Electronic Supplementary Material (ESI) for Soft Matter.
This journal is © The Royal Society of Chemistry 2020

# Clickable Iron Oxide NPs Based on Catechol Derived Ligands: Synthesis and Characterization.

Esther Pozo-Torres[a,‡], Carlos Caro[a,b,‡], Ashish Avasthi[b], Jose María Páez-Muñoz[b], María Luisa García-Martín[b,c,*], Inmaculada Fernández[a,*], Manuel Pernia Leal[a,*].

[a]Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, 41012 Seville (Spain)

[b]BIONAND, Andalusian Centre for Nanomedicine and Biotechnology, C/ Severo Ochoa, 35, Junta de Andalucía, Universidad de Málaga, 29590 Campanillas Málaga, Spain

[c]Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, 29590 Málaga, Spain

‡Esther Pozo-Torres and Carlos Caro contributed equally to this work.

* email: mpernia@us.es; inmaff@us.es; mlgarcia@bionand.es

Index

2.3. FTIR

2.4. TGA

2.5. Relaxivities

2.6. *in vitro* cell evaluation.

## 1. Experimental Section

**1.1. Materials**. Chemicals and solvents were obtained from commercial suppliers (Sigma Aldrich, Acros Organics and Fisher Scientific) and used as received. Iron (III) chloride, Sodium Oleate, Oleic acid 99%, 1-octadecene, gallic acid, 3,4-dihydroxycinnamic acid 98%, poly ethylene glycol 1500 Da (1.5 kDa), tetraethylene glycol, 1-octadecene, triethylamine, 4-dimethylaminopyridine, dicyclohexyl carbodiimide (DCC), *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*,*N*-Diisopropylethylamine (DIPEA), 1-hydroxybenzotriazole (HOBt), hydrochloric acid (HCl), Sodium sulfate ($Na_2SO_4$), 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle Medium (DMEM). As solvents, Milli-Q water (18.2 MΩ, filtered with filter pore size 0.22 μM) from Millipore, toluene, ethanol, acetone, dimethylsulphoxide (DMSO), hexane, chloroform, dichloromethane and tetrahydrofuran were used anhydrous and HPLC grade.

### 1.2. Synthesis of the Nanoparticles.

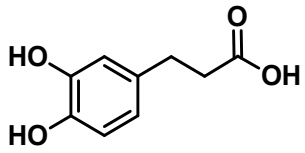
*Synthesis of Iron Oleate.* The synthesis was carried out following a previously published procedure. [Park et al., Nat. Mat. 3 (2004) 891–89] A mixture of iron chloride (10.8 g, 40 mmol) and sodium oleate (36.5 g, 120 mmol) was dissolved in ethanol (80 ml), distilled water (60 ml) and hexane (140 ml). The resulting solution was heated to 60 °C for 4 h under a $N_2$ atmosphere. Next, the mixture was cooled down to room temperature

and the organic phase was separated and washed 3 times with distilled water. Finally, solvents were evaporated in the rotavapor resulting in a wax-like compound.

*Synthesis of Magnetic Nanoparticles.* Iron oleate previously prepared (1 g, 1.1 mmol), oleic acid (0.3 g, 1 mmol) and 5.15 g of 1-octadecene were heated to 320ºC for 1 h (heating ramp of 3°C/min) under an inert atmosphere. Then, the mixture was cooled down to room temperature and washed several times with ethanol-acetone (1:1) as precipitating agents, and subsequently centrifuged. Finally, the nanoparticles were re-suspended in toluene.

**1.3 Synthesis of the Ligands.**

**Synthesis of dihydrocaffeic acid.**




A solution of caffeic acid (8.9 mmol, 1.6g) and Pd/C (0.1 g) in methanol (20 mL), in a round-bottom flask, under hydrogen atmosphere was stirred overnight at room temperature. The mixture was filtered through a plug of celite and the filtrate evaporated in vacuum. The crude was purfied by flash column chromatography, using AcOEt as eluent, to give the dihydrocaffeic acid (1.378g, 96%) as a yellowish solid. $^{1}$H NMR (500 MHz MeOD) δ (ppm): 6.66 (d, *J*=7.9 Hz, 1H), 6.64 (d, J= 2.1, 1H), 6.52 (dd, *J*= 8.3 Hz, *J*=2.0Hz, 1H), 2.75 (t, *J*=7.7Hz, 2H), 2.51 (t, *J*=7.7Hz, 2H). $^{13}$C NMR (125MHz, $CDCl_3$) δ (ppm): 177.0, 146.2, 144.6, 133.8, 120.5, 116.4, 37.2, 31.5. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_9H_{10}O_4$ Na 205.0491; found 205.0466. FTIR peaks ($cm^{-1}$): 1466 (C-H bend vibration), 1359 (C-H bend vibration), 1341 (C-H bend vibration), 1307 (anti-symmetric stretch vibration), 1268 (C-O stretch vibration), 1238

(C-O stretch vibration), 1092 (C-O-C stretch vibration), 942 (CH out-of-plane bending vibration).

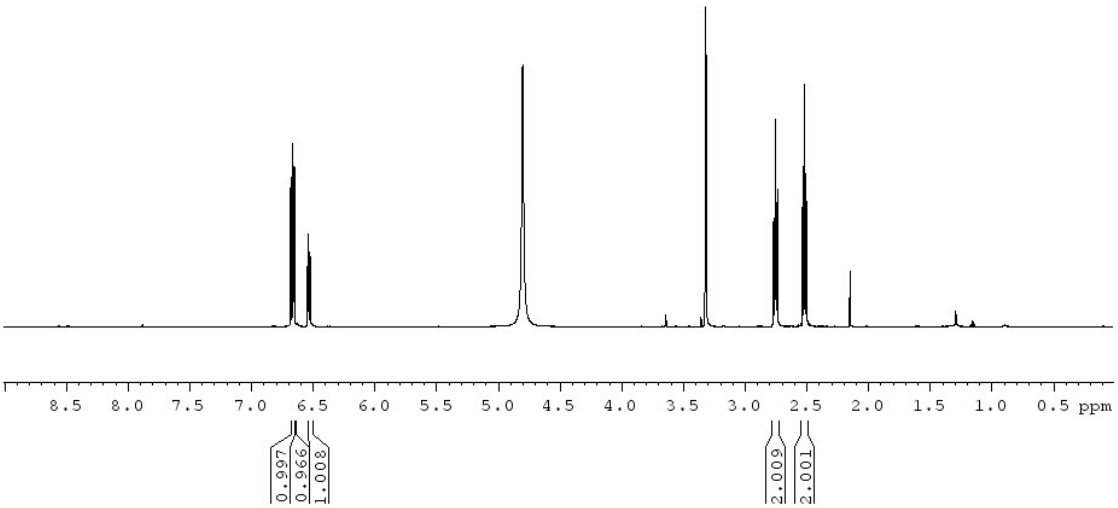



Figure S1. $^{1}H$ NMR (500 MHz MeOD) of dihydrocaffeic acid.

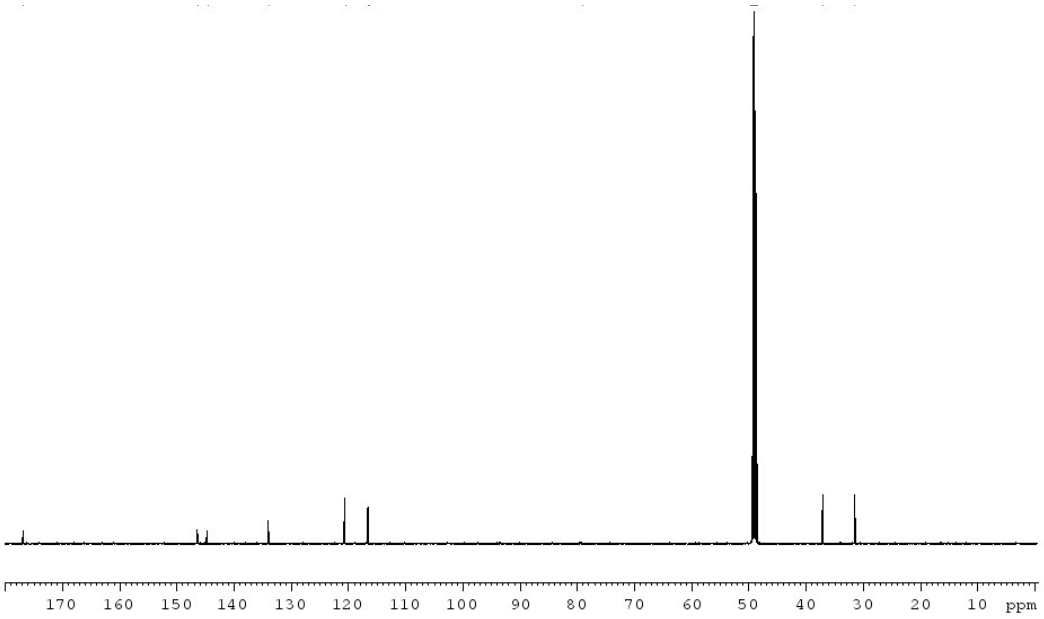
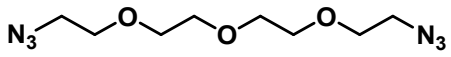



Figure S2. $^{13}C$ NMR (125 MHz, MeOD) of dihydrocaffeic acid.

**Synthesis of $N_3$-TEG-$N_3$ (8)**



To a solution of tetraethylene glycol (48 g, 0.25 mol), $Et_3N$ (85 mL, 0.62 mol) and THF (200 mL) at 0ºC was added dropwise methanesulfonyl chloride (44 mL, 0.57 mol). The reaction mixture was then allowed to warm to room temperature and then it was stirred vigorously overnight. The solution was diluted with $CH_2Cl_2$ (250 mL) and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic solution was dried over $Na_2SO_4$, and the solvents were removed under vacuum. The crude was taken to the next step without any further purification.

To a solution of dimesylated compound in EtOH was added $NaN_3$ (48.2 g, 0.74 mol). The mixture was heated at reflux overnight, cooled down to room temperature and concentrated in the rota-evaporator. The residue was diluted with $CH_2Cl_2$ (250 mL), washed with brine (50 mL), and dried over $Na_2SO_4$. Solvent was removed under vacuum to yield the crude product. The crude was purified by silica gel chromatography ($CH_2Cl_2$/MeOH, 20:1) to give **8** as a yellowish liquid (56 g, 97%). $^1$H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.67-3.62 (m, 12H), 3.38-3.30 (m, 4H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 70.8, 70.1, 50.8. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_8H_{16}N_6O_3Na$ 267.7776; found 267.1172.

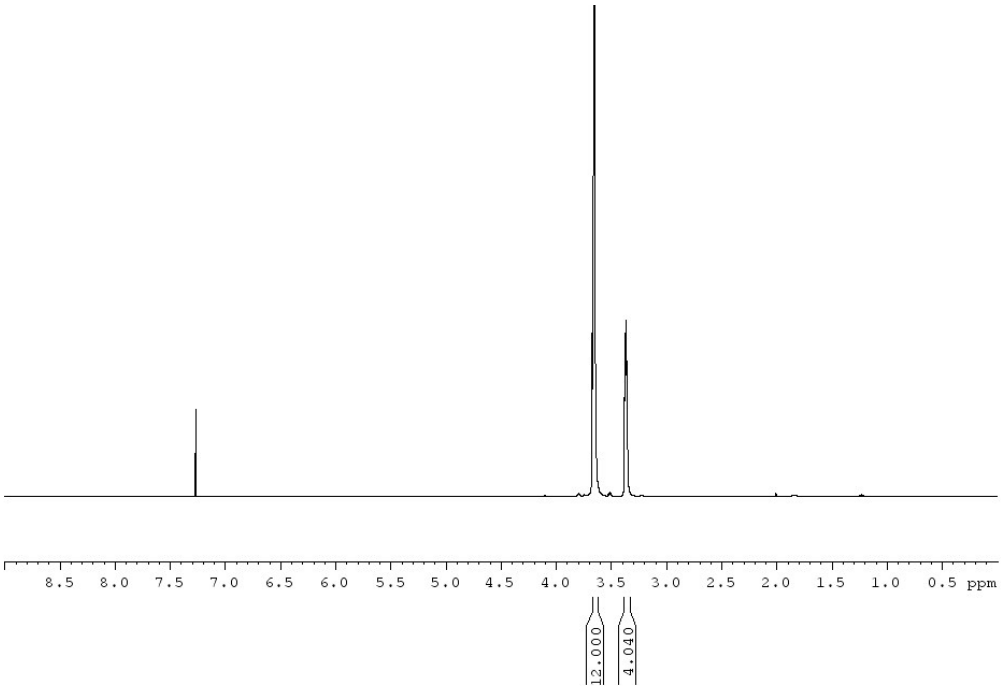



Figure S3. $^{1}H$ NMR (500 MHz, $CDCl_3$) of $N_3$-TEG-$N_3$ (**8**).

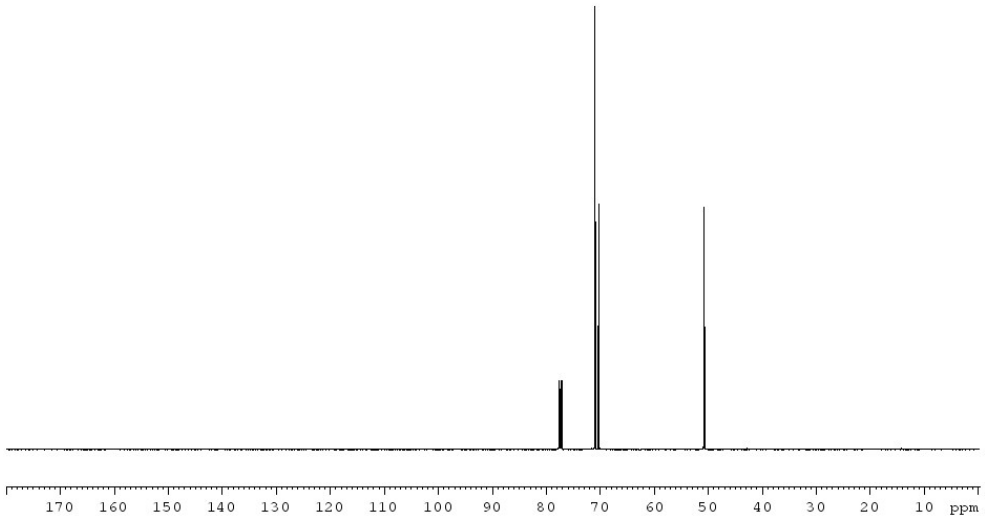
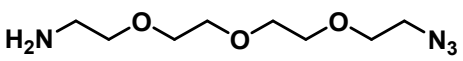



Figure S4. $^{13}C$ NMR (125 MHz, $CDCl_3$) of $N_3$-TEG-$N_3$ (**8**).

**Synthesis of $H_2N$-TEG-$N_3$ (9).**




The spacer $H_2N$-TEG-$N_3$ **(9)** was synthesized following the protocol reported by Mattoussi and col.[1] Briefly, to a solution of $N_3$-TEG-$N_3$ (**8**) (15 g, 61 mmol) in

EtOAc/HCl (1M) at 0°C was added dropwise a solution of $Ph_3P$ (17.7, 67.6 mmol) in EtOAc (20 mL). The mixture was heated slowly to room temperature and stirred for 10 h. Then, the aqueous phase was separated, washed with EtOAc, and it was taken to basic pH by adding KOH at 0°C. The desired product was extracted from the aqueous phase with DCM and the organic layer was dried with anhydrous $Na_2SO_4$. The solvent was rota-evaporated to give **9** as a yellowish liquid (10 g, 77%). $^1$H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.57-3.46 (m, 11H), 3.43 (t, *J*=5.1Hz, 2H), 3.26 (t, *J*=5.0Hz, 2H), 2.79 (t, *J*=5.0Hz, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 132.6, 131.8, 131.7, 128.3, 74.6, 71.6, 70.4, 70.4, 70.3, 69.9, 69.7. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_8H_{19}N_4O_3$ 219.1452; found 219.1446.

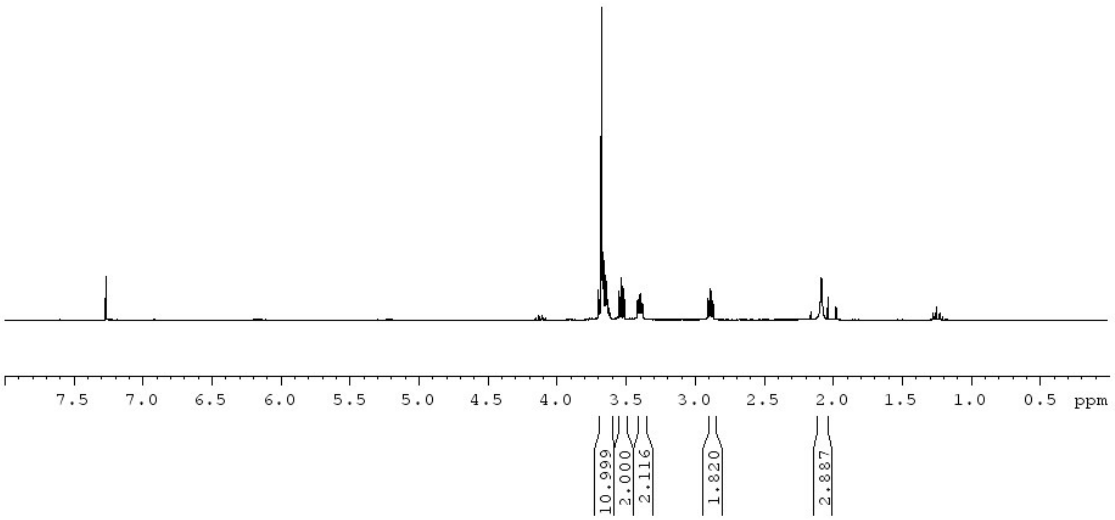



Figure S5. $^1$H NMR (500 MHz, $CDCl_3$) of $H_2N$-TEG-$N_3$ **(9)**.

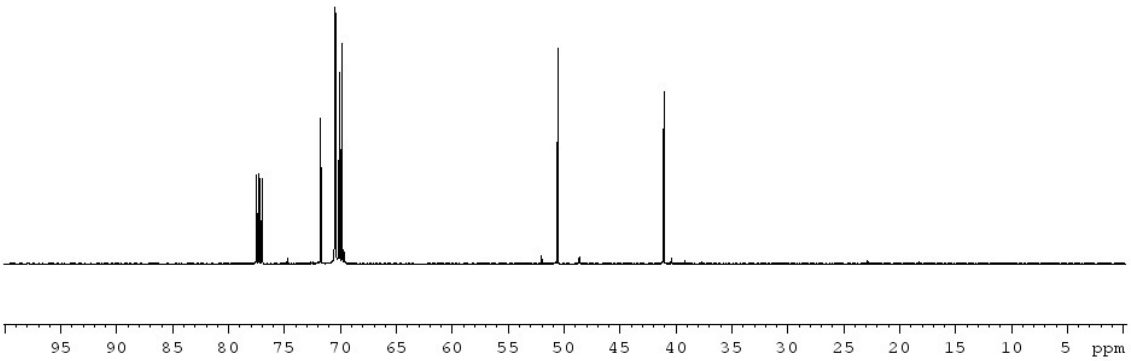
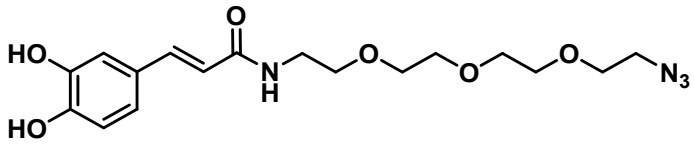



Figure S6. $^{13}C$ NMR (125 MHz, $CDCl_3$) of $H_2N$-TEG-$N_3$ **(9)**.

**Synthesis of CA-TEG-$N_3$ (1).**




CA-TEG-$N_3$ (**1**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols starting from **CA** (180 mg, 1 mmol) and **9** (218 mg, 1 mmol). The crude was purified by silica gel chromatography (DCM/MeOH 30:1 v/v) obtaining **1** as brownish syrup (102 mg, 27%). $^1H$ NMR (500 MHz, $CDCl_3$) δ (ppm): 7.46 (d, *J*=15.4Hz, 1H), 6.85 (t, *J*=10.2Hz, 4H), 6.19 (d, *J*=15.4Hz, 1H), 3.69-3.46 (m, 14H), 3.32 (t, *J*=5.0Hz, 2H). $^{13}C$ NMR (125MHz, $CDCl_3$) δ (ppm): 167.5, 147.0, 144.7, 141.9, 127.4, 121.7, 117.7, 115.8, 114.7, 70.7, 70.7, 70.6, 70.3, 70.1, 69.9, 50.8, 39.8. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{17}H_{24}N_4O_6$ Na 403.1588; found 403.1581.

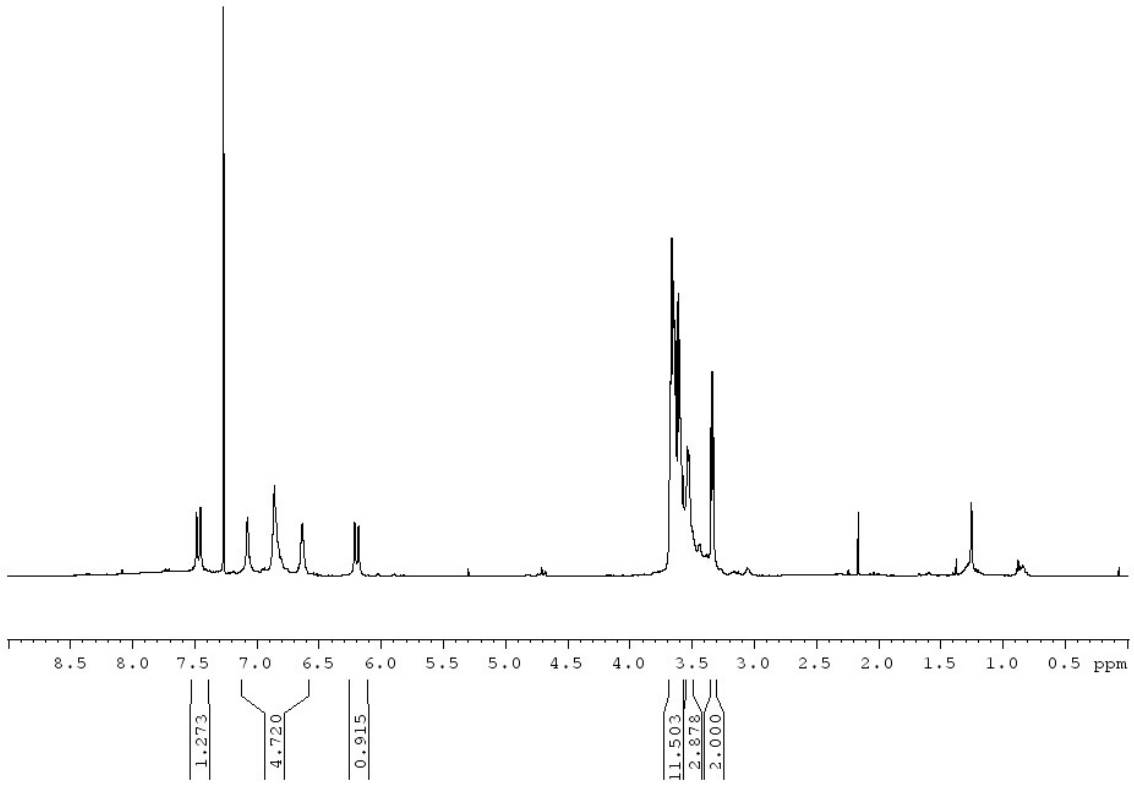



Figure S7. $^{1}H$ NMR (500 MHz, $CDCl_3$) of CA-TEG-$N_3$ (**1**).

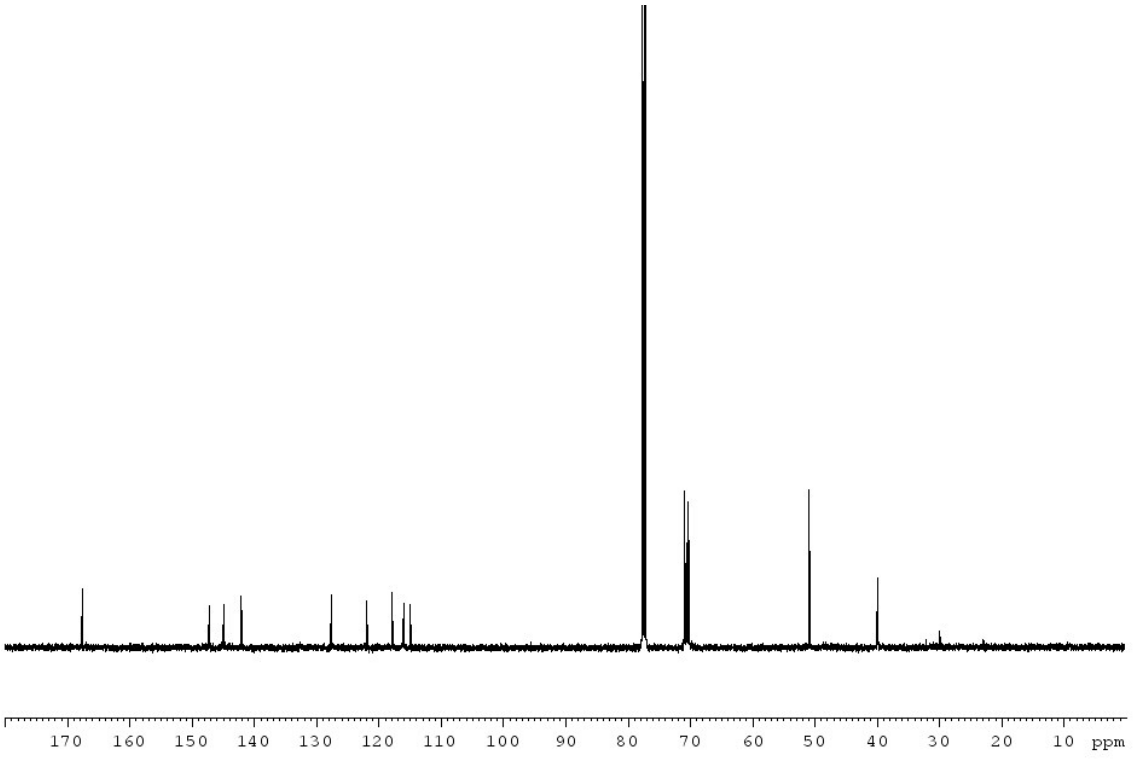
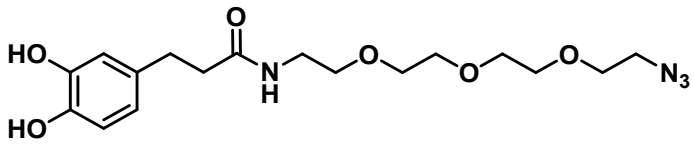



Figure S8. $^{13}C$ NMR (125 MHz, $CDCl_3$) of CA-TEG-$N_3$ (**1**).

**Synthesis of DHCA-TEG-$N_3$ (2).**




DHCA-TEG-$N_3$ (**2)** was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols starting from DH**CA** (182 mg, 1 mmol) and **9** (218 mg, 1 mmol). Purification by silica gel chromatography (DCM/MeOH 30:1 v/v) rendered **2** as brownish syrup (189 mg, 49%). $^1$H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.77 (d, *J* = 8.21 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 6.57 (dd, *J* = 8.1 Hz, *J* = 1.9 Hz, 1H), 3.69-3.59 (m, 8H), 3.58-3.52 (m, 2H), 3.46-3.41 (m, 2H), 3.46-3.32 (m, 4H), 2.81 (t, *J* = 7.1 Hz, 2H), 2.43 (t, *J* = 7.1 Hz, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ (ppm): 144.2, 143.1, 132.9, 120.4, 115.7, 70.8, 70.7, 70.1, 69.9, 50.8, 39.5, 38.6, 31.2. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{17}H_{24}N_4O_6$ Na 405.1745, found 405.1740

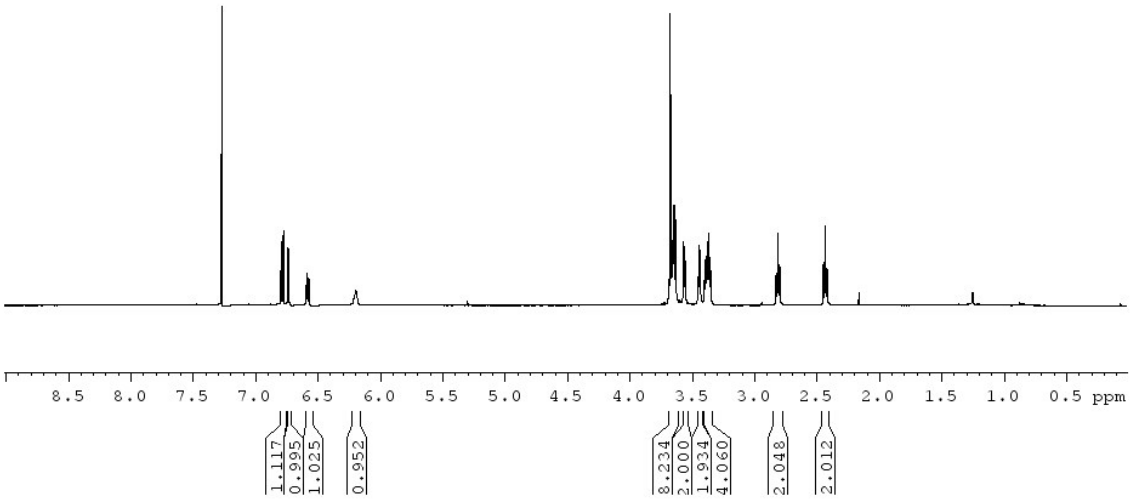



Figure S9. $^1$H NMR (500 MHz, $CDCl_3$) of DHCA-TEG-$N_3$ (**2**).

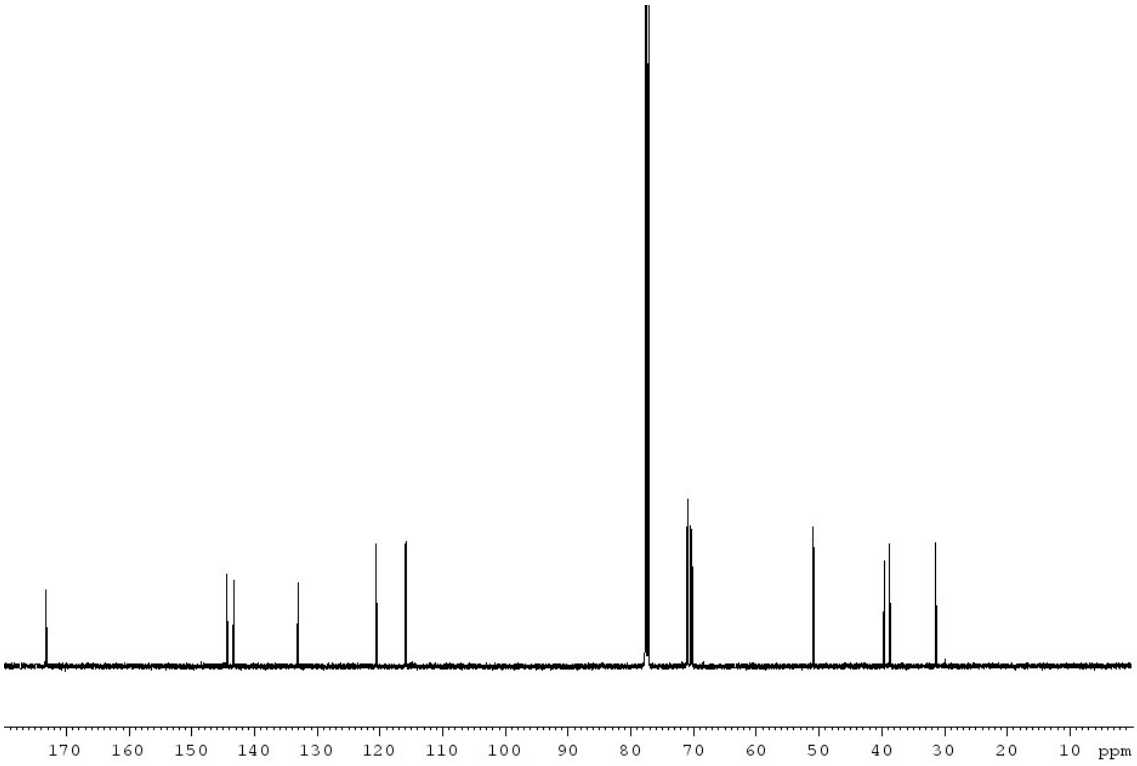
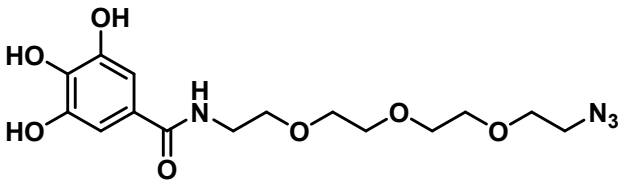



Figure S10. $^{13}C$ NMR (125 MHz, $CDCl_3$) of DHCA-TEG-$N_3$ (**2**).

**Synthesis of GA-TEG-$N_3$ (3).**




GA-TEG-$N_3$ (**3**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols starting from **GA** (170 mg, 1 mmol) and **9** (218 mg, 1 mmol). The desired compound was not possible to purify through chromatography and it was used in the following step without previous purification. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{15}H_{23}N_4O_7$ 371.1555, found 371.1561

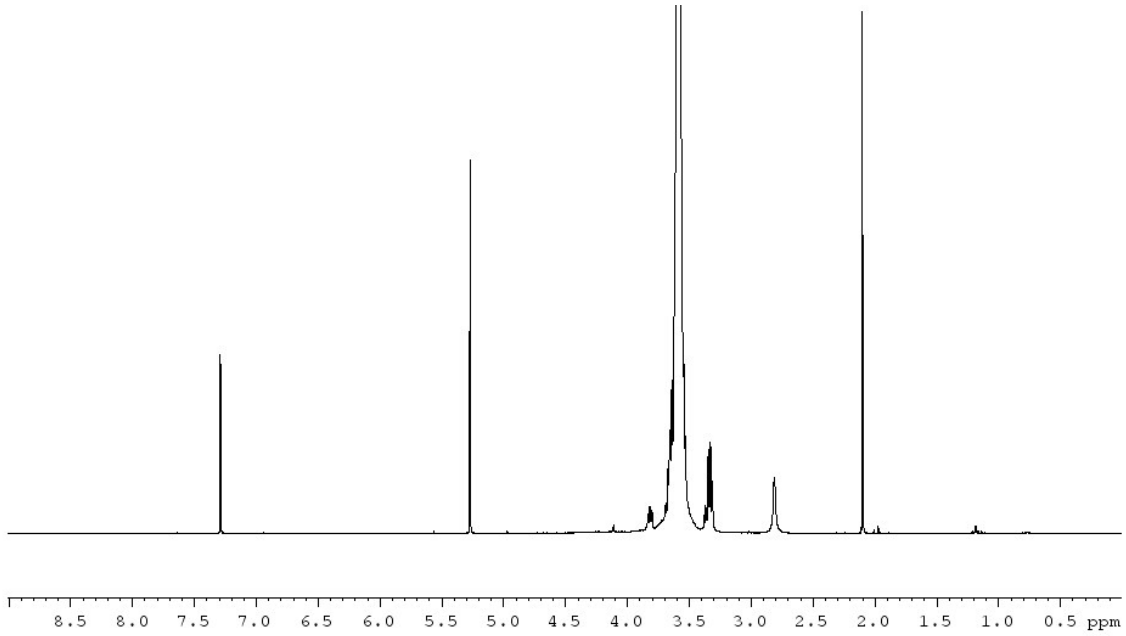
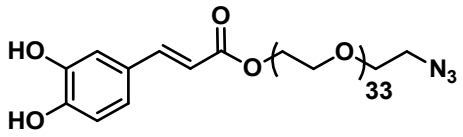



Figure S11. $^{1}$H NMR (500 MHz, $CDCl_3$) of HO-PEG-$N_3$ **(12)**.

**Synthesis of CA-PEG-$N_3$ (4).**




CA-PEG-$N_3$ (**4**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols starting from **CA** (180 mg, 1 mmol) and **12** (1500 mg, 1 mmol). Purification by silica gel chromatography (from DCM/MeOH 10:1 v/v to DCM/MeOH/HCOOH 5:1:0.5) rendered the desired product **4** mixed with the unreacted spacer PEG1500. However, $^{1}$H NMR spectroscopy confirmed the presence of desired product **4**. $^{1}$H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.70-7.60 (m, 1H), 7.25-6.70 (m, 2H), 6.55-6.40 (m, 1H), 6.32-6.19 (m, 1H), 4.15-4.12, (m, 2H), 3.90-3.48 (m, $CH_2$-PEG), 3.43-3.40 (m, 2H), 3.24-3.17 (m, 2H). FTIR peaks ($cm^{-1}$): 3000-2700 $cm^{-1}$ (O-H stretch vibration), 2111 $cm^{-1}$ (N=N=N stretch vibration), 1357 (C-H bend vibration),

1341 (C-H bend vibration), 1275 (C-O stretch vibration), 1241 (C-O stretch vibration), 1100 (C-O-C stretch vibration), 958 (CH out-of-plane bending vibration).

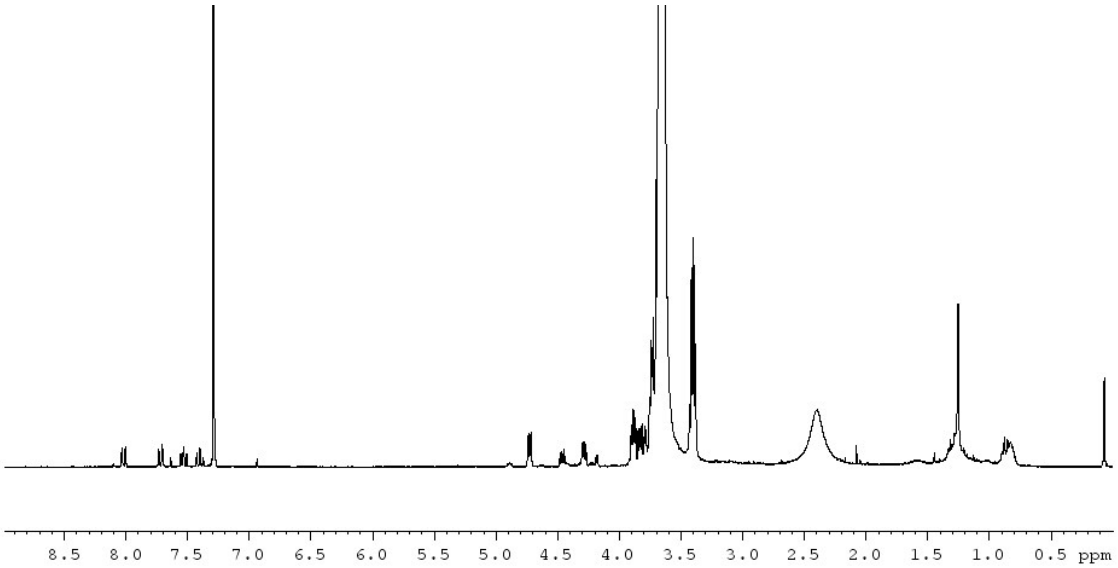
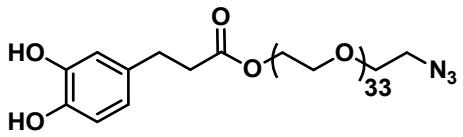



Figure S12. $^{1}$H NMR (500 MHz, $CDCl_3$) of CA-PEG-$N_3$ (**4**).

**Synthesis of DHCA-PEG-$N_3$ (5).**




DHCA-PEG-$N_3$ (**5**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols DH**CA** (182 mg, 1 mmol) and **12** (1500 mg, 1 mmol). $^{1}$H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.80-6.71 (m, 2H), 6.57-6.53 (m, 1H), 4.66 (bs, 1H), 4.23-4.19, (m, 2H), 3.89-3.64 (m, $CH_2$-PEG), 3.31-3.24 (m, 2H), 2.92-2.81 (m, 2H), 2.68-2.54 (m, 2H). FTIR peaks ($cm^{-1}$): 3000-2700 $cm^{-1}$ (O-H stretch vibration), 2118 $cm^{-1}$ (N=N=N stretch vibration), 1467 (C-H bend vibration), 1359 (C-H bend vibration), 1341 (C-H bend vibration), 1276 (C-O stretch vibration), 1240 (C-O

stretch vibration), 1105 (C-O-C stretch vibration), 961 (CH out-of-plane bending vibration).

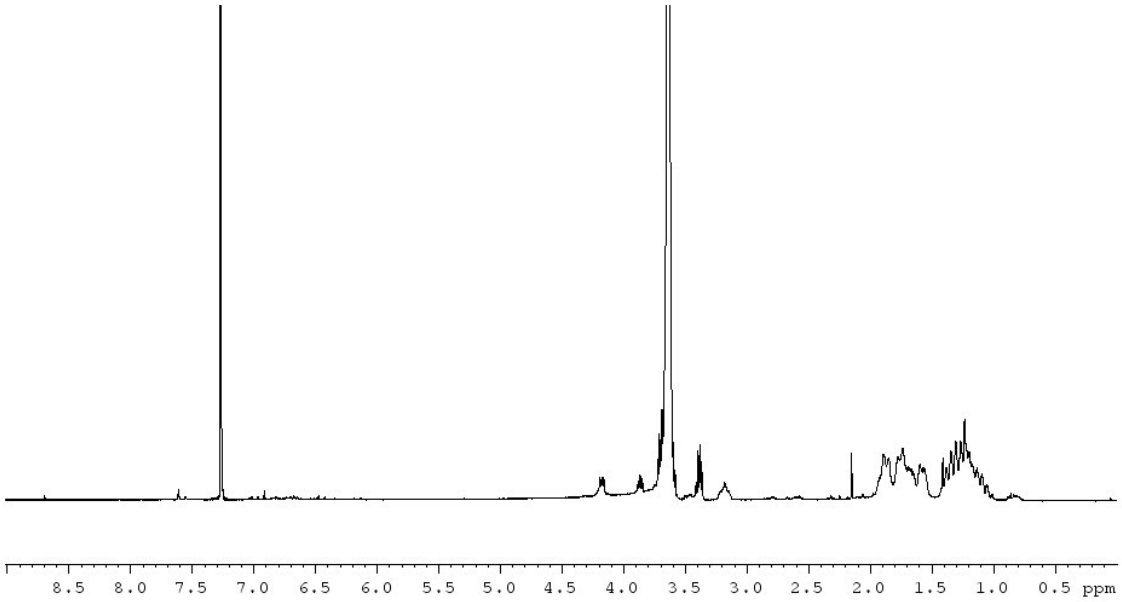
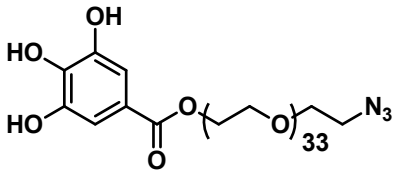



Figure S13. $^{1}H$ NMR (500 MHz, $CDCl_3$) of DHCA-PEG-$N_3$ (**5**).

**Synthesis of GA-PEG-$N_3$. (6).**




GA-PEG-$N_3$ (**6**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols **GA** (170 mg, 1 mmol) and **12** (1500 mg, 1 mmol). $^{1}H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 8.1 (s, 1H), 4.33-4.13 (m, 2H), 3.88-3.58 (m, $CH_2$-PEG), 3.41-3.36 (m, 2 H). FTIR peaks ($cm^{-1}$): 3000-2700 $cm^{-1}$ (O-H stretch vibration), 2105 $cm^{-1}$ (N=N=N stretch vibration), 1466 (C-H bend vibration), 1359 (C-H bend vibration), 1340 (C-H bend vibration), 1279 (C-O stretch vibration), 1238 (C-O

stretch vibration), 1102 (C-O-C stretch vibration), 956 (CH out-of-plane bending vibration).

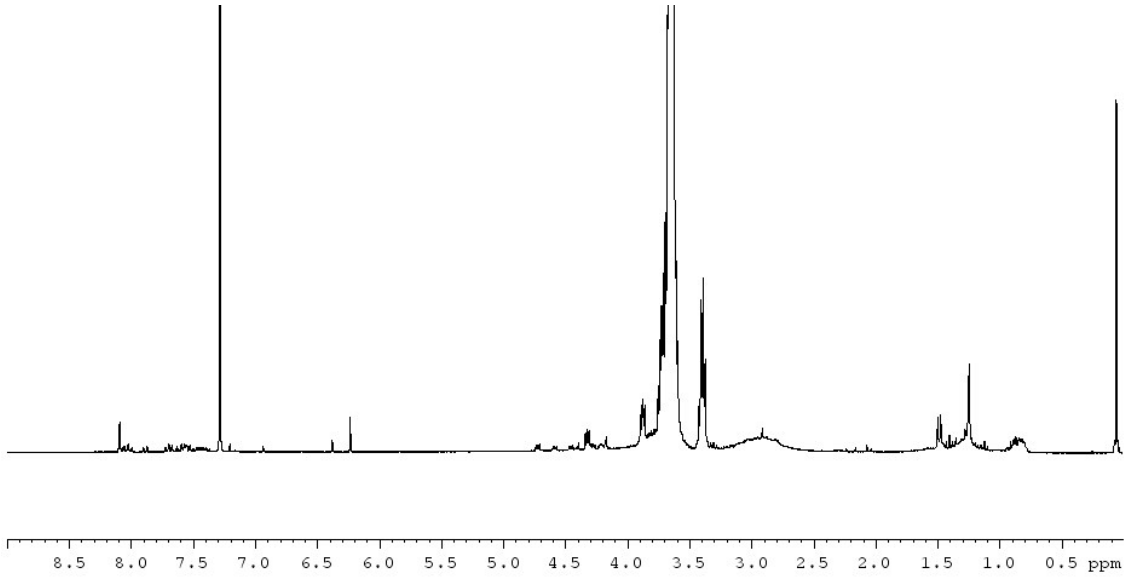
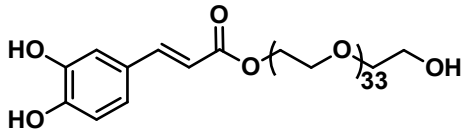



Figure S14. $^{1}H$ NMR (500 MHz, $CDCl_3$) of GA-PEG-$N_3$ (**6**).

**Synthesis of CA-PEG-OH (13).**




CA-PEG-OH (**13**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols **CA** (180 mg, 1 mmol) and **PEG** (1500 mg, 1 mmol). $^{1}H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 7.63-7.55 (m, 1H), 7.20-7.02 (m, 1H), 6.96-6.77 (m, 1H), 6.60-6.48 (m, 1H), 6.32-6.19 (m, 1H), 4.20-4.17, (m, 2H), 3.78-3.59 (m, $CH_2$-PEG), 3.22-3.15 (m, 2H). FTIR peaks ($cm^{-1}$): 3000-2700 $cm^{-1}$ (O-H stretch vibration), 1466 (C-H bend vibration), 1359 (C-H bend vibration), 1341 (C-H bend vibration), 1268 (C-O stretch vibration), 1238 (C-O stretch vibration), 1092 (C-O-C stretch vibration), 942 (CH out-of-plane bending vibration).

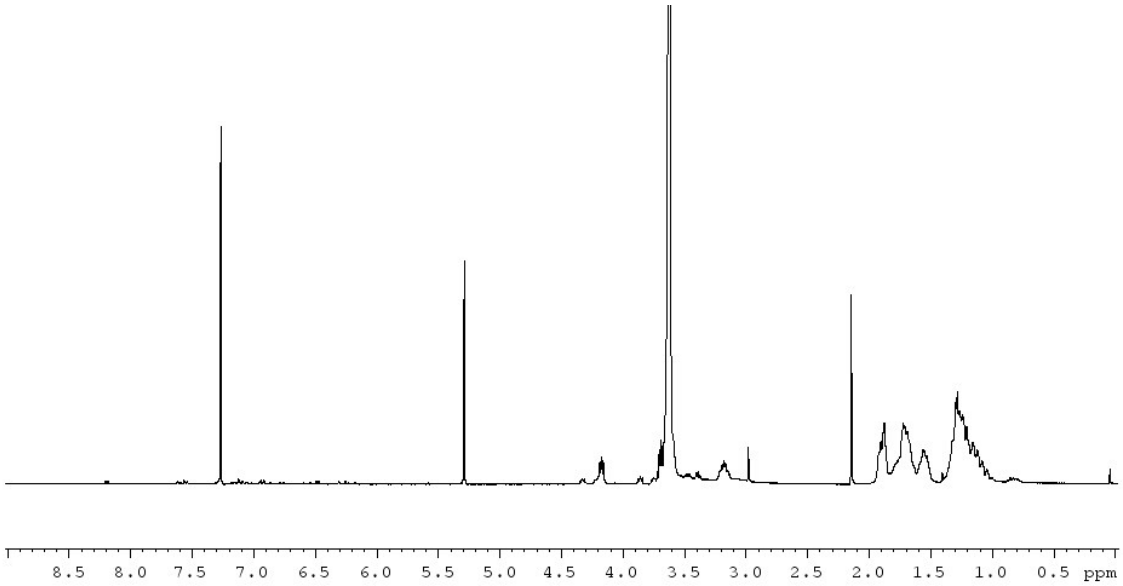
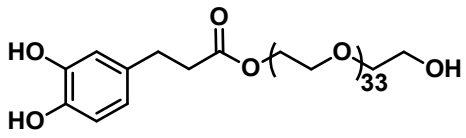



Figure S15. $^{1}H$ NMR (500 MHz, $CDCl_3$) of CA-PEG-OH (**13**).

**Synthesis of DHCA-PEG-OH (14).**




DHCA-PEG-OH (**14**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols DH**CA** (182 mg, 1 mmol) and **PEG** (1500 mg, 1 mmol). $^{1}H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 6.95-6.64 (m, 2H), 6.52-6.46 (m, 1H), 4.22-4.16, (m, 2H), 3.71-3.53 (m, $CH_2$-PEG), 3.20-3.13 (m, 2H), 2.89-2.76 (m, 2H), 2.65-2.54 (m, 2H). FTIR peaks ($cm^{-1}$): 3000-2700 $cm^{-1}$ (O-H stretch vibration), 1466 (C-H bend vibration), 1359 (C-H bend vibration), 1341 (C-H bend vibration), 1279 (C-O stretch vibration), 1238 (C-O stretch vibration), 1092 (C-O-C stretch vibration), 945 (CH out-of-plane bending vibration).

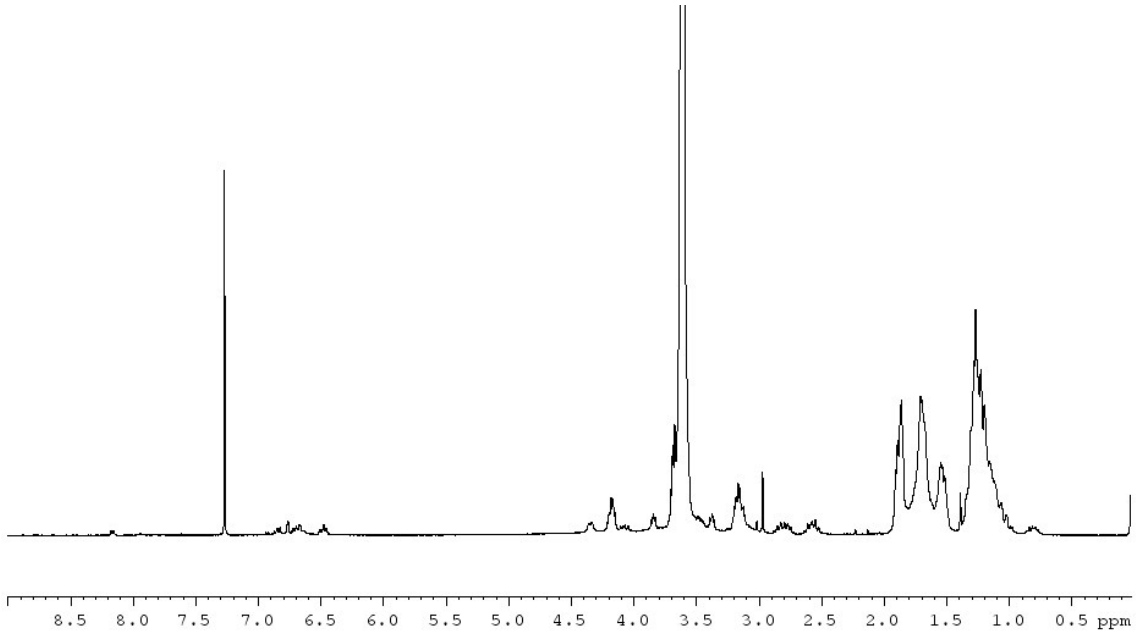
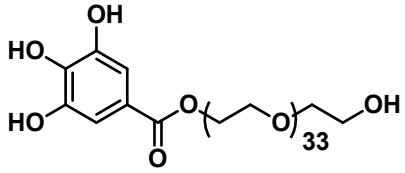



Figure S16. $^{1}H$ NMR (500 MHz, $CDCl_3$) of DHCA-PEG-OH (**14**).

**Synthesis of GA-PEG-OH (15).**




GA-PEG-OH (**15**) was synthesized following the general synthetic protocol for the incorporation of PEG spacers to catechols **GA** (170 mg, 1 mmol) and **PEG** (1500 mg, 1 mmol). $^{1}H$ NMR spectroscopy confirmed the desired product **15**. $^{1}H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 6.92 (s, 2H), 4.15-4.12 (m, 2H), 3.67-3.54 (m, $CH_2$-PEG), 3.18-3.10 (m, 2H). FTIR peaks ($cm^{-1}$): 3000-2700 $cm^{-1}$ (O-H stretch vibration), 1466 (C-H bend vibration), 1359 (C-H bend vibration), 1341 (C-H bend vibration), 1268 (C-O stretch vibration), 1238 (C-O stretch vibration), 1092 (C-O-C stretch vibration), 942 (CH out-of-plane bending vibration).

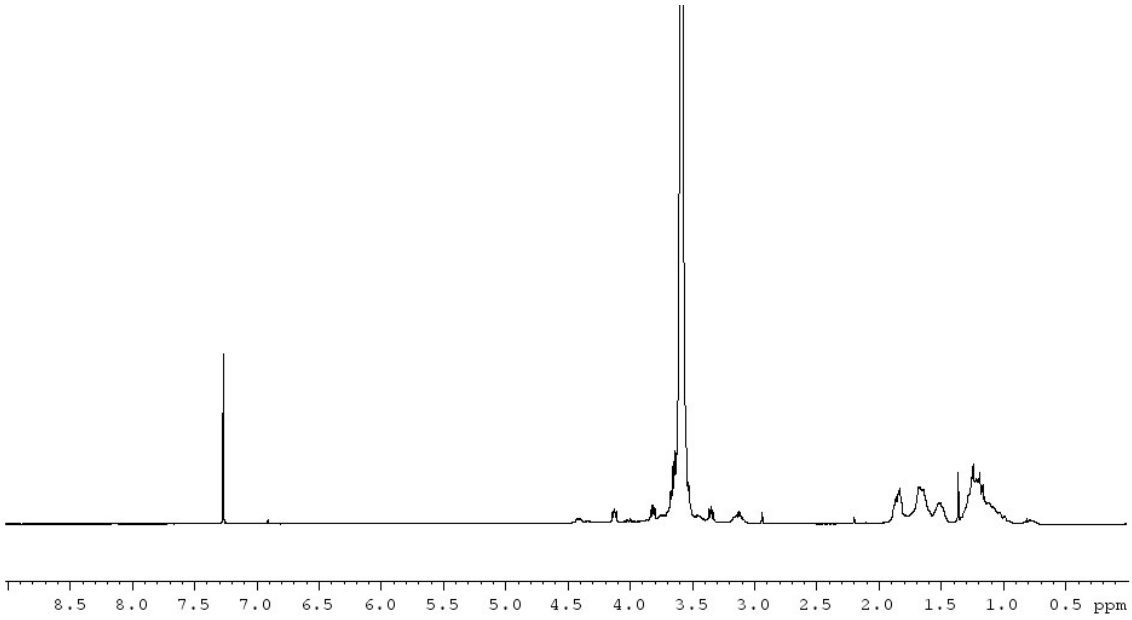



Figure S17. $^{1}H$ NMR (500 MHz, $CDCl_3$) of GA-PEG-OH (**15**).

**Synthesis of nanoemulsions based on DHAC-PEG-$N_3$ ligand.**

Briefly, DHCA-PEG-$N_3$ ligand (7.5 mg, 5 µmol) was dissolved in $CHCl_3$ (1 mL) in a glass vial. Then, $CHCl_3$ was evaporated using a nitrogen flow, and the ligand was dissolved in distilled $H_2O$ (1 mL). The mixture was ultrasonicated at 80ºC for 15 min, filtered through at syringe filter (0.22 µm) and stored at 4ºC. The resulting nanoemulsions showed a TEM diameter of 86 nm, whereas the hydrodynamic diameter, measured by DLS, resulted in 142 nm. (DLS: 141.8 ± 24.3 nm, PDI: 0.8)

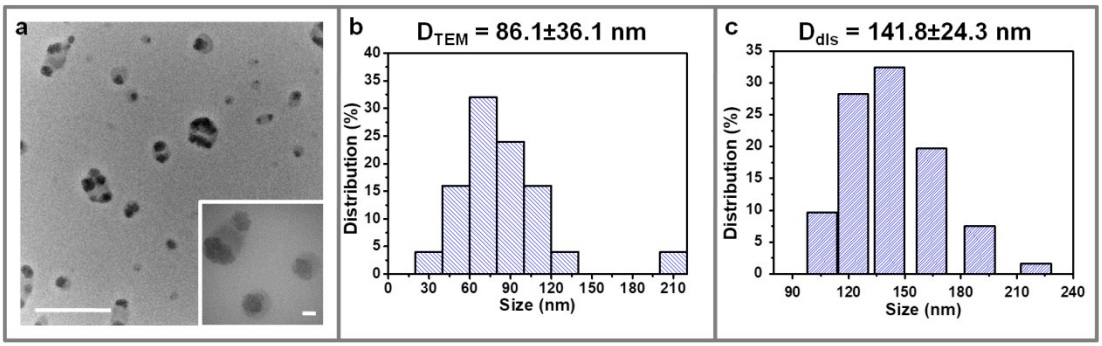



Figure S18. Left, Representative TEM images of nanoemulsions. Scale bars correspond to 500 nm for low magnification TEM images and 50 nm for the insets. Right, Size

distribution histogram. Diameter is expressed as the mean ± SD, by measuring at least 100 particles.

### 1.4. Characterization Methods.

**Nuclear Magnetic Resonance Spectroscopy (NMR).** $^{1}$H-NMR and $^{13}$C NMR spectra of samples prepared in $CDCl_3$ and MeOD were recorded on a NMR Bruker Ascend 300 and 500 MHz spectrometers.

**Inductively Coupled Plasma High Resolution Mass Spectroscopy (ICP-HRMS)**. Fe concentration was determined by ICP-HRMS. Magnetic NPs were digested with aqua regia (a mixture of three parts of $HNO_3$ and one part of HCl). Briefly, 2.5 mL of aqua regia were added to 25 µL of a solution of nanoparticles in a volumetric flask. The mixture was left overnight. Then, milli-Q water was added to complete the total volume of 25 mL.

**Cell Culture**. Mouse microglia cell line HFF-1 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37 ºC in an incubator with a humidified atmosphere with 5 % $CO_2$.

## 2. Results

### 2.1. TEM Characterization

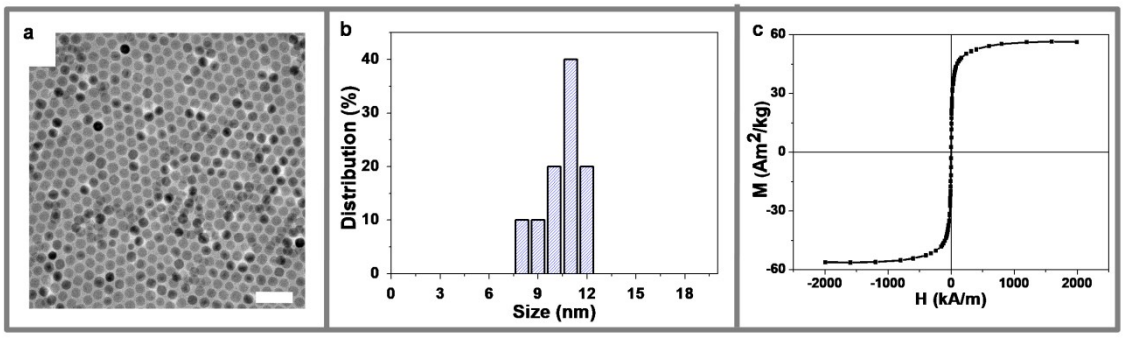


Figure S19. a) Representative TEM image of the oleic acid capping iron oxide NPs. Scale bar corresponds to 50 nm. b) Histogram of the TEM diameters of the inorganic core measured on at least 100 nanoparticles. c) Field-dependent magnetization curves (M–H) measured at 298 K for oleic acid capping iron oxide NPs.

## 2.2. DLS

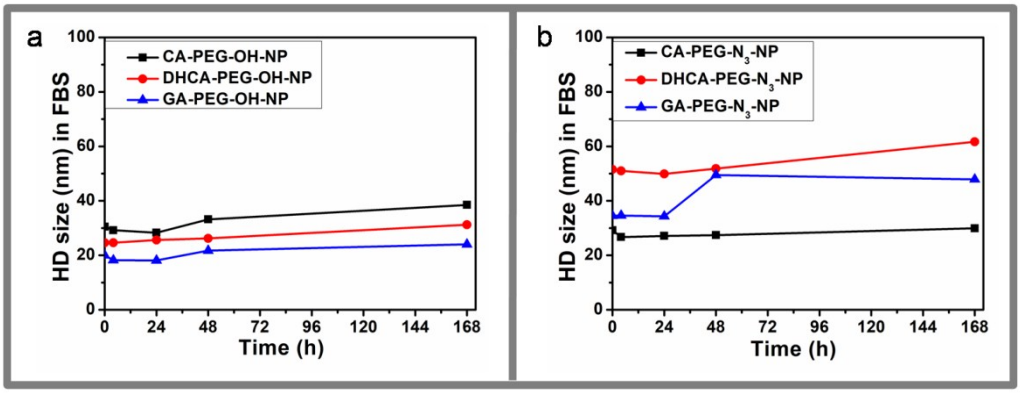



Figure S20. Stability assay based on the HD sizes of the NPs functionalized with (a) catechol PEG-OH derived ligands and (b) with catechol PEG-$N_3$ derived ligands.

## 2.3. FTIR

| Sample | Wavenumber ($cm^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| CA-PEG-OH-NP | 3000-2700 | - | 1467 | 1359 | 1342 | 1279 | 1238 | 1099 | 960 |
| DHCA-PEG-OH-NP | 3000-2700 | - | 1467 | 1361 | 1342 | 1278 | 1239 | 1100 | 960 |
| GA-PEG-OH-NP | 3000-2700 | - | 1465 | 1361 | 1342 | 1278 | 1239 | 1099 | 960 |
| CA-PEG-$N_3$-NP | 3000-2700 | 2106* | 1466 | 1359 | 1340 | 1279 | 1238 | 1102 | 956 |
| DHCA-PEG-$N_3$-NP | 3000-2700 | 2111* | - | 1357 small | 1341 | 1275 small | 1241 | 1100 | 958 |
| GA-PEG-$N_3$-NP | 3000-2700 | 2118* | 1467 | 1359 | 1341 | 1276 | 1240 | 1105 | 961 |

Tabla S1.

* Related to $N_3$ peaks.

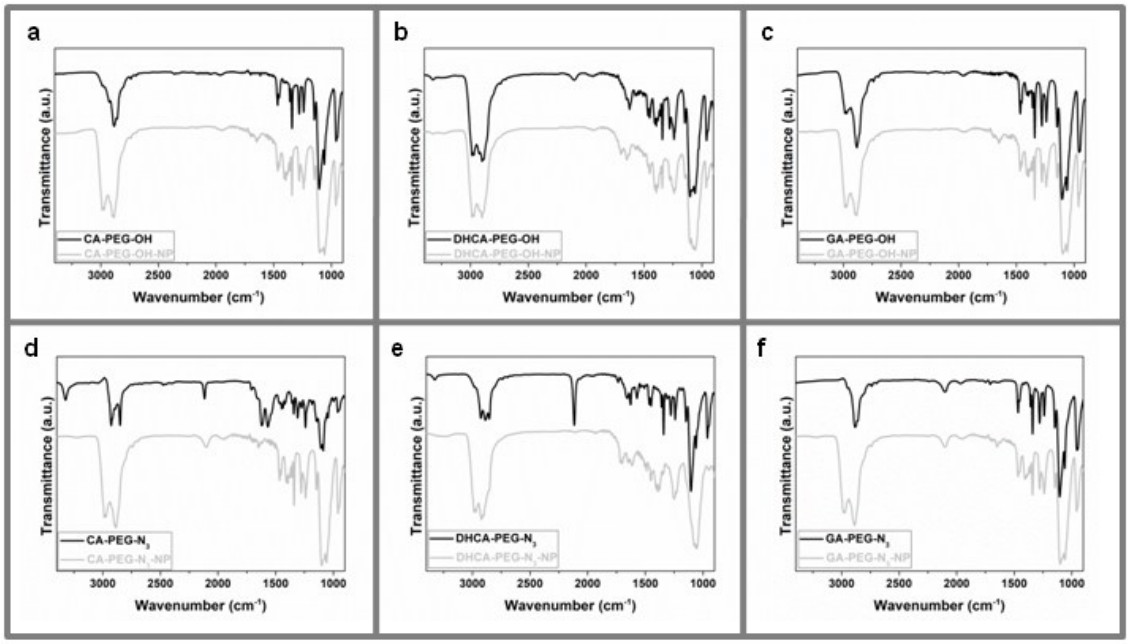



Figure S21. FTIR spectra of the corresponding ligands (black lines) and functionalized NPs (grey lines).

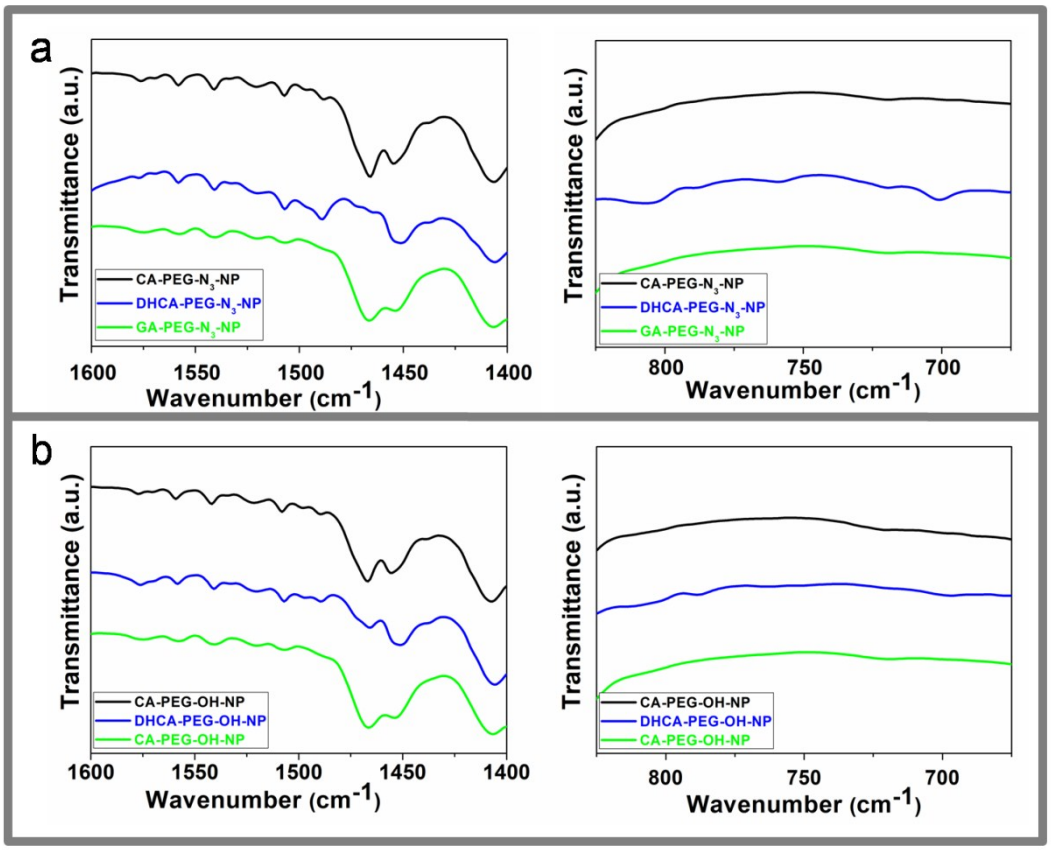


Figure S22. FTIR spectra of the functionalized NPs with: a) catechol PEG $N_3$ derived ligands and b) catechol PEG OH derived ligands.

## 2.4. Thermo-Gravimetric Analysis (TGA)

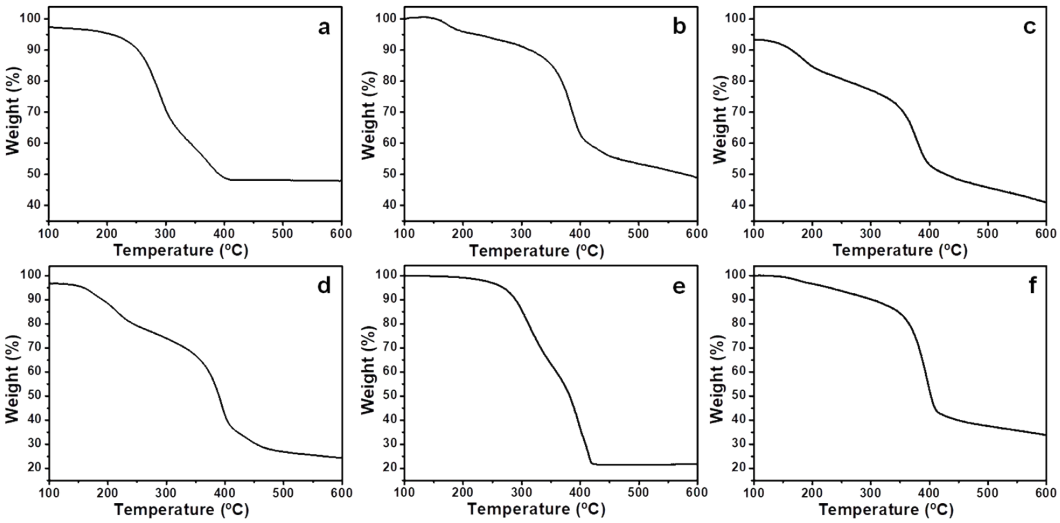



Figure S23. Thermo-gravimetric analysis of a) CA-PEG-OH-NPs; b) DHCA-PEG-OH-NPs, c) GA-PEG-OH-NPs, d) CA-PEG- $N_3$-NPs, e) DHCA-PEG- $N_3$-NPs and e) GA-PEG- $N_3$-NPs.

## 2.2. Relaxivities

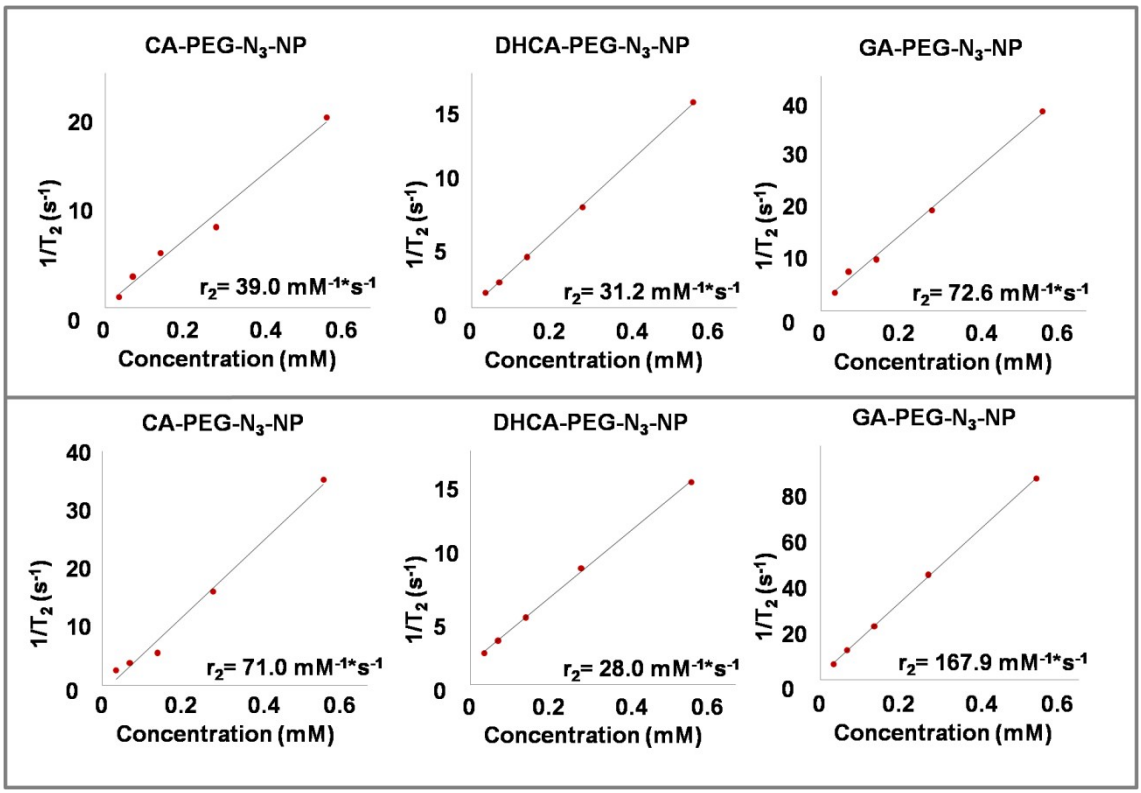



Figure S24. Plot of 1/T over Fe concentration of functionalized NPs: Low magnetic field (1.5 T), upper panel, and high magnetic field (9.4 T), lower panel.

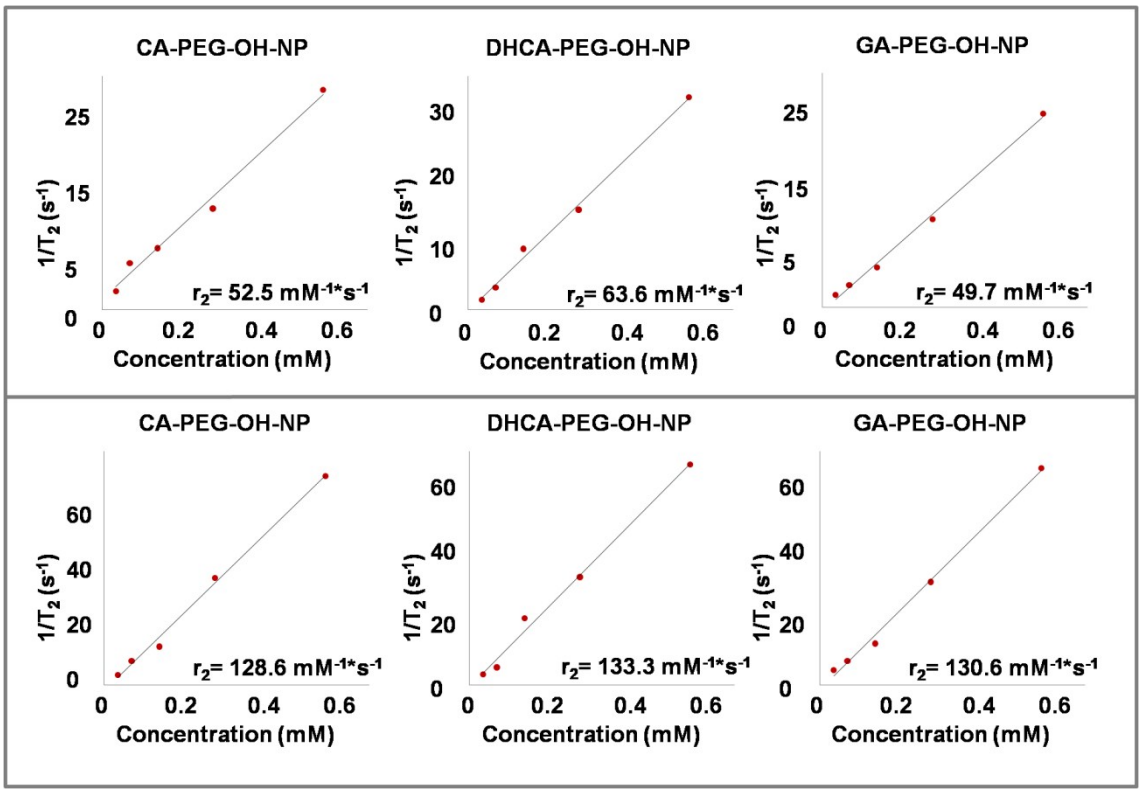



Figure S25. Plot of 1/T over Fe concentration of functionalized NPs: Low magnetic field (1.5 T), upper panel, and high magnetic field (9.4 T), lower panel.

### 2.3. In vitro cell evaluation.

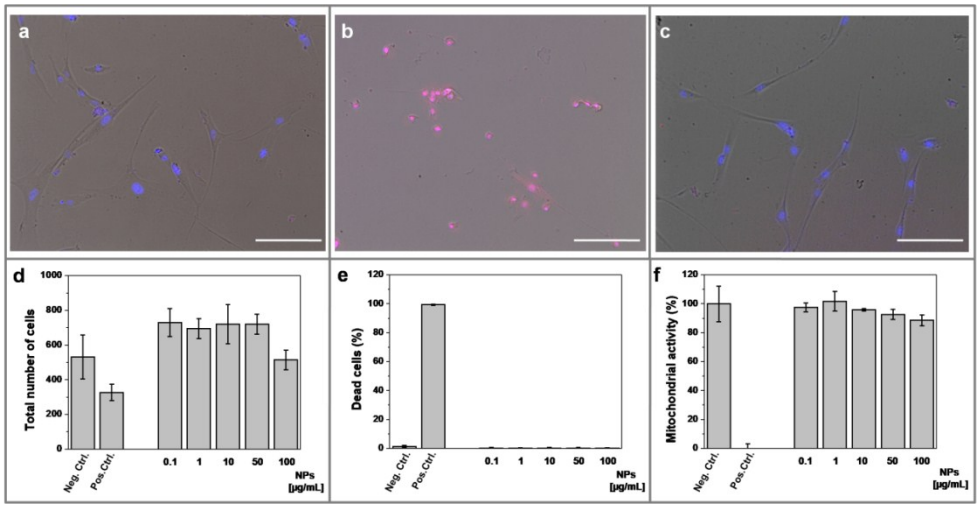



Figure S26. Live/dead viability assays on HFF-1 fibroblasts: a) negative control, b) positive control, c) cells exposed to 100 µg/mL of CA-PEG-OH-NPs. The images show the merge of bright field (grey), DAPI (blue) and TO-PRO-3 Iodine (red). Scale bar is

50 µm. d) Total number of cells per well, e) percentage of dead cells and f) cytotoxicity effects of CA-PEG-OH-NPs by the MTT assay of HFF-1 cells exposed to increasing concentration of MNPs from 0.1 µg/mL to 100 µg/mL.

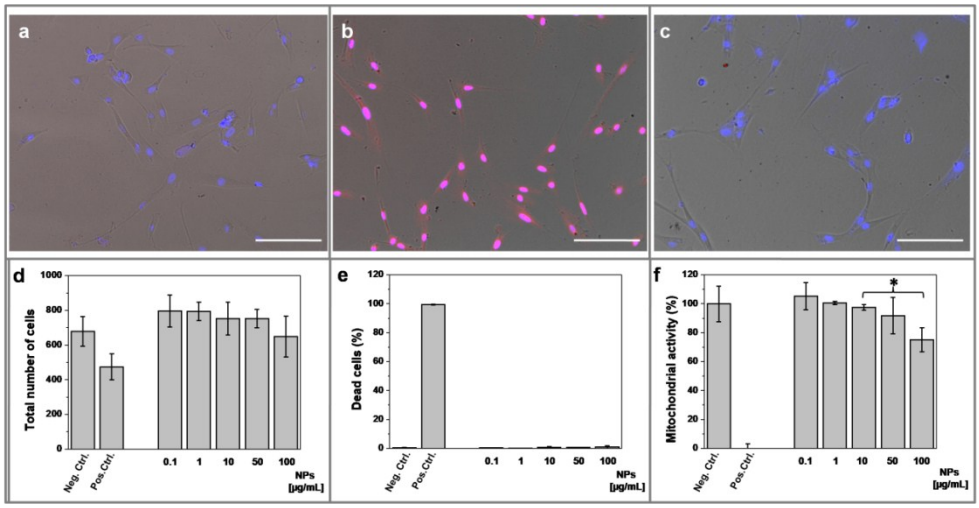



Figure S27. Live/dead viability assays on HFF-1 fibroblasts: a) negative control, b) positive control, c) cells exposed to 100 µg/mL of DHCA-PEG-OH-NPs. The images show the merge of bright field (grey), DAPI (blue) and TO-PRO-3 Iodine (red). Scale bar is 50 µm. d) Total number of cells per well, e) percentage of dead cells and f) cytotoxicity effects of DHCA-PEG-OH-NPs by the MTT assay of HFF-1 cells exposed to increasing concentration of MNPs from 0.1 µg/mL to 100 µg/mL.

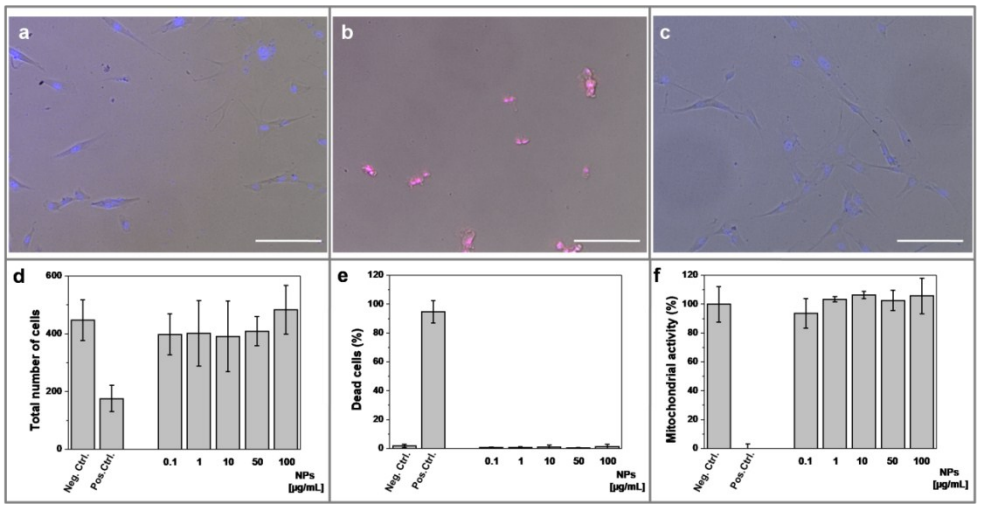



Figure S28. Live/dead viability assays on HFF-1 fibroblasts: a) negative control, b) positive control, c) cells exposed to 100 µg/mL of GA-PEG-OH-NPs. The images show the merge of bright field (grey), DAPI (blue) and TO-PRO-3 Iodine (red). Scale bar is 50 µm. d) Total number of cells per well, e) percentage of dead cells and f) cytotoxicity effects of GA-PEG-OH-NPs by the MTT assay of HFF-1 cells exposed to increasing concentration of MNPs from 0.1 µg/mL to 100 µg/mL.

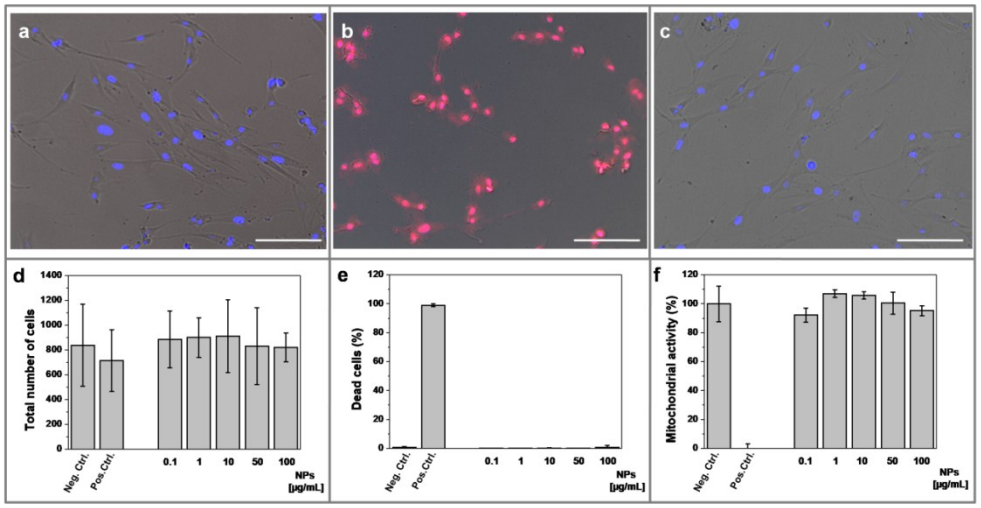



Figure S29. Live/dead viability assays on HFF-1 fibroblasts: a) negative control, b) positive control, c) cells exposed to 100 µg/mL of CA-PEG-$N_3$-NPs. The images show the merge of bright field (grey), DAPI (blue) and TO-PRO-3 Iodine (red). Scale bar is 50 µm. d) Total number of cells per well, e) percentage of dead cells and f) cytotoxicity

effects of CA-PEG-$N_3$-NPs by the MTT assay of HFF-1 cells exposed to increasing concentration of MNPs from 0.1 μg/mL to 100 μg/mL.

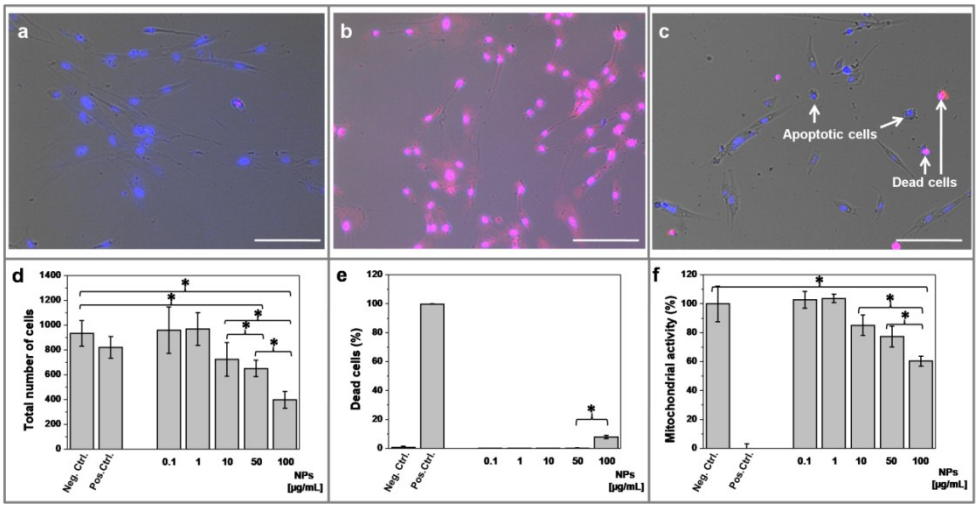



Figure S30. Live/dead viability assays on HFF-1 fibroblasts: a) negative control, b) positive control, c) cells exposed to 100 μg/mL of DHCA-PEG-$N_3$-NPs. The images show the merge of bright field (grey), DAPI (blue) and TO-PRO-3 Iodine (red). Scale bar is 50 μm. d) Total number of cells per well, e) percentage of dead cells and f) cytotoxicity effects of DHCA-PEG-$N_3$-NPs by the MTT assay of HFF-1 cells exposed to increasing concentration of MNPs from 0.1 μg/mL to 100 μg/mL.

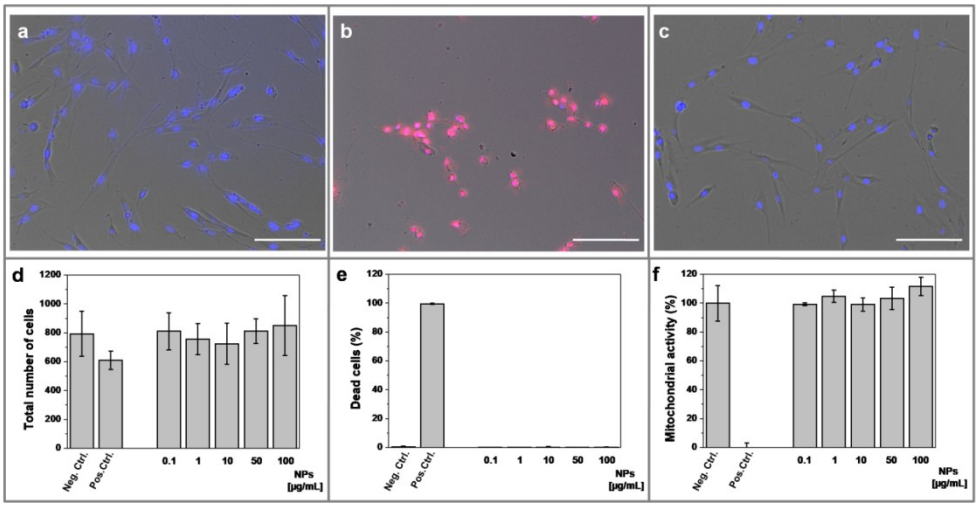



Figure S31. Live/dead viability assays on HFF-1 fibroblasts: a) negative control, b) positive control, c) cells exposed to 100 µg/mL of GA-PEG-$N_3$-NPs. The images show the merge of bright field (grey), DAPI (blue) and TO-PRO-3 Iodine (red). Scale bar is 50 µm. d) Total number of cells per well, e) percentage of dead cells and f) cytotoxicity effects of GA-PEG-$N_3$-NPs by the MTT assay of HFF-1 cells exposed to increasing concentration of MNPs from 0.1 µg/mL to 100 µg/mL.

1. H. Mattoussi, K. Susumu, H. T. Uyeda and I. L. Medintz, *Journal of Biomedicine and Biotechnology*, 2007, **2007**.