stored at −20 °C until analysis.

### 5.2 Plasma and tissue pretreatment

Tissues (heart, liver, kidney, spleen, and tumor) were homogenized in a mixture of 0.2 mg of tissue to 0.8 mL of blank plasma. The tissue homogenates were stored at -20 ºC until analysis. For plasma and tissue homogenate samples, 10% DMSO in 800 μL methanol were added to 200 μL of plasma or homogenate samples. After vortex-mix for 2 min, the samples were centrifuged at 10,000 rpm for 15 min. The supernatant was transferred into

another tube and evaporated under a stream of nitrogen at 30 ºC. The residue was reconstituted with 200 µL mobile phase and centrifuged again. 20 µL of the supernatant was injected into the HPLC system for analysis. HPLC analyses were performed according to the described on item 2.