SUPPORTING INFORMATION FOR :

A water soluble probe with near infra-red two-photon absorption and polarity-induced fluorescence for cerebral vascular imaging

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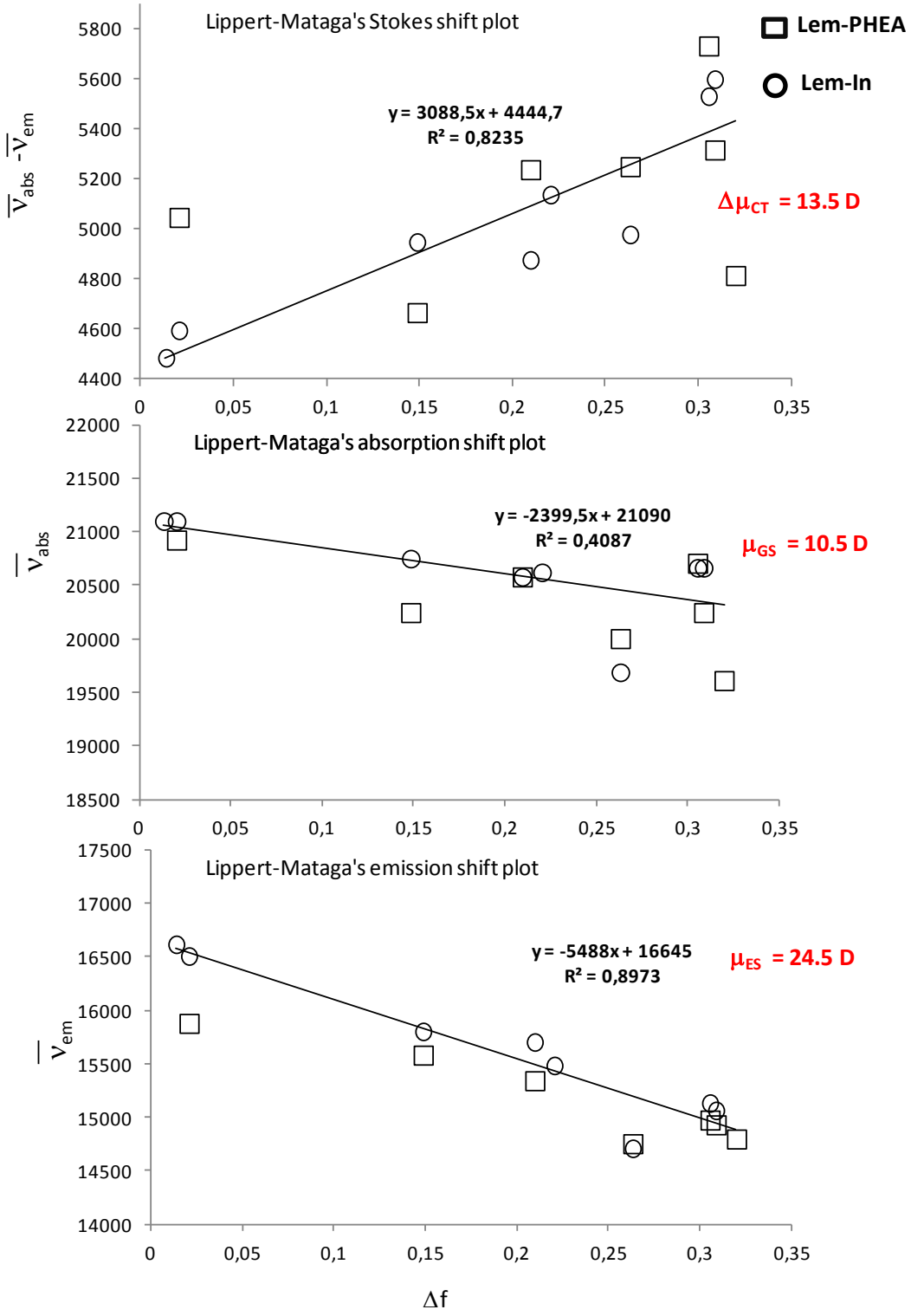
Figure S1. Plot of emission Stokes shift (top) absorption shift (center), and emission shift(bottom)t to Lippert-Mataga polarity scale



Linearization is performed on Lem-In data only. An Onsager's radius of 8,5 A is considered for the molecule, on the basis of comparison with literature values for a closely related compound (compound 1f in reference 1, calculated from crystal structure packing parameters) [1]

Figure S2. Plot of the fluorescence lifetime vs emission energy, and fit of the evolution to an exponential model

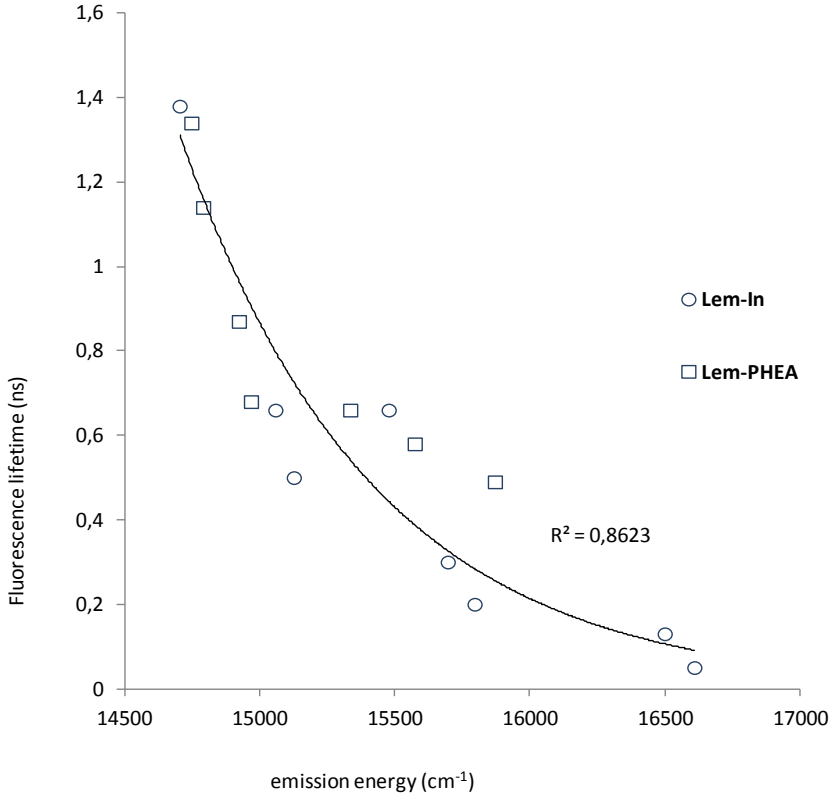
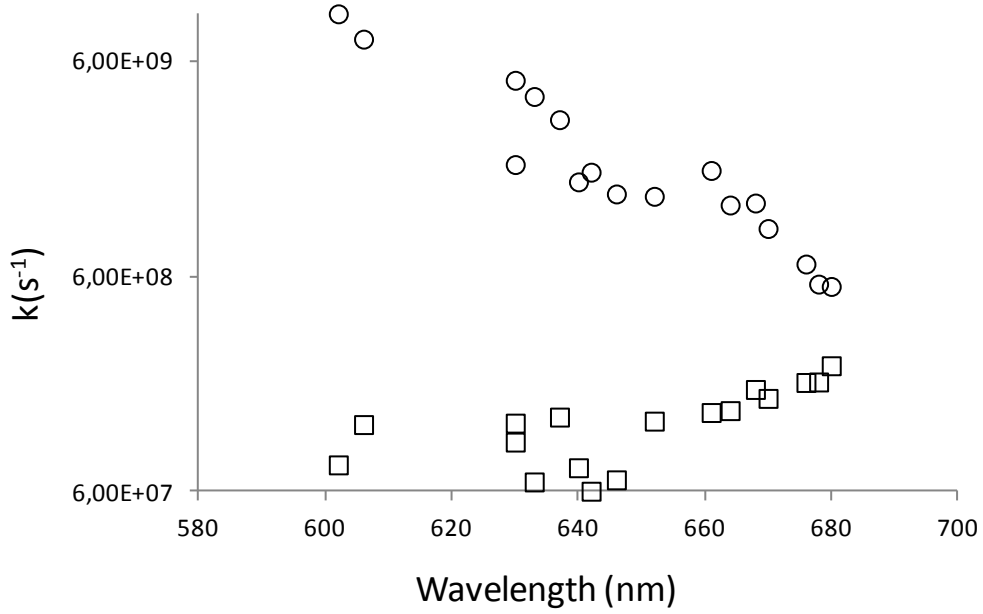


Figure S3 Excited state decay's kinetic constants *vs* emission wavelength



Plot of the evolution of the radiative (k_r : □) and non radiative (k_{nr} : ○) kinetic constants for the excited state decay *vs* emission wavelength. The *y* axis is in a logarithmic scale, where $k_r = \phi_f/\tau_f$, and $k_{nr} = 1/t_f - k_r = (1- \phi_f)/\tau_f$

Scheme S1 bond numbering for the values in Table S-1

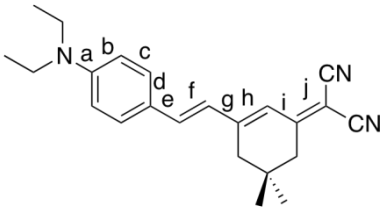


Table S1

Distance	S_0 (Theo)	S_1 (Theo)	S_0 (Exp)
a	1.372	1.358	1.379
b	1.417	1.426	1.395
c	1.380	1.370	1.380
d	1.405	1.427	1.404
e	1.452	1.417	1.444
f	1.353	1.397	1.334
g	1.446	1.404	1.448
h	1.365	1.409	1.364
i	1.430	1.397	1.418
j	1.377	1.416	1.364
MAD	0.009		
BLA	0.061	0.028	0.057

Comparison between theoretical (PCM-CAM-B3LYP) and experimental (XRD) structures for the ground-state (and excited-state for theory) distance along the conjugated path. MAD is the mean absolute deviation, whereas BLA is the computed bond length alternation. All values are in Å.

Figures S4-S5

In order to assess the possibility of rotation at the excited-state, we have performed rigid-rotor scans of the dialkylamino and dicyano terminal groups (see dihedral in in S3) using the CAM-B3LYP functional within a LR-PCM solvent model approach. As can be seen in S4, a maximal energy value for deformation close to 90° have been systematically predicted for the ϕ_1, irrespective of the solvent or state (GS *versus* ES). On the contrary for ϕ_2, we found an extra (less stable minima) for the perpendicular structure only in toluene. For the records, we underline that 1) SS-PCM calculations failed to converge in several cases (hence explaining the use of the LR-PCM model); 2) calculations performed with a range-separated hybrids presenting a phyisically correct asymprotic behavior, namely ωB97X-D, lead to similar trends for ϕ_2.

Figure S4 Tested dihedral angle for excited-state twists.

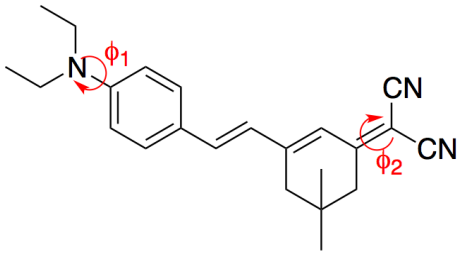


Figure S5 Evolution of the relative energies of the GS and ES in toluene and DMSO, for twist around ϕ_1 and ϕ_2.

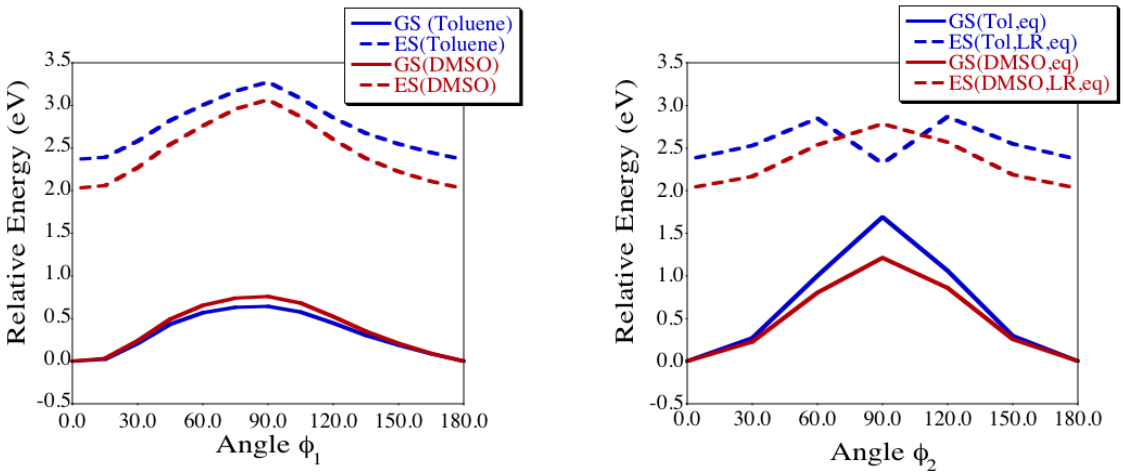
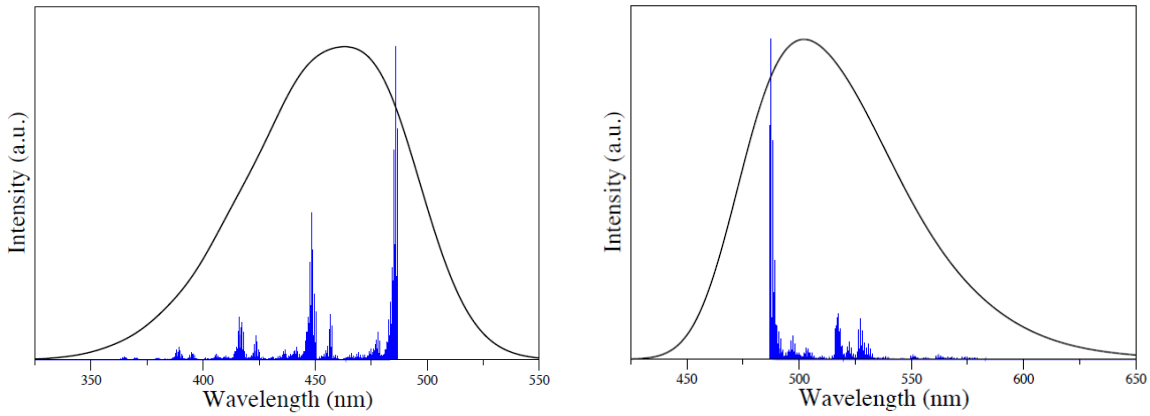
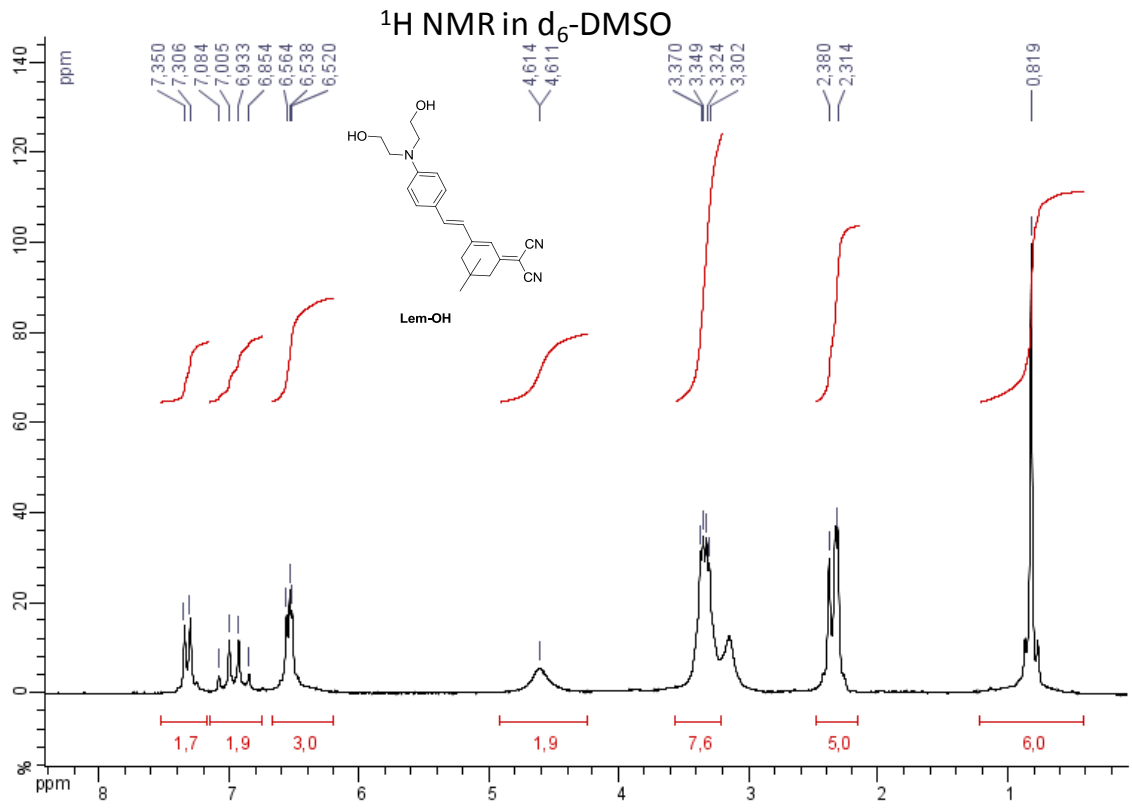
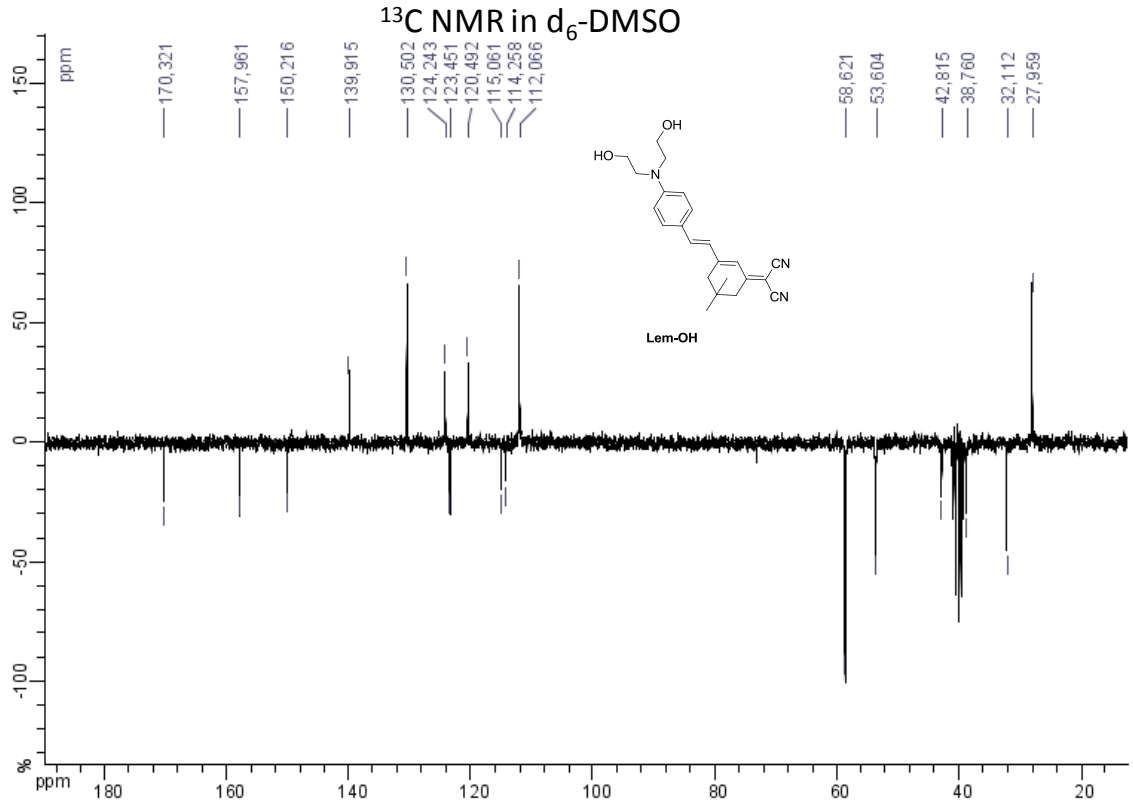


Figure S6 stick and convoluted vibrationally resolved calculated absorption (left) and emission (right) spectra for Lem in DMSO

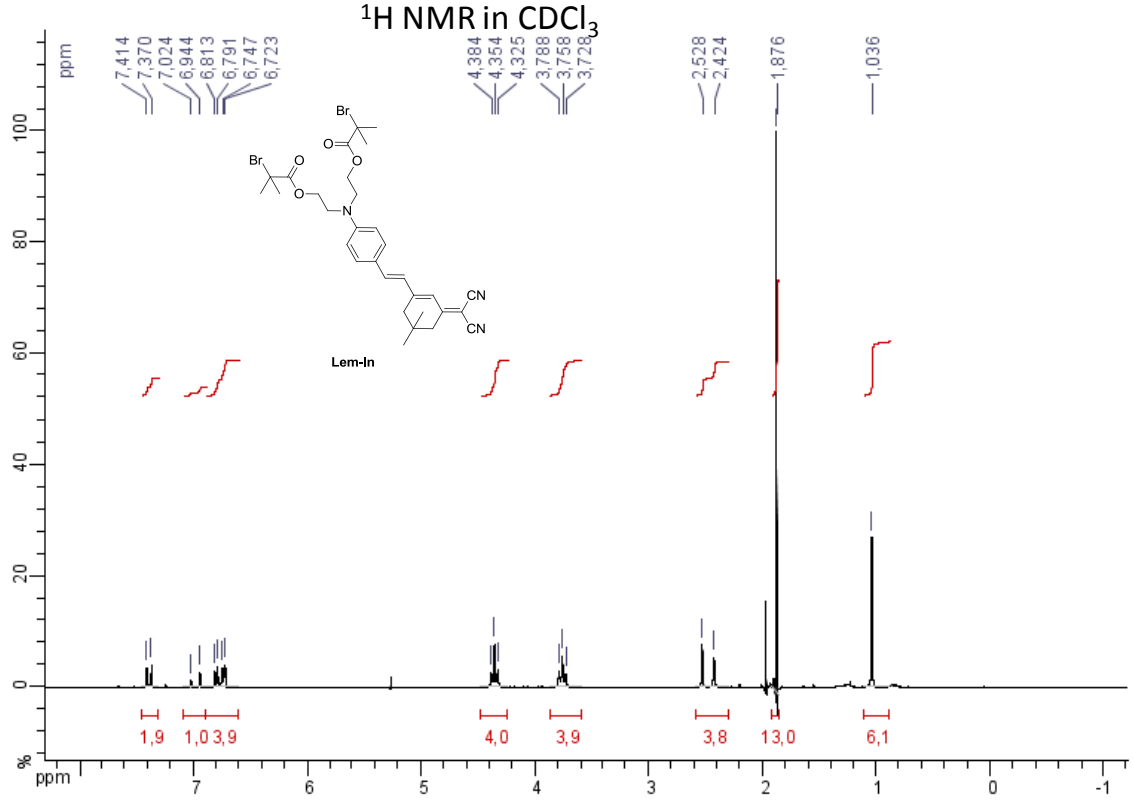


Figures S7. ^{1}H and ^{13}C NMR spectra of all new compounds in this paper

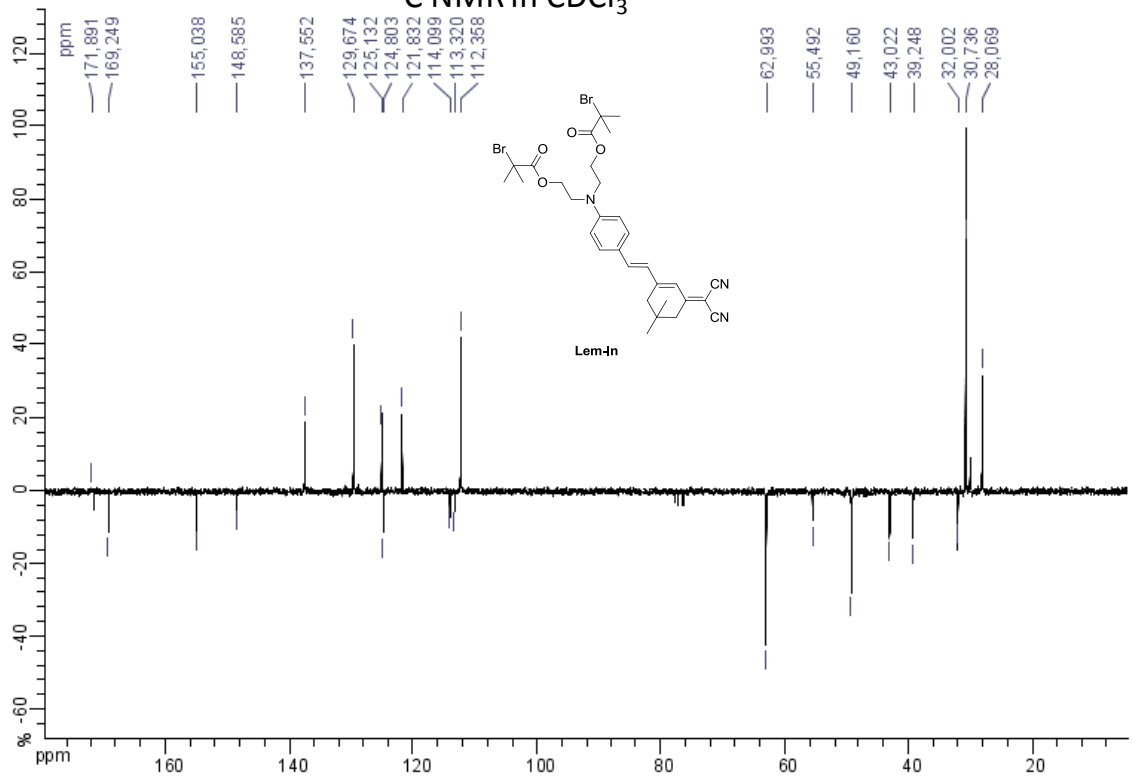




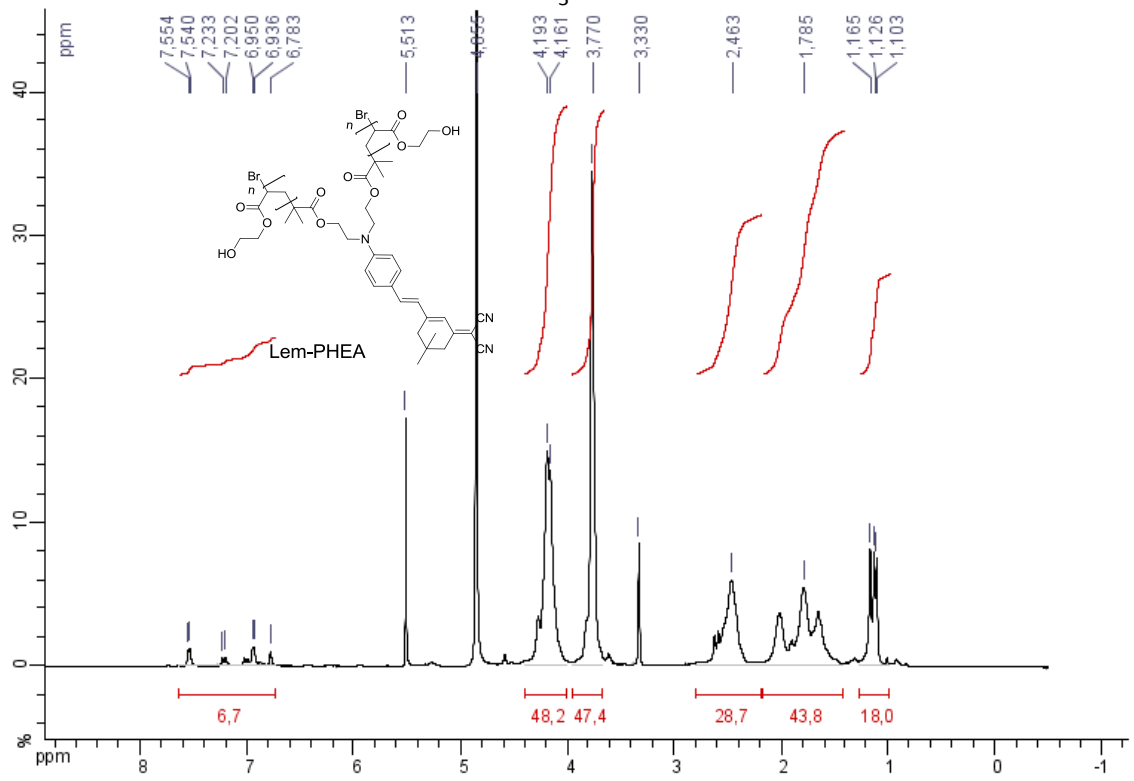
^{1}H NMR in $CDCl_3$



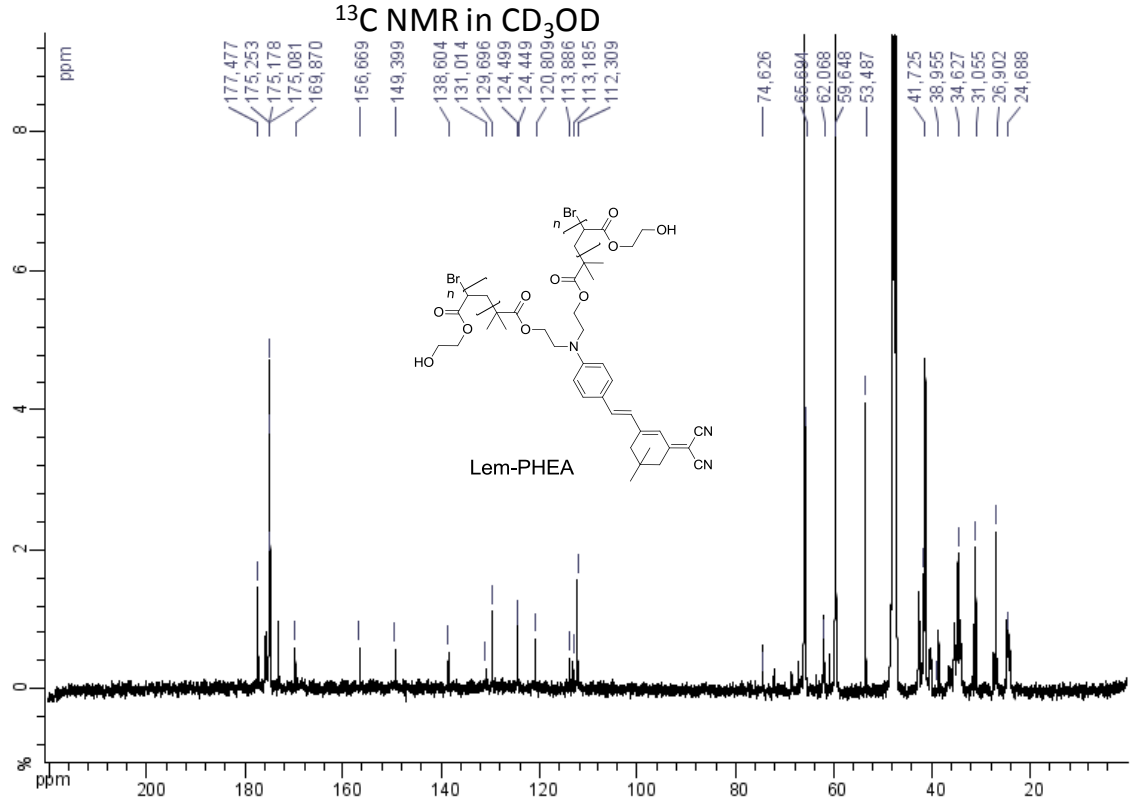
^{13}C NMR in $CDCl_3$



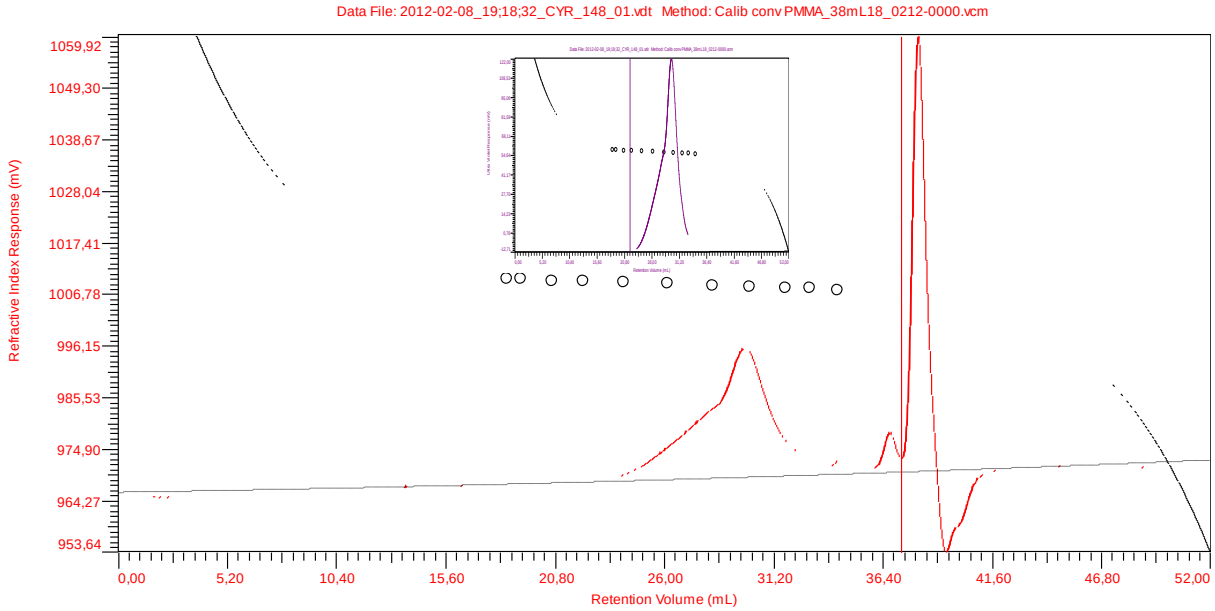
^{1}H NMR in CD_3OD



^{13}C NMR in CD_3OD



Figures S8. GPC traces of Lem-PHEA (detection : RI(main), UV (inset))



Mn = 4737
Mw/Mn (PDI) = 2.09

Figure S9: *In vivo* measurements of the red and near infrared emission of Lem-PHEA in the cerebral cortex vasculature of a CD1 mouse.

S8a) The cutoff wavelength of the dichroic mirror in front of the 20x objective was 760 nm and the cutoff wavelength of the dichroic mirror in between the near infrared and red channel was 650 nm. In near infrared PMT (without emission filter), all fluorescence emissions larger than 650 and smaller than 760 nm were measured, whereas in the red PMT (with emission filter) photon wavelengths between 576 and 660 nm were detected. The emission spectrum of Lem-PHEA is superposed. **S8b-d)** The fluorescence intensity profile of a vein showed approximately a 2.5x higher intensity profile in the near IR than in the red channel.

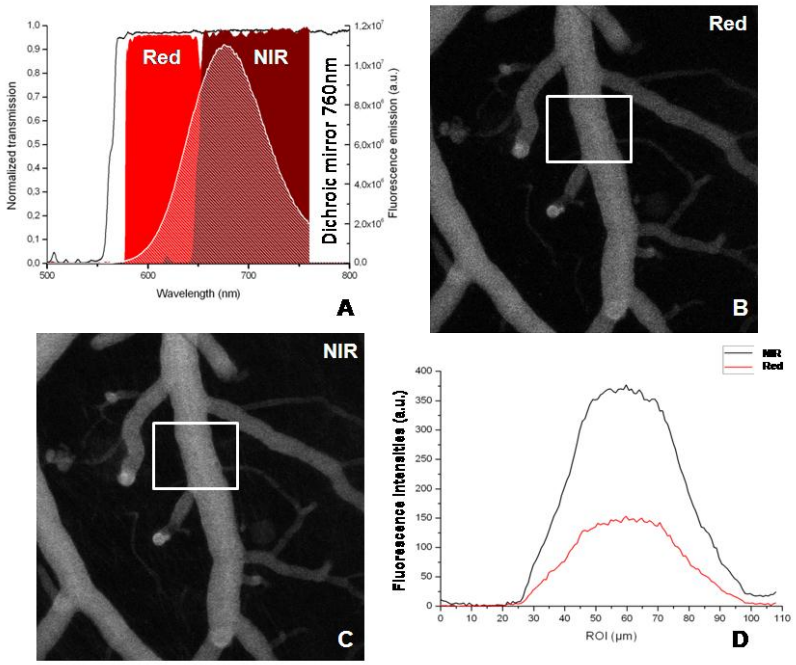
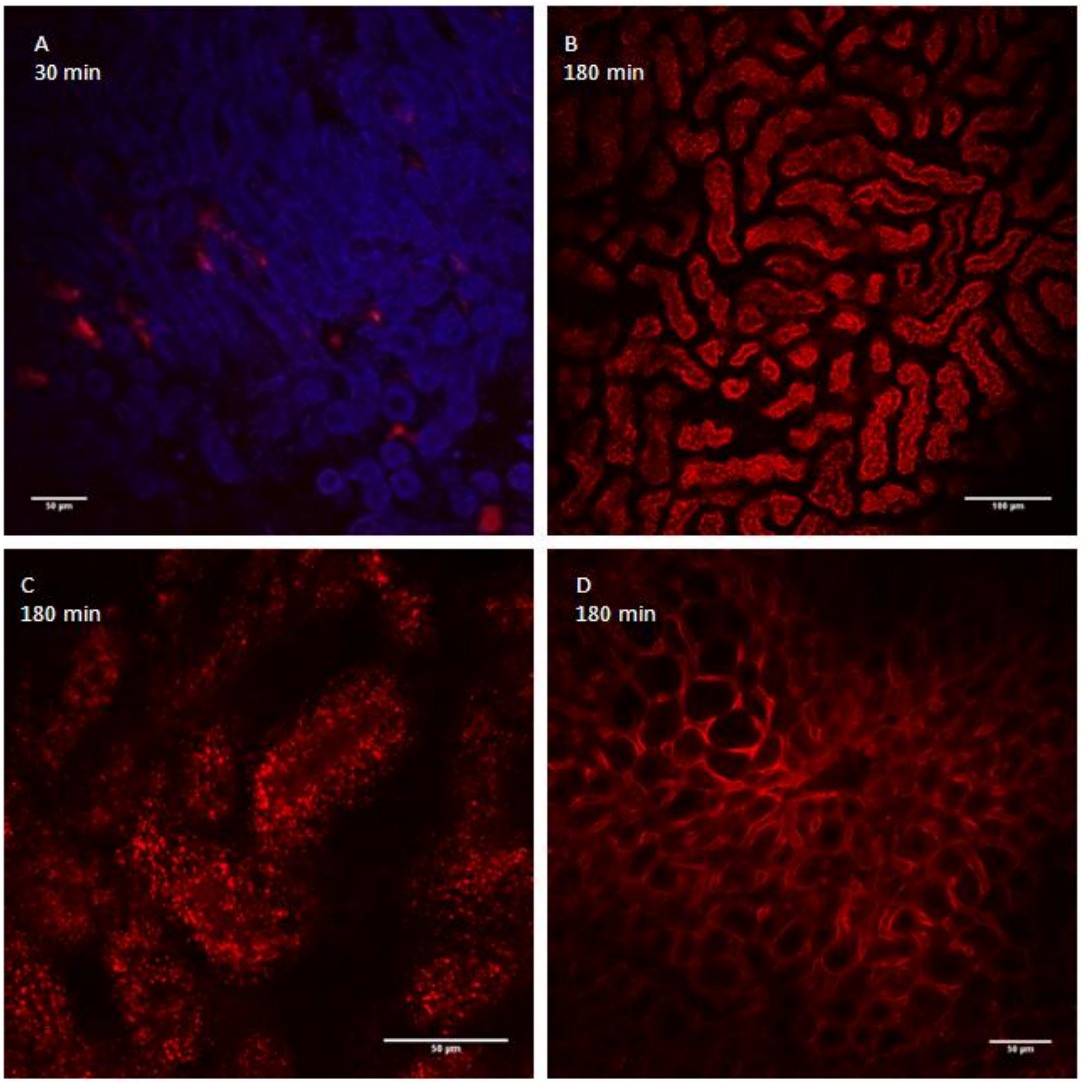


Figure S10: Local biodistribution of Lem-PHEA in the kidneys and liver.

S9a) Two-photon image of a kidney slice below the cortex showing the blue endofluorescence of the tubules (excitation wavelength 740 nm) and the red fluorescence of Lem-PHEA that accumulated in the renal tubules as early as 30 min after i.v. injection. Scale bar = 50 µm. **S9b)** Two-photon image of the renal cortex in situ (whole kidney) at 2 hours after dye injection. The dye was only present in the tubules and absent in the blood vessels. The excitation wavelength was 950 nm. Scale bar = 100 µm. **S9c)** Detail of the tubules in the renal cortex at 2h after dye injection at an excitation wavelength of 950 nm. Scale bar = 50 µm. **S9d)** Two-photon image of a liver slice at 2h after dye injection. Lem-PHEA stayed in the vascular compartment and no dye accumulation was observed in hepatic cells.



1. **J. Massin, W. Dayoub, J.-C. Mulatier, C. Aronica, Y. Bretonnière and C. Andraud, *Chem. Mater.*, 2010, 23, 862-873.**