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Supporting information

for

An albumin-binding Gd-HPDO3A contrast agent for improved intravascular retention

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Content:

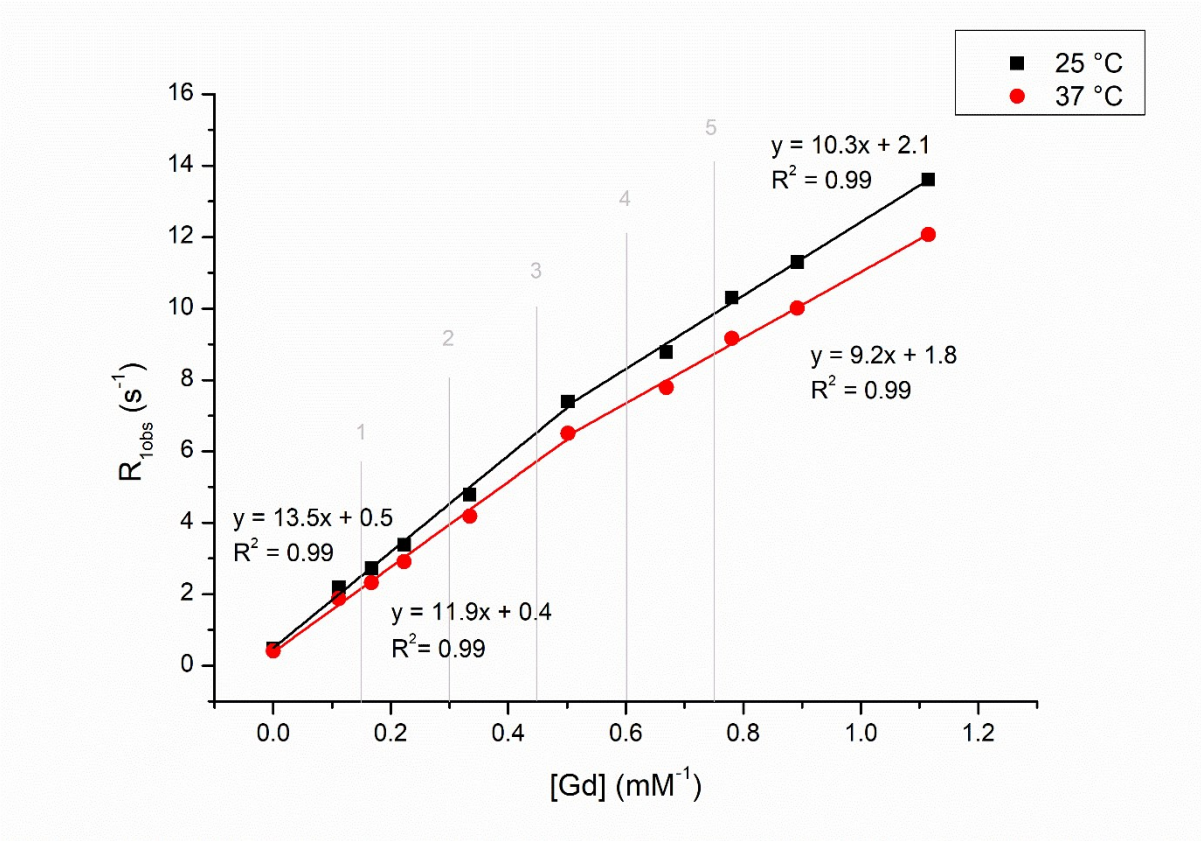
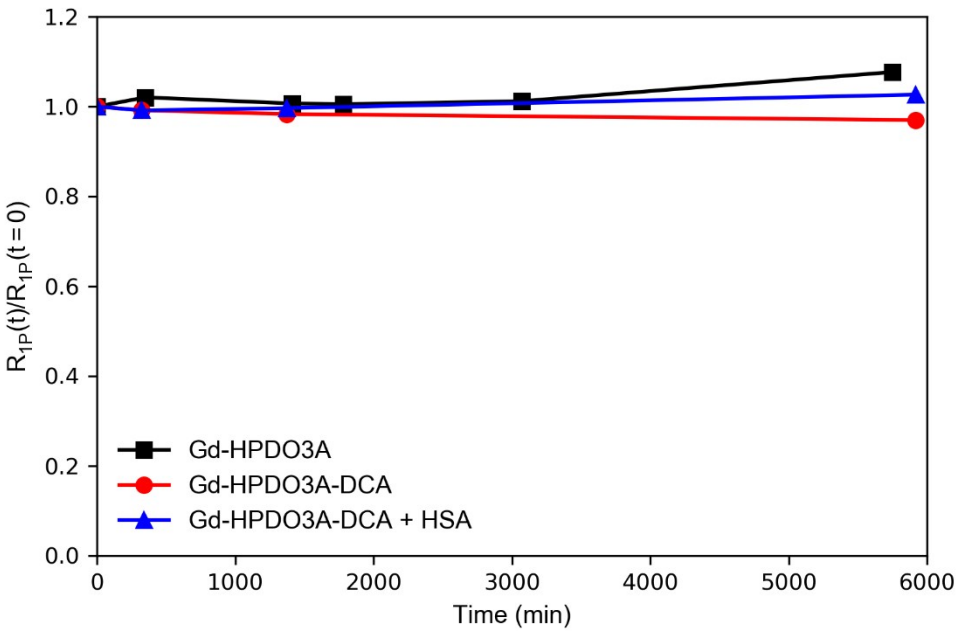


Figure S1: Proton relaxation rate (R_{1obs}) of 0.15 mM HSA in 50 mM PBS as a function of increasing Gd-HPDO3A-DCA concentration. Measured at 0.47 T, pH 7.4 at 298K and 310 K.

Figure S2: Transmetalation of Gd complexes with 1 eq. Zinc in 67 mM phosphate buffer at 310 K and pH 7.4, measured at 0.47 T.



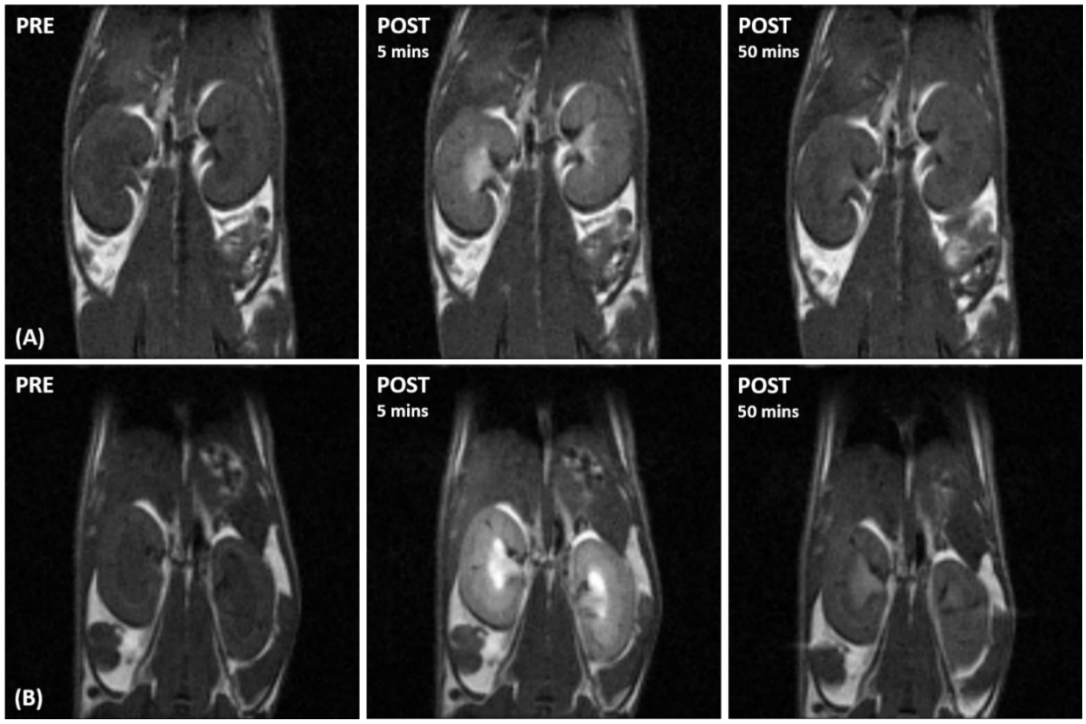


Figure S3: Representative T1-weighted MSME for healthy tissues: kidneys and liver (partially observable) obtained pre and after injection with A) Gd-HPDO3A and B) Gd-HPDO3A-DCA, images show left to right pre-contrast, and post-contrast at 5 and 50 min.

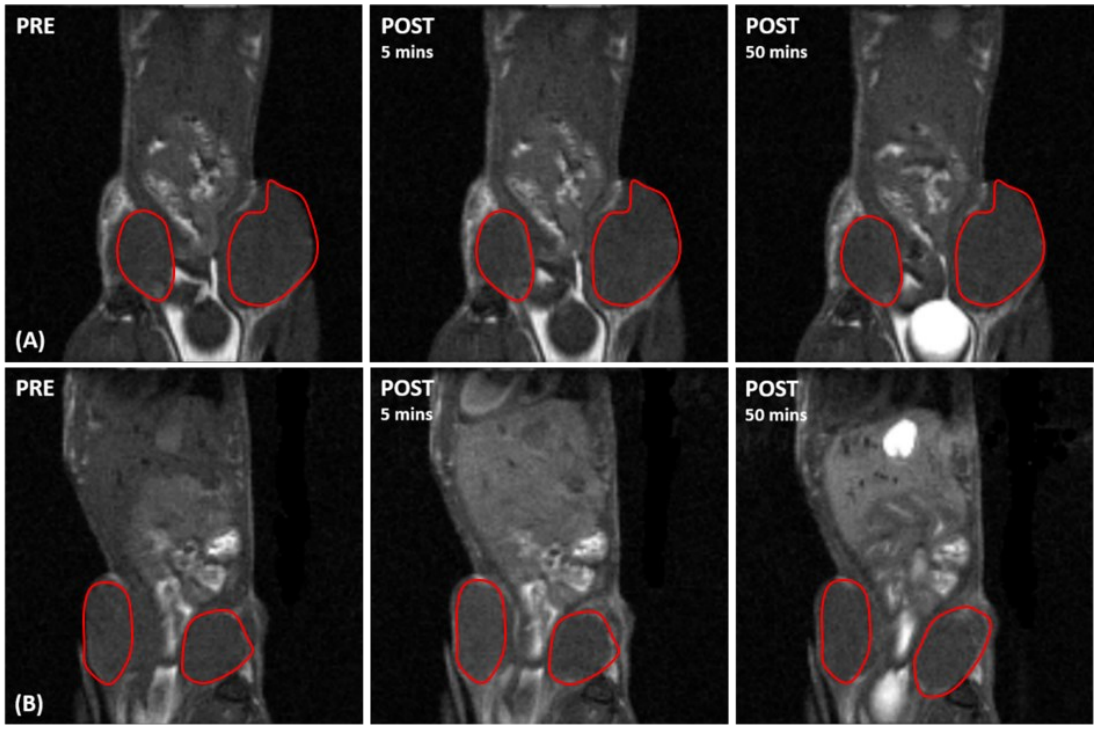


Figure S4: Representative T1-weighted MSME for healthy tissues: tumours (red lines) and liver obtained pre and after injection with A) Gd-HPDO3A and B) Gd-HPDO3A-DCA, images show left to right pre-contrast, and post-contrast at 5 and 50 min.

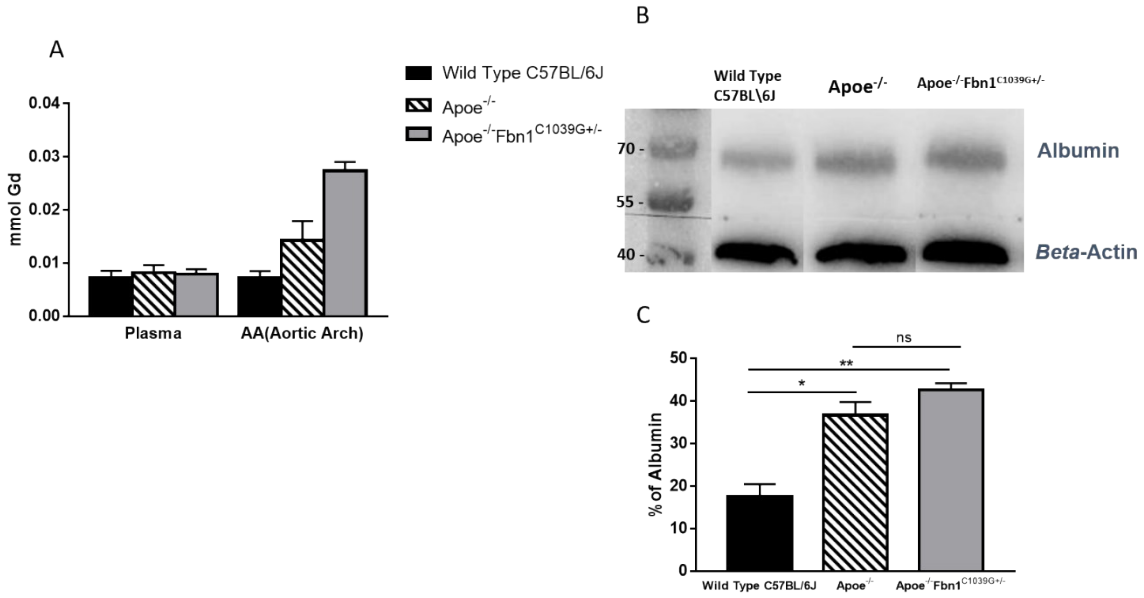


Figure S5: A) Quantification of gadolinium concentration by ICP-MS in plasma and in the aortic arch of three species of mouse models. B) Western Blot (n=2 per group) and C) percentage of albumin deposition in the vessel wall in the three species of mouse models.

*P<0.05; **P<0.01; ***P<0.001

NMR spectra, HRMS and Chromatograms of compounds A,C,D,E, Gd-HPDO3A-DCA and Eu-HPDO3A-DCA

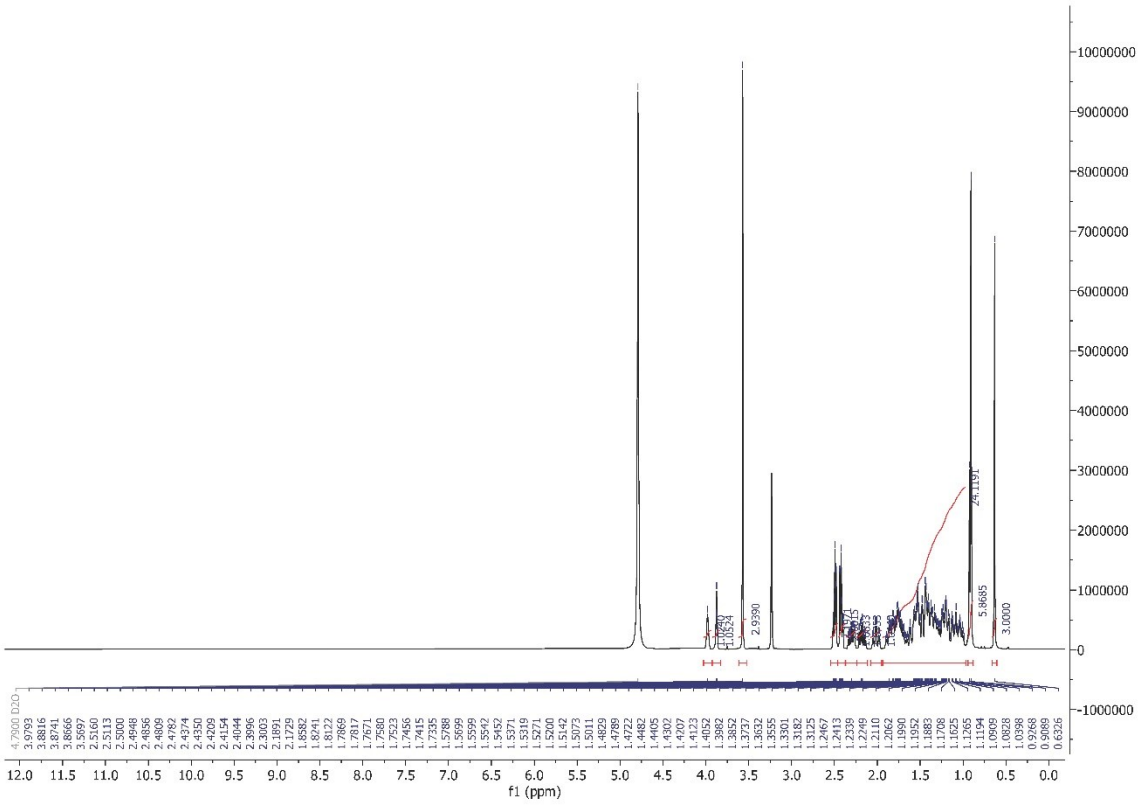


Figure S6: ^{1}H NMR spectrum of compound **A** in CD_3OD at 400 MHz and 298 K.

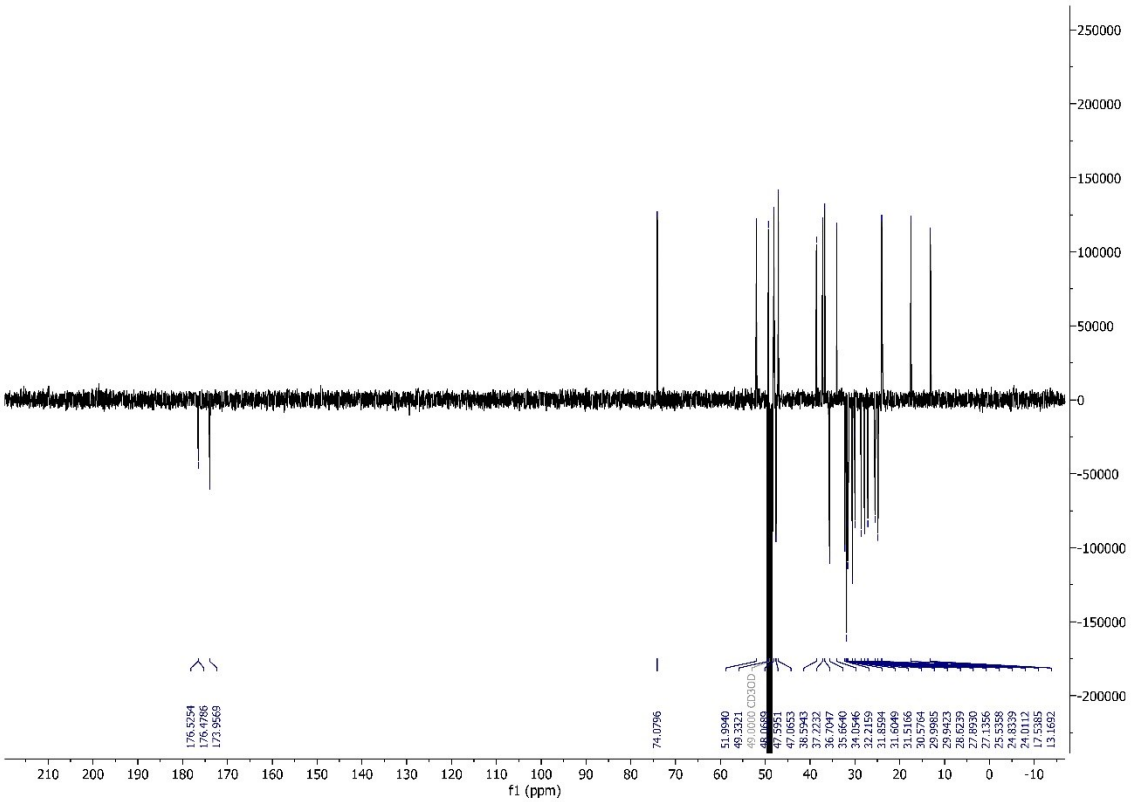


Figure S7: ^{13}C APT NMR spectrum of compound **A** in CD_3OD at 400 MHz and 298 K.

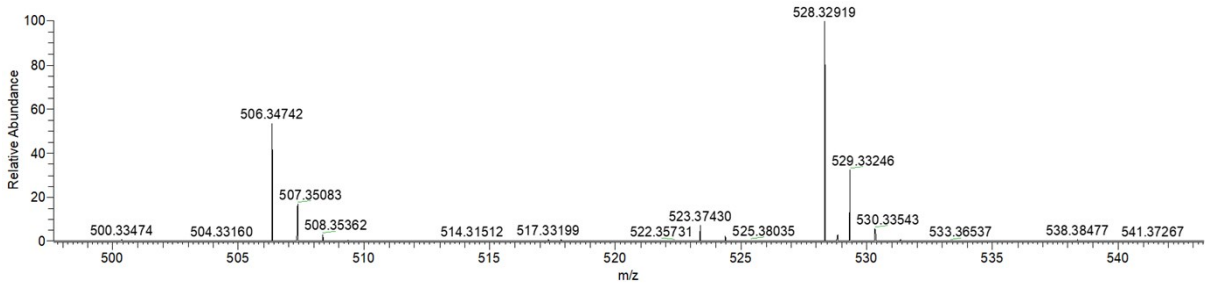


Figure S8: HRMS spectrum of compound **A**.

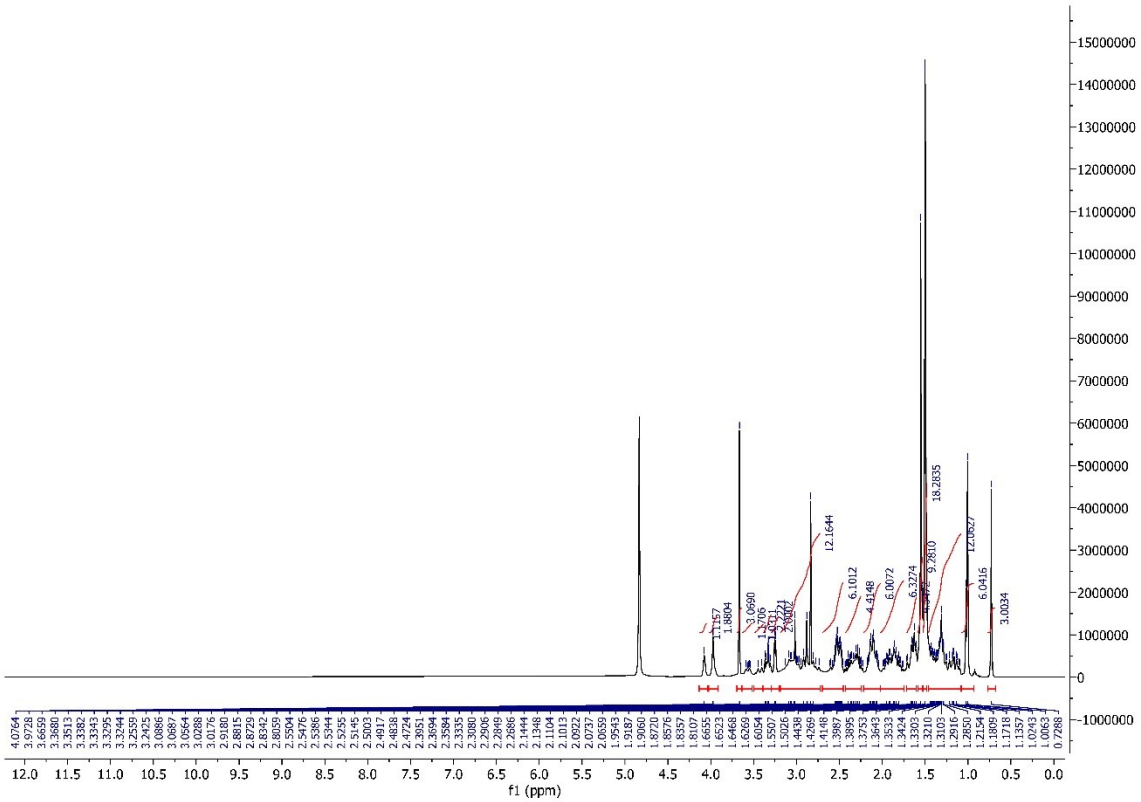


Figure S9: ^{1}H NMR spectrum of compound **C** in CD_3OD at 400 MHz and 298 K.

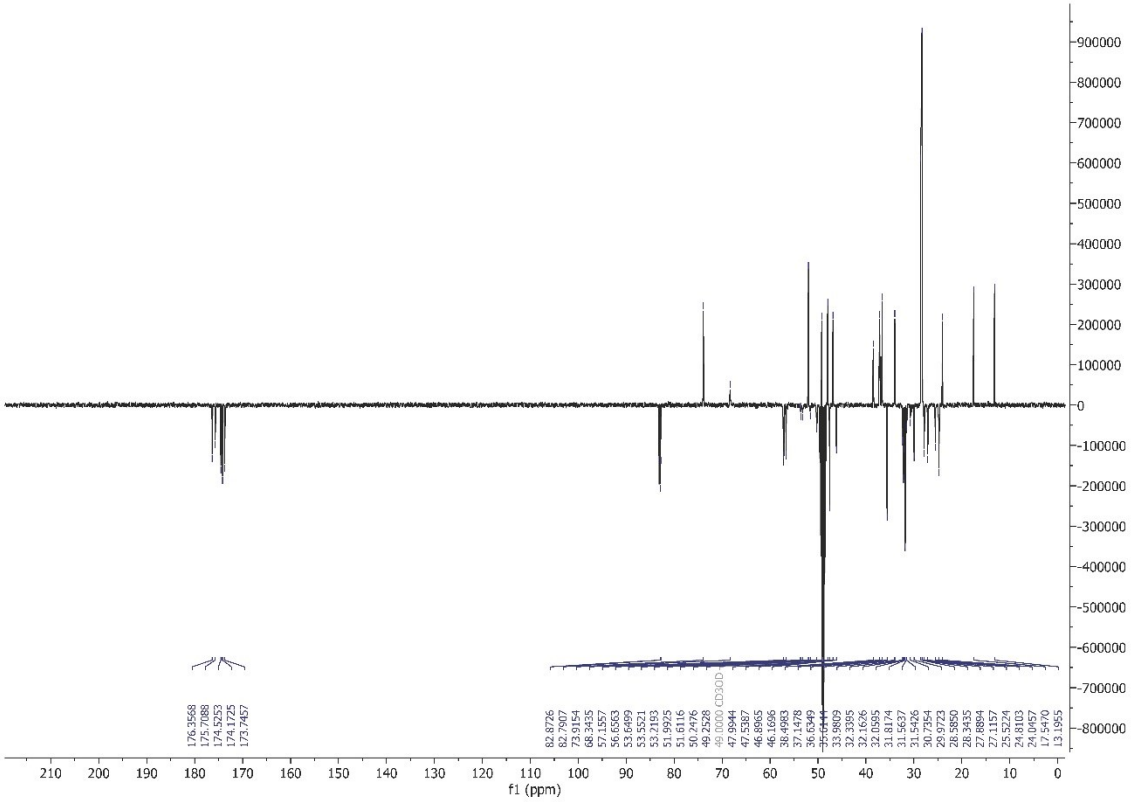


Figure S10: ^{13}C APT NMR spectrum of compound **C** in CD_3OD at 400 MHz and 298 K.

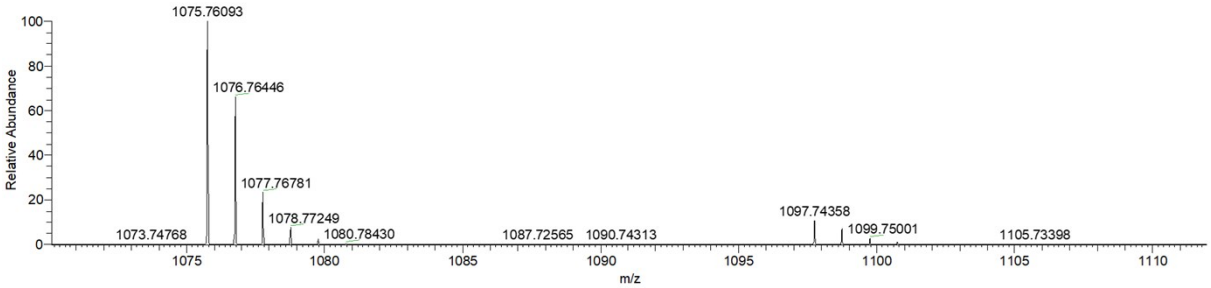


Figure S11: HRMS spectrum of compound **C**.

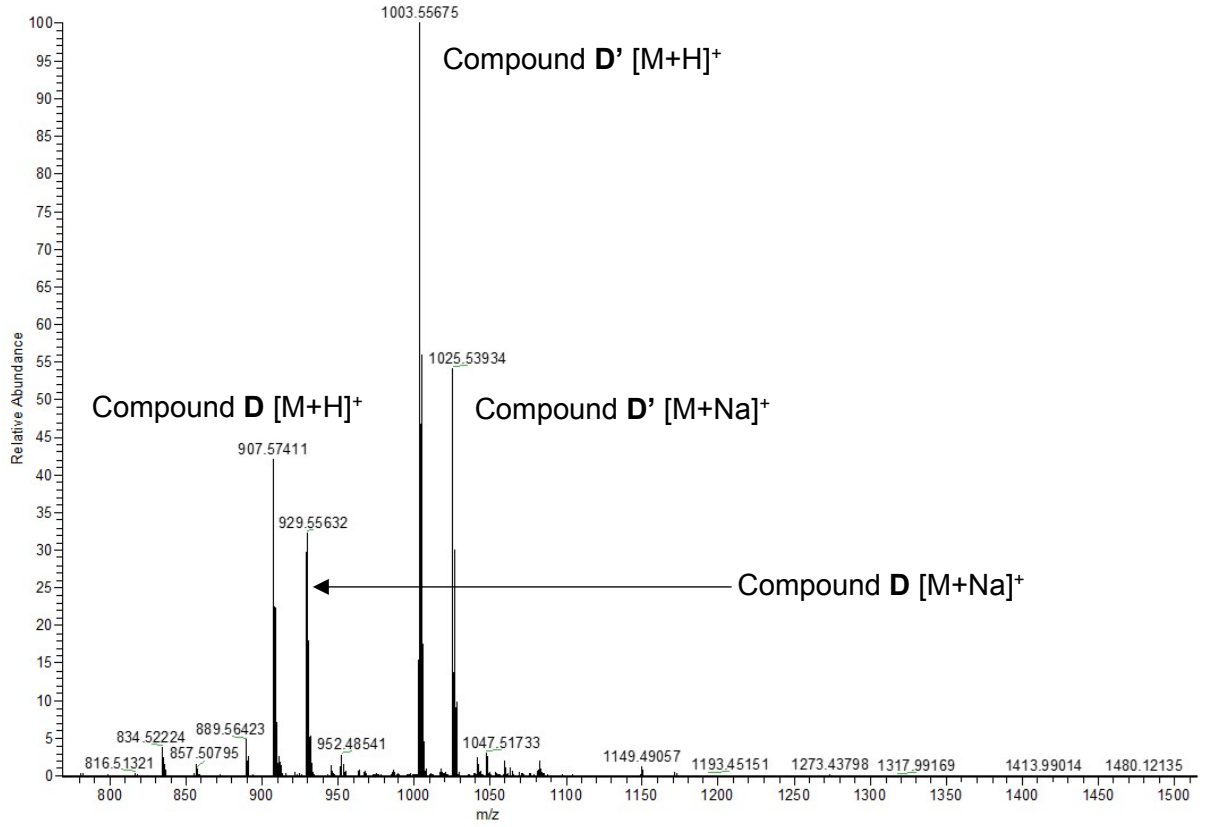
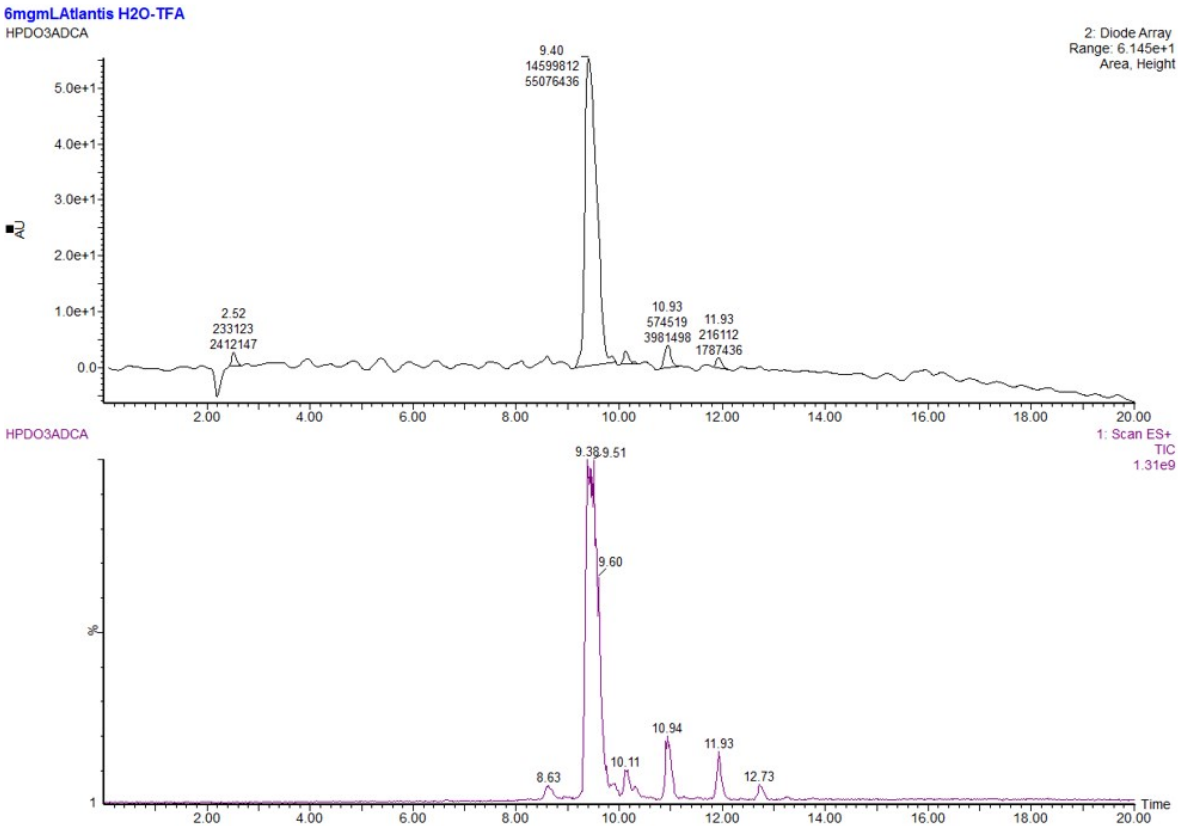


Figure S12: HRMS spectrum of compound **D** and **D'**.

a)



b)

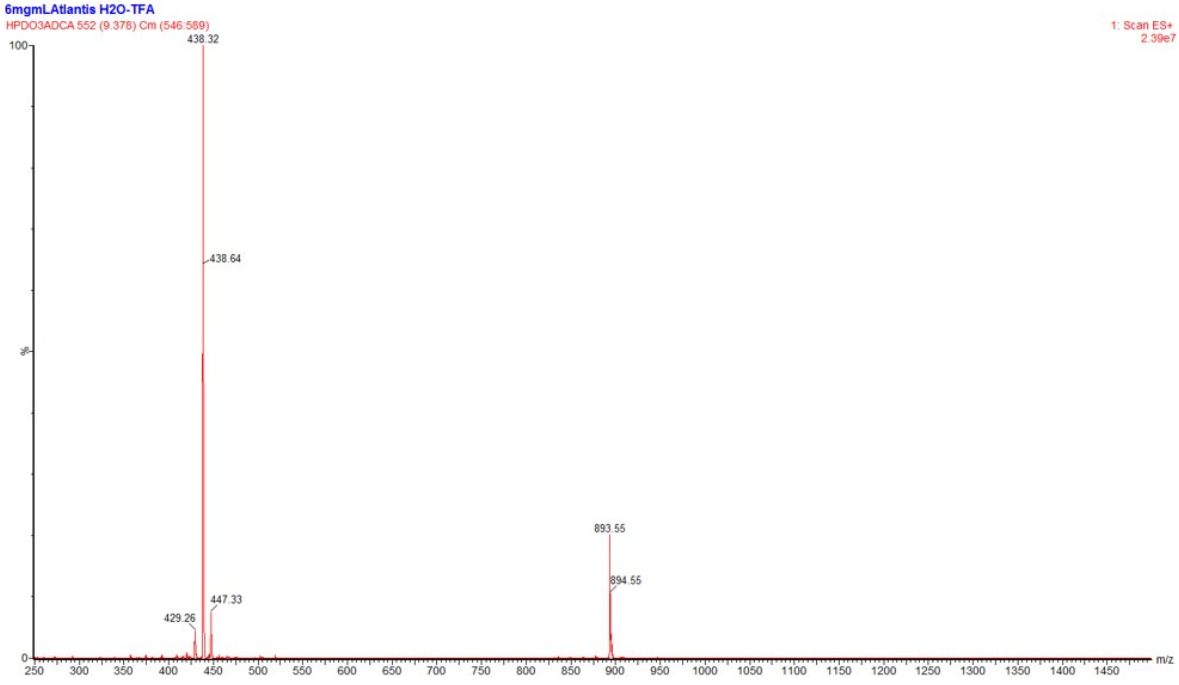


Figure S13: a) Chromatogram Diode Array (200-400 nm) and TIC ESI+, purity >90% and b) Mass spectrum in ESI+ of peak at 9.4 min of compound **E**.

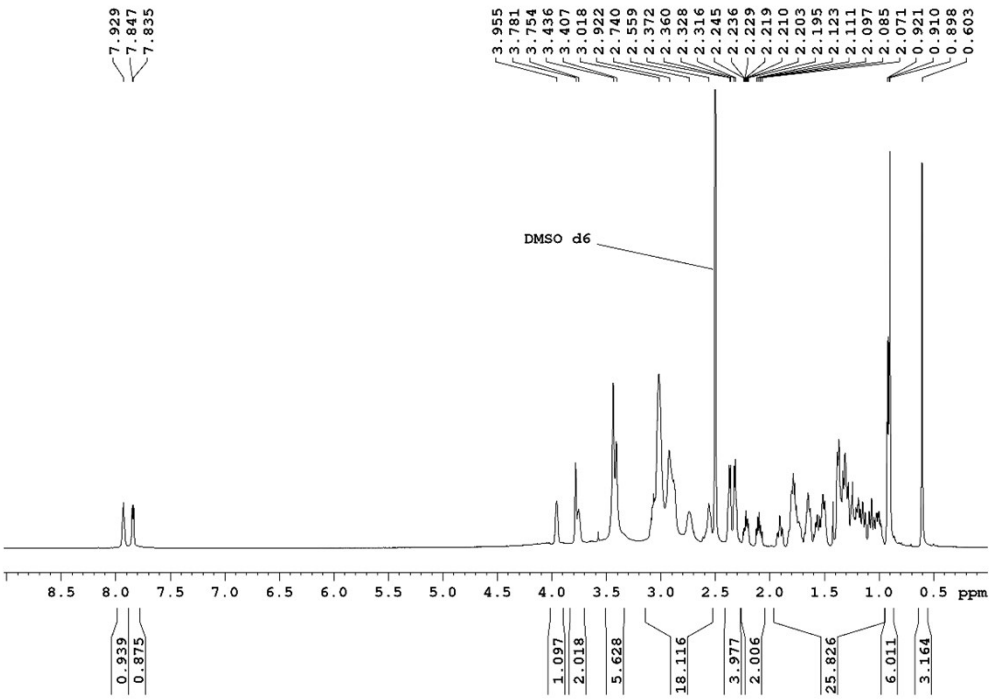


Figure S14: ^{1}H-NMR spectrum of compound **E** in DMSO d_6 at 600 MHz and 298 K.

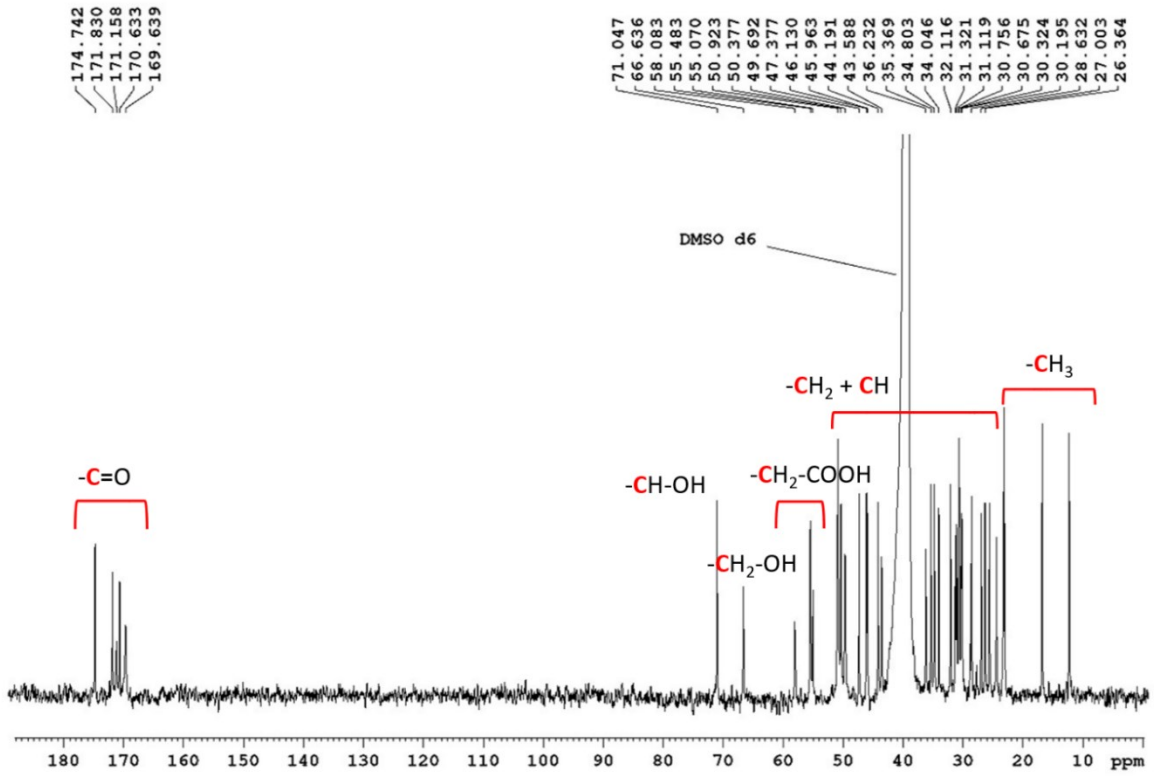


Figure S15: ^{13}C-NMR spectrum of compound **E** in DMSO d_6 at 600 MHz and 298 K.

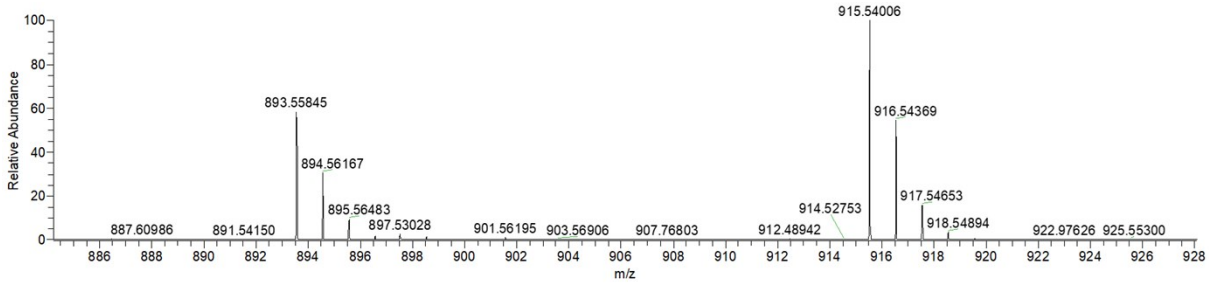
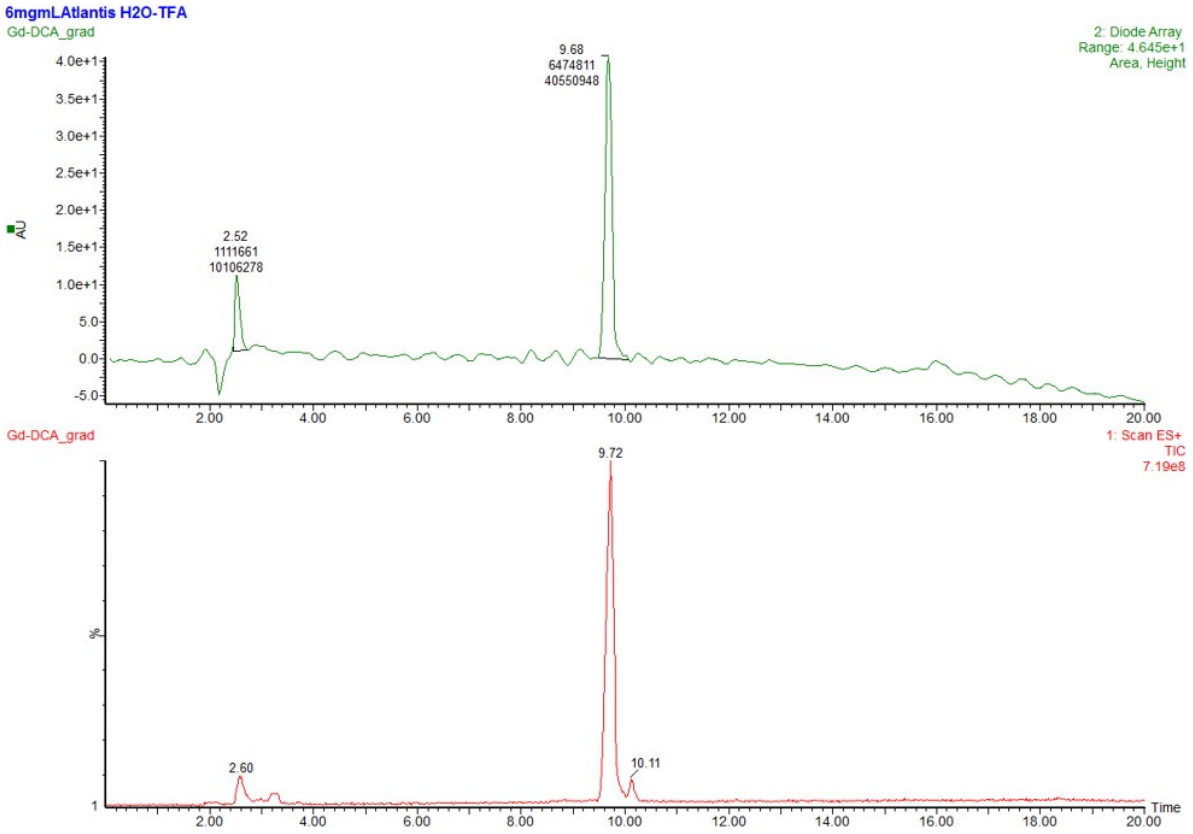


Figure S16: HRMS spectrum of compound **E**.

a)



b)

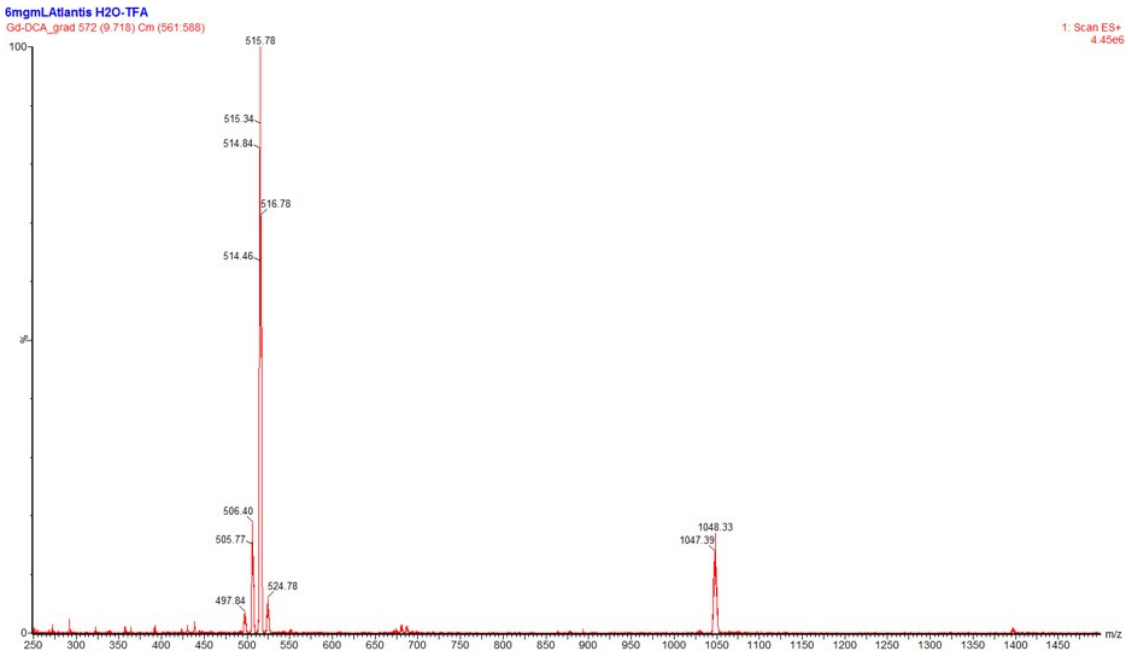
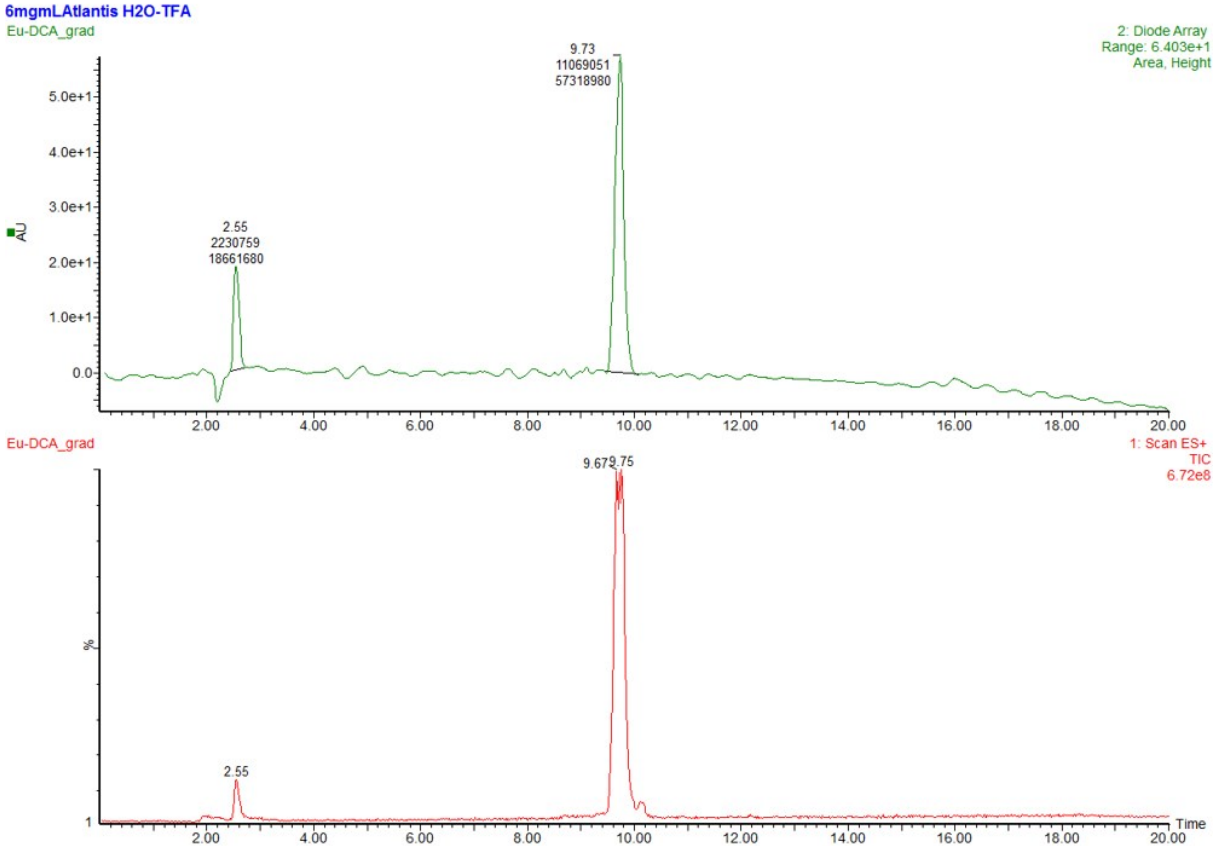


Figure S17: a) Chromatogram Diode Array (200-400 nm) and TIC ESI+and b) Mass spectrum in ESI+ of peak at 9.72 min of **Gd-HPDO3A-DCA**.

a)


6mgmLAtlantis H2O-TFA
Eu-DCA_grad
2: Diode Array
Range: 6.403e+1
Area, Height
9.73
11069051
57318980
2.55
2230759
18661680
AU
Eu-DCA_grad
1: Scan ES+
TIC
6.72e8
9.679.75
2.55
%
Time

b)


6mgmLAtlantis H2O-TFA
Eu-DCA_grad 574 (9.753) Cm (560:590)
1: Scan ES+
7.86e6
513.28
512.28
504.27
503.27
522.34
1043.45
1042.39
%
m/z

Figure S18: a) Chromatogram Diode Array (200-400 nm) and TIC ESI+ and b) Mass spectrum in ESI+ of peak at 9.68 min of **Eu-HPDO3A-DCA**.

Proton relaxation enhancement fitting equations

HSA binding parameters were determined using the proton relaxation enhancement method. The water proton relaxation rate (R_1^{obs}) of Gd-HPDO3A-DCA (0.1 mM) was measured as a function of increasing HSA concentration (0-1mM) in 50 mM PBS at 298 K and 310 K, 21.5 MHz, pH 7.4. R_1^{obs} was defined as the following:

$$R_{1obs} = r_{1A}[Gd-L] + r_{1B}[Gd-L-HSA] + R_{1d} \quad (1)$$

The diamagnetic contribution (R_{1d}) was determined as a function of increasing HSA concentration and subtracted from the observed relaxation rate to give the paramagnetic relaxation rate (R_{1P}). The data were then fitted to the following equilibrium, with the association constant defined as follows:

$$Gd-L + HSA \leftrightarrow Gd-L-HSA \quad (2)$$

$$K_a = [Gd-L-HSA]/[Gd-L][nHSA] \quad (3)$$

where *n* is the number of independent binding sites and K_a the apparent binding constant. A second titration was carried out to estimate *n*, where the concentration of HSA was constant (0.2 mM) and the concentration of the Gd complex was increased from 0 – 1 mM.

For competitive binding experiments, a solution of equimolar Gd complex and HSA in 50 mM PBS was prepared. Increasing concentrations of the drug were added up to 40-50 times excess, maintaining a constant Gd and HSA concentration. For ibuprofen and warfarin, 0.2 mM Gd and HSA were used, with a maximum drug concentration of 10 mM. Due to the low aqueous solubility of mitoxantrone, a maximum drug concentration of 4 mM was used with 0.1 mM Gd and HSA. Proton relaxation measurements on the solutions were performed at 21.5 MHz and 310 K.