Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for:

Synthesis and physicochemical characterisation of new squalenoyl amphiphilic gadolinium complexes as nanoparticle contrast agents

Mohammad Othman[1], Didier Desmaële[2], Patrick Couvreur[1], Luce Vander Elst[3], Sophie Laurent[3], Robert N. Muller[3], Claudie Bourgaux[1], Estelle Morvan[2], Thierry Pouget[4], Sinda Lepêtre-Mouelhi[2], Philippe Durand[5] and Ruxandra Gref[1]*.

[1]Univ. Paris-Sud XI, Faculté de Pharmacie, UMR CNRS 8612, 92296 Châtenay-Malabry Cedex, France. [2]Univ. Paris-Sud XI, Faculté de Pharmacie, UMR CNRS 8076 BIOCIS, 92296 Châtenay-Malabry Cedex, France. [3]Univ. Mons, Laboratoire de RMN et d'Imagerie Moléculaire, 19, avenue Maistriau, B-7000 Mons, Belgique. [4]LVMH Recherche Parfums et Cosmétiques, Département Innovation Matériaux et Technologies, 185 Avenue de Verdun, F-45804 Saint Jean de Braye, France. [5]CNRS, UPR 2301, (ICSN), 91198 Gif-sur-Yvette, France.

Contents:

1. Methods section

- **Determination of the amount of Gd^{3+} complex by using the Evans' method:**

The amount of SQ-Gd^{3+} complex was determined by Evans' method [Corsi et al. 2001]. For this, *tert*-butanol (25 μL) was added as standard to samples of SQ-Gd^{3+} (500 μL). ^{1}H NMR spectra were acquired at (400 MHz, 298 K) in the presence of an inner insert cell containing an internal standard consisting of D_2O (100 μL) and *tert*-butanol (25 μL). By measuring the difference of the chemical shift, Δδ in ppm, between the signals of *tert*-butanol from the two solutions, it was possible to calculate the exact amount of the paramagnetic agent present in the solution, using equation (1), in which c is the concentration of the Gd^{3+} complex, s=1/3 (for cryomagnet), T is the absolute temperature in degrees Kelvin (K), μ_{eff} is the magnetic moment of the lanthanide metal (7.94 for Gd^{3+}).

$$\Delta\delta = \frac{4000\pi cs}{T}\cdot\left(\frac{\mu_{eff}}{2.84}\right)^2 \qquad \text{Eq. (1)}$$

- **Preparation of 4% (w/v) Human Serum Albumin (HSA) and SQ-Gd^{3+}/HSA Samples.** HSA (2g) was dissolved in Milli Q water (10 mL) to prepare 20% (w/v). Once the HSA was dissolved enough H_2O was added to dilute the protein to 8 % (w/v), this solution was mixed with a same volume of SQ-Gd complex in water (i.e., 2mM) solution to prepare 1 mM SQ-Gd^{3+} complex with 4% HSA.

2. Variable-temperature ^{17}O measurements: The observed transverse relaxation rates R_2^{obs} were calculated from the signal width at half-height ($\Delta\nu_{1/2}$): $R_2^{obs}=\pi\Delta\nu_{1/2}$. The value of R_2^{p} was obtained by subtracting the diamagnetic contribution of water (figure 1). The fitting of the data was performed as previously described [Muller et al. 1999].

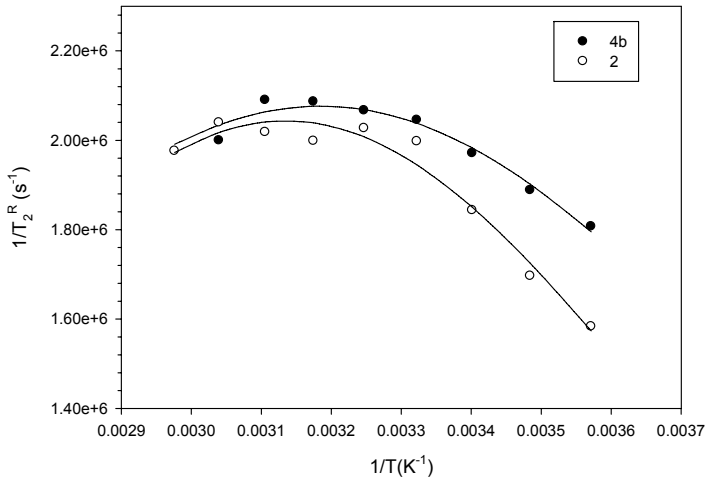


Figure 1. ^{17}O transverse relaxometry of SQ-Gd^{3+} complexes ● **4b** and ○ **2**. The lines correspond to the theoretical fitting of the data points.

3. **Illustration of two amphiphilic molecules organized in supramolecular Nanoassemblies according to the geometry of the molecule.**

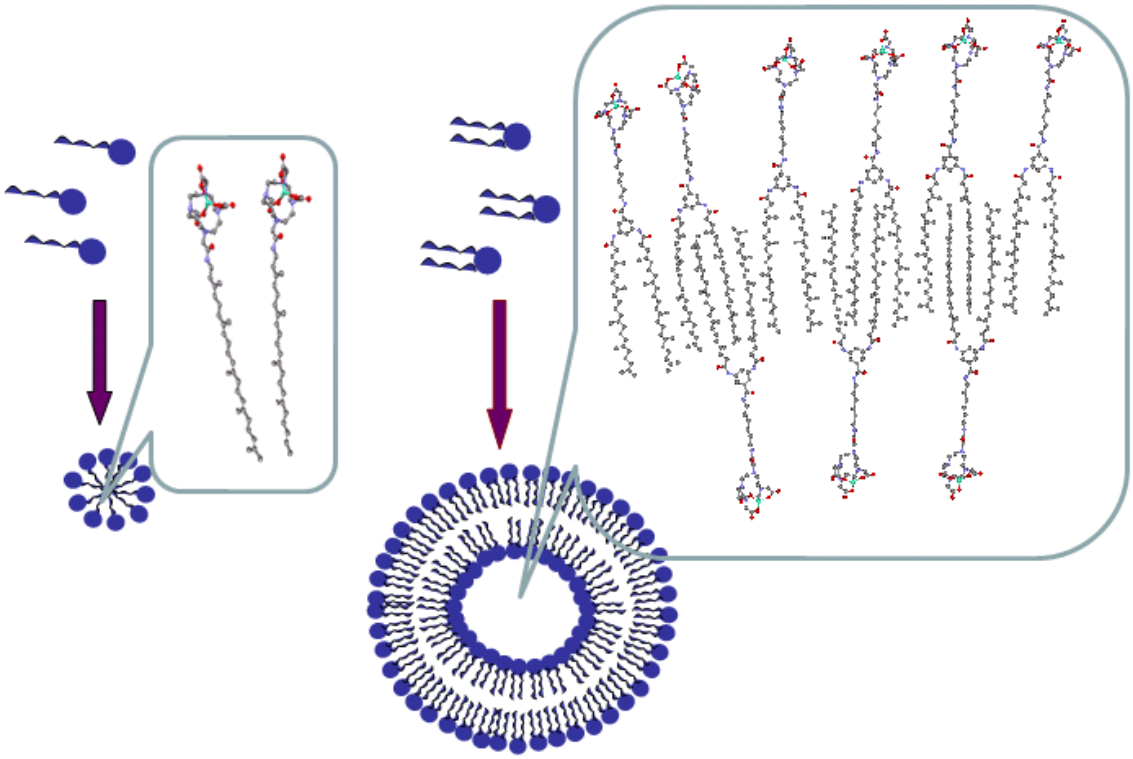


Figure 2. Schematic representation of micelles (Gd^{3+} chelate coupled to one chain of squalene e.g. "the complex SQ-Gd^{3+} **2**") and liposome (Gd^{3+} chelate coupled to two chains of squalene "the complex SQ-Gd^{3+} **4c**") obtained from two amphiphilic active agents (according to the geometry hydrophilicity/ hydrophobicity ratio which could lead to micellar or liposomal forms.

4. **Characterization of the synthesized SQ-Gd^{3+} complexes by dynamic light scattering.**

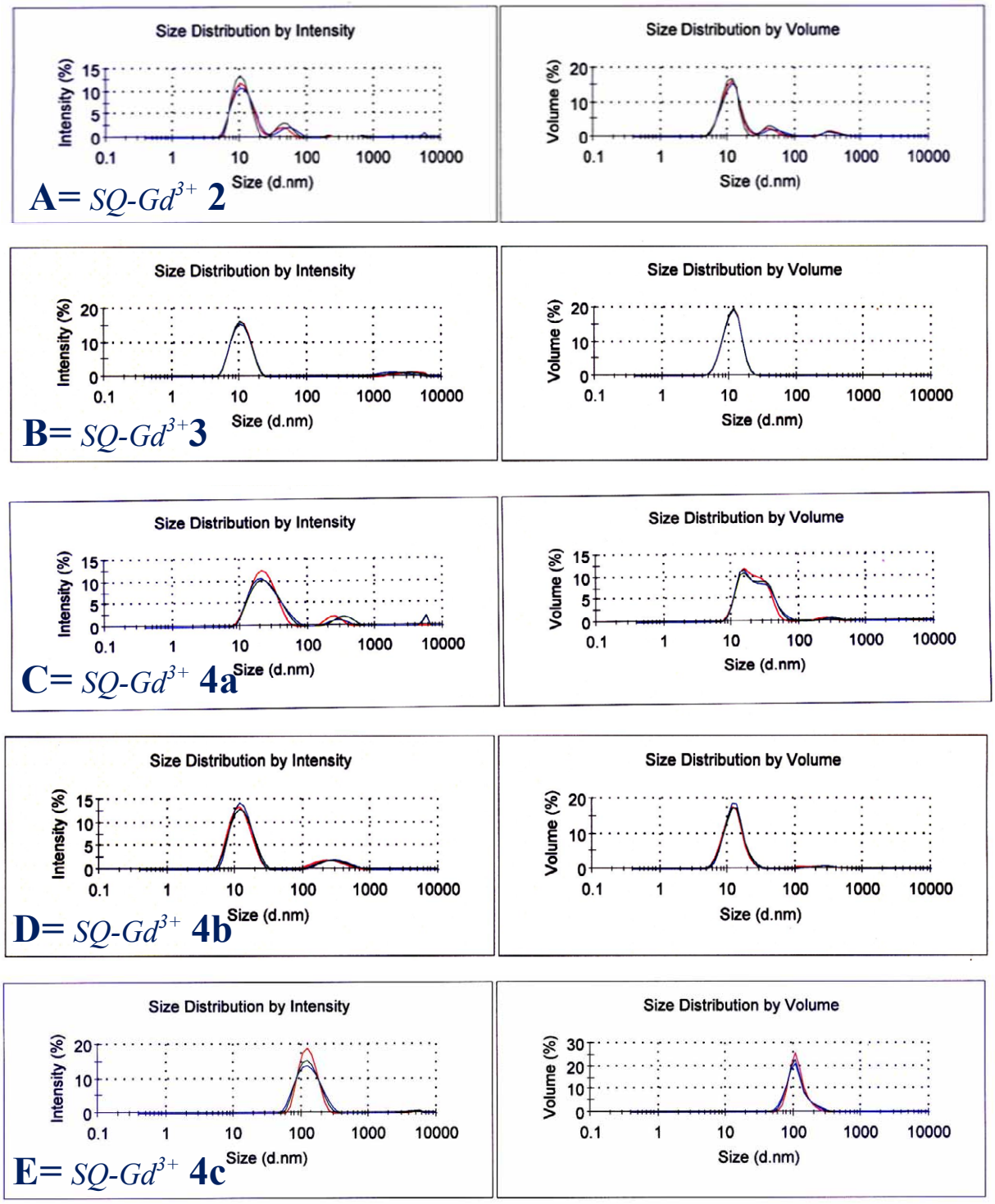


Figure 3. Size distributions by intensity and by volume of nanoassemblies of ***A)*** SQ-Gd^{3+} **2,** d = 11 nm, polydispersity index (PDI = 0.3), ***B)*** SQ-Gd^{3+} **3,** d = 12nm, PDI = 0.26, ***C)*** SQ-Gd^{3+} **4a,** d = 22 nm, (PDI = 0.34), ***D)*** SQ-Gd^{3+} **4b,** d = 14 nm, PDI = 0.29, ***E)*** SQ-Gd^{3+} **4c,** d = 110 nm, PDI = 0.15.

5. CMC of SQ-Gd^{3+} complexes estimated by pyrene.

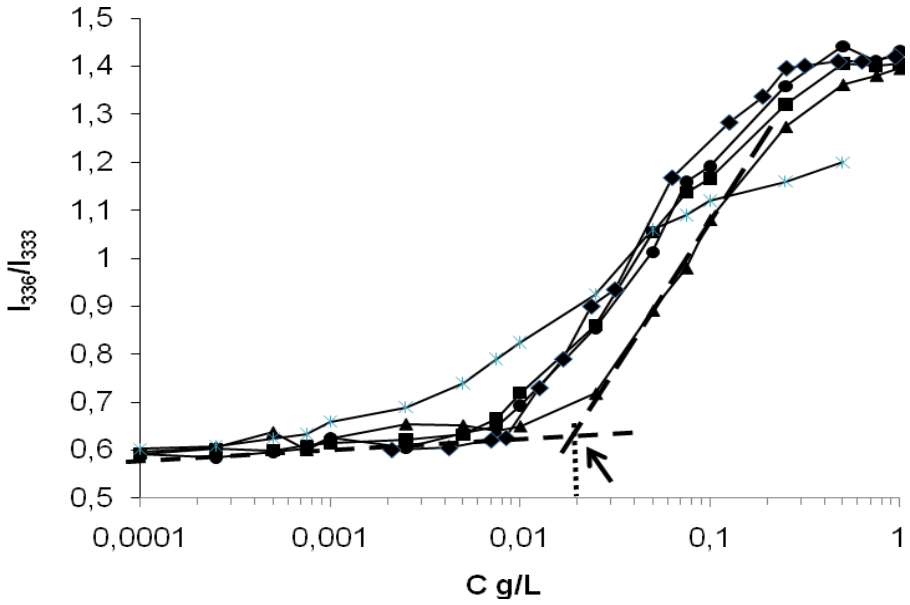


Figure 4. Experimental determination of CMC of SQ-Gd^{3+} complexes , changes in the I_{336} nm/I_{333} nm ratio of pyrene fluorescence intensity as a function of logarithm of SQ-Gd^{3+} complex concentrations (from 1×10^{-4} to 1 g/L); SQ-Gd^{3+} ■ **2,** ♦ **3,** ▲ **4a,** ● **4b,** * **4c**. The arrow indicates to the estimated CMC of SQ-Gd^{3+} **4a** as an example.

6. Gd^{3+} number in nanoassemblies (NAs) (micelle or liposome like structures).

The payload of SQ-Gd^{3+} in micelles was determined by using equations (2-5), **with the assumption that the micelles have spherical forms and the density (ρ) of the nanoassembly (NAs) equal to 1**. The volume of a sphere is presented in eq. 2, in which V_{NAs} and d are the volume and the mean hydrodynamic diameter of a spherical particle, respectively. In eq.3, ρ_{NAs} and W_{NAs} are the density and the weight (g) of NAs respectively. In eq.4: W_{NAs} is assumed to be equal to V_{NAs}.

In eq.5: N_{ca} is the number of Gd^{3+} complex in NAs, M_w is the molecular weight (g), and NA is Avogadro's number.

These equations are based on the particle size.

$$V_{NAs} = \frac{1}{6}\pi d^3 \qquad \text{Eq.2}$$

$$\rho_{NAs} = \frac{W_{NAs}}{V_{NAs}} \qquad \text{Eq.3}$$

If $\rho_{NAs} = 1$

$$W_{NAs} = V_{NAs} \qquad \text{Eq.4}$$

$nm^3 = 10^{-21}\ cm^3$

$$N_{ca} = \frac{W_{NAs}}{M_w} N_A \qquad \text{Eq.5}$$

{π =3.1416, NA=6.022045×10^{23} Mol^{-1}}

The calculated numbers of SQ-Gd^{3+} 2-4b were between 120 and 540 molecules in one micelle.

To evaluate **the amount of SQ-Gd^{3+} included into liposome-like supramolecular NAs**, by combination the data obtained by DLS, cryo-TEM, and SAXS measurements; the volume of the membrane of a liposome could be calculated from the difference between the volumes of two spheres: The volume of an inner sphere with a diameter d_1=100 nm, subtracted from that one of an outer sphere with a diameter $d_2 = d_1 + 11 = 111$ nm (from SAXS results the thickness of the membrane was estimated to be equal to 11 nm) according to the equations (6, then 4 and 5), the number of **4C** was calculated . Mw = 1556.21

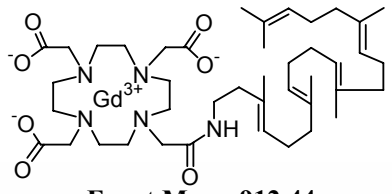
$$\Delta V_{NAs} = \frac{1}{6}\pi(d_2^3 - d_1^3) \qquad \text{Eq.6}$$

The obtained volume of the membrane of **4c** was $\Delta V_{NAs} = 1.925\times10^5 nm^3$

Thus the N_{4c} was found to be about 74,480 molecules/ particle.

7. High resolution mass spectra HRMS of the synthesized SQ-Gd^{3+} complexes.

SQ-Gd^{3+} complex 2



Exact Mass: 912,44
Mol. Wt.: 912,27

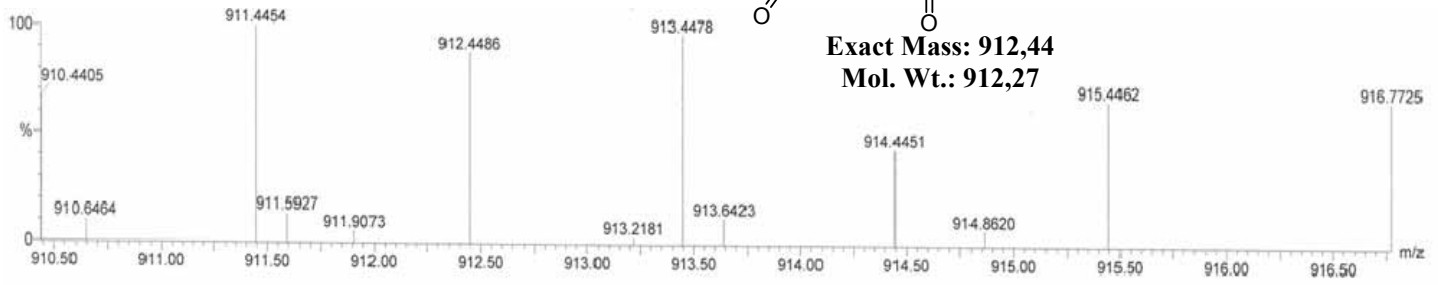
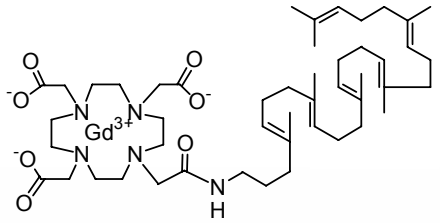


Figure 5. High Resolution Mass Spectrum (HRMS), ESI/TOF (+) of SQ-Gd^{+3} complex **2**, located around m/z = 913.4478 $[M+H]^+$, showing the characteristic isotopic pattern of the Gd^{+3}.

SQ-Gd^{3+} complex 3



Exact Mass: 994,51
Mol. Wt.: 994,41

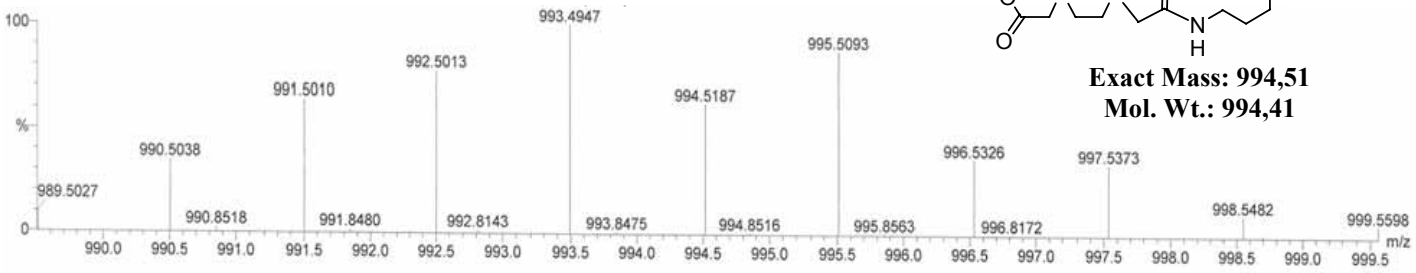


Figure 6. ESI/TOF (-) Mass spectrum of SQ-Gd^{3+}complex **3**, located around m/z = 993.4947 $[M-H]^-$ showing the characteristic isotopic pattern of the Gd^{+3}.

SQ-Gd^{3+} complex 4a

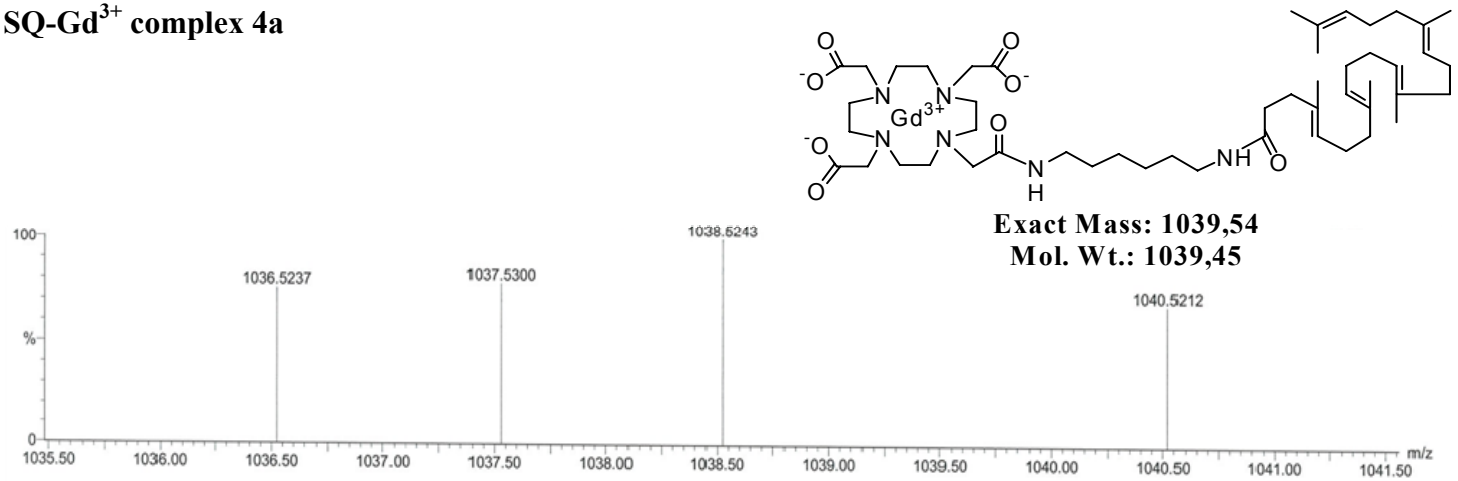


Figure 7. HRMS, ESI/TOF (-) of SQ-Gd^{3+}complex **4a**, located around m/z = 1038.5243 $[M-H]^-$ showing the characteristic isotopic pattern of the Gd^{+3}

SQ-Gd^{3+} complex 4b

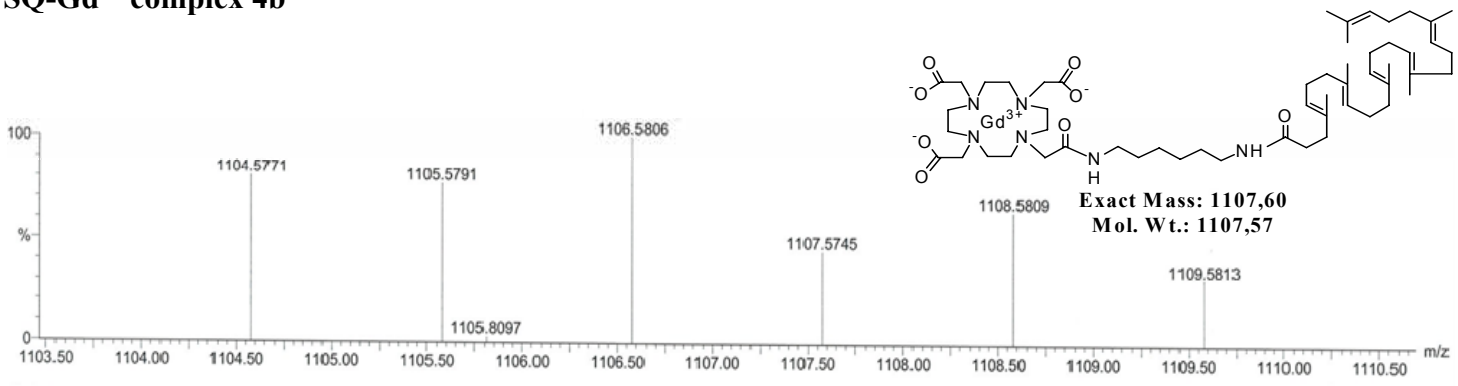


Figure 8. HRMS, ESI/TOF (-) of SQ-Gd^{3+}complex **4b**, located around m/z = 1106.5806 $[M-H]^-$ showing the characteristic isotopic pattern of the Gd^{+3}

SQ-Gd^{3+} complex 4c

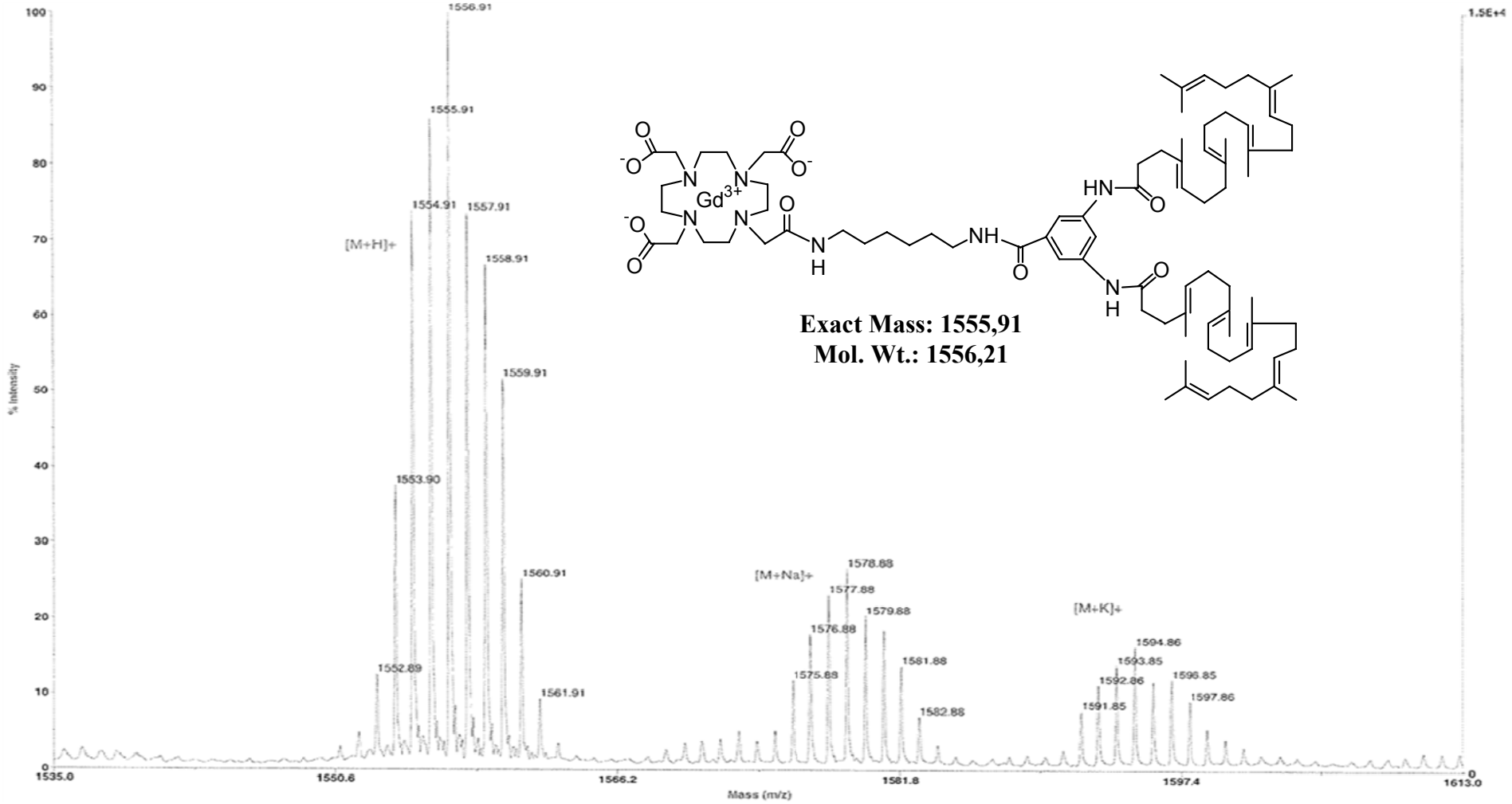


Figure 9. HRMS, MALDI/TOF of SQ-Gd^{3+} complex **4c**, located around m/z = 1556.91456 $[M+H]^+$, 1578.88 $[M+Na]^+$ and 1594.86 $[M+K]^+$ showing the characteristic isotopic pattern of the Gd^{+3}

1. D. M. Corsi, C. Platas-Iglesias, H. Van Bekkum, *Magn. Reson. Chem.* 2001, **39,** 723 – 726.
2. R.N. Muller, B. Raduchel, S. Laurent, J. Platzek, C. Pierart, P. Mareski, L. Vander Elst. *Eur. J. Inorg. Chem.*, 1999, **11**, 1949-1955.