Supplementary material

Synthesis of long-wavelength-absorbing photosensitizer/ nanoparticle conjugates and their in vitro PDT evaluation on colorectal cancer cell lines

Luce Janice Ndzimbou[a], Rayan Chkair[a], Gautier M.A. Ndong Ntoutoume[a], Mona Diab-Assaf[b], Guillaume Chemin[a], Bertrand Liagre[a], Frédérique Brégier[a] and Vincent Sol[a,*]

[a] Univ. Limoges, LABCiS, UR 22722, F-87000 Limoges, France.

[b] Doctoral School of Sciences and Technology, Lebanese University, Hadath El Jebbeh, Beyrouth 21219, Lebanon.

1. **Materials**

All chemicals and reagents were obtained from Sigma-Aldrich, Alfa Aesar, VWR, TCI, Acros Organics or Thermo Scientific and used without further purification. *Spirulina maxima* dry powder was purchased from Eurl Claudine Vallée-ZA du Bon René Chanzeaux (France). HCT116 and HT-29 colorectal cancer cells line were purchased from the American Type Culture Collection (ATCC-LGC Standards, Mosheim, France). Microcrystalline cellulose was purchased from Sigma-Aldrich and used for the cellulose nanocrystals synthesis.

Thin-layer chromatography (TLC) was done on silica gel 60 F_{254} aluminium sheets (Merck KGaA, Darmstadt Germany) for reaction monitoring. Preparative TLC plates used for the purification were prepared with Silica gel 60 ($PF_{254,}$ Merck KGaA, Darmstadt, Germany). Column chromatography was performed over silica gel 60 (0.015-0.040 mm, Merck KGaA, Darmstadt, Germany).

UV-Visible spectra were obtained with an Analytik Jena Specord 210 spectrophotometer equipped with quartz cuvettes (1 cm path-length, Hellma Analytics).

^{1}H NMR and ^{13}C spectra were recorded on a Brucker DPX 500 spectrometer, operating at 500 and 125.75 MHz, for ^{1}H NMR and ^{13}C, respectively. Chemical shifts (δ in ppm) were reported relative to tetramethylsilane as the reference as δ values and the coupling constant *J* in Hz.

Mass Spectrometry (MS). Ultra-high resolution mass analyses using electrospray ionization technique were performed with an LTQ-Orbitrap-XL (Thermo Fischer) operated in positive ion mode.

The FTIR-Attenuated Total Reflection (ATR) spectroscopy was recorded from 600 to 4000 cm^{-1} using Perkin Elmer FT-IR/NIR spectrometer Frontier.

Nanoparticle size and zeta potential (ζ) measurements: average hydrodynamic diameter and polydispersity index (PDI) were measured by dynamic light scattering (DLS) using Zetasizer Nano-ZS (Malvern Instruments, UK). Each solution was analyzed at 25 °C at a scattering angle

of 173 °. Zeta potential was performed through electrophoretic light scattering at 25 °C, 149 V. Distilled water was used as a dispersant and samples were measured in triplicate.

PDT was carried out *in vitro* using a 650 nm (30 J/cm^2) delivered from the light source PDT TP-1 (Cosmedico Medizintechnik GmbH, Schwenningen, Germarny).

2. Figures

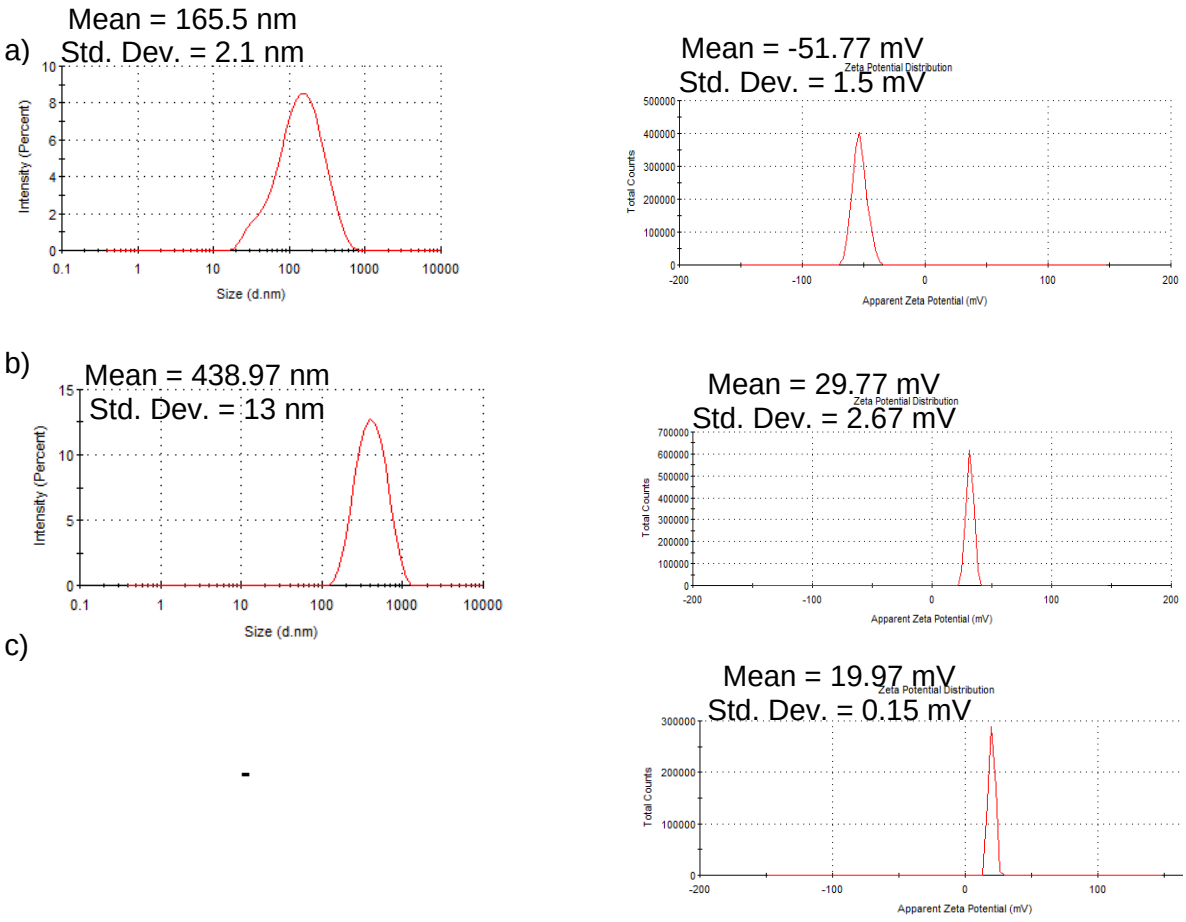


Fig. S1: Particle size distribution (DLS) and zeta potential of CNCs (a), CNCs/β-CD^{+} (b) and CNCs/β-CD^{+}/PIA (c).

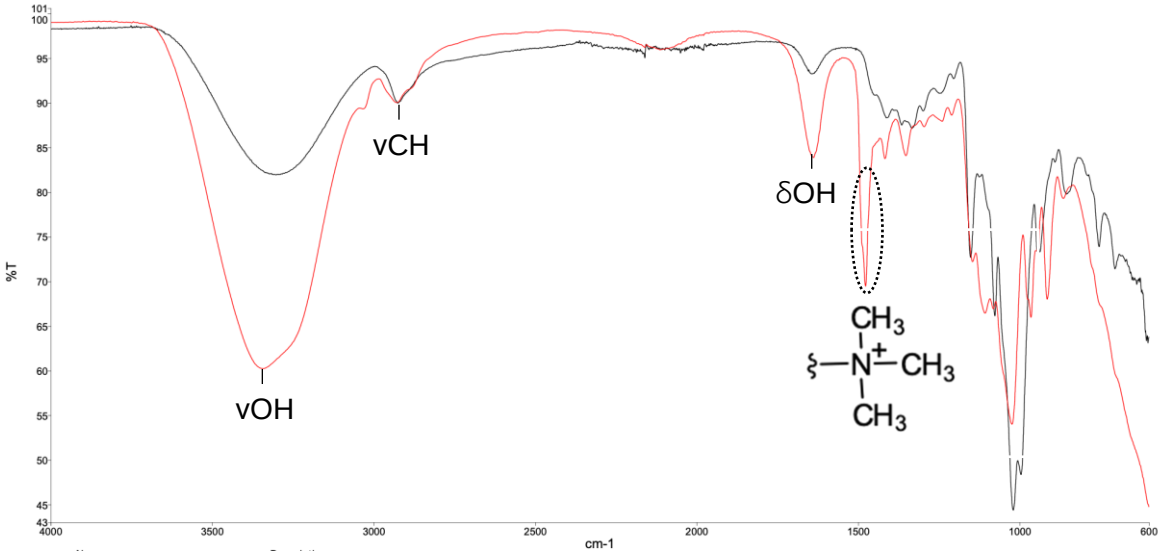


Fig. S2: FTIR-ATR spectra of β-cyclodextrin (black) and cationic β-cyclodextrin (red).

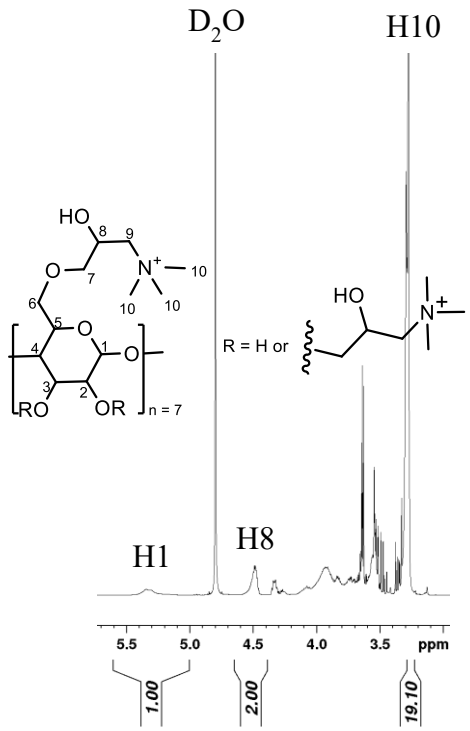


Fig. S3: ^{1}H NMR spectrum of cationic β-cyclodextrin in D_2O, degree of Substitution = 2.

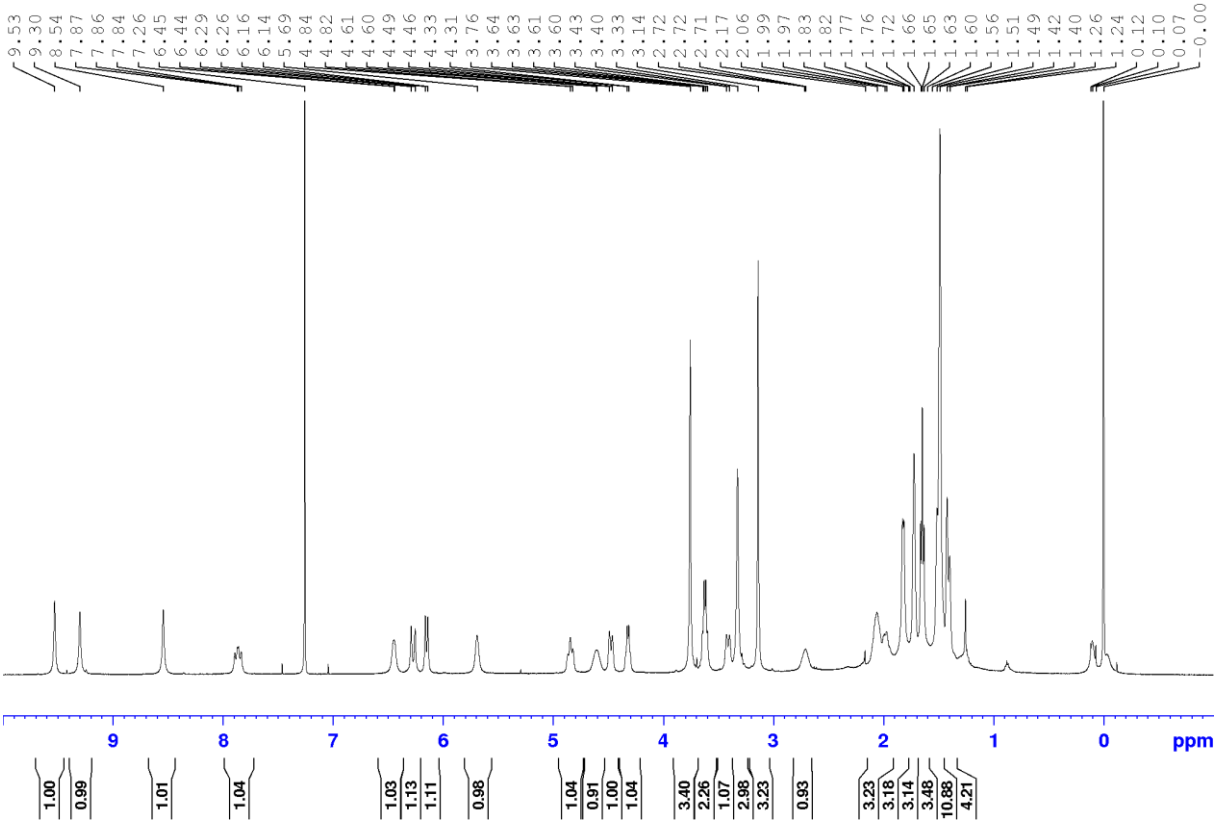


Fig. S4: ^{1}H NMR spectrum of PIA in $CDCl_3$.

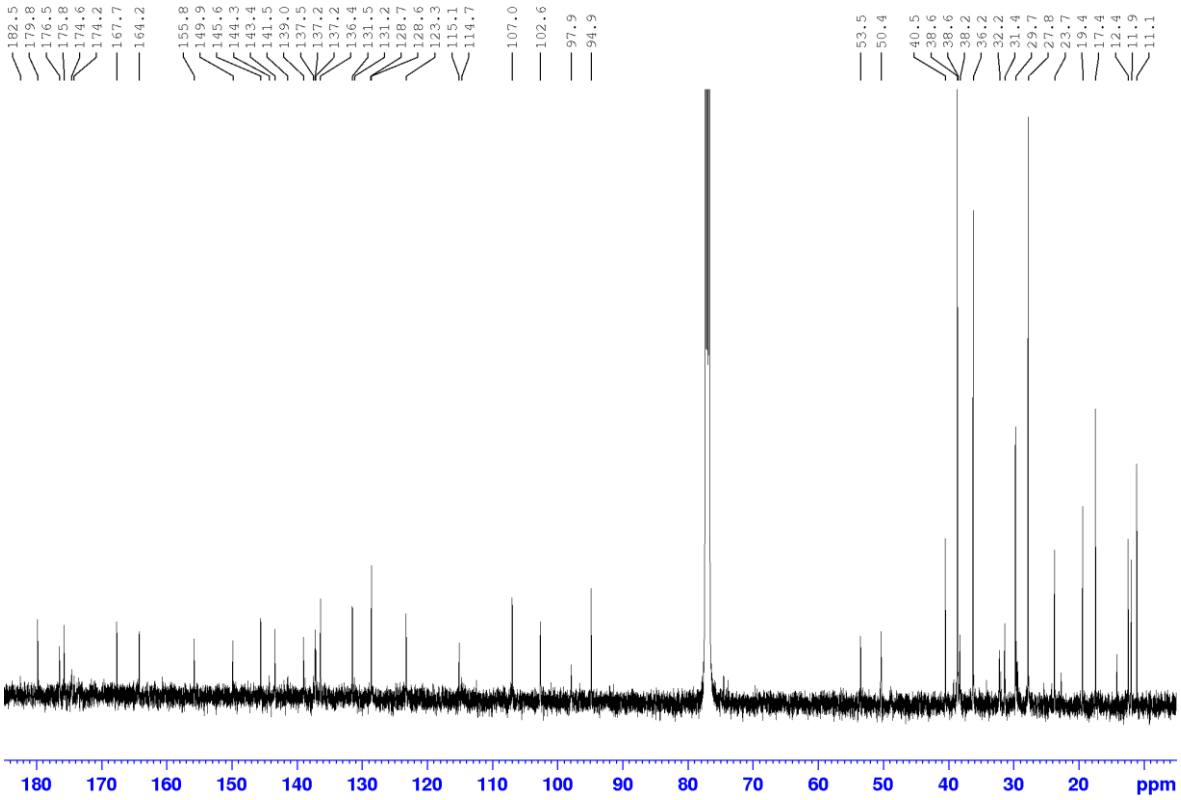


Fig. S5: ^{13}C NMR spectrum of PIA in $CDCl_3$.

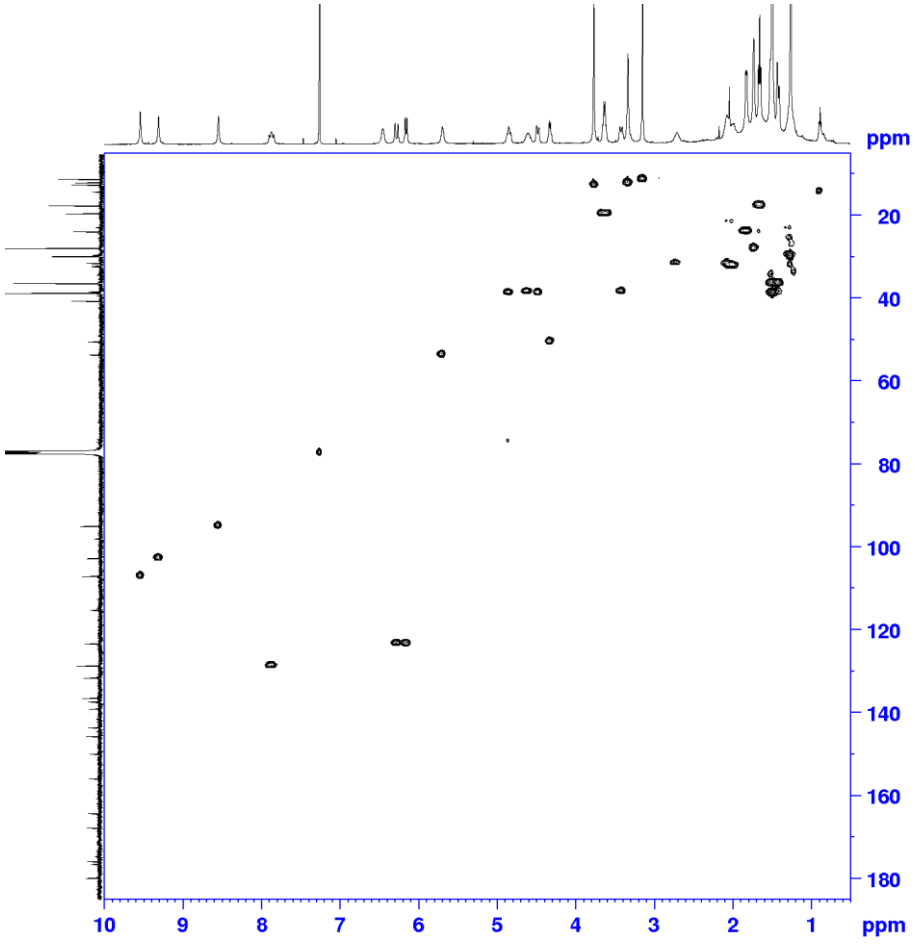
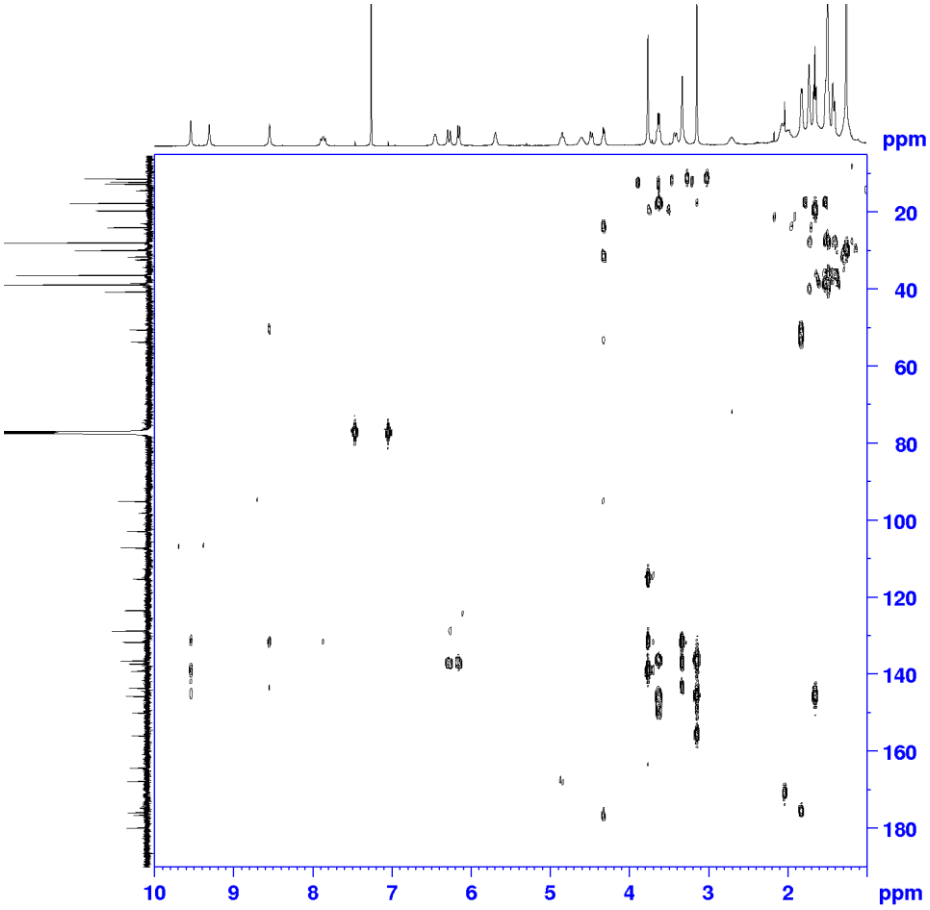


Fig. S6: ^{1}H-^{13}C HSQC spectrum of PIA recorded in $CDCl_3$.



88

89 **Fig. S7: ^{1}H-^{13}C HMBC spectrum of PIA recorded in $CDCl_3$.**

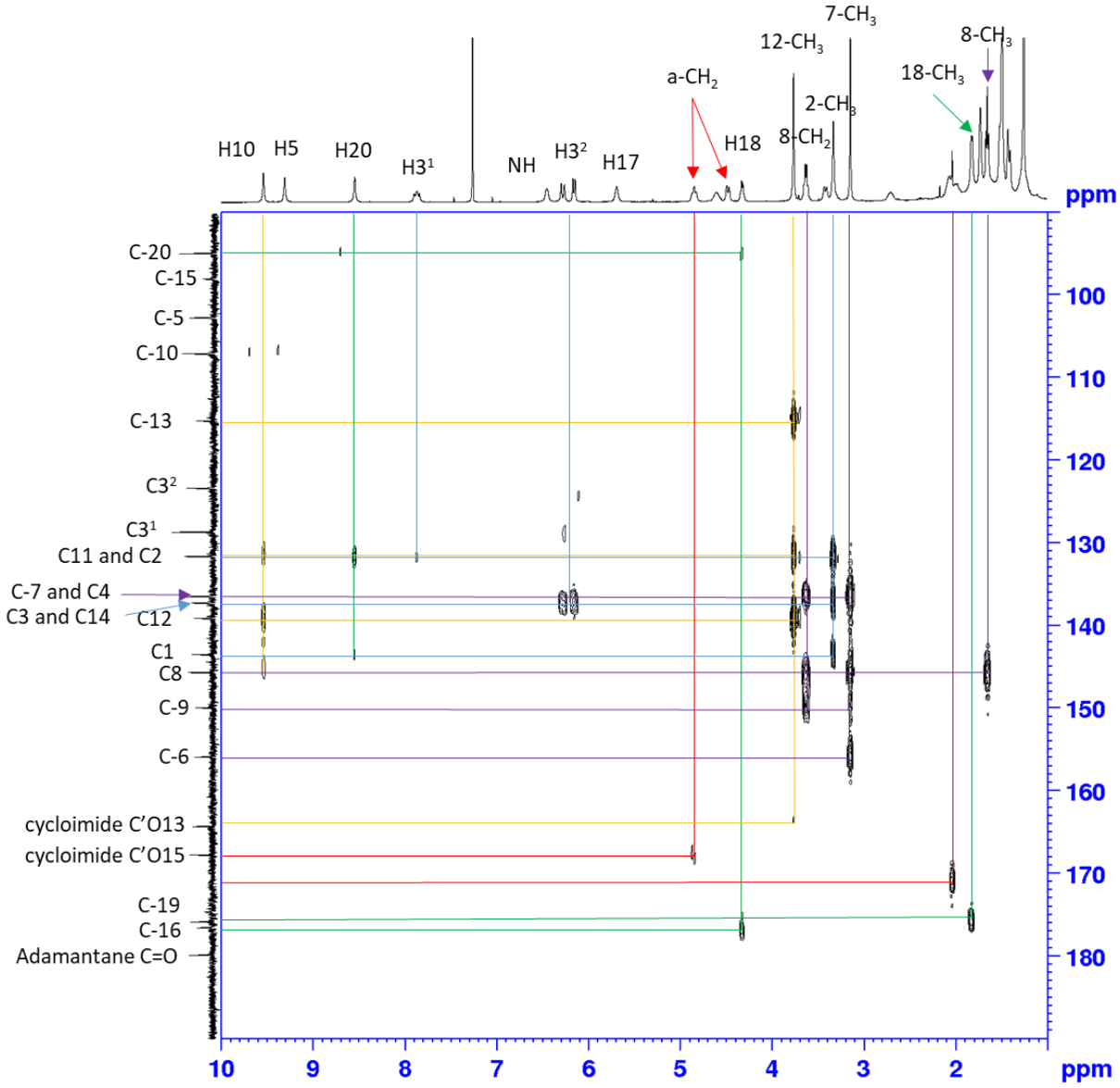
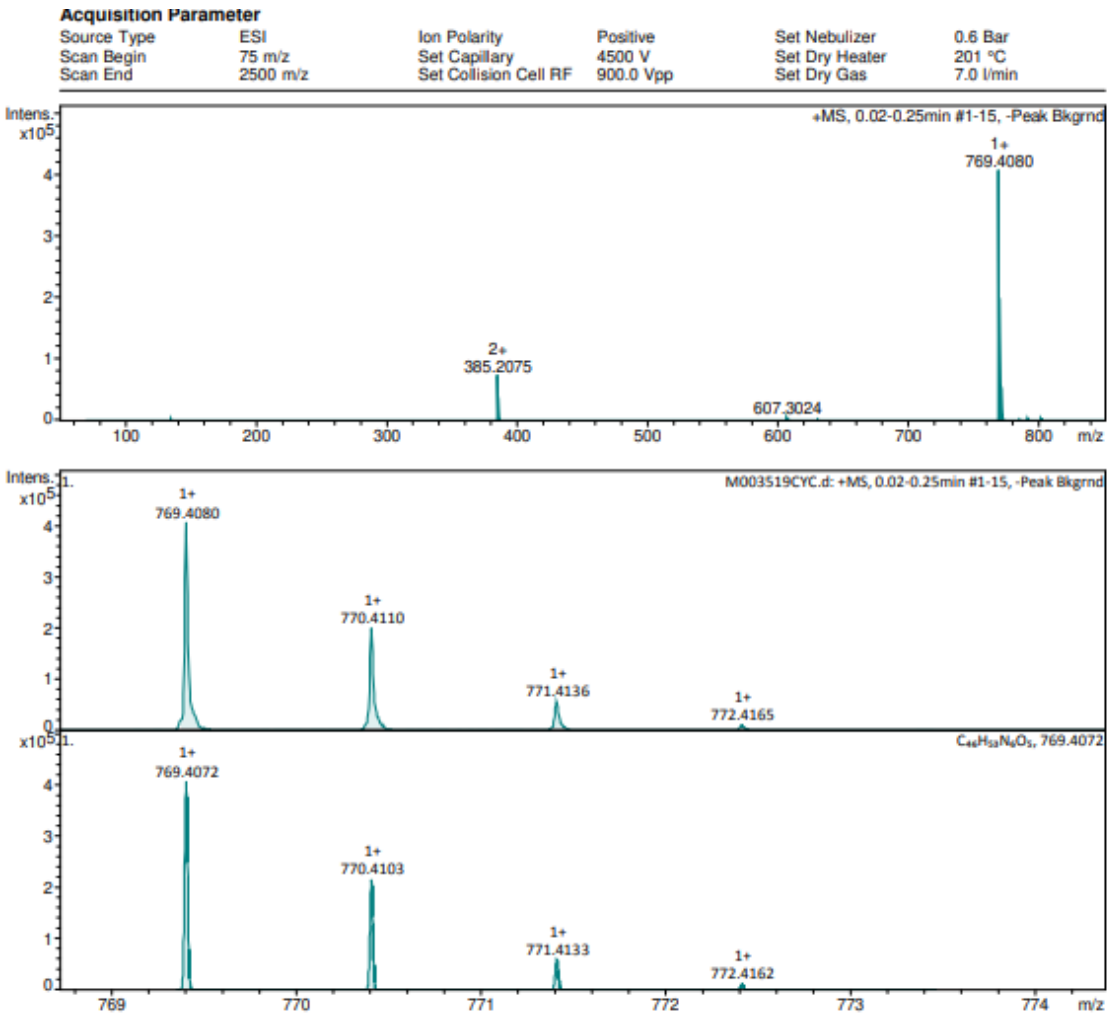
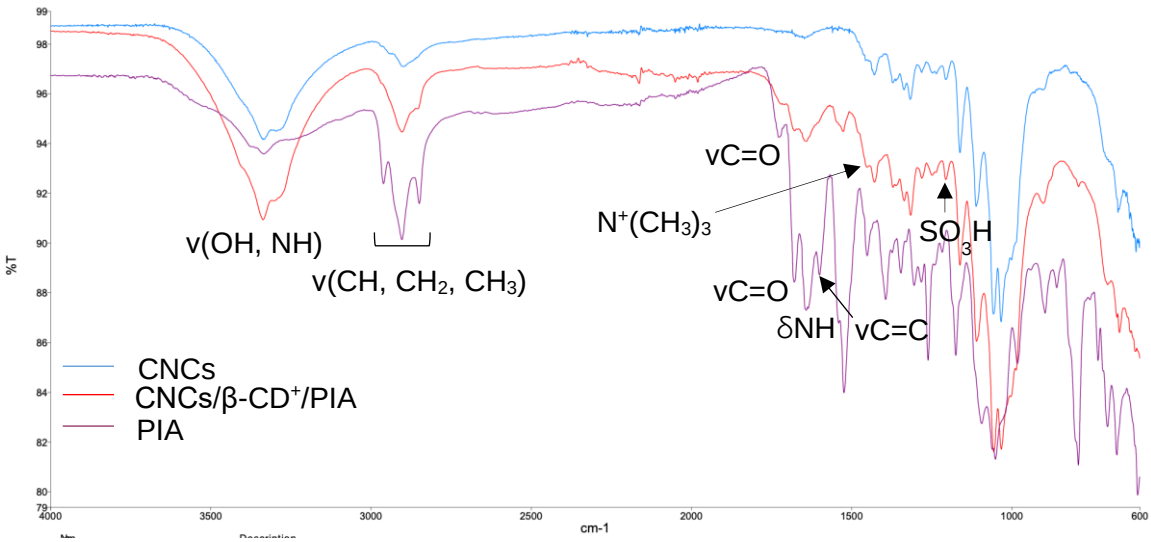


Fig. S8: Zoom of the ^{1}H-^{13}C HMBC spectrum in which the main correlations are drawn to allow interpretation of ^{13}C NMR.



92

93 **Fig. S9: HRMS (ESI) mass spectrum of PIA.**



94

95 **Fig. S10: FTIR/ATR overlay spectra of CNCs, CNCs/β-CD⁺/PIA complex and PIA.**

96

97