Supporting Information

Dendritic maleimide functionalization of core-shell (γ-Fe_2O_3/polymer) nanoparticles for efficient bio-immobilization

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1) General information: materials and characterization methods

Materials

Solvents and starting materials were obtained commercially from Acros, Alfa Aesar or Aldrich (reagent grade) and were used without further purification. The solvents were purchased anhydrous (DMF, DMSO, Dioxane, THF, $CHCl_3$, EtOAc, AcOH) or were dried according to standard procedures over dehydrating agents and distilled under an inert atmosphere (CH_2Cl_2: CaH_2; MeOH: CaH_2; Toluene: Na/benzophenone). 300 nm γ-Fe_2O_3/Polymer core-shell superparamagnetic nanoparticles (Carboxyl-Adembeads), COOH: 350 $\mu mol.g^{-1}$ of particles, were purchased from Ademtech SA ; 15 nm Streptavidin Gold Nanoparticles (SA-Au NPs) from BBI ; 20 nm Amino PEGylated Gold Nanoparticles (H_2N-Au NPs) from Polysciences Inc.; and Thiol-PEG-Biotin (HS-PEG-Biot), from Polypure.

General characterization methods for organic compounds

The ^1H and ^{13}C spectra were recorded on Bruker Avance 300 FT NMR spectrometer (^1H: 300.13 MHz, ^{13}C: 75.46 MHz) or on Bruker Avance II 400 FT NMR spectrometer (^1H: 400.13 MHz, ^{13}C: 100.62 MHz). Chemical shifts, δ, are represented in part per million (ppm) and coupling constants, *J*, in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singulet, d = doublet, t = triplet, q = quartet, and m = multiplet.

Mass spectra were performed on a QStar Elite Mass Spectrometer equipped with an electrospray ionization (ESI) source (operated in a positive or negative ion mode).

Elemental analyses were carried out in elemental analysis department at CNRS-Vernaison, France. For column chromatography, Merck silica gel 60 (230–400 mesh) was used.

ATR FTIR experiments

The ATR spectra were recorded on a Thermo-Scientific Nicolet iS50 FT-IR spectrometer equipped with a DLaTGS detector (KBr window) using the diamond iS50 ATR accessory. Each spectrum was obtained in the 4000-400 cm^{-1} spectral range, at a resolution of 4 cm^{-1}, by coadding 500 scans.

UV-Vis experiments

UV-Vis spectra were recorded between 200 and 800 nm using a PerkinElmer UV/Vis spectrophotometer Lambda 650.

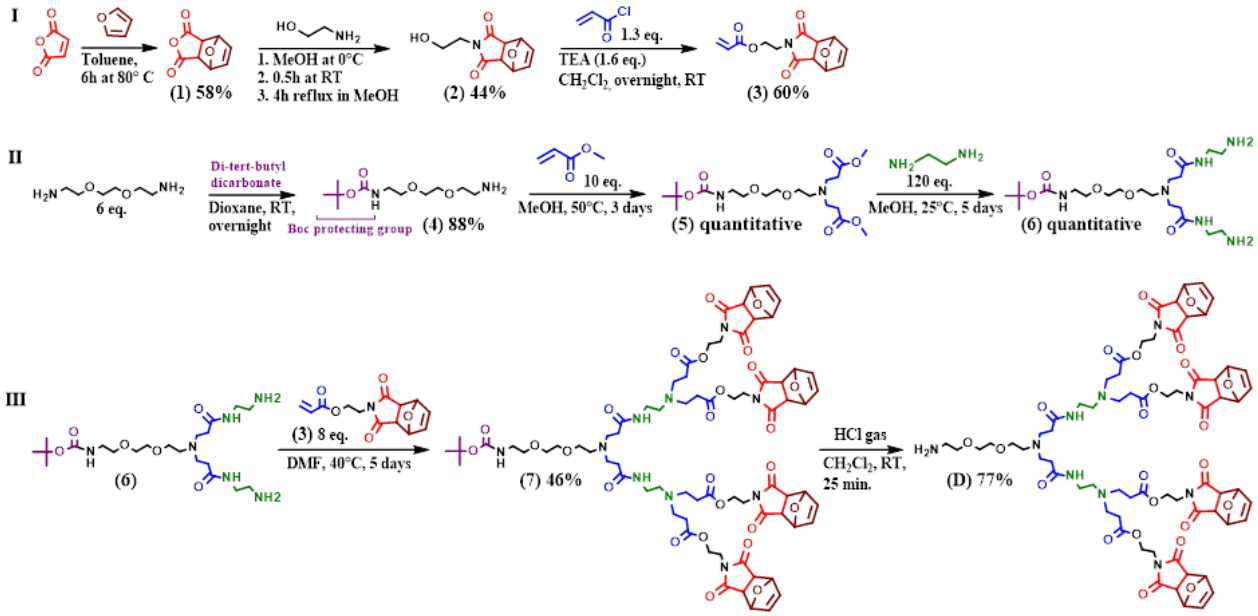
Transmission Electron Microscopy (TEM)

The microscopy was done at the Bordeaux Imaging Center - Bordeaux University a Core facility of the French network "France Bio Imaging". The help of Sabrina LACOMME and Etienne GONTIER is acknowledged. TEM images were obtained on Electronic Microscope HITACHI H7650, (120 Kv, high resolution, equipped with a GATAN 11MPx camera). The samples were prepared by deposition of MNPs dispersed in ethanol or water (5-10 µL, 0.25-0.5 mg/mL) on carbon coated copper grids and dried in the air.

Zeta Potential

Zeta Potential measurements were carried out on Horiba Scientific nanoparticle analyzer SZ-100. The measurements were performed at 25°C for diluted aqueous suspension of MNPs at pH varying from 3.5 to 9.0.

2. Synthesis of precursors and dendron D



Scheme S1. Synthesis of dendron D.

Synthesis of compound 1

Maleic anhydride (30.0 g, 0.31 mol) was suspended in 150 mL of toluene, the suspension was warmed up to 80°C and 33 mL of furan (0.46 mol, 1.3 eq.) were added. The turbid solution was stirred for 6 h and then cooled to room temperature without stirring. White crystals, which precipitated out of solution after 1 h, were collected by vacuum filtration, washed with petroleum ether (2×30 mL) and dried under vacuum to yield compound **1** (58 %) as small white needles.

^{1}H NMR (400 MHz, DMSO) δ 6.58 (s, 2H, C*H*=C*H*), 5.35 (s, 2H, C*H*O), 3.31 (s, 2H, C*H*).

^{13}C NMR (101 MHz, DMSO) δ 171.53 (s, 2C, *C*=O), 136.85 (s, 2C, *C*H=*C*H), 81.64 (s, 2C, *C*HO), 49.08 (s, 2C, *C*H).

FT IR (cm^{-1}) = 1858, 1780 ($\nu_{C=O}$ five membered ring anhydrides); 921, 878, 849, 822 (maleic anhydride ring deformation).

Synthesis of compound 2

Compound **1** (10.00 g, 60.19 mmol) was dispersed in 20 mL of MeOH and the suspension cooled to 0 °C. Then, a solution of ethanolamine (3.6 mL, 60.19 mmol, 1 eq.) in 20 mL of MeOH was added dropwise during 10 min. The resulting clear yellow solution was stirred first, for 5 min. at 0 °C, then 30 min. at room temperature, and finally refluxed for 4 h. At the end of reaction the flask was cooled to room temperature, and after 2 h the product began to crystallize. The mixture

was stored in the freezer overnight, and the precipitated crystals were collected by vacuum filtration (yield: 44 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 2H, C*H*=C*H*), 5.26 (s, 2H, C*H*), 3.70 (dt, *J* = 10.0, 3.5 Hz, 4H, -CH_2-N-, HO-CH_2-), 2.88 (s, 2H, C*H*), 2.40 (s, 1H, -O*H*).

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.89 (s, 2C, N-(*C*=O)-), 136.62 (s, 2C, *C*H=*C*H), 81.08 (s, 2C, O*C*H), 60.30 (s, 1C, -*C*H_2-OH), 47.60 (s, 2C, *C*H), 41.84 (s, 1C, -*C*H_2-N).

MS (ESI, m/z) $[M+Na]^+$ calc. – 232.0580, found – 232.0587

FT IR (cm^{-1}) = 3473 (ν OH); 2932 (ν_{as} CH_2); 2866 (ν_s CH_2); 1768, 1683 ($\nu_{C=O}$ cyclic imides in five membered ring); 918,875, 851 (maleic anhydride ring deformation).

Synthesis of compound 3

A mixture formed of compound **2** (2.00 g, 9.56 mmol, 1 eq. in 40 mL of freshly distilled CH_2Cl_2) and anhydrous TEA (2.2 mL, 1.55 g, 15.3 mmol, 1.6 eq.) was cooled to 0 °C and 1 mL of acryloyl chloride (1.13 g, 12.43 mmol, 1.3 eq.) were added dropwise. The mixture was stirred at room temperature overnight under argon atmosphere. The solvent, excess of acryloyl chloride and TEA were evaporated under vacuum. The resulting residue was re-solubilized in 150 mL of CH_2Cl_2, washed with brine (3×100 mL), dried over $MgSO_4$, filtrated and concentrated in vacuum. Then, the obtained residue was purified by column chromatography on silica gel (AcOEt 100%) to yield compound **3** (60 %) as white crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 2H, C*H*=C*H*), 6.37 (d, *J* = 17.3 Hz, 1H, CH_2=), 6.06 (dd, *J* = 17.3, 10.5 Hz, 1H, =C*H*-), 5.83 (d, *J* = 11.9 Hz, 1H, CH_2=), 5.25 (s, 2H, C*H*), 4.30 (t, *J* = 5.4 Hz, 2H, -O-CH_2-), 3.80 (t, *J* = 5.4 Hz, 2H, -CH_2-N-), 2.86 (s, 2H, C*H*).

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.07 (s, 2C, -N-(*C*=O)-), 165.84 (s,1 C, -(*C*=O)-O-), 136.66 (s, 2C, *C*H=*C*H), 131.41 (s, 1C, *C*H_2=), 128.07 (s, 1C, =*C*H-), 81.02 (s, 2C, -*C*H-O), 60.81 (s, 2C, -O-*C*H_2-), 47.56 (s, 2C, -*C*H-), 37.89 (s, 2C, -*C*H_2-N-).

MS (ESI, m/z) $[M+Na]^+$ calc. – 286.0685, found – 286.0692

EA calc.: C, 59.31; H, 4.98; N, 5.32; O, 30.39; found: C, 59.64; H, 5.03; N, 5.27; O, 29.66

FT IR (cm^{-1}) = 2907 (ν_{as} CH_2); 1773, 1690 ($\nu_{C=O}$ cyclic imides in five membered ring); 1723 ($\nu_{C=O}$ ester); 914,873, 850, 824 (maleimide ring deformation).

Synthesis of compound 4

A solution of di-*tert*-butyl dicarbonate (11.25 g, 0.05 mol, 1 eq. in 125 mL of anhydrous dioxane) was added dropwise to a solution of 2,2-(ethylenedioxy)bis(ethylamine) (44 mL, 45.75 g, 0.31 mol, 6 eq. in 125 mL of anhydrous dioxane). The mixture was stirred at room temperature overnight under argon atmosphere. Afterwards, the solvent was evaporated, the residue was solubilized in 400 mL of CH_2Cl_2 and washed with H_2O (3×200 mL; to remove the excess of 2,2-(ethylenedioxy)bis(ethylamine)). The organic phase was dried over $MgSO_4$, filtrated and

concentrated under vacuum. A column chromatography on basic alumina was then performed (AcOEt 100%) to yield compound **4** (88 %) as pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (s, 1H, N*H*), 3.59 (s, 4H, -O-CH_2-CH_2-O-), 3.55 - 3.47 (m, 4H, -O-CH_2-), 3.29 (dd, *J* = 10.4, 5.2 Hz, 2H, CH_2-NH-), 2.86 (t, *J* = 5.2 Hz, 2H, -CH_2-NH_2), 1.57 (s, 2H, -NH_2), 1.42 (s, 9H, -CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.08 (s, 1C, O-(*C*=O)-NH-), 79.18 (s, 1C, C_q), 73.28 (s, 2C, -O-*C*H_2-), 70.26 (s, 2C, -O-*C*H_2-*C*H_2-O-), 41.70 (s, 1C, -*C*H_2-NH_2), 40.38 (s, 1C, -*C*H_2-NH-), 28.46 (s, 3C, *C*H_3).

MS (ESI, m/z) $[M+H]^+$ calc. – 249.1818, found – 249.1808

EA calc.: C, 53.20; H, 9.74; N, 11.28; O, 25.77; found: C, 52.73; H, 9.61; N, 10.84; O, 26.35

FT IR (cm^{-1}) = 3361 (ν_{as} NH_2); 2974 (ν_{as} CH_3); 2926 (ν_{as} CH_2); 2866 (ν_s CH_2); 1704 ($\nu_{C=O}$ O(C=O)NH); 1518 (ν_{CN}, δ_{NH} C-N-H) amide II.

Synthesis of compound 5

To a solution of compound **4** (6.00 g, 24.16 mmol in 100 mL freshly distilled MeOH), 22 mL of methyl acrylate (20.8 g, 241.6 mmol, 10 eq.) were added. The mixture was stirred for 3 days at 50 °C. At the end of reaction the excess of methyl acrylate and MeOH was evaporated under vacuum to yield compound **5** (quantitatively) as yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.07 (s, 1H, N*H*), 3.64 (s, 6H, -O-CH_3), 3.56 (s, 4H, -O-CH_2-CH_2-O-), 3.51 (t, *J* = 5.4 Hz, 4H, -O-CH_2-), 3.28 (dd, *J* = 10.4, 5.2 Hz, 2H, -CH_2-NH-), 2.81 (t, *J* = 7.1 Hz, 4H, -N-CH_2-), 2.66 (t, *J* = 6.1 Hz, 2H, -CH_2-N-), 2.44 (t, *J* = 7.1 Hz, 4H, -CH_2-COO), 1.41 (s, 9H, -CH_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.03 (s, 2C, -(*C*=O)-O-), 156.09 (s, 1C, -(*C*=O)-NH-), 79.22 (s, 1C, C_q), 70.43 (d, *J* = 10.1 Hz, 2C, -O-*C*H_2-*C*H_2-O-), 69.79 (s, 2C, -*C*H_2-O-), 53.36 (s, 1C, -*C*H_2-N-), 51.63 (s, 2C, -O-*C*H_3), 50.04 (s, 2C, -N-*C*H_2), 40.48 (s, 1C, -*C*H_2-NH-), 32.68 (s, 2C, -*C*H_2-COO-), 28.52 (s, 2C, -*C*H_3).

MS (ESI, m/z) $[M+H]^+$ calc. – 421.2544, found – 421.2554; MS $[M+Na]^+$ calc. – 443.2431, found – 443.2441

EA calc.: C, 54.27; H, 8.63; N, 6.66; O, 30.44; found: C, 54.66; H, 8.54; N, 6.60; O, 30.64

FT IR (cm^{-1}) = 3374 (ν_{NH} O(C=O)NH); 2976 (ν_{as} CH_3); 2951 (ν_{as} CH_2); 2867 (ν_s CH_2); 1735 ($\nu_{C=O}$ ester); 1711 ($\nu_{C=O}$ O(C=O)NH); 1513 (ν_{CN}, δ_{NH} C-N-H) amide II.

Synthesis of compound 6

To a solution of compound **5** (11.00 g, 26.16 mmol in 100 mL freshly distilled MeOH), 140 mL of ethylene diamine (126g, 2.09 mol, 80 eq.) were added. The mixture was stirred for 5 days at 25 °C. At the end of reaction the excess of ethylene diamine and MeOH was evaporated under vacuum to yield compound **6** (quantitatively) as a very viscous brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 2H, -N*H*-), 5.65 (s, 1H, -N*H*-), 3.56 – 3.47 (m, 8H, -CH_2-O-CH_2-CH_2-O-CH_2-), 3.24 (dd, *J* = 11.7, 5.8 Hz, 6H, -CONH-CH_2-), 2.81 – 2.69 (m, 8H, N- CH_2-, -CH_2-NH_2), 2.62 (t, *J* = 5.3 Hz, 2H, -CH_2-N), 2.33 (t, *J* = 6.2 Hz, 4H, -CH_2-CONH-), 2.03 (s, 4H, -NH_2), 1.40 (s, 9H, -CH_3).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.93 (s, 2C, -(*C*=O)NH), 155.26 (s, 1C, -O(*C*=O)NH), 78.22 (s, 1C, *Cq*), 69.32 (s, 2C, -O-*C*H_2-*C*H_2-O-), 68.34 (s, 2C, -O-*C*H_2-), 52.77 (s, 1C, -*C*H_2-N), 50.01 (s, 2C, -N-*C*H_2-), 41.14 (s, 1C, -OCONH-*C*H_2-), 40.46 (s, 2C, -*C*H_2-NH_2), 39.38 (s, 2C, -CONH-*C*H_2-), 33.36 (s, 2C, -*C*H_2-CONH -), 27.52 (s, 3C, -*C*H_3).

MS (ESI, m/z) [MH^+] calc. – 477.3395, found – 477.3398; MS [MNa^+] calc. – 499.3240, found – 499.3243

EA calc.: C, 52.92; H, 9.31; N, 17.63; O, 20.14; found: C, 52.12; H, 9.20; N, 16.88 O, 21.15

FT IR (cm^{-1}) = 3295 (ν_{NH} (C=O)NH); 2970 (ν_{as} CH_3); 2929 (ν_{as} CH_2); 2866 (ν_s CH_2); 1702 ($\nu_{C=O}$ O(C=O)NH); 1644 ($\nu_{C=O}$) amide I; 1537 (ν_{CN}, δ_{NH} C-N-H) amide II.

Synthesis of compound 7

To a solution of compound **6** (1.81 g, 3.80 mmol in 20 mL of anhydrous DMF) a solution of compound **3** (8.00 g, 30.39 mmol, 8 eq. in 40 mL of anhydrous DMF) was added. The reaction mixture was stirred at 40°C during 5 days. At the end of reaction, the solvent was evaporated and the residue was purified relying on the difference of solubility of the mixture in toluene. The compound **7** is not soluble in toluene while the starting material (compound **3**) added in a large excess is. Thus, the residue was solubilized in a minimal volume of CH_2Cl_2 and added dropwise to a large volume of toluene, and then the solution volume was reduced twice. After two days the precipitated gluey mass was separated from the solution by decantation and the procedure was repeated again (at least three times). By this procedure compound **7** was obtained in 46 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 8H, C*H*=C*H*), 5.24 (s, 8H, C*H*), 4.20 (s, 8H, -(C=O)O-CH_2-), 3.73 (t, *J* = 5.3 Hz, 8H, -CH_2-N), 3.58 (s, 4H, -O-CH_2-CH_2-O-), 3.51 (t, 4H, -CH_2-O-,-O-CH_2-), 3.27 (dd, *J* = 10.0, 5.0 Hz, 6H, -NH-CH_2-), 2.85 (d, *J* = 19.2 Hz, 12H, C*H*, -N-CH_2-), 2.71 (t, *J* = 6.7 Hz, 8H, -N-CH_2-), 2.50 (t, *J* = 5.8 Hz, 6H, -CH_2-N-), 2.42 – 2.29 (m, 12H, -CH_2-(C=O)O, -CH_2-(C=O)NH), 1.43 (s, 9H, -CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 176.25 (s, 8C, -N(*C*=O)$_2$), 172.42 (s, 6C, -(*C*=O)O-, -(*C*=O)NH-), 156.21 (s, 1C, -O(*C*=O)NH-), 136.72 (s, 8C, *C*H=*C*H), 81.09 (s, 8C, *C*H), 79.24 (s, 1C, C_q), 70.39 (s, 4C, -*C*H_2-O-*C*H_2-*C*H_2-O-*C*H_2-), 60.86 (s, 4C, -(C=O)O-*C*H_2-), 52.87 (d, *J* = 18.6 Hz, 3C, -*C*H_2-N), 50.29 (s, 2C, N-*C*H_2-), 48.91 (s, 4C, N-*C*H_2-), 47.65 (s, 8C, *C*H), 40.51 (s, 1C, -O(C=O)NH-*C*H_2-), 37.94 (s, 4C, -*C*H_2-N), 37.24 (s, 2C, (C=O)NH-*C*H_2-), 32.43 (s, 6C, -*C*H_2-(C=O)NH-, -*C*H_2-(C=O)O-), 28.60 (s, 3C, -*C*H_3).

MS (ESI, m/z) $[M]^{2+}$ calc. – 765.3321, found – 765.3329; MS [M] calc. – 1529.6570, found – 1529.6210; MS $[M+Na]^+$ calc. – 1551.6345, found – 1551.5985

EA calc.: C, 57.32; H, 6.33; N, 9.16; O, 27.20; found: C, 57.76; H, 6.39; N, 9.03; O, 26.96

FT IR (cm^{-1}) = 3372 (ν_{as} NH_2); 2958 (ν_{as} CH_3); 2923 (ν_{as} CH_2); 2869 (ν_s CH_2); 1774, 1695 ($\nu_{C=O}$ cyclic imides in five membered ring); 1730 ($\nu_{C=O}$ ester); 1640 ($\nu_{C=O}$) amide I; 1527 (ν_{CN}, δ_{NH} C-N-H) amide II; 915,876, 853, 824 (maleimide ring deformation)

Synthesis of dendron D

Compound **7** (3.00g) was solubilized in freshly distilled CH_2Cl_2 (40 mL) and gaseous HCl was bubbled through the solution during 25 min. At the end of the reaction, the solvent, *t*-butanol and excess of HCl were evaporated under vacuum. Then, the residue was solubilized in 100 mL of

H_2O, washed with CH_2Cl_2 (3×100 mL) and dried in vacuum to yield dendron **D** (77 %) as a yellow-orange very viscous oil.

^{1}H NMR (300 MHz, D_2O) δ 6.65 (s, 8H, C*H*=C*H*), 5.32 (s, 8H, C*H*), 4.36 (t, *J* = 5.1 Hz, 8H, -(C=O)O-CH_2-), 3.94 (d, *J* = 4.4 Hz, 2H, O-CH_2-), 3.83 (dd, *J* = 9.4, 4.7 Hz, 10H, -CH_2-O-, -CH_2-N-), 3.78 (s, 4H, -O-CH_2-CH_2-O-), 3.72 – 3.65 (m, 4H, -(C=O)NH-CH_2-), 3.55 (dd, *J* = 13.8, 6.9 Hz, 14H, -N-CH_2-, -CH_2-N), 3.43 (t, *J* = 5.7 Hz, 4H, -CH_2-N), 3.28 – 3.22 (t, 2H, -CH_2-NH_2), 3.16 (s, 8H, C*H*), 2.91 (d, *J* = 6.4 Hz, 12H, -CH_2-(C=O)NH-, -CH_2-(C=O)O-).

^{13}C NMR (75 MHz, D_2O) δ 179.31 (s, 8C, -N(*C*=O)$_2$), 172.81 (s, 2C, (*C*=O)NH), 172.61 (s, 4C, (*C*=O)O), 136.56 (s, 8C, *C*H=*C*H), 81.16 (s, 8C, *C*H), 69.79 (d, *J* = 19.5 Hz, 2C, -O-*C*H_2-*C*H_2-O-), 66.54 (s, 1C, -*C*H_2-O-), 64.09 (s, 1C, -O-*C*H_2-), 61.88 (s, 4C, (C=O)O-CH_2-), 53.09 (s, 2C, -*C*H_2-N), 52.91 (s, 1C, -*C*H_2-N), 49.85 (s, 8C, N-*C*H_2-), 49.14 (s, 2C, N-*C*H_2-), 47.54 (s, 8C, *C*H), 39.17 (s, 1C, -*C*H_2-NH_2), 37.11 (s, 4C, -*C*H_2-N), 34.53(s, 2C, -CH_2-(C=O)NH-), 28.77 (s, 4C, -CH_2-(C=O)O-), 28.23 (s, 2C, -CH_2-(C=O)NH-).

MS (FD, m/z) $[M+H]^+$ calc. – 1429.6051, found – 1429.6008

EA (M×5H_2O×4HCl) calc: C, 49.04; H, 6.17; Cl, 8.52; N, 8.41; O, 27.86; found: C, 48.76; H, 6.16; Cl, 9.58; N, 8.72; O, 26.92.

FT IR (cm^{-1}) = 3389 (ν_{as} NH_2); 2956 (ν_{as} CH_2); 2866 (ν_s CH_2); 1772, 1693 ($\nu_{C=O}$ cyclic imides in five membered ring); 1735 ($\nu_{C=O}$ ester); 1551 (ν_{CN}, δ_{NH} C-N-H) amide II; 1184 cm^{-1} (C-N-C stretch); 914, 877, 852, 824 (maleimide ring deformation)

3. General grafting and maleimide deprotection procedures

Grafting of dendron D on core-shell γ-Fe_2O_3/Polymer MNPs

First, 5 mg of γ-Fe_2O_3/Polymer MNPs were dispersed in 1 mL of 50 mM MES/0.3 % PF solution.[a] Then, 2.85 mg of EDC[b] (corresponding to 8.5 eq. per equivalent of available COOH groups on γ-Fe_2O_3/Polymer MNPs, which is ~350 μmol COOH/g) solubilized in 1 mL of 50 mM MES/0.3 % PF solution and 5.99 mg of NHS[c] (3.5 eq. per equivalent of EDC quantity) solubilized in 1 mL of 50 mM MES/0.3 % PF solution were added. Subsequently, 10 equivalents of dendron D (per equivalent of available COOH groups on γ-Fe_2O_3/Polymer MNPs) solubilized in 1 mL of 50 mM MES/0.3 % PF solution were loaded in the grafting reaction. The total reaction volume was brought up to 5 mL (corresponding to the final concentration of 1 mg of MNPs/mL of solution) and then sonicated (~10 sec, 60W). The grafting proceeded in a thermomixer (300 rpm) at 25°C for 16 h. During all the grafting period the particles remained well dispersed and no sedimentation or aggregation was observed. At the end of the grafting, MNPs were separated from the reaction mixture by magnetic decantation, washed with: MES (1x5mL), H_2O (1×5mL), EtOH (3×5mL), re-dispersed in EtOH (5 mg/mL) and stored in the fridge.

Maleimide group deprotection

5 mg of the modified MNPs (**γ-Fe_2O_3/Pol@D**) were dispersed in 0.5 mL of anhydrous DMSO and the cleavage of furan protection proceeded in a thermomixer (300 rpm) at 99 °C during 5 h. At the end of deprotection reaction the resulting maleimide functionalized MNPs (**γ-Fe_2O_3/Pol@D-Mal**) were separated from the reaction mixture by magnetic decantation, washed with EtOH (5 × 1 mL), dispersed in EtOH (5 mL) and stored in the fridge.

[a] MES: 4-Morpholineethanesulfonic acid and, PF: Pluronic F-127
[b] N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide
[c] N-hydroxysuccinimide

4. Immobilization tests

Covalent coupling of thiol modified biotin (HS-PEG-Biot) with the maleimide functionalized MNPs

1 mg of maleimide functionalized MNPs (**γ-Fe_2O_3/Pol@D-Mal**) was dispersed in 0.375 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH=7.1 with 1 M HCl solution), and 2.76 mg of HS-PEG-Biot solubilized in 0.125 mL of 0.01 M PBS[d]/0.05% Tween-20 buffer solution were added to the MNPs' suspension. The reaction mixture was incubated in a thermomixer (300 rpm) at 37°C for 16 h. During the incubation period the particles remained well dispersed and no sedimentation or aggregation was observed. At the end of incubation reaction, MNPs (**γ-Fe_2O_3/Pol@D@Biot**) were separated from the reaction mixture by magnetic decantation, washed with 0.01 M PBS/0.05% Tween-20 buffer solution, then re-dispersed in 1 mL of the same buffer solution and stored in the fridge.

Immobilization of 15 nm SA-Au NPs on biotin functionalized MNPs

First, the storage buffer solution (Tris buffer saline pH = 8.2, containing 1 % BSA, 15 mM NaN_3 and 30 % glycerol) of 15 nm SA-Au NPs was replaced with 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH = 7.1 with 1 M HCl solution) according to the following procedure: 1 mL of SA-Au NPs (13.65 μg/mL of protein, 1.4×10^{12} particles/mL) was transferred into a conical tube and 1 mL of 0.01 M PBS/0.05% Tween-20 buffer solution was added. The particles were vortexed and then centrifuged during 1 h at the speed of 9000 rpm. The solution was separated from the particles with a pipet. Then, the particles were re-dispersed in 2 mL of same buffer solution, vortexed and centrifuged during 1 h at the same speed. The procedure was repeated twice and then particles were dispersed in 1.4 mL (corresponding to 1.00×10^{12} part/mL) of the same buffer solution and stored in the fridge.

To 0.05 mg of biotin modified MMPs (**γ-Fe_2O_3/Pol@D@Biot**), 2.00×10^{11} SA-Au NPs (15 nm) dispersed in 0.2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (pH = 7.1) were added (corresponding to 4.0×10^{12} SA-Au NPs/mg of functionalized MNPs). The reaction mixture was incubated in a thermomixer (300 rpm) at 37°C for 16 h. and no sedimentation or aggregation was observed during this period. Afterwards, MNPs were separated from the reaction mixture by magnetic decantation and the solutions of SA-Au NPs were analyzed by UV-Vis spectroscopy. The resulting MNPs (**γ-Fe_2O_3/Pol@D@Biot@SA-Au**) were washed with water (5×1 mL), then re-dispersed 0.2 mL of water, and stored in the fridge.

[d] PBS: Phosphate buffered saline

Incubation of biotin functionalized MNPs with 20 nm H_2N-PEG-Au NPs

First, 20 nm H_2N-Au NPs were dispersed in 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH = 7.1 with 1 M HCl solution) according to the following procedure: 0.2 mL of H_2N-Au NPs' aqueous solution (corresponding to 6.8×10^{12} H_2N-Au NPs) were transferred into a conical tube and 2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution was added. The dispersion was vortexed and then centrifuged during 40 min. at the speed of 8500 rpm. Then, the solution was separated from the particles with a pipet. The particles were then dispersed in 2 mL of same buffer solution, vortexed and centrifuged during 40 min at the same speed. The procedure was repeated one more time and then particles were dispersed in 6.8 mL of buffer solution and stored in the fridge (1×10^{12} particles/mL).

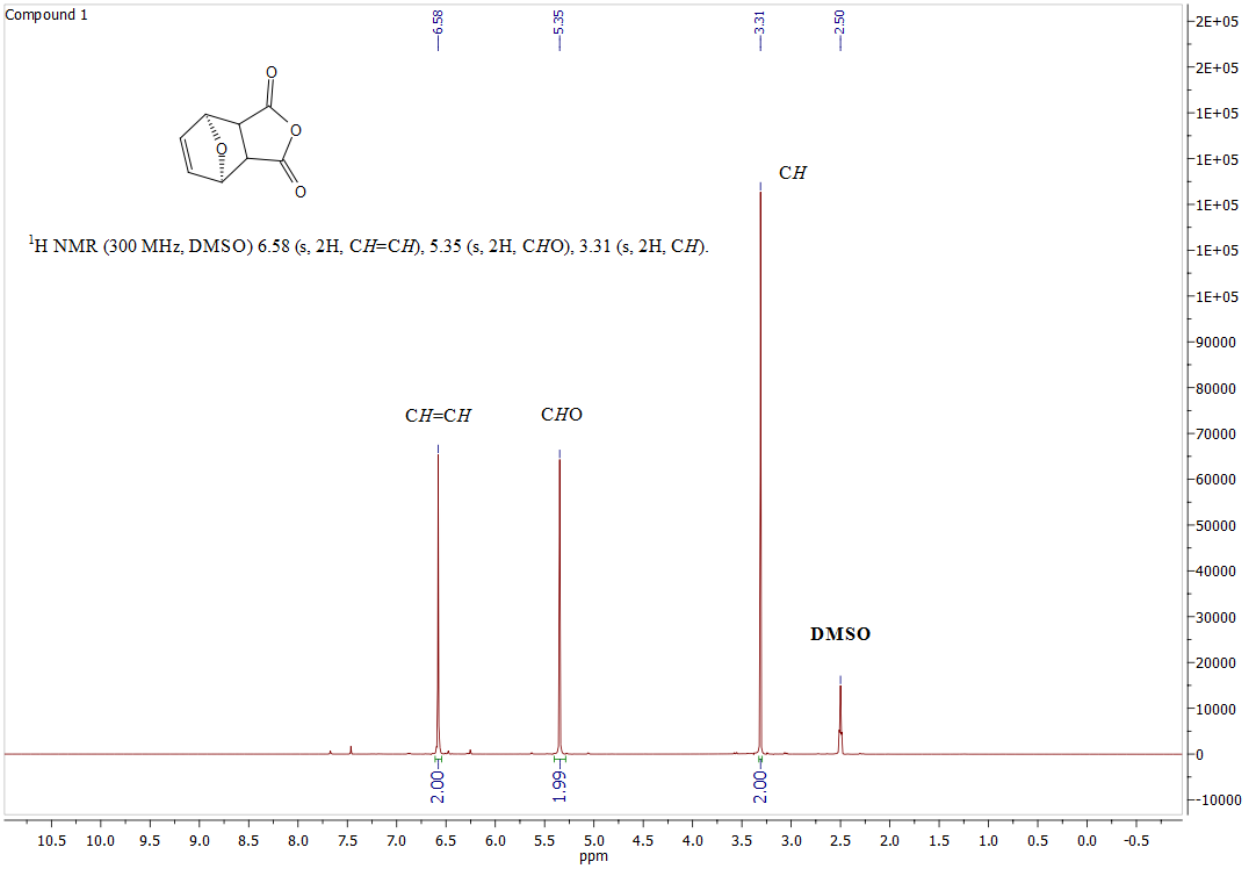
Subsequently, 0.05 mg of biotin functionalized MNPs (**γ-Fe_2O_3/Pol@D@Biot**) were incubated with 2.00×10^{11} H_2N-PEG-Au NPs of 20 nm dispersed in 0.2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (pH = 7.1). The incubation proceeded in a thermomixer (300 rpm) at 37°C during 16 h. At the end of the incubation time, MNPs were separated from the reaction mixture, and the solutions of H_2N-PEG-Au NPs were removed, while MNPs were washed with water (5×1 mL), then re-dispersed 0.2 mL of water, and stored in the fridge.

Incubation of maleimide functionalized MNPs with 20 nm H_2N-PEG-Au NPs

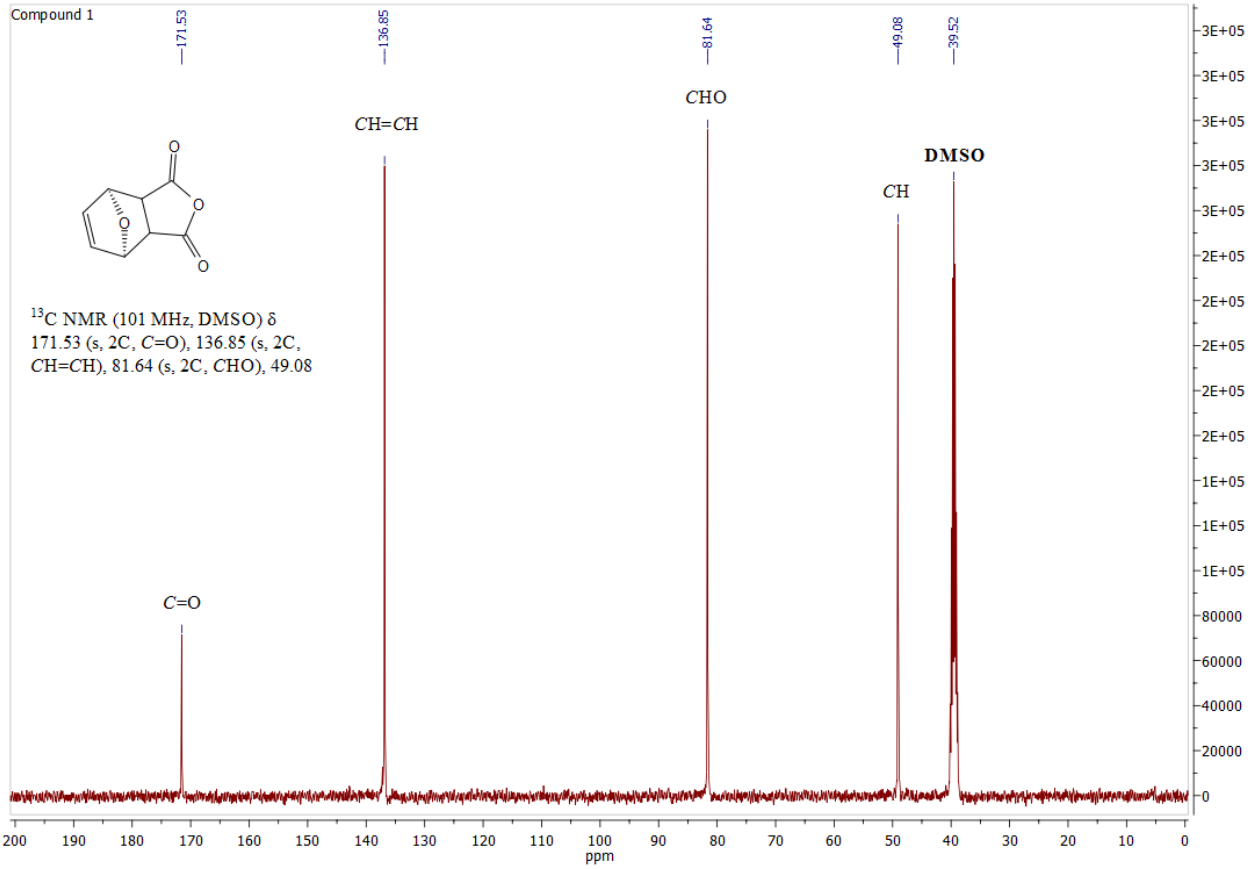
To 0.05 mg of native or maleimide functionalized MMPs (**γ-Fe_2O_3/Pol@D-Mal**), were added 2.00×10^{11} H_2N-PEG-Au NPs (20 nm) dispersed in 0.2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH = 7.1 with 1 M HCl solution). The reaction mixtures were incubated in a thermomixer (300 rpm) at 37°C for 16 h. No sedimentation or aggregation was observed during this period. Afterwards, MNPs were separated from the reaction mixture, and the solutions of H_2N-PEG-Au NPs were analyzed by UV-Vis spectroscopy, while MNPs were washed with water (5×1 mL), then re-dispersed 0.2 mL of water, and stored in the fridge.

5. Characterization of the compounds

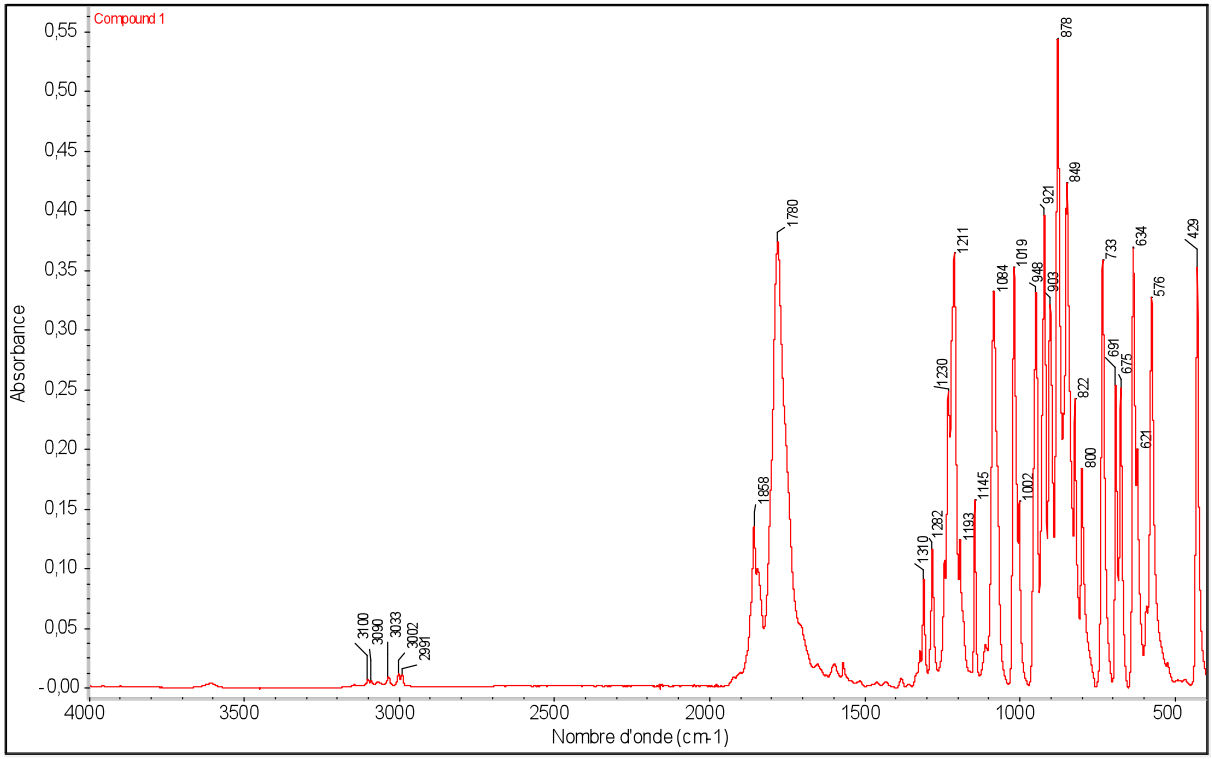
Compound 1



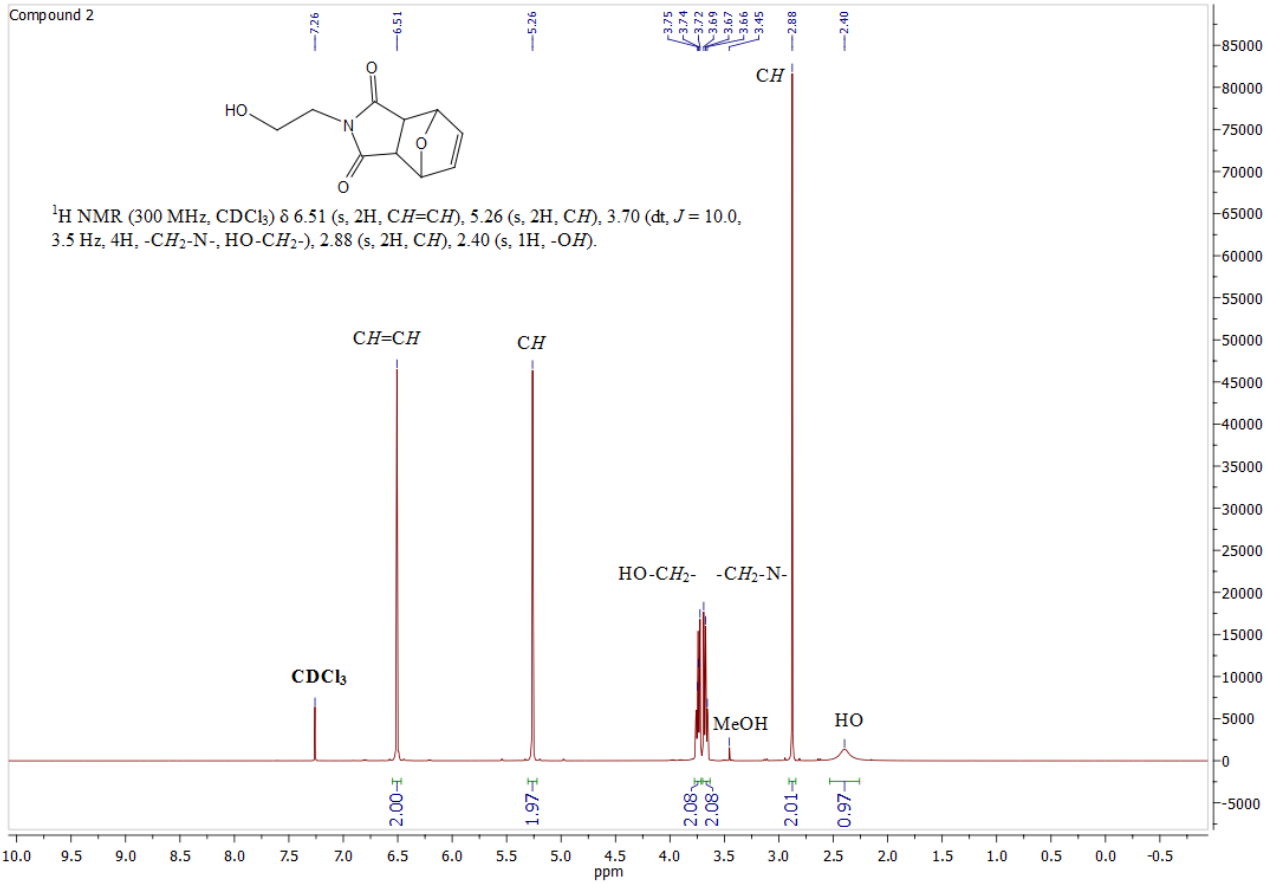
^{1}H NMR (300 MHz, DMSO) 6.58 (s, 2H, C*H*=C*H*), 5.35 (s, 2H, C*H*O), 3.31 (s, 2H, C*H*).



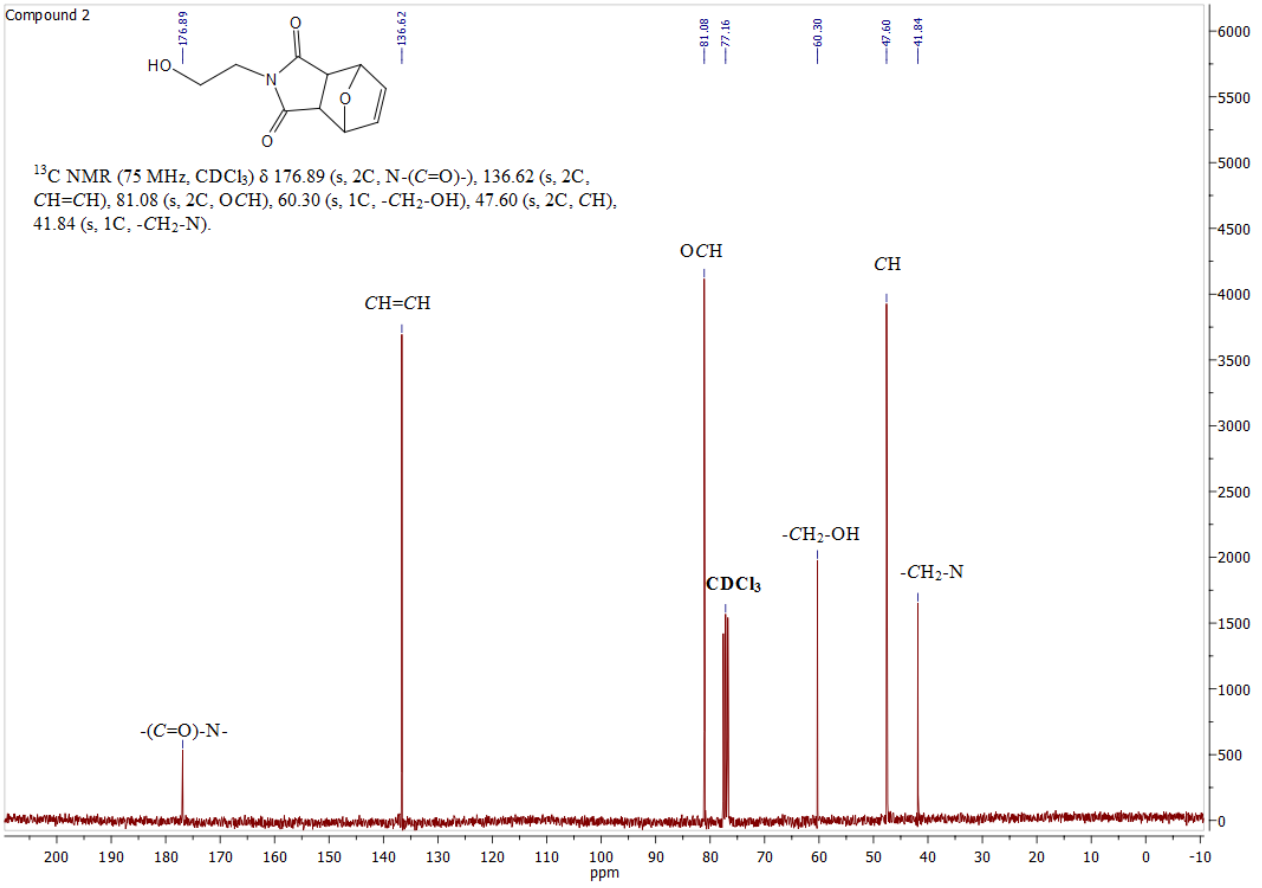
^{13}C NMR (101 MHz, DMSO) δ
171.53 (s, 2C, *C*=O), 136.85 (s, 2C,
*C*H=*C*H), 81.64 (s, 2C, *C*HO), 49.08


Compound 1
Absorbance
Nombre d'onde (cm-1)
0,55
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
-0,00
4000
3500
3000
2500
2000
1500
1000
500
3100
3090
3033
3002
2991
1858
1780
1310
1282
1230
1211
1193
1145
1084
1019
1002
948
921
903
878
849
822
800
733
691
675
634
621
576
429

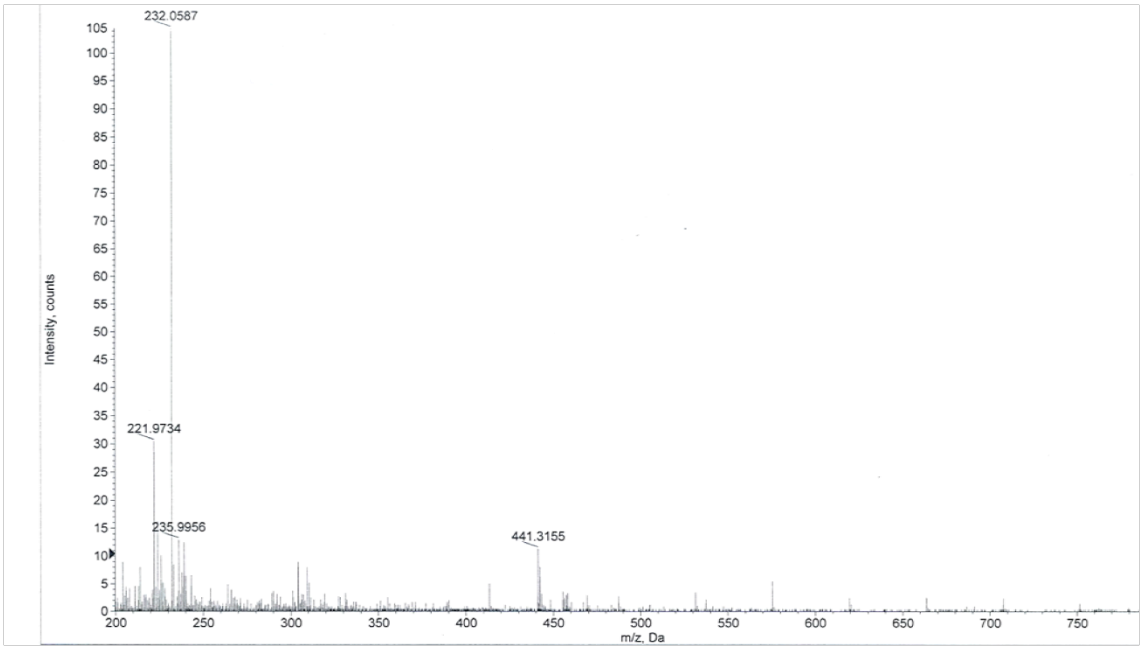
Compound 2

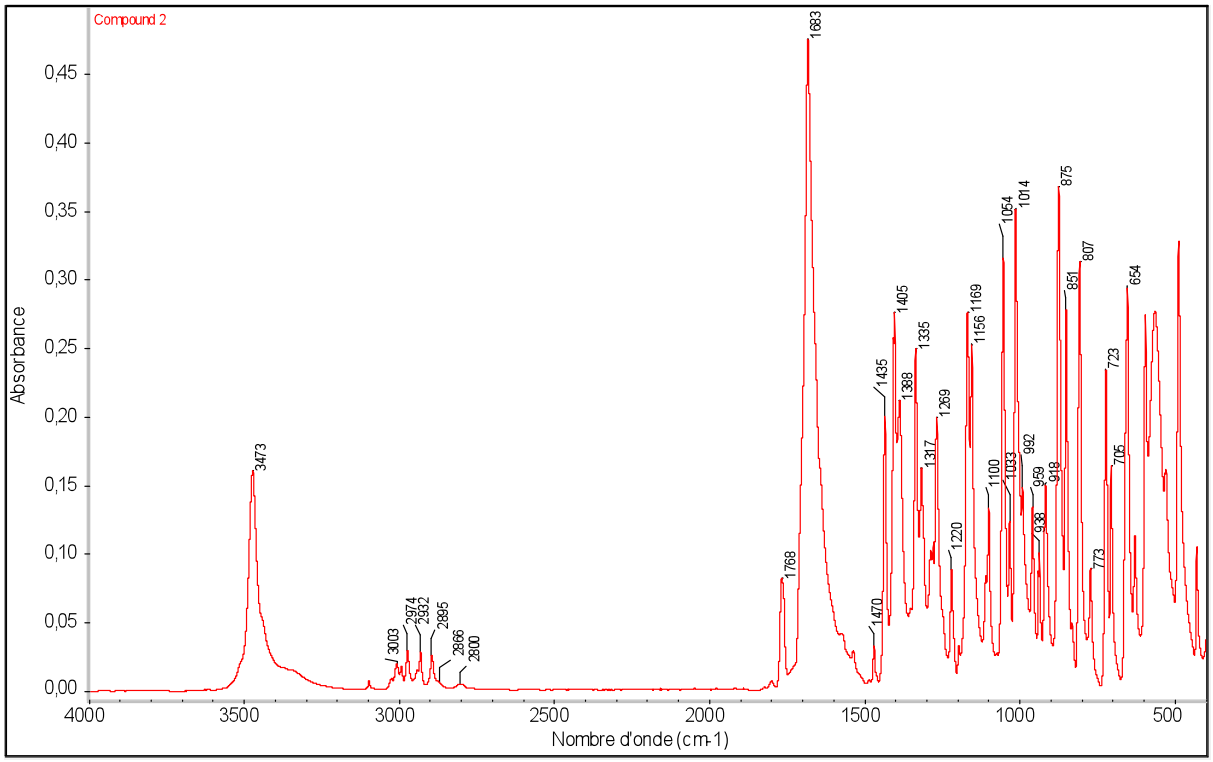


^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 2H, C*H*=C*H*), 5.26 (s, 2H, C*H*), 3.70 (dt, *J* = 10.0, 3.5 Hz, 4H, -CH_2-N-, HO-CH_2-), 2.88 (s, 2H, C*H*), 2.40 (s, 1H, -O*H*).

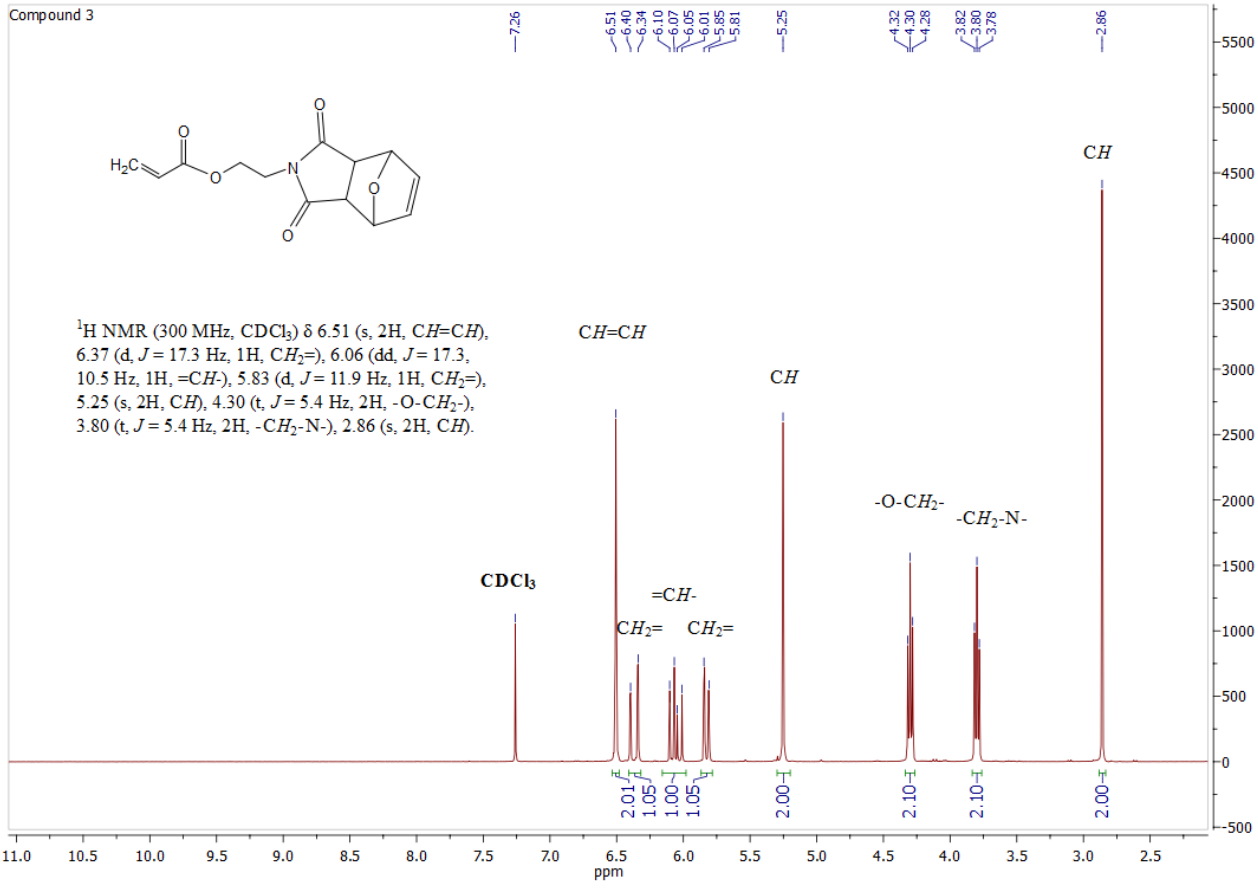


^{13}C NMR (75 MHz, $CDCl_3$) δ 176.89 (s, 2C, N-(*C*=O)-), 136.62 (s, 2C, *C*H=*C*H), 81.08 (s, 2C, O*C*H), 60.30 (s, 1C, -*C*H_2-OH), 47.60 (s, 2C, *C*H), 41.84 (s, 1C, -*C*H_2-N).

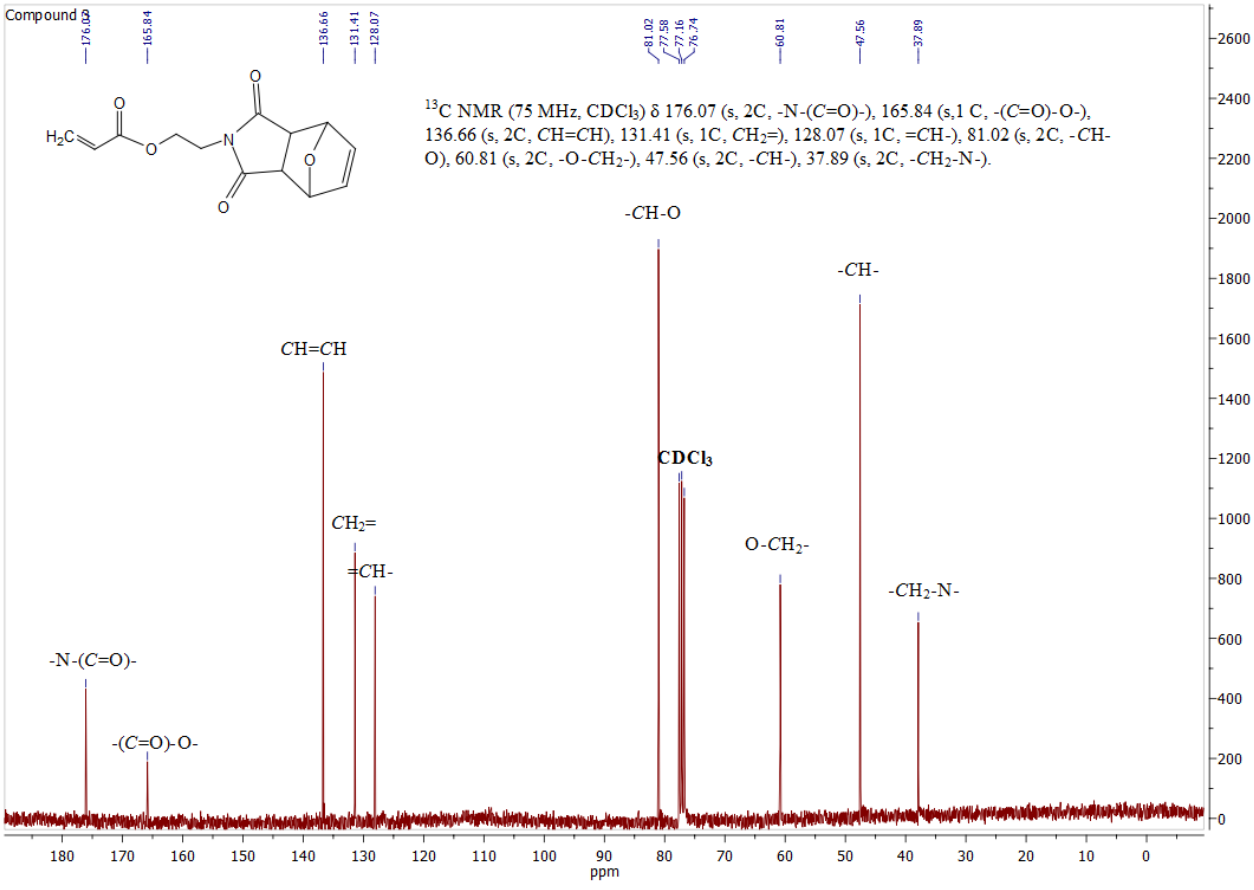

232.0587
221.9734
235.9956
441.3155
Intensity, counts
m/z, Da


Compound 2
Absorbance
Nombre d'onde (cm-1)
3473
3003
2974
2932
2895
2866
2800
1768
1683
1470
1435
1405
1388
1335
1317
1269
1220
1169
1156
1100
1054
1033
1014
992
959
938
918
875
851
807
773
723
705
654

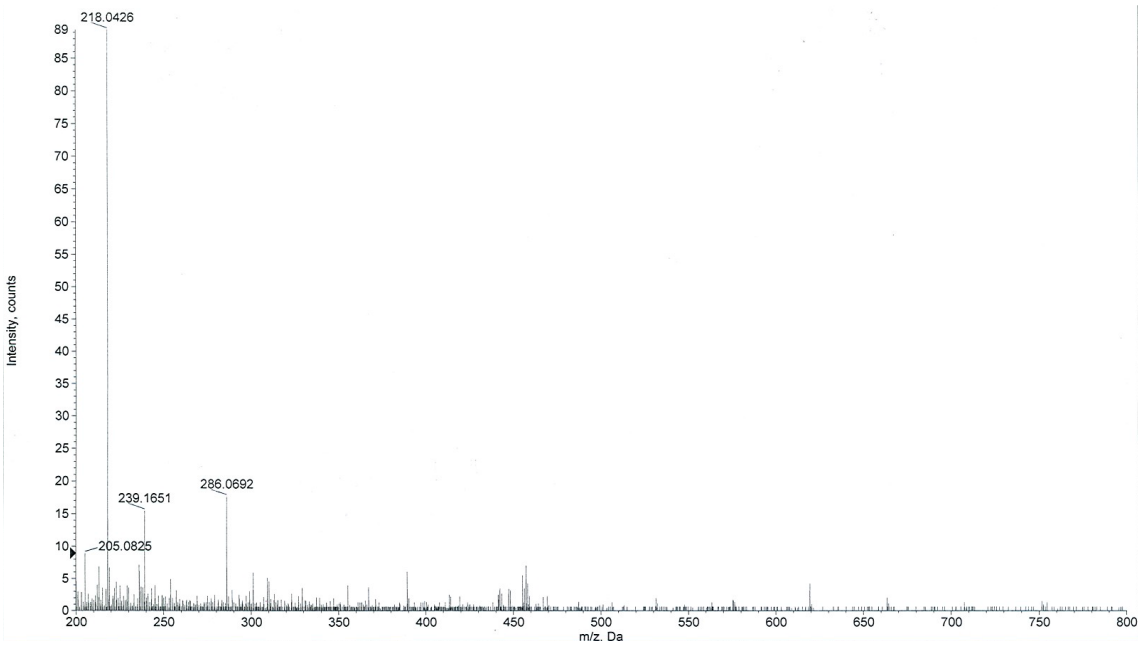
Compound 3

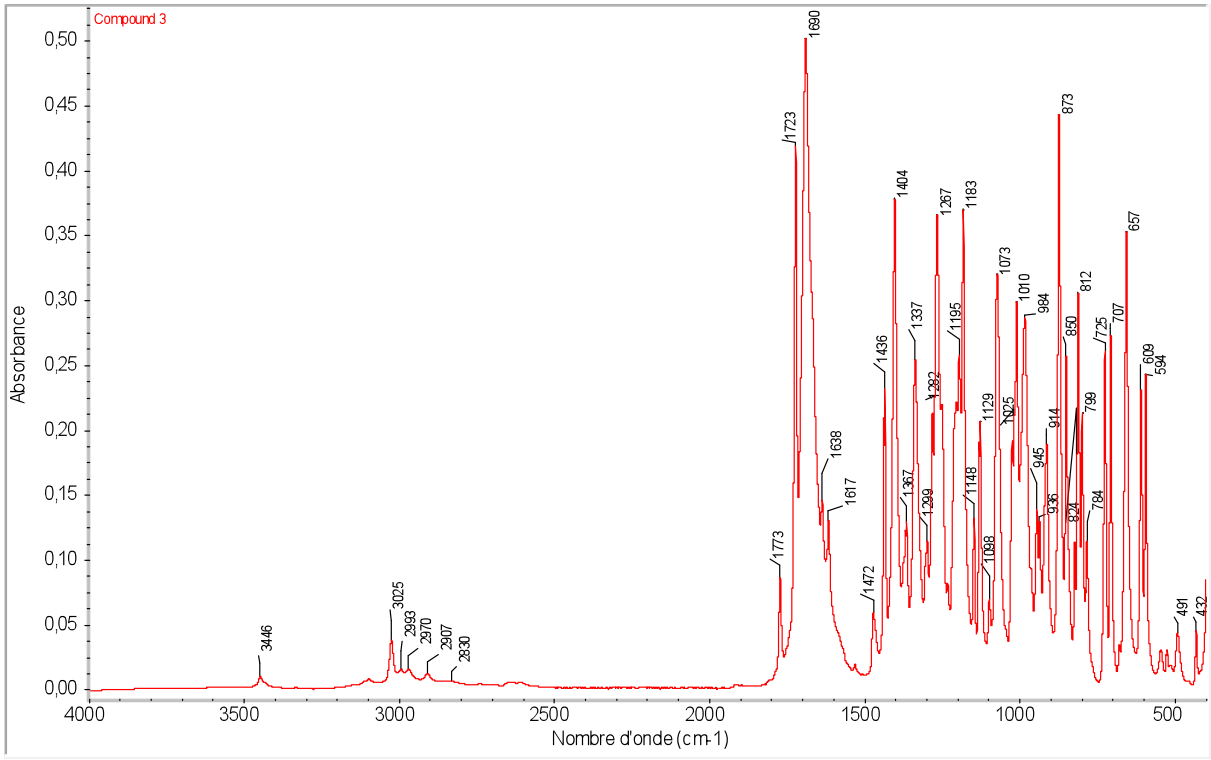


^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 2H, C*H*=C*H*), 6.37 (d, *J* = 17.3 Hz, 1H, CH_2=), 6.06 (dd, *J* = 17.3, 10.5 Hz, 1H, =C*H*-), 5.83 (d, *J* = 11.9 Hz, 1H, CH_2=), 5.25 (s, 2H, C*H*), 4.30 (t, *J* = 5.4 Hz, 2H, -O-CH_2-), 3.80 (t, *J* = 5.4 Hz, 2H, -CH_2-N-), 2.86 (s, 2H, C*H*).

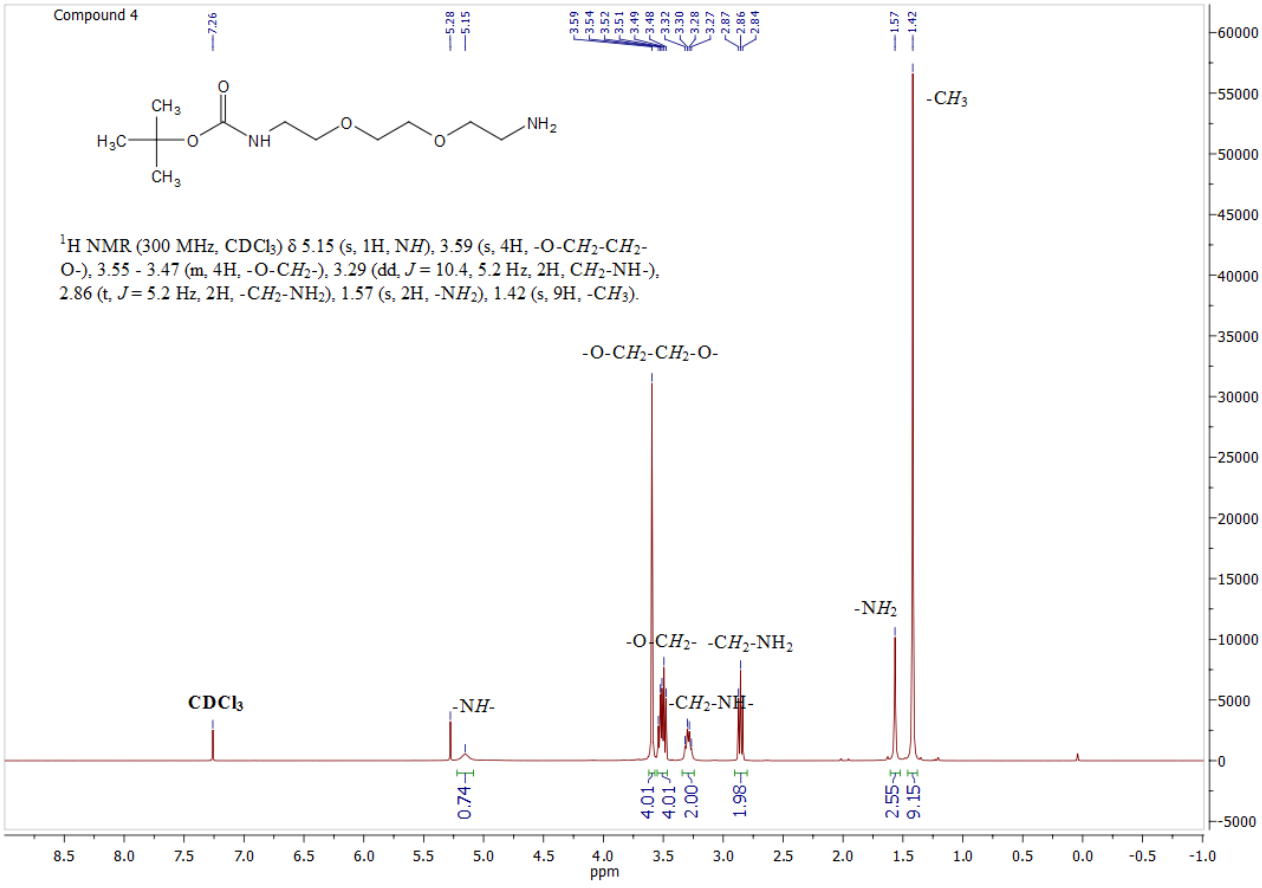


^{13}C NMR (75 MHz, $CDCl_3$) δ 176.07 (s, 2C, -N-(*C*=O)-), 165.84 (s,1 C, -(*C*=O)-O-), 136.66 (s, 2C, *C*H=*C*H), 131.41 (s, 1C, *C*H_2=), 128.07 (s, 1C, =*C*H-), 81.02 (s, 2C, -*C*H-O), 60.81 (s, 2C, -O-*C*H_2-), 47.56 (s, 2C, -*C*H-), 37.89 (s, 2C, -*C*H_2-N-).

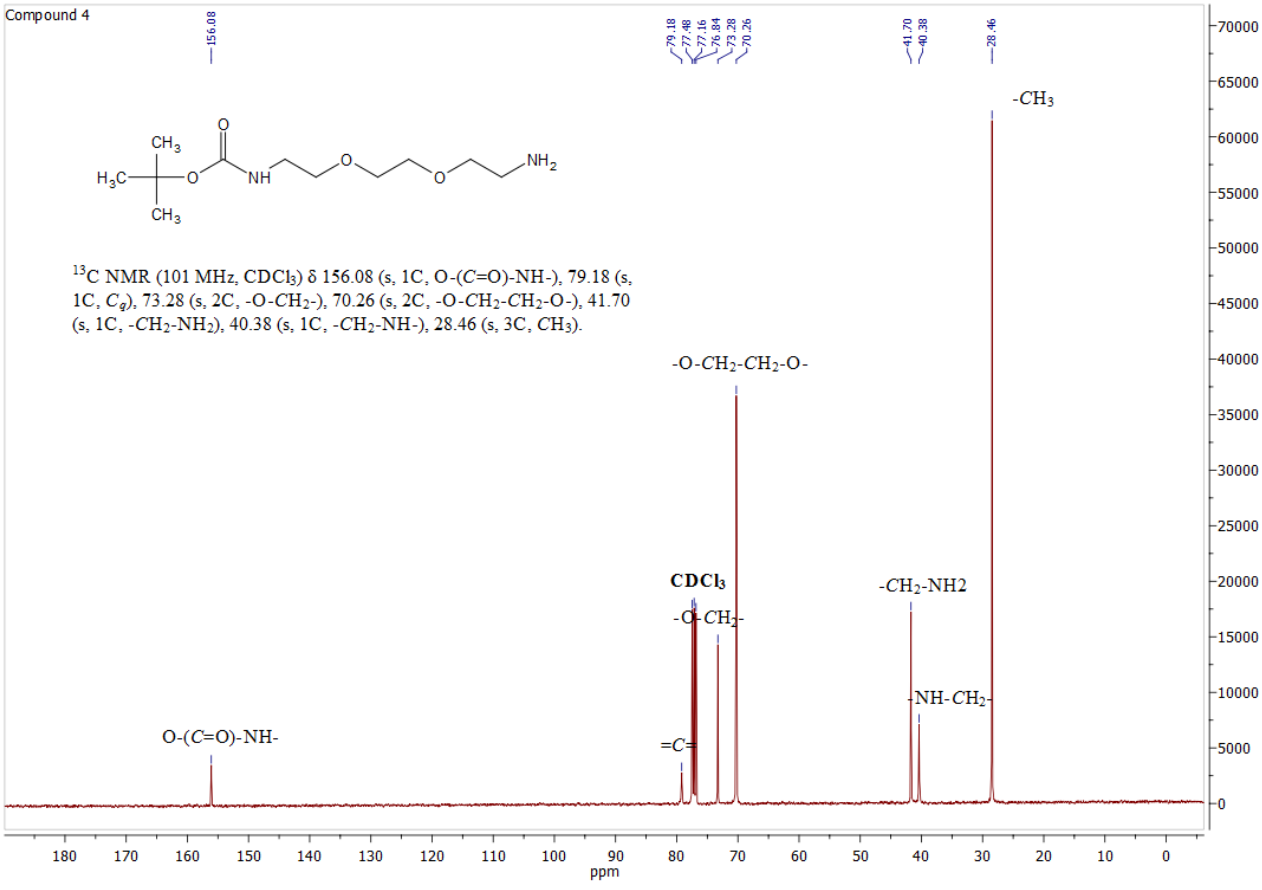

218.0426
239.1651
286.0692
205.0825
Intensity, counts
m/z, Da


Compound 3
Absorbance
Nombre d'onde (cm-1)
3446
3025
2993
2970
2907
2830
1773
1723
1690
1638
1617
1472
1436
1404
1367
1337
1299
1267
1195
1183
1148
1129
1098
1073
1025
1010
984
945
914
873
850
812
799
784
725
707
657
609
594
491
432

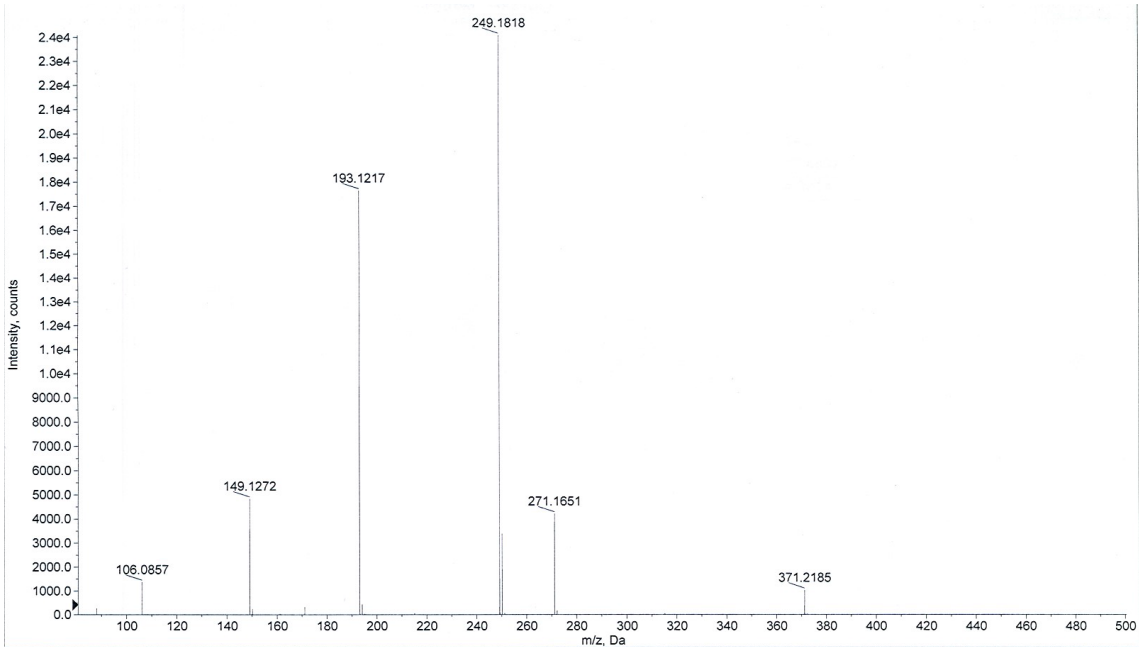
Compound 4

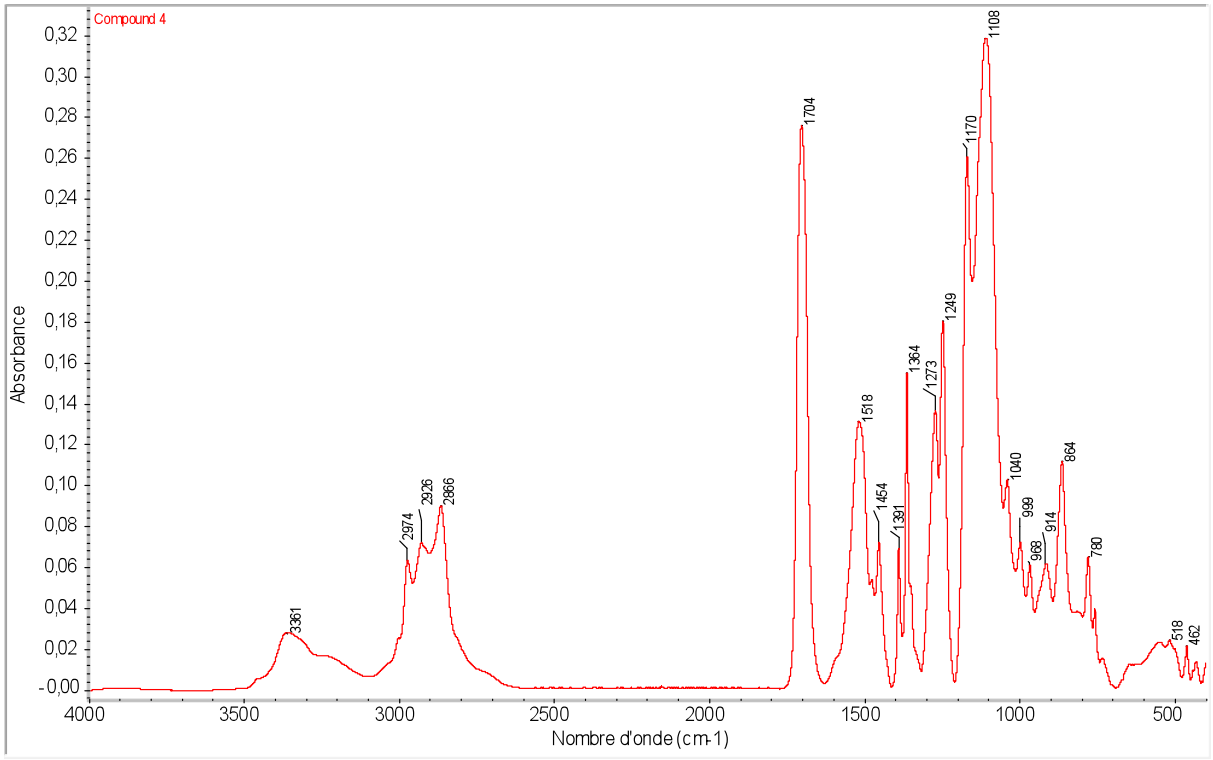


^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (s, 1H, N*H*), 3.59 (s, 4H, -O-C*H*$_2$-C*H*$_2$-O-), 3.55 - 3.47 (m, 4H, -O-C*H*$_2$-), 3.29 (dd, *J* = 10.4, 5.2 Hz, 2H, C*H*$_2$-NH-), 2.86 (t, *J* = 5.2 Hz, 2H, -C*H*$_2$-NH$_2$), 1.57 (s, 2H, -N*H*$_2$), 1.42 (s, 9H, -C*H*$_3$).

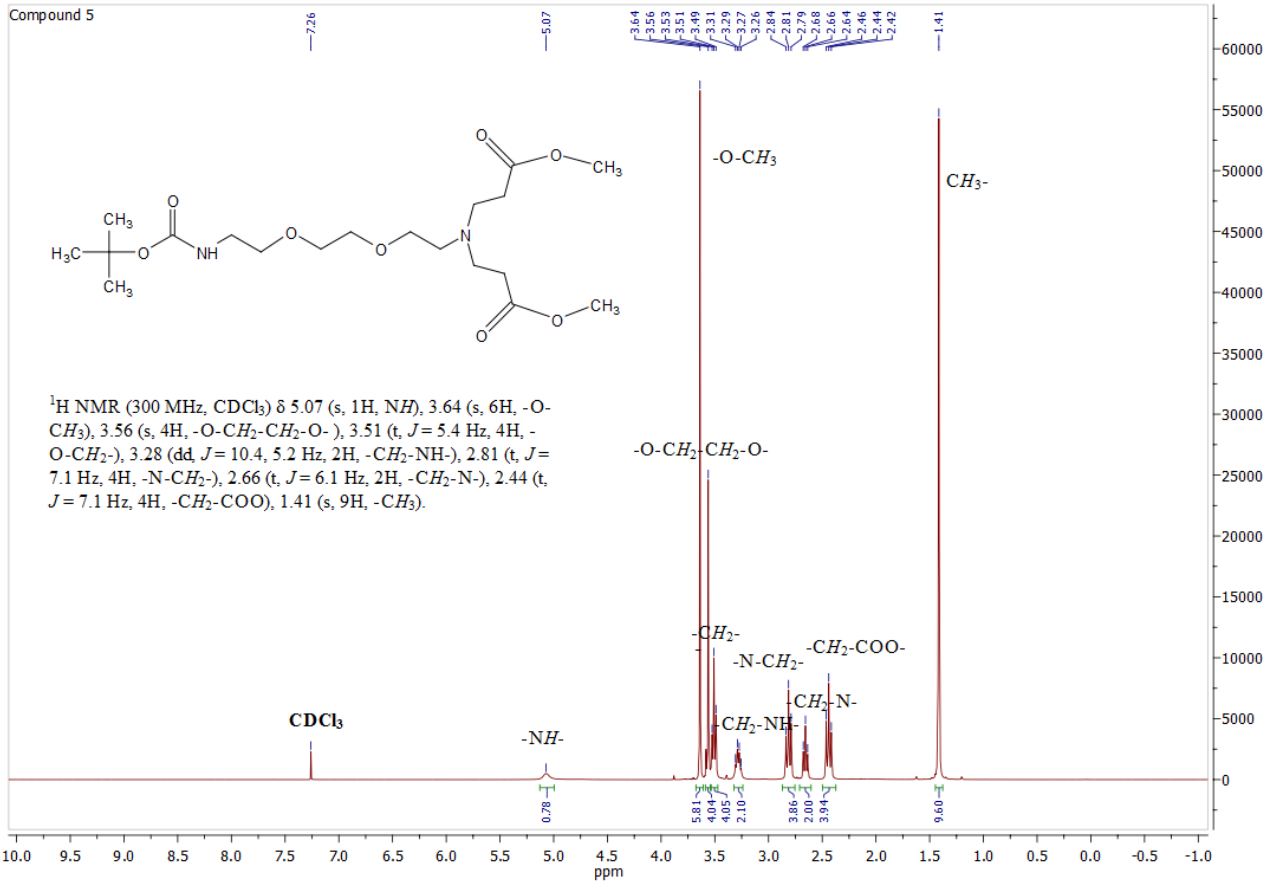


^{13}C NMR (101 MHz, $CDCl_3$) δ 156.08 (s, 1C, O-(*C*=O)-NH-), 79.18 (s, 1C, C_q), 73.28 (s, 2C, -O-*C*H$_2$-), 70.26 (s, 2C, -O-*C*H$_2$-*C*H$_2$-O-), 41.70 (s, 1C, -*C*H$_2$-NH$_2$), 40.38 (s, 1C, -*C*H$_2$-NH-), 28.46 (s, 3C, *C*H$_3$).

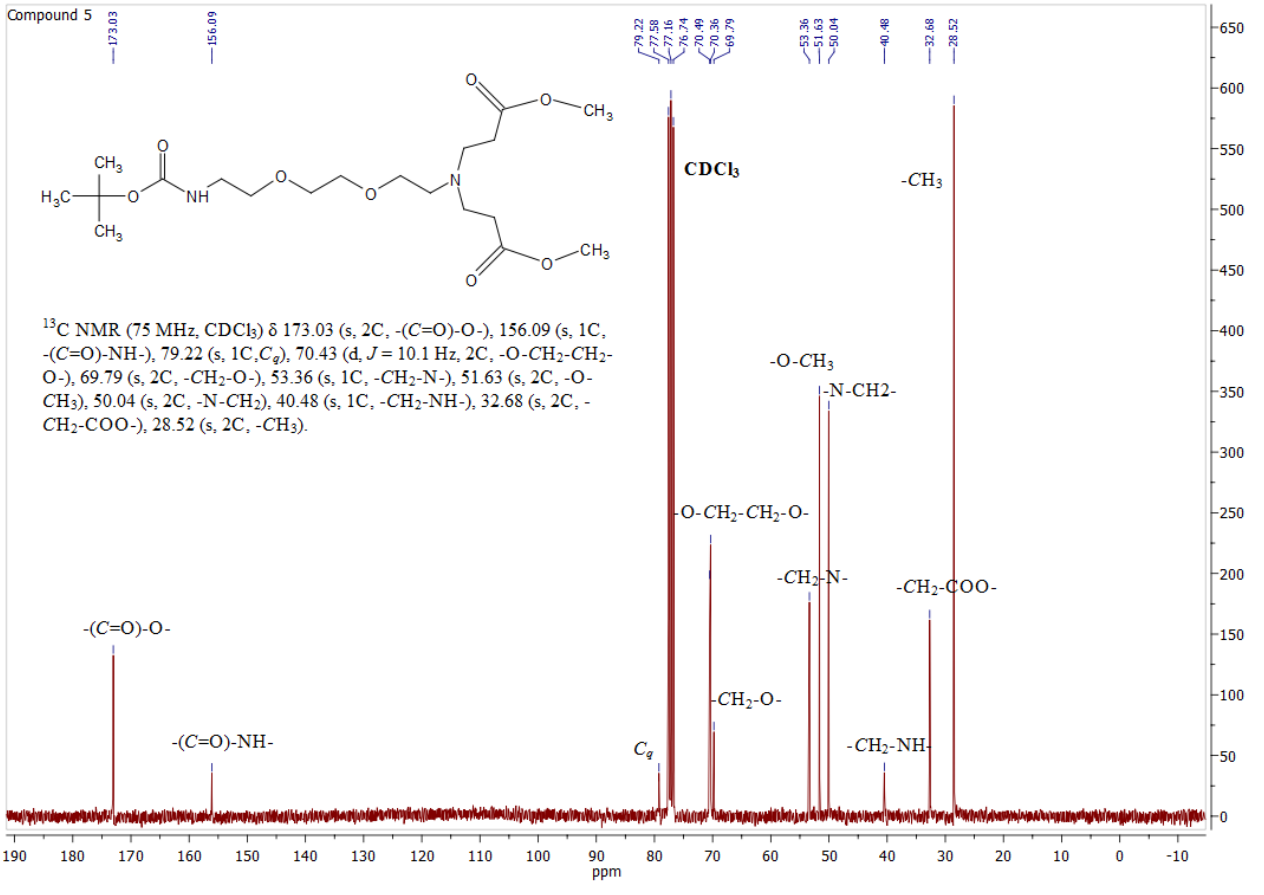

249.1818
193.1217
149.1272
271.1651
106.0857
371.2185
Intensity, counts
m/z, Da


Compound 4
Absorbance
Nombre d'onde (cm-1)
3361
2974
2926
2866
1704
1518
1454
1391
1364
1273
1249
1170
1108
1040
999
968
914
864
780
518
462

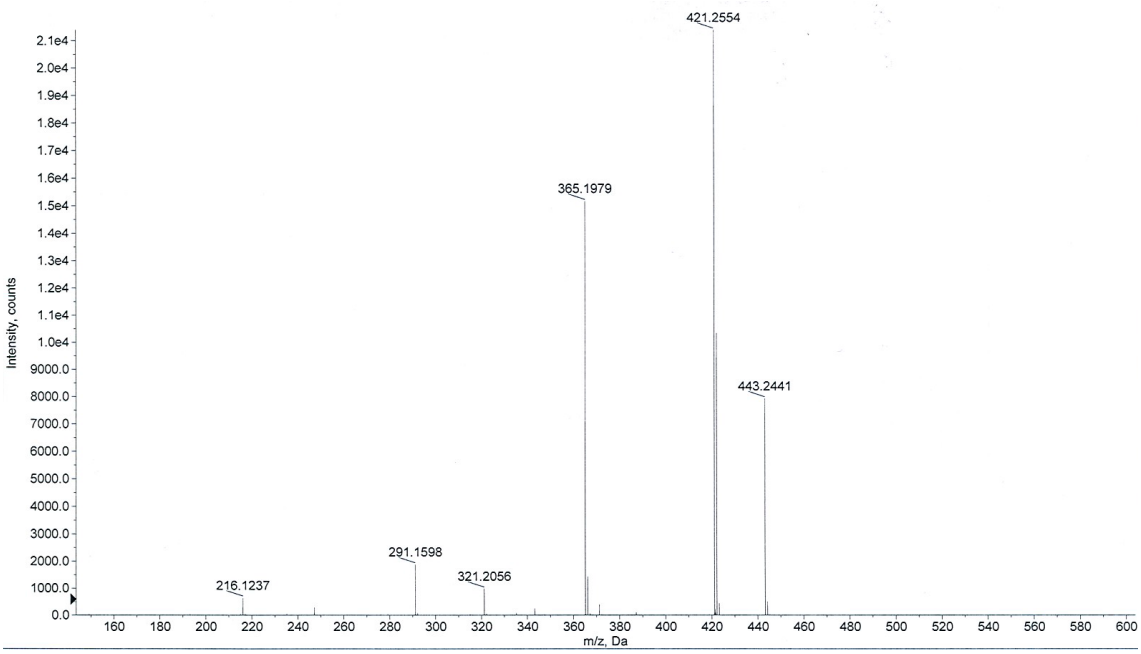
Compound 5

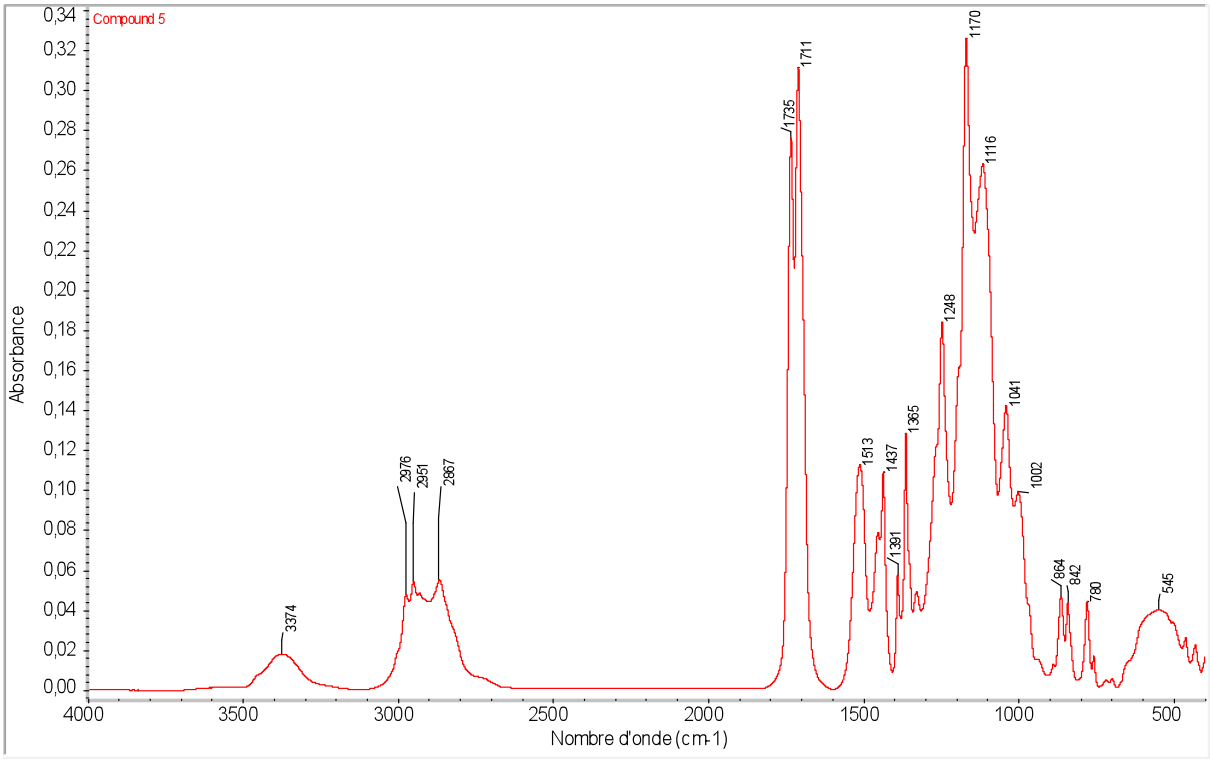


^{1}H NMR (300 MHz, $CDCl_3$) δ 5.07 (s, 1H, N*H*), 3.64 (s, 6H, -O-CH_3), 3.56 (s, 4H, -O-CH_2-CH_2-O-), 3.51 (t, J = 5.4 Hz, 4H, -O-CH_2-), 3.28 (dd, J = 10.4, 5.2 Hz, 2H, -CH_2-NH-), 2.81 (t, J = 7.1 Hz, 4H, -N-CH_2-), 2.66 (t, J = 6.1 Hz, 2H, -CH_2-N-), 2.44 (t, J = 7.1 Hz, 4H, -CH_2-COO), 1.41 (s, 9H, -CH_3).

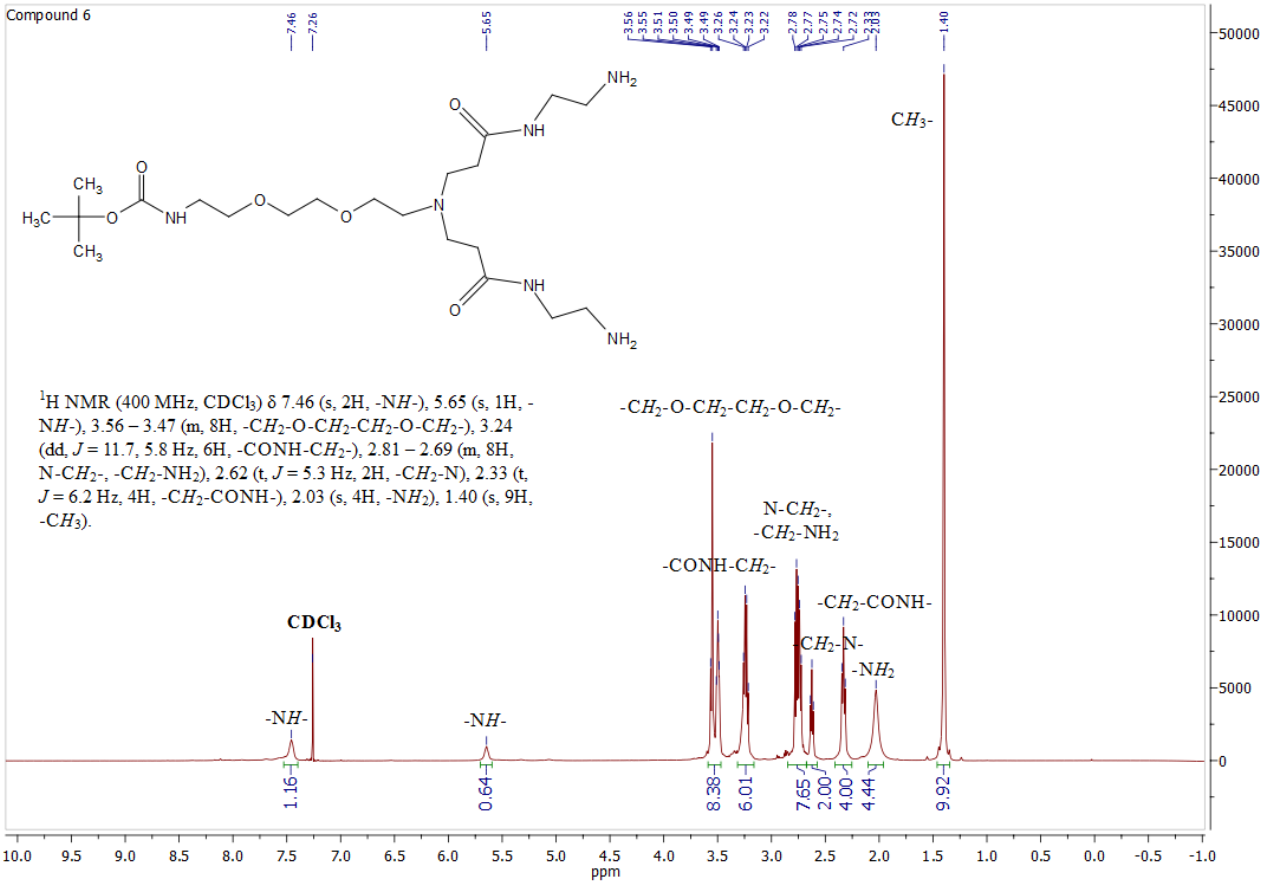


^{13}C NMR (75 MHz, $CDCl_3$) δ 173.03 (s, 2C, -(*C*=O)-O-), 156.09 (s, 1C, -(*C*=O)-NH-), 79.22 (s, 1C,C_q), 70.43 (d, J = 10.1 Hz, 2C, -O-CH_2-CH_2-O-), 69.79 (s, 2C, -CH_2-O-), 53.36 (s, 1C, -CH_2-N-), 51.63 (s, 2C, -O-CH_3), 50.04 (s, 2C, -N-CH_2), 40.48 (s, 1C, -CH_2-NH-), 32.68 (s, 2C, -CH_2-COO-), 28.52 (s, 2C, -CH_3).

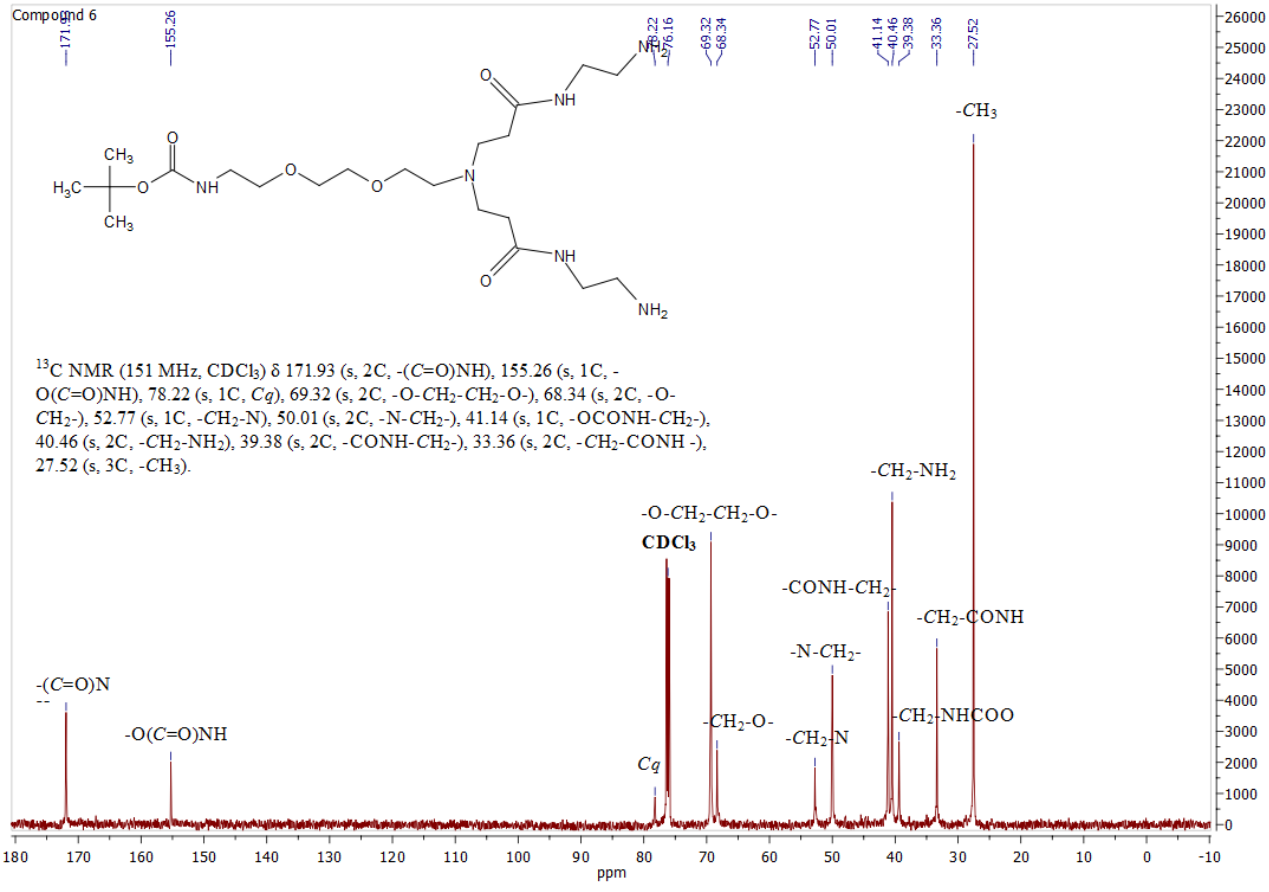

421.2554
365.1979
443.2441
291.1598
321.2056
216.1237
Intensity, counts
m/z, Da


Compound 5
Absorbance
Nombre d'onde (cm-1)
3374
2976
2951
2867
1735
1711
1513
1437
1391
1365
1248
1170
1116
1041
1002
864
842
780
545

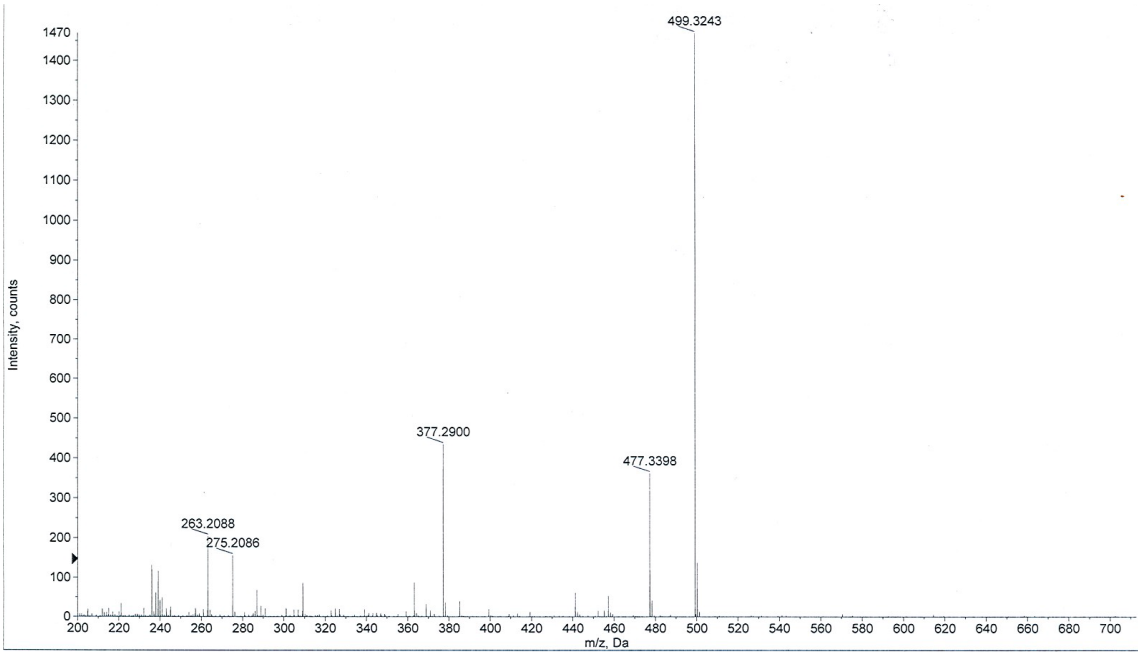
Compound 6

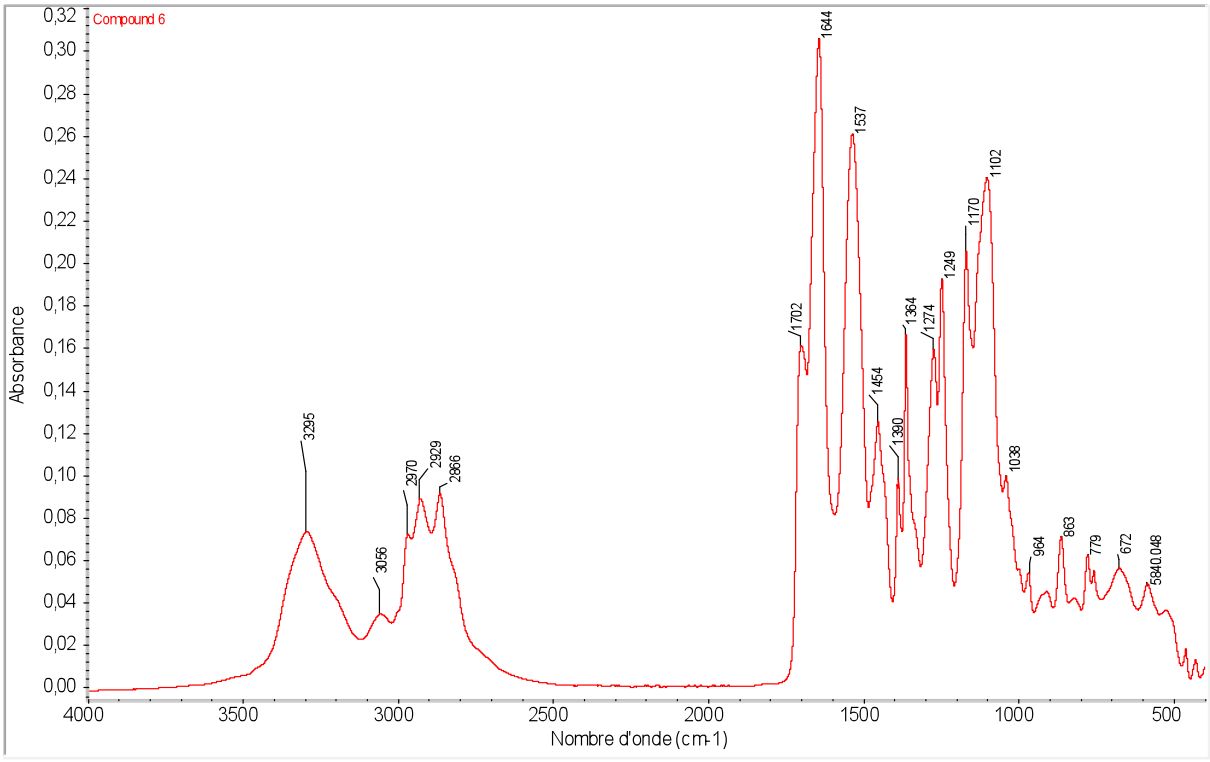


^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 2H, -N*H*-), 5.65 (s, 1H, -N*H*-), 3.56 – 3.47 (m, 8H, -C*H*$_2$-O-C*H*$_2$-C*H*$_2$-O-C*H*$_2$-), 3.24 (dd, *J* = 11.7, 5.8 Hz, 6H, -CONH-C*H*$_2$-), 2.81 – 2.69 (m, 8H, N-C*H*$_2$-, -C*H*$_2$-NH$_2$), 2.62 (t, *J* = 5.3 Hz, 2H, -C*H*$_2$-N), 2.33 (t, *J* = 6.2 Hz, 4H, -C*H*$_2$-CONH-), 2.03 (s, 4H, -N*H*$_2$), 1.40 (s, 9H, -C*H*$_3$).

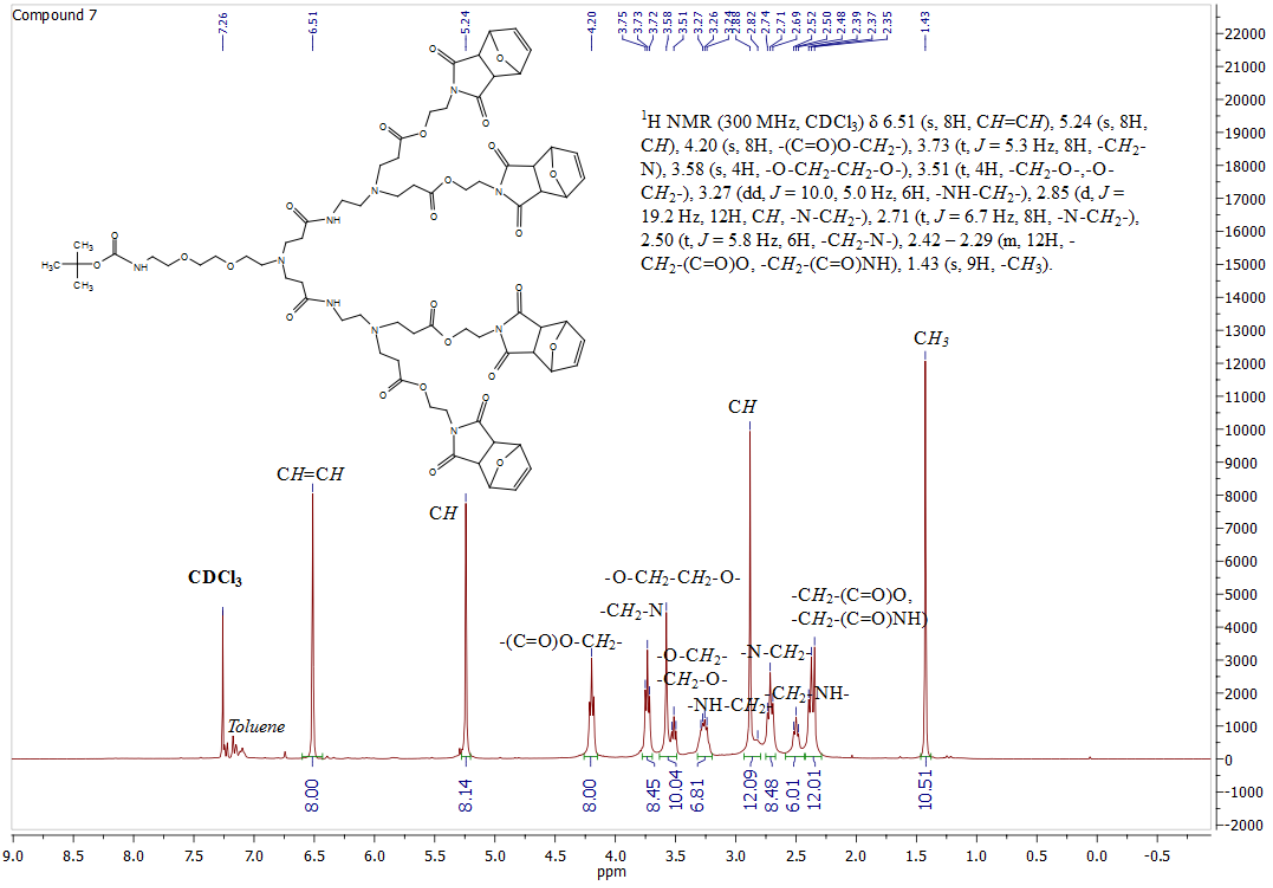


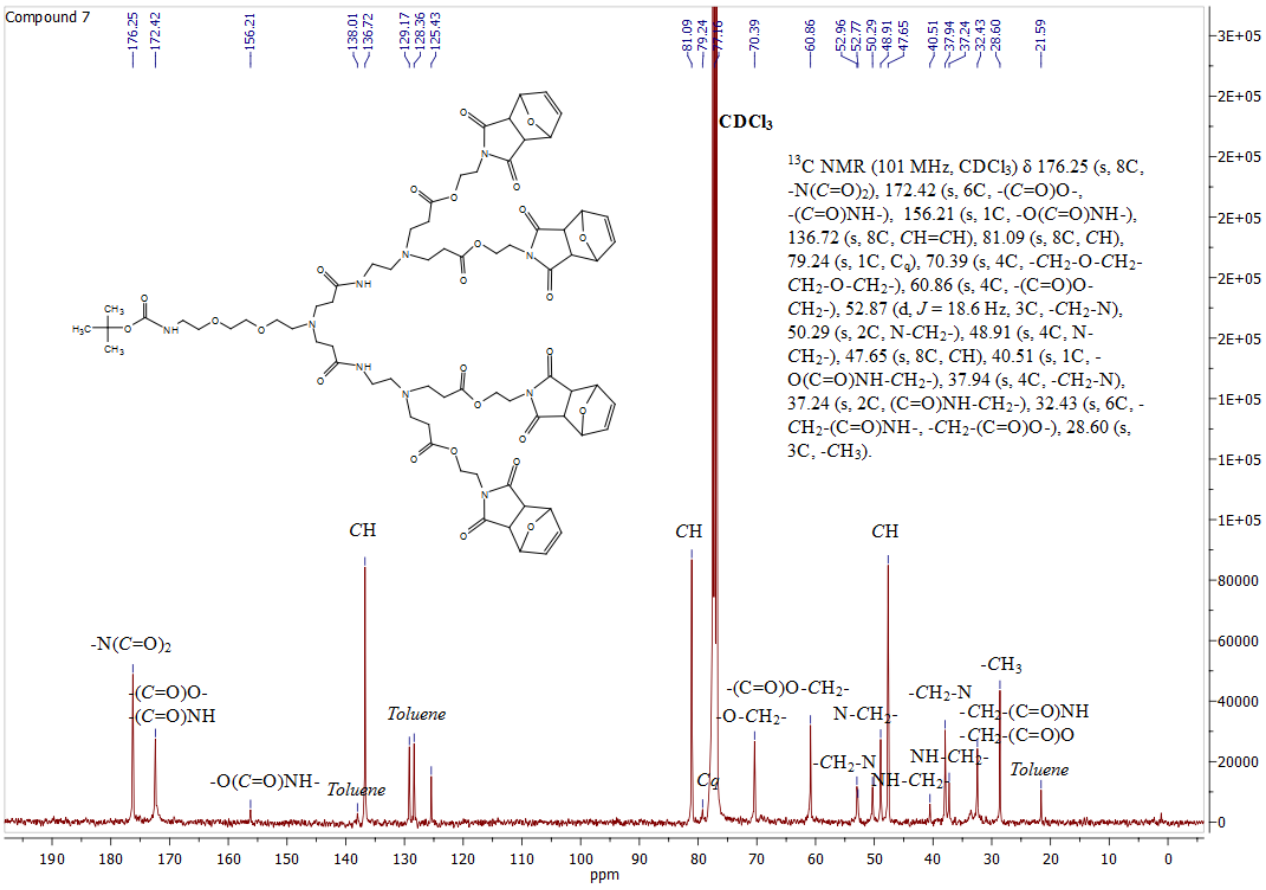
^{13}C NMR (151 MHz, $CDCl_3$) δ 171.93 (s, 2C, -(*C*=O)NH), 155.26 (s, 1C, -O(*C*=O)NH), 78.22 (s, 1C, *Cq*), 69.32 (s, 2C, -O-*C*H$_2$-*C*H$_2$-O-), 68.34 (s, 2C, -O-*C*H$_2$-), 52.77 (s, 1C, -*C*H$_2$-N), 50.01 (s, 2C, -N-*C*H$_2$-), 41.14 (s, 1C, -OCONH-*C*H$_2$-), 40.46 (s, 2C, -*C*H$_2$-NH$_2$), 39.38 (s, 2C, -CONH-*C*H$_2$-), 33.36 (s, 2C, -*C*H$_2$-CONH -), 27.52 (s, 3C, -*C*H$_3$).

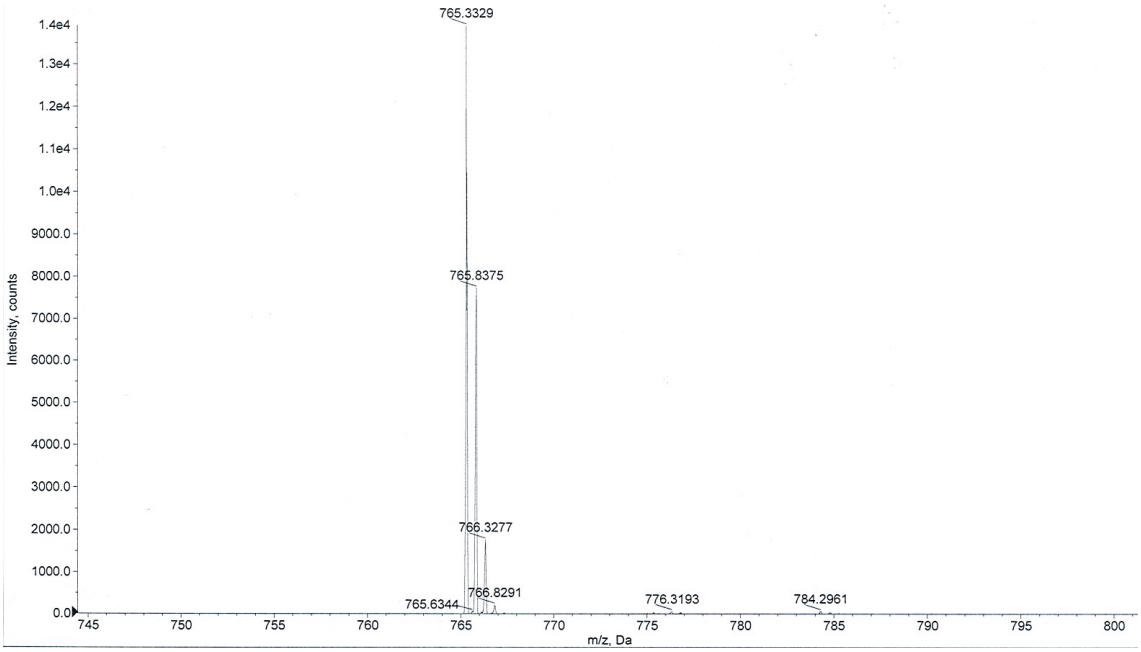

499.3243
377.2900
477.3398
263.2088
275.2086
Intensity, counts
m/z, Da

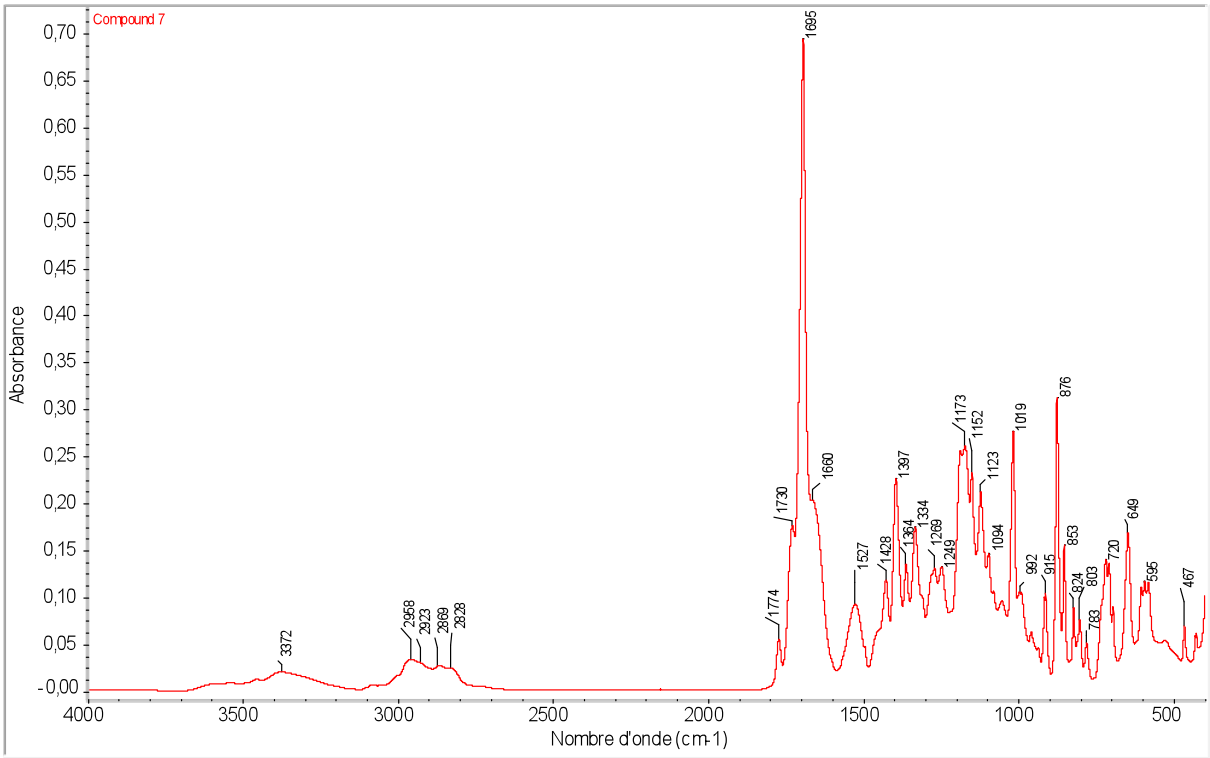

Compound 6
Absorbance
Nombre d'onde (cm-1)
3295
3056
2970
2929
2866
1702
1644
1537
1454
1390
1364
1274
1249
1170
1102
1038
964
863
779
672
5840.048

Compound 7

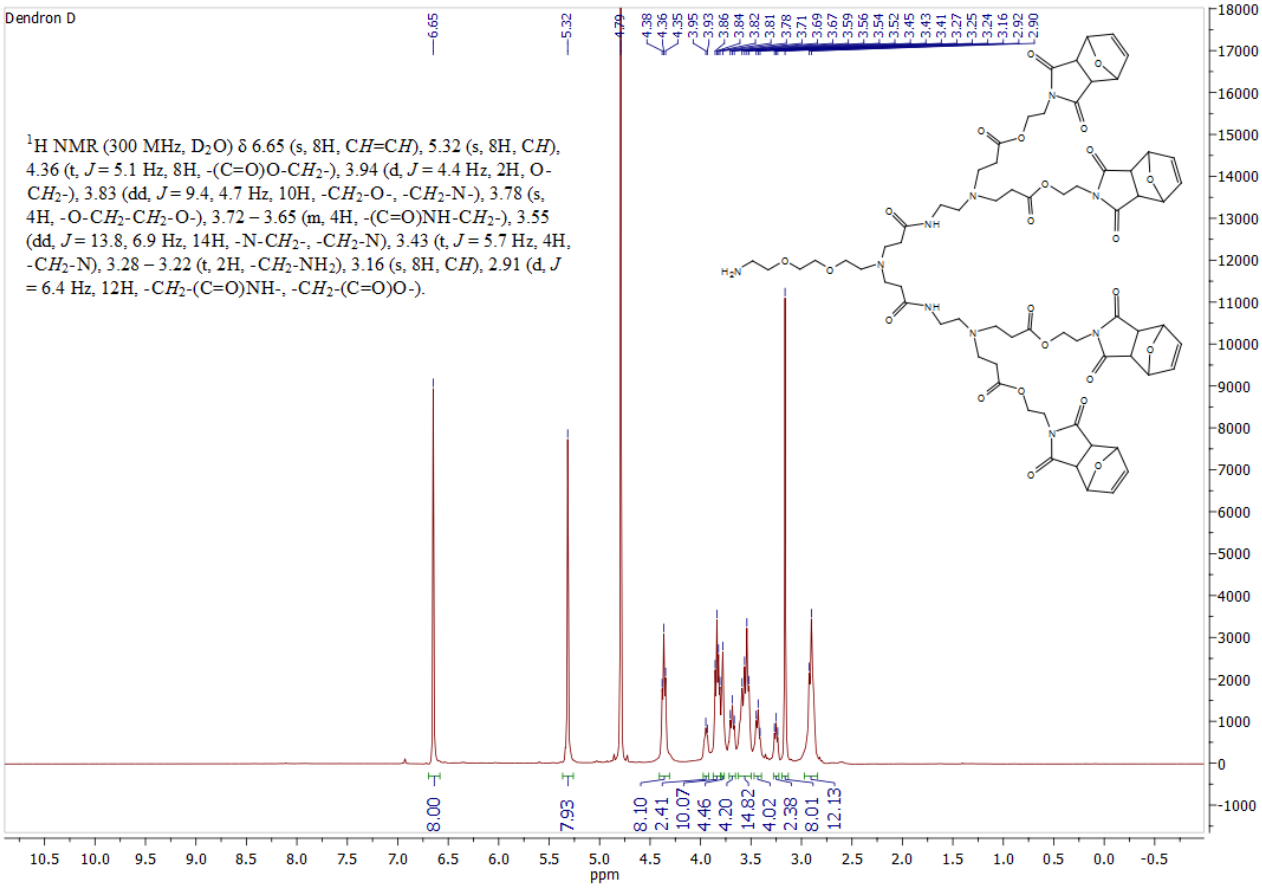

Compound 7
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 8H, CH=CH), 5.24 (s, 8H, CH), 4.20 (s, 8H, -(C=O)O-CH_2-), 3.73 (t, J = 5.3 Hz, 8H, -CH_2-N), 3.58 (s, 4H, -O-CH_2-CH_2-O-), 3.51 (t, 4H, -CH_2-O-,-O-CH_2-), 3.27 (dd, J = 10.0, 5.0 Hz, 6H, -NH-CH_2-), 2.85 (d, J = 19.2 Hz, 12H, CH, -N-CH_2-), 2.71 (t, J = 6.7 Hz, 8H, -N-CH_2-), 2.50 (t, J = 5.8 Hz, 6H, -CH_2-N-), 2.42 – 2.29 (m, 12H, -CH_2-(C=O)O, -CH_2-(C=O)NH), 1.43 (s, 9H, -CH_3).
CH_3
CH
CH=CH
CH
$CDCl_3$
-O-CH_2-CH_2-O-
-CH_2-N
-CH_2-(C=O)O,
-CH_2-(C=O)NH
-(C=O)O-CH_2-
-O-CH_2-
-CH_2-O-
-NH-CH_2-
-N-CH_2-
-CH_2-NH-
Toluene
8.00
8.14
8.00
8.45
10.04
6.81
12.09
8.48
6.01
12.01
10.51
ppm


Compound 7
^{13}C NMR (101 MHz, $CDCl_3$) δ 176.25 (s, 8C, -N(C=O)$_2$), 172.42 (s, 6C, -(C=O)O-, -(C=O)NH-), 156.21 (s, 1C, -O(C=O)NH-), 136.72 (s, 8C, CH=CH), 81.09 (s, 8C, CH), 79.24 (s, 1C, C_q), 70.39 (s, 4C, -CH_2-O-CH_2-CH_2-O-CH_2-), 60.86 (s, 4C, -(C=O)O-CH_2-), 52.87 (d, J = 18.6 Hz, 3C, -CH_2-N), 50.29 (s, 2C, N-CH_2-), 48.91 (s, 4C, N-CH_2-), 47.65 (s, 8C, CH), 40.51 (s, 1C, -O(C=O)NH-CH_2-), 37.94 (s, 4C, -CH_2-N), 37.24 (s, 2C, (C=O)NH-CH_2-), 32.43 (s, 6C, -CH_2-(C=O)NH-, -CH_2-(C=O)O-), 28.60 (s, 3C, -CH_3).
$CDCl_3$
CH
CH
CH
-N(C=O)$_2$
-(C=O)O-
-(C=O)NH
-O(C=O)NH-
Toluene
Toluene
C_q
-(C=O)O-CH_2-
-O-CH_2-
-CH_2-N
N-CH_2-
-CH_2-N
NH-CH_2-
-CH_3
-CH_2-(C=O)NH
-CH_2-(C=O)O
Toluene
ppm


765.3329
765.8375
766.3277
766.8291
765.6344
776.3193
784.2961
Intensity, counts
m/z, Da


Compound 7
Absorbance
Nombre d'onde (cm-1)
3372
2958
2923
2869
2828
1774
1730
1695
1660
1527
1428
1397
1364
1334
1269
1249
1173
1152
1123
1094
1019
992
915
876
853
824
803
783
720
649
595
467

Dendron D


Dendron D
^{1}H NMR (300 MHz, D_2O) δ 6.65 (s, 8H, C*H*=C*H*), 5.32 (s, 8H, C*H*), 4.36 (t, *J* = 5.1 Hz, 8H, -(C=O)O-CH_2-), 3.94 (d, *J* = 4.4 Hz, 2H, O-CH_2-), 3.83 (dd, *J* = 9.4, 4.7 Hz, 10H, -CH_2-O-, -CH_2-N-), 3.78 (s, 4H, -O-CH_2-CH_2-O-), 3.72 – 3.65 (m, 4H, -(C=O)NH-CH_2-), 3.55 (dd, *J* = 13.8, 6.9 Hz, 14H, -N-CH_2-, -CH_2-N), 3.43 (t, *J* = 5.7 Hz, 4H, -CH_2-N), 3.28 – 3.22 (t, 2H, -CH_2-NH_2), 3.16 (s, 8H, C*H*), 2.91 (d, *J* = 6.4 Hz, 12H, -CH_2-(C=O)NH-, -CH_2-(C=O)O-).
6.65
5.32
4.79
4.38
4.36
4.35
3.95
3.93
3.86
3.84
3.82
3.81
3.78
3.71
3.69
3.67
3.59
3.56
3.54
3.52
3.45
3.43
3.41
3.27
3.25
3.24
3.16
2.92
2.90
8.00
7.93
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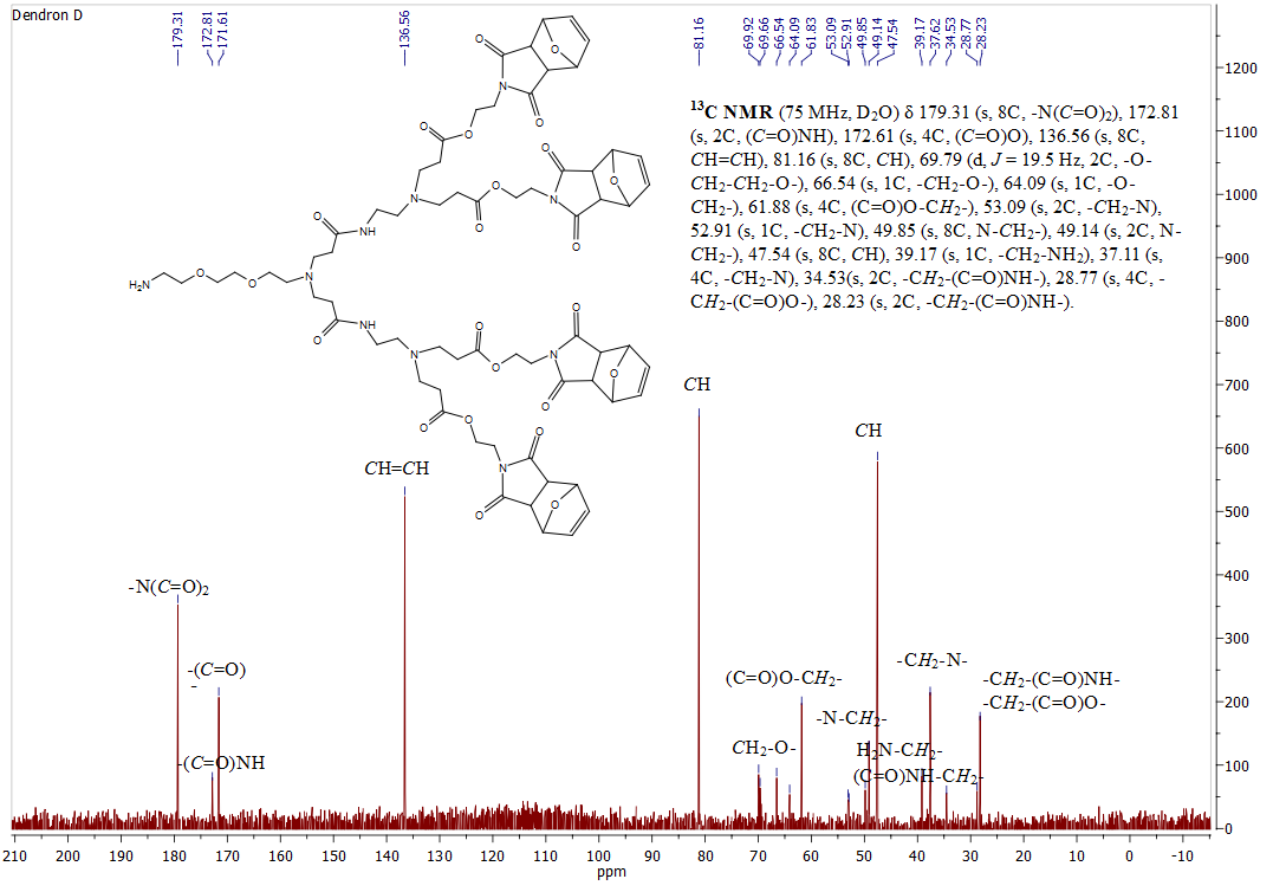
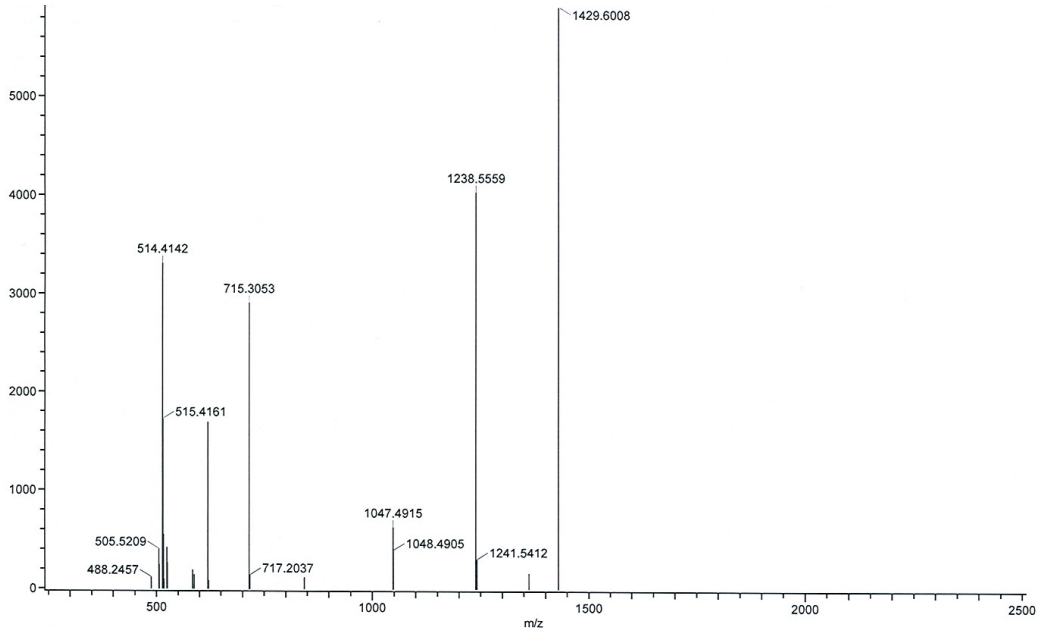
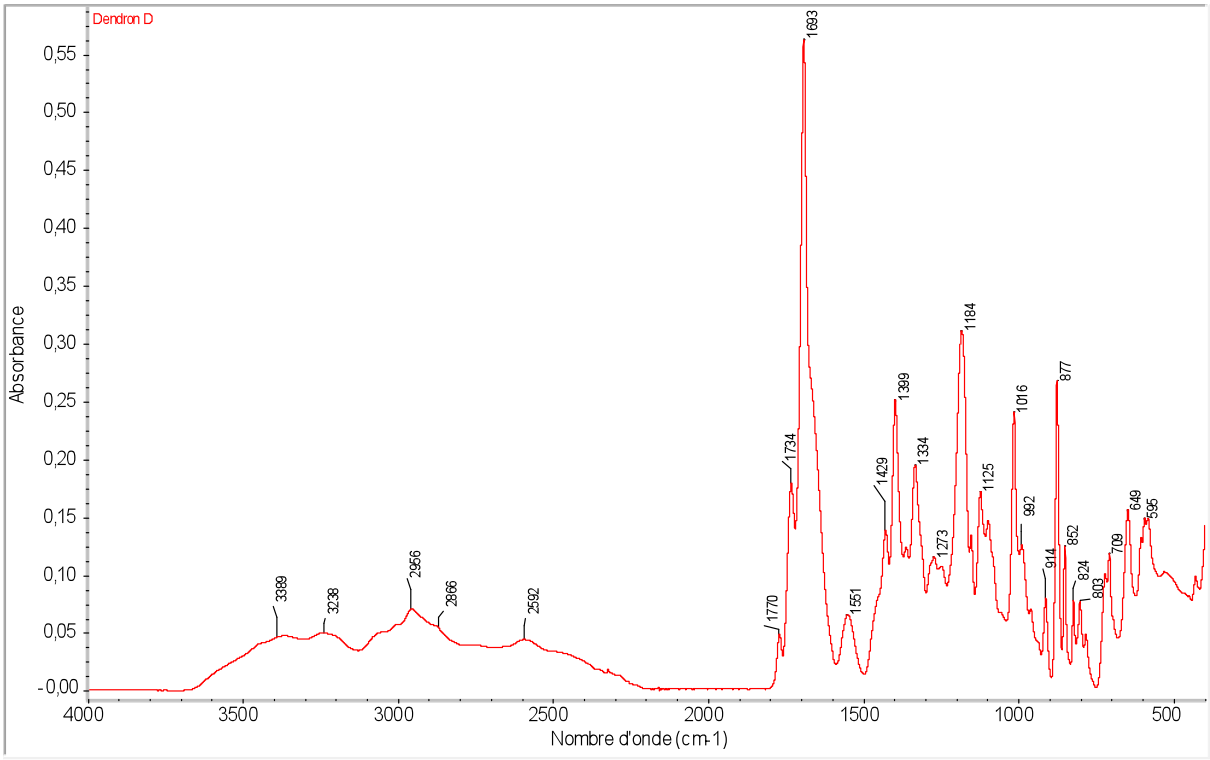
2.41
10.07
4.46
4.20
14.82
4.02
2.38
8.01
12.13
ppm


Dendron D
^{13}C NMR (75 MHz, D_2O) δ 179.31 (s, 8C, -N(*C*=O)$_2$), 172.81 (s, 2C, (*C*=O)NH), 172.61 (s, 4C, (*C*=O)O), 136.56 (s, 8C, *C*H=*C*H), 81.16 (s, 8C, *C*H), 69.79 (d, *J* = 19.5 Hz, 2C, -O-CH_2-CH_2-O-), 66.54 (s, 1C, -*C*H_2-O-), 64.09 (s, 1C, -O-*C*H_2-), 61.88 (s, 4C, (C=O)O-*C*H_2-), 53.09 (s, 2C, -*C*H_2-N), 52.91 (s, 1C, -*C*H_2-N), 49.85 (s, 8C, N-*C*H_2-), 49.14 (s, 2C, N-*C*H_2-), 47.54 (s, 8C, *C*H), 39.17 (s, 1C, -*C*H_2-NH_2), 37.11 (s, 4C, -*C*H_2-N), 34.53(s, 2C, -*C*H_2-(C=O)NH-), 28.77 (s, 4C, -*C*H_2-(C=O)O-), 28.23 (s, 2C, -*C*H_2-(C=O)NH-).
179.31
172.81
171.61
136.56
81.16
69.92
69.66
66.54
64.09
61.83
53.09
52.91
49.85
49.14
47.54
39.17
37.62
34.53
28.77
28.23
CH
CH
CH=CH
-N(C=O)2
-(C=O)-
-(C=O)NH
(C=O)O-CH2-
-N-CH2-
CH2-O-
-CH2-N-
H2N-CH2-
(C=O)NH-CH2-
-CH2-(C=O)NH-
-CH2-(C=O)O-
ppm


1429.6008
1238.5559
514.4142
715.3053
515.4161
1047.4915
1048.4905
505.5209
488.2457
717.2037
1241.5412
5000
4000
3000
2000
1000
0
500
1000
1500
2000
2500
m/z


Dendron D
Absorbance
0,55
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
-0,00
4000
3500
3000
2500
2000
1500
1000
500
Nombre d'onde (cm-1)
3389
3238
2956
2866
2592
1770
1734
1693
1551
1429
1399
1334
1273
1184
1125
1016
992
914
877
852
824
803
709
649
595

6. Figures

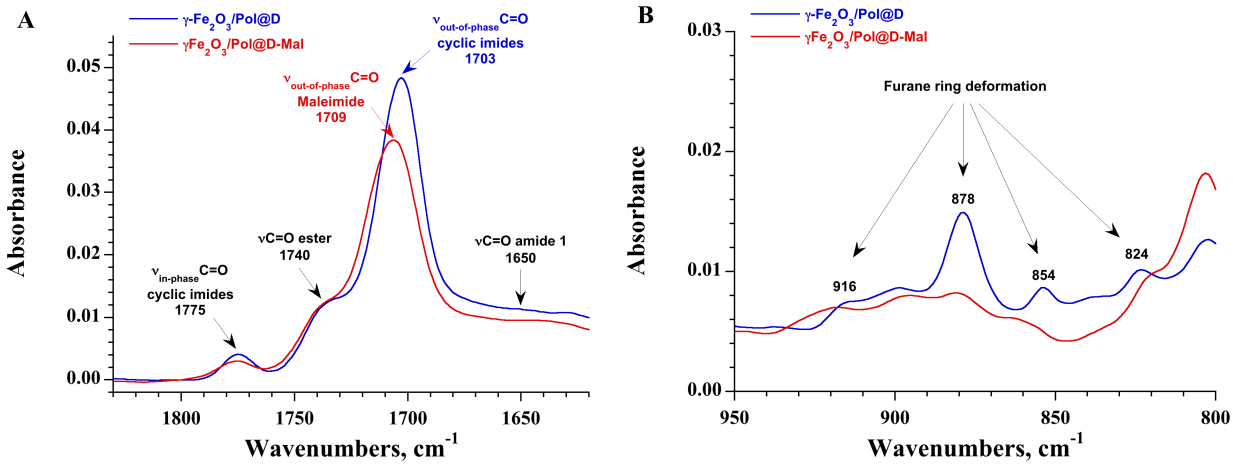


Fig. S1. ATR FTIR spectra of **γ-Fe_2O_3/Pol@D** and **γ-Fe_2O_3/Pol@D-Mal** in the A) 1830 - 1620 cm^{-1} and B) 950 - 800 cm^{-1} spectral ranges, after subtraction of the native **γ-Fe_2O_3/Pol-COOH** data.

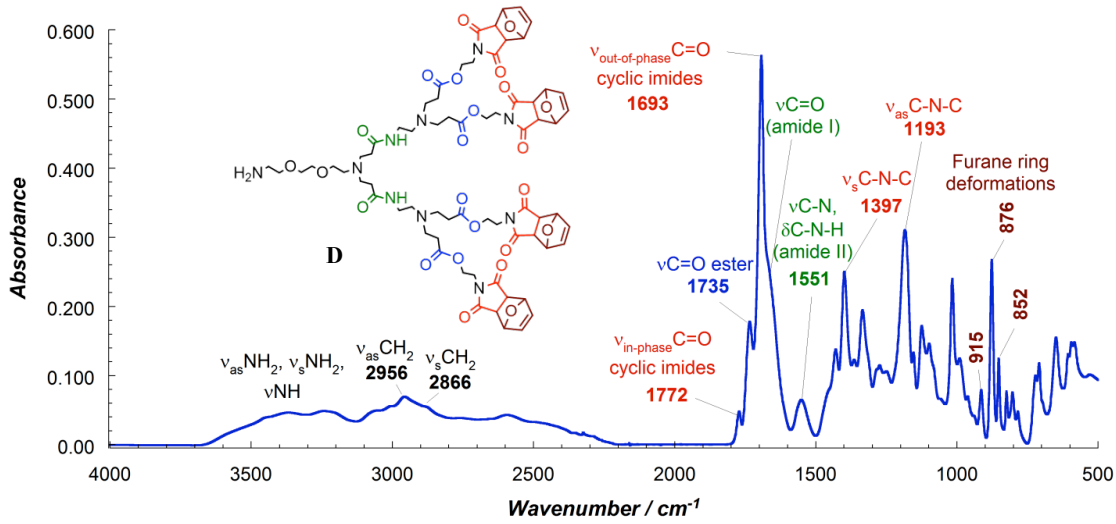


Fig. S2. ATR FT-IR spectrum of dendron **D**.

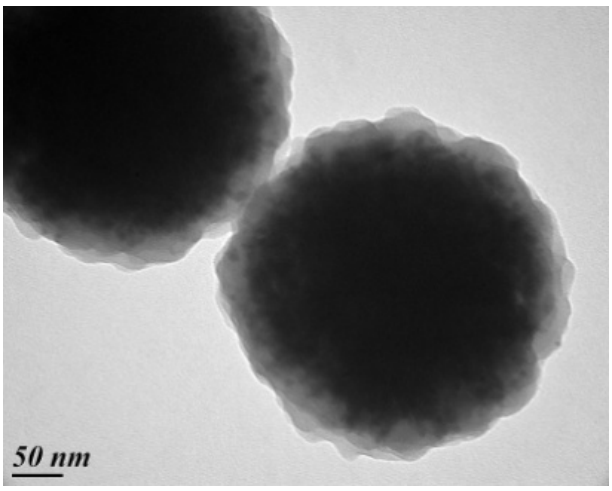


Fig. S3. TEM image of native **γ-Fe_2O_3/Polymer** MNPs.

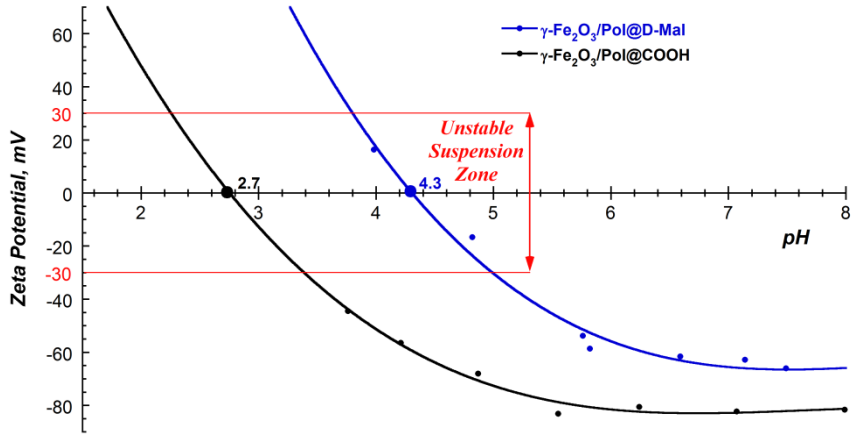


Fig. S4. pH effect on the surface charge of native **γ-Fe_2O_3/Pol-COOH** MNPs (solid line in black) and maleimide functionalized **γ-Fe_2O_3/Pol@D-Mal** MNPs

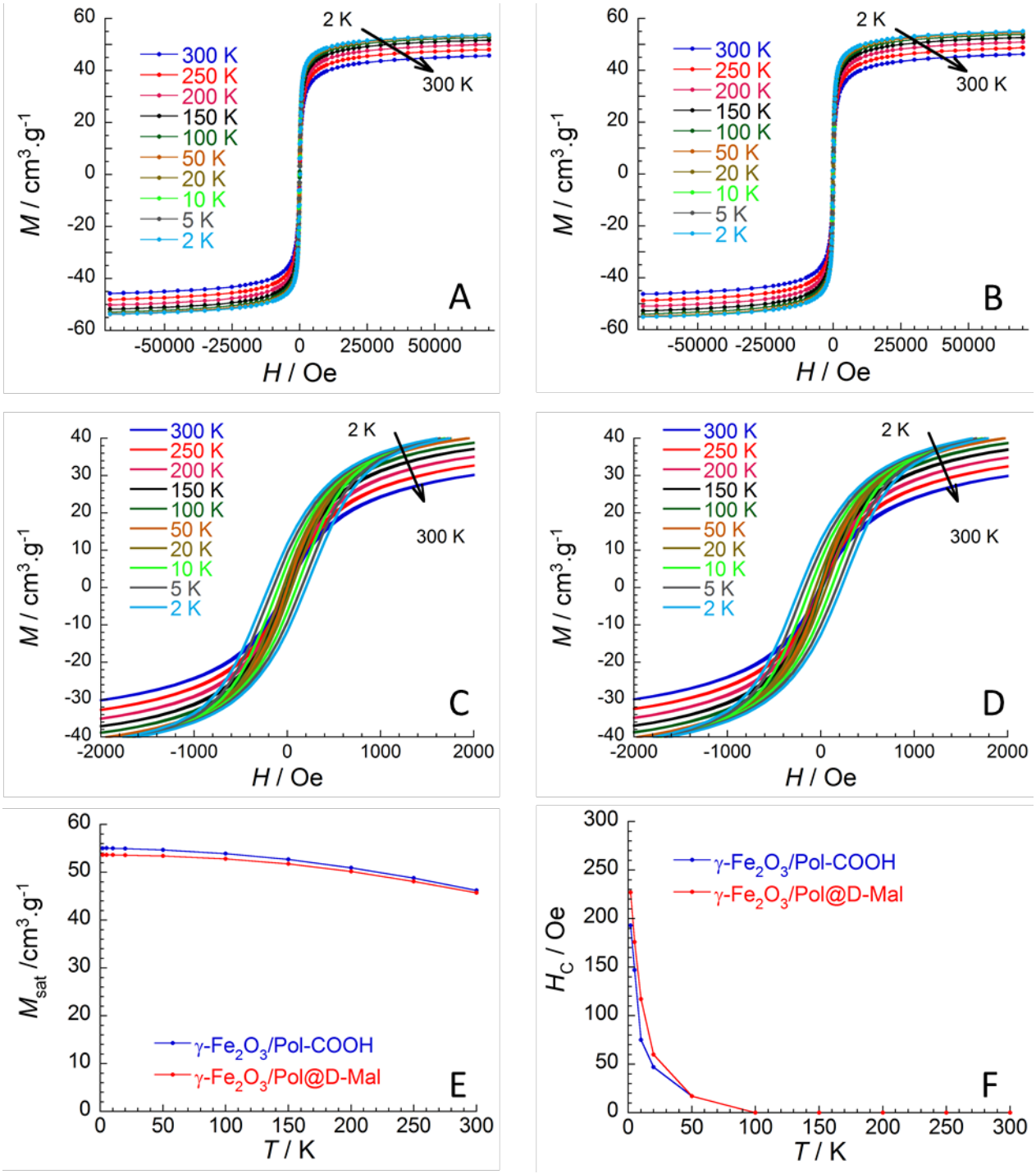


Fig. S5. Field dependence of magnetization at different temperatures for **γ-Fe_2O_3/Pol-COOH** (A, C) and **γ-Fe_2O_3/Pol@D-Mal** (B, D), where *M* is the magnetization of the sample and *H* is the applied magnetic field. Temperature dependence of the magnetization saturation (E) and of the coercive field (F) for **γ-Fe_2O_3/Pol-COOH** (blue curves) and **γ-Fe_2O_3/Pol@D-Mal** (red curves).

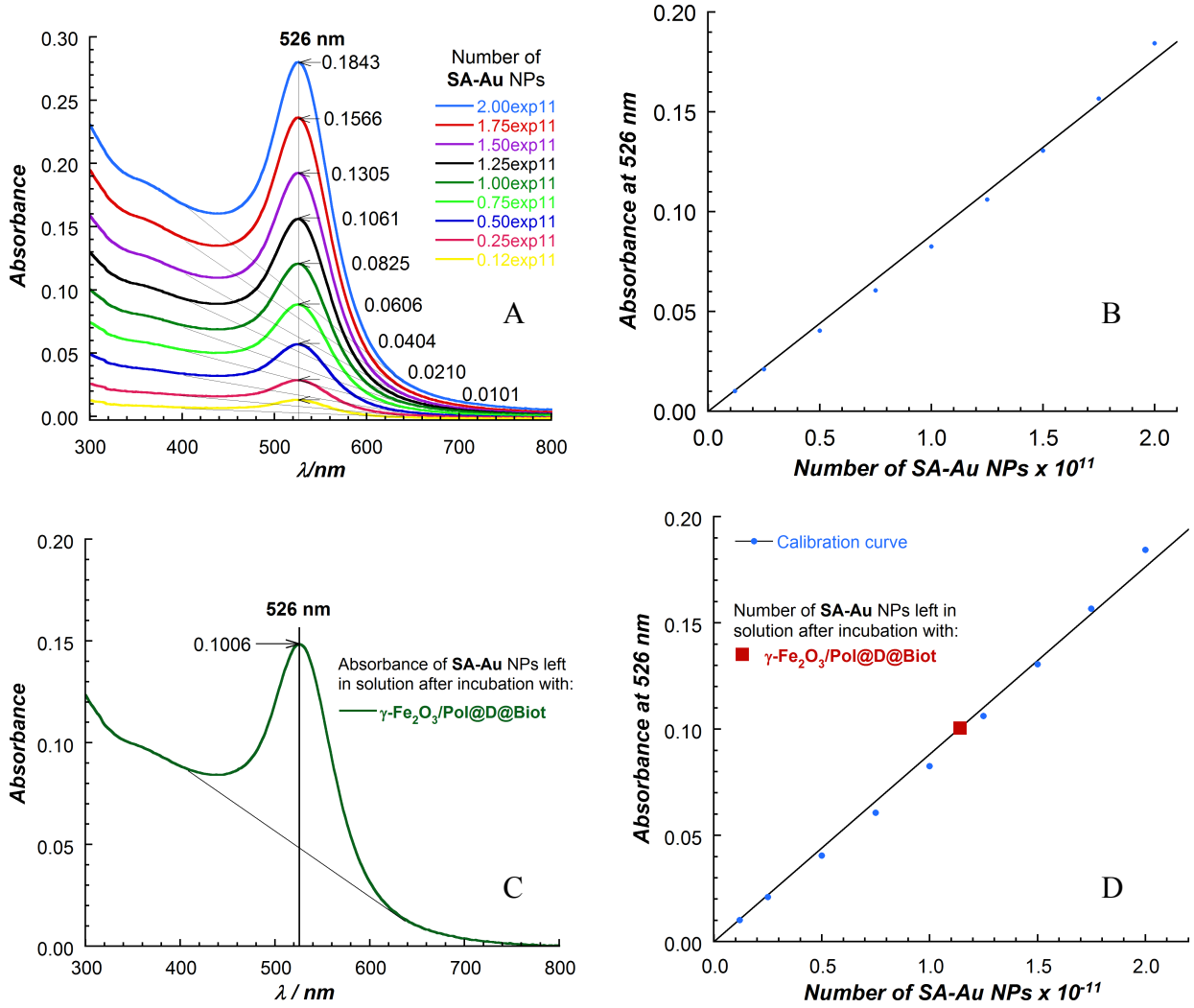


Fig. S6. Calibration plot (A) and calibration curve (B) of 15 nm SA-Au NPs. Absorption spectrum of the 15 nm SA-Au NPs' solution, recovered after the immobilization reactions (C) and (D) dependence of Absorbance at 526 nm as function of 15 nm SA-Au NPs number left in solution after the immobilization reaction.

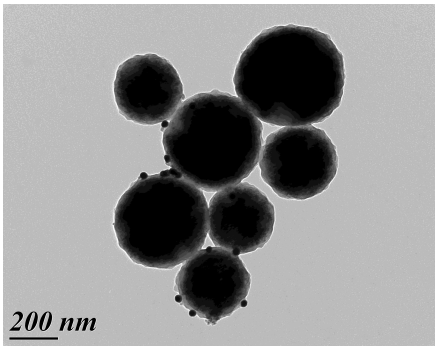


Fig. S7. TEM image of **γ-Fe_2O_3/Pol@D-Mal** MNPs recovered after their incubation with 20 nm H_2N-PEG-Au NPs.