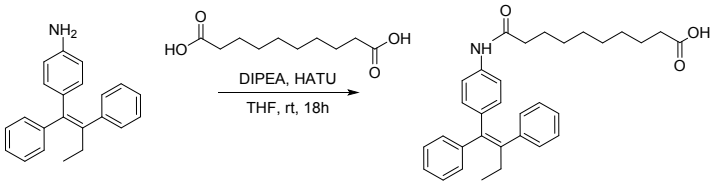
Chemistry

General

All reactions were carried out in oven-dried glassware and dry solvents under nitrogen atmosphere. Unless otherwise stated, all solvents were purchased from Sigma Aldrich and used without further purification. Substrates and reagents were purchased from Sigma Aldrich and used as received. Thin layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by a solution of *p*-anisaldehyde with heating. Flash chromatography was performed using silica gel (240-400 mesh, Merck). ^{1}H-NMR spectra were recorded on Bruker DRX-400 and Bruker DRX-300 instruments and are reported relative to residual $CHCl_3$ and CD_3OD. ^{13}C-NMR spectra were recorded on the same instruments (100 and 75 MHz) and are reported relative to $CDCl_3$ and CD_3OD. Chemical shifts (δ) for proton and carbon resonances are quoted in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard. MS spectra were recorded using electrospray ionization (ESI) technique on a Waters Micromass Q-Tof micro mass spectrometer and HR-ESI mass spectra were recorded on FT-ICR APEX^{II} (Bruker Daltonics). IR spectra were recorded on a Jasco FT-IR 4100 spectrometer using CH_2Cl_2 in NaCl rectangular windows.

Experimental procedures

Preparation of 6

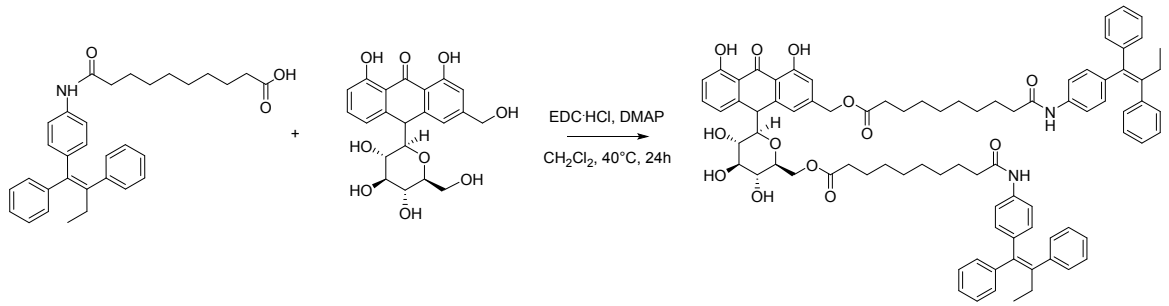


To a solution of sebacic acid (0.270 g, 1.336 mmol) in THF (20 mL) HATU (0.558 g, 1.469 mmol) and DIPEA (0.345 g, 2.672 mmol) are added and the reaction mixture is stirred for 30 minutes. Then **6** (0.400 g, 1.1336 mmol) was added and the reaction mixture was stirred at rt overnight. The solvent is removed under reduced pressure, AcOEt is added and it is washed with water and brine. The organic layer is then dried over Na_2SO_4 and concentrated under reduced pressure to obtain **6**. The crude is purified by flash chromatography (AcOEt/Hex) to obtain pure **6** (0.270 g, Yield: 42%).

^{1}H-NMR($CDCl_3$, 300 MHz): δ(ppm)= 7.33-7.02 (12H, m), 6.80 (2H, d, J=7.8) 4.78 (1H, bs), 2.43 (2H, q, J = 7.6 Hz), 2.30 (2H, t, J = 7.7 Hz), 2.25 (2H, t, J = 7.5 Hz), 1.75-1.55 (4H, m), 1.40-1.25 (8H, m), 0.92 (3H, t, J = 7.7 Hz).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ(ppm)= 175.3, 174.6, 145.1, 143.2, 143.1, 141.3, 139.7, 135.4, 131.9 (2C), 129.6 (2C), 129.4 (2C), 128.2 (2C), 127.7 (2C), 127.3, 126.3, 116.1 (2C), 37.58, 34.98, 31.80, 30.81 (4C), 26.96, 26.22, 13.60.

Preparation of 1



To a solution of **6** (0.085 g, 0.176 mmol) in CH_2Cl_2 (10 mL) DMAP (0.032 g, 0.262 mmol) and EDC·HCl (0.050 g, 0.262 mmol) are added and the reaction mixture is stirred for 30 minutes. Then aloin (0.091 g, 0.269 mmol) is added and the reaction mixture is stirred at 40°C overnight. The solvent is removed under reduced pressure to give **1** without further purification (0.021 g, Yield: 8%).

IR (film) *v*: 3053, 1638, 1724 cm^{-1}

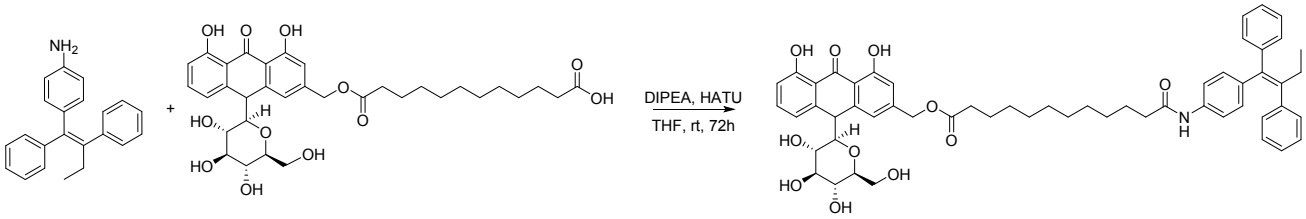
^{1}H-NMR (CD_3OD, 400 MHz): δ(ppm)= 7.58-7.47 (2H, m), 7.37-7.34 (3H, m), 7.30-6.95 (24H, m), 6.89-6.87 (1H, m), 6.81-6.79 (3H, m), 5.16-5.13 (2H, m), 4.90-4.86 (1H, m), 4.67-4.64 (1H, m), 3.68-3.41 (4H, m), 3.13-3.04 (2H, m), 2.51-2.25 (12H, m), 1.74-1.52 (8H, m), 1.31-1.25 (16H, m), 0.92 (6H, t, J = 7.3 Hz)

^{13}C-NMR (CD_3OD, 100 MHz): δ(ppm)= 194.0, 174.5, 173.8, 173.1 (2C), 162.0, 161.8, 145.2, 144.9, 144.7, 143.4 (2C), 142.2 (2C), 141.9 (2C), 138.8 (2C), 138.4 (2C), 136.2 (2C), 135.9, 130.8 (3C), 130.4, 129.4 (7C), 129.1 (3C), 127.9 (4C), 127.6 (3C), 127.5 (2C), 127.0, 126.4 (2C), 125.9 (2C), 119.7, 118.7 (3C), 117.3, 116.9, 85.25, 85.06, 80.33, 79.31, 68.80, 64.79, 61.49, 44.40, 33.74 (2C), 33.58 (2C), 28.76 (8C), 28.52 (2C), 25.42 (2C), 24.62, 24.43, 12.42 (2C)

MS (ESI) = 1372.31 $(M+Na^+)^+$

HRMS (ESI): m/z $(M+Na^+)^+$ calcd for $C_{85}H_{92}N_2O_{13}Na$: 1371.6489; found: 1371.6497

Preparation of 3



To a solution of **8** (0.080 g, 0.130 mmol) in THF (30 mL) are added HATU (0.050 g, 0.130 mmol) and DIPEA (0.034 g, 0.266 mmol) and the reaction mixture is stirred for 30 minutes. Then **5** (0.039 g, 0.130 mmol) is added and the reaction mixture was stirred at rt for 72h. The solvent is removed under reduced pressure to obtain crude **3**. The crude is purified by flash chromatography (CH_2Cl_2/MeOH) to obtain pure **3** (0.077 g Yield: 65%).

IR (film) *v*: 3208, 1737, 1603 cm^{-1}

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm)= 7.45-6.76 (19H, m), 4.84-4.64 (2H, m), 4.53 (1H, m), 4.12-4.08 (1H, m), 3.74-3.68 (2H, m), 3.20-2.80 (4H, m), 2.41 (2H, q, J= 7.4), 2.31-2.26 (2H, m), 2.15-2.12 (2H, m), 1.70-1.57 (2H, m), 1.53-1.42 (2H, m), 1.32-1.24 (12H, m), 0.89 (3H, t, J = 7.4 Hz)

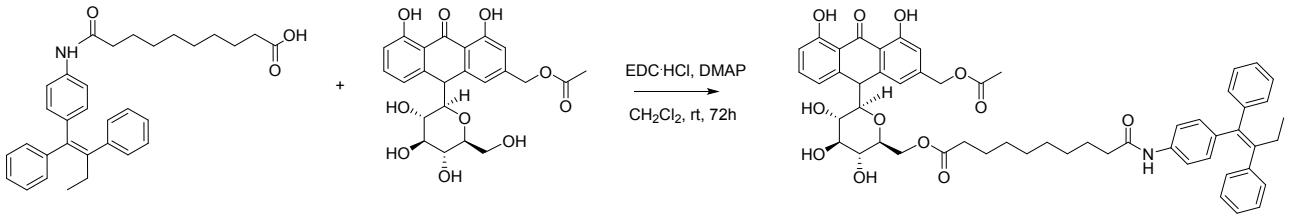
^{13}C-NMR ($CDCl_3$, 75 MHz): δ(ppm)= 195.8, 175.5, 174.9, 163.3, 163.0, 147.4, 145.1, 143.8 (2C), 143.6, 142.8, 140.5, 140.1, 137.9, 132.5 (2C), 132.2, 131.2 (2C), 130.8 (2C), 129.6 (2C), 129.3 (2C), 128.1, 127.6, 121.6,

120.4 (2C), 119.5, 118.8, 118.2, 114.9, 114.4, 86.31, 80.09, 79.29, 71.82, 71.47, 64.85 (2C), 45.83, 38.33, 35.12, 30.85 (6C), 30.51, 27.26, 26.16, 13.50

MS (ESI) = 935.05 $(M+Na^+)^+$

HRMS (ESI): m/z $(M+Na^+)^+$ calcd for $C_{55}H_{61}NO_{11}Na$: 934.4136; found: 934.4145

Preparation of 2



To a solution of **6** (0.080 g, 0.166 mmol) in CH_2Cl_2 (18 mL) are added EDC·HCl (0.038 g, 0.199 mmol) and DMAP (0.025 g, 0.199 mmol) and the reaction mixture is stirred for 30 minutes. Then **9** (0.076 g, 0.166 mmol) is added and the reaction mixture is stirred at rt for 72h. The solvent is removed under reduced pressure to obtain crude **2**. The crude is purified by flash chromatography (CH_2Cl_2/MeOH) to obtain pure **2** (0.030 g Yield: 20%).

IR (film) *v*: 3361, 1731, 1637 cm^{-1}

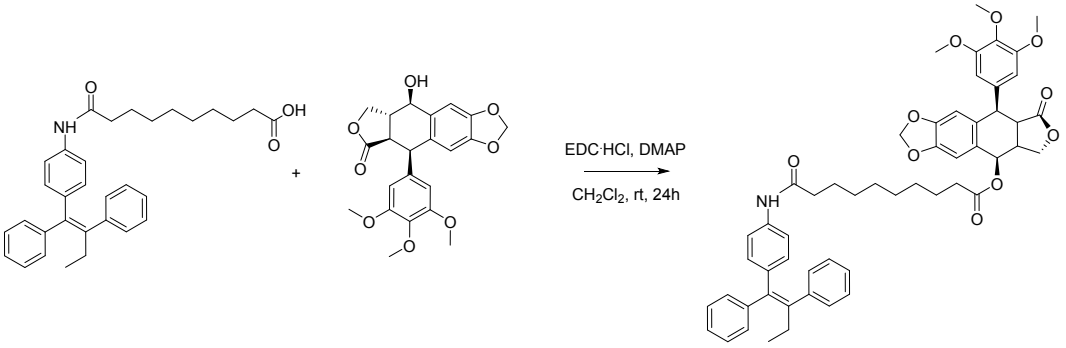
^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm)= 7.53-7.50 (1H,m), 7.38-7.35 (2H, m), 7.30-7.09 (12H, m), 6.90-6.88 (2H, m), 6.80 (2H, d, *J* = 8.4 Hz), 5.15 (2H, d, *J* = 3.6 Hz), 4.88 (1H, m, HSQC), 4.65 (1H, s), 3.56 (1H, d, *J* = 12 Hz), 3.53 (1H, d, *J* = 9.6 Hz), 3.44 (1H, dd, *J* = 12 Hz *J* = 5.2 Hz), 3.12-3.01 (3H, m), 2.47 (2H, q, *J* = 7.6 Hz), 2.37 (2H, t, *J* = 7.2 Hz), 2.31-2.26 (2H, m), 2.16 (3H, s), 1.67-1.57 (4H, m), 1.37-1.27 (8H, m), 0.924 (3H, t, *J* = 7.2 Hz).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ(ppm)= 194.7, 174.8, 173.9, 173.8, 162.3, 162.2, 145.8, 144.1, 142.8, 142.6 (2C), 142.0, 139.4, 139.1, 136.9, 135.8, 131.4 (2C), 130.1 (2C), 129.7 (2C), 128.5 (2C), 128.2 (3C), 127.0, 126.8, 119.4 (2C), 119.1, 117.9 (2C), 116.3, 114.6, 85.70, 81.06, 79.93, 69.60 (2C), 65.60, 62.13, 45.03, 37.19, 34.36, 29.42 (4C), 29.16, 26.08, 25.08, 20.03, 13.06.

MS (ESI) = 949.09 $(M+Na^+)^+$

HRMS (ESI): m/z $(M+Na^+)^+$ calcd for $C_{55}H_{59}NO_{12}Na$: 948.3929; found: 948.3938

Preparation of 4



To a solution of **6** (0.059 g, 0.122 mmol) in dry CH_2Cl_2 (10 mL), EDC·HCl (0.028, g, 0.147 mmol) and DMAP (0.011 g, 0.086 mmol) are added. Then, aloin (0.051 g, 0.122 mmol) is added in the reaction mixture and

stirred at rt for 5 h. HCl 1M (9 mL) is then added and the aqueous phase is washed with AcOEt (4 x 8 mL). The combined organic extracts are dried over Na_2SO_4 and evaporated under reduced pressure. The crude is purified by flash chromatography (AcOEt/Hex) to provide **4** (0.086 g, 80 %) as an orange solid.

IR (film) *v*: 3327, 1779, 1732, 1667 cm^{-1}

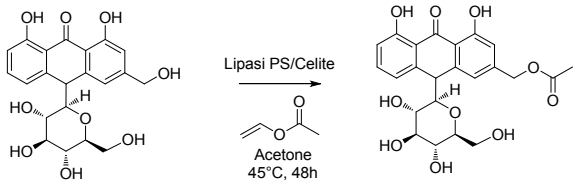
^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm)= 7.38-7.09 (12H, m), 6.80 (2H, d, J = 8.5 Hz), 6.78 (1H,s), 6.53 (1H, s), 6.37 (2H, s), 5.95 (2H, d, J = 4.5 Hz), 5.88 (1H, d, J = 8.9 Hz), 4.59 (1H, d, J = 4.0), 4.36-4.31 (1H, m), 4.22-4.18 (1H, m), 3.78 (3H, s), 3.72 (6H, s), 2.92 (1H, dd, J = 14 Hz, J = 4.5 Hz), 2.82-2.77 (1H, m), 2.55-2.29 (4H, m), 2.22 (2H, t, J = 7.5 Hz), 1.81-1.51 (4H, m), 1.48-1.18 (8H, m), 0.93 (3H, t, J = 7.7 Hz).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ(ppm)= 174.9, 174.4, 171.8, 153.3 (2C), 148.8, 148.3, 144.1, 142.9, 142.7, 139.5, 138.8, 137.7, 136.4, 135.5, 133.0, 132.0 (2C), 130.3 (2C), 130.2 (2C), 129.0, 128.8 (2C), 128.6 (2C), 127.3, 126.8, 119.2 (2C), 110.4, 108.7 (2C), 107.8, 102.3, 74.06, 72.15, 61.43, 56.76 (2C), 46.30, 44.40, 39.53, 38.27, 35.03, 29.81 (5C), 26.16, 25.69, 14.22.

MS (ESI) = 903.06 $(M+Na^+)^+$

HRMS (ESI): m/z $(M+Na^+)^+$ calcd for $C_{54}H_{57}NO_{10}Na$: 902.3875; found: 902.3883

Preparation of 7



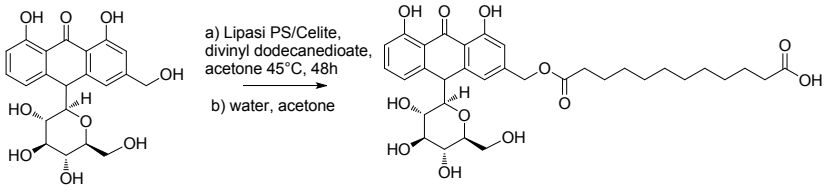
Aloin (100 mg, 0.24 mmol) and vinyl acetate (33 µL, 0.36 mmol) are dissolved in 15 mL of dry acetone under nitrogen atmosphere. Molecular sieves 4Å (30 mg) and lipase PS supported on Celite® (80 mg, 1 % w / w lipase / celite) are then added to the resulting solution which is incubated at 45 °C and 210 rpm for 48 h. After that, the formation of the desired product is checked by TLC ($CHCl_3$/MeOH/HCOOH = 8:2:0.1, UV) and the crude mixture filtered, concentrated *in vacuo* and purified by flash column chromatography on silica gel (AcOEt/MeOH/H_2O = 9.5:0.5:0.1), affording the purified product as a yellow solid (85 mg, 80%).

^{1}H-NMR (CD_3OD, 400 MHz): δ(ppm)= 7.47 (1H, ddd, J = 8.3, 7.6, 5.8 Hz), 7.04-7.00 (2H, m), 6.86-6.81 (2H, m), 5.16 (2H, d, J = 3.6 Hz), 4.50 (1H, d, J = 1.9 Hz), 3.59-3.55 (1H, m), 3.42-3.37 (2H, m), 3.29-3.25 (1H, m), 3.00-2.88 (3H, m), 2.17 (1H, s), 2.15 (2H, s)

^{13}C-NMR (CD_3OD, 100 MHz): δ(ppm)= 194.0, 171.6, 171.13, 171.03, 161.77, 161.69, 161.55, 161.51, 145.4, 145.01, 144.88, 144.2, 142.0, 141.6, 135.7, 135.0, 119.9, 118.58, 118.51, 117.4, 117.2, 116.82, 116.63, 115.7, 115.5, 114.1, 113.8, 85.18, 85.01, 80.26, 80.17, 78.53, 78.50, 70.60, 70.58, 70.50, 70.46, 64.96, 64.91, 61.88, 61.83, 60.2, 44.45, 44.38, 19.50, 19.44, 13.1;

MS (ESI) = 483.1 $(M+Na^+)^+$

Preparation of 8



To a solution of aloin (100 mg, 0.24 mmol) in dry acetone (15 mL) divinyl dodecanedioate (101 mg, 0.36 mmol) and Novozyme 435 (30 mg) are added. The resulting heterogeneous mixture is incubated at 45 °C and 210 rpm for 48h. After that, 5 µL water are added to the mixture which is incubated again for additional 2 h, checking the formation of the target acid by TLC ($CHCl_3$/MeOH/HCOOH = 8:2:0.1, UV). The crude mixture is then filtered, concentrated *in vacuo* and purified by flash column chromatography on silica gel (AcOEt/MeOH/H_2O = 9.5:0.5:0.1) affording the purified product as a yellow oil (60 mg, 40%).

^{1}H-NMR (CD_3OD, 400 MHz): δ(ppm)= 7.50 (1H, ddd, J = 8.3, 7.5, 6.3 Hz), 7.06 (2H, q, J = 6.0 Hz), 6.89-6.84 (2H, m), 5.16 (2H, d, J = 3.7 Hz), 4.59 (1H, d, J = 1.5 Hz), 3.58 (1H, dd, J = 11.7, 1.9 Hz), 3.43-3.39 (2H, m), 3.33 (2H, dt, J = 3.3, 1.6 Hz), 3.28 (1H, t, J = 8.6 Hz), 3.03-2.97 (1H, m), 2.94-2.89 (2H, m), 2.47-2.37 (4H, m), 1.70-1.58 (4H, m), 1.29 (12H, br. s)

^{13}C-NMR (CD_3OD, 100 MHz): δ(ppm)= 194.1, 173.68, 173.60, 170.9, 161.84, 161.75, 161.62, 161.59, 145.5, 145.08, 145.04, 144.4, 142.1, 135.8, 135.0, 119.9, 118.60, 118.59, 117.5, 117.24, 117.22, 116.88, 116.70, 115.8, 115.5, 114.1, 113.9, 85.0, 80.30, 80.20, 78.55, 78.53, 70.62, 70.53, 70.46, 64.74, 64.71, 61.89, 61.85, 44.5, 33.6, 33.2, 29.08, 29.03, 28.91, 28.88, 28.74, 28.65, 24.7, 24.3

MS (ESI) = 653.3 $(M+Na^+)^+$.

Nanoparticle preparation

Nanoparticle suspensions were prepared by a nanoprecipitation method1. Briefly, compounds **1**-**4** were dissolved in either methanol or tetrahydrofuran (THF) (2 mg/mL) and the solution was added dropwise to ultrapure water under stirring (270 rpm) in order to have a final acqueous suspension 1 mg/mL. Finally the organic solvent was evaporated under reduced pressure.

Nanoparticle characterization.

Particle Size and ζ-Potential Analyses

The hydrodynamic diameter of nanoparticles was investigated by dynamic light scattering (DLS) measurements, performed with Zetasizer Nano ZS ZEN3600 (Malvern Instruments Ltd,Worcestershire, UK), equipped with an He-Ne laser (λ = 632.8 nm) working at 4 mW. A disposable cuvette with an optical lenght of 1 cm was used and the backscattered light (173 °) was collected. The samples were prepared with an average concentration of 200 µg/mL in ultrapure water, and they were allowed to equilibrate at 25 °C for 30 seconds before the analysis. The hydrodynamic diameter was derived using Stokes-Einstein equation, considering a viscosity of the medium of 0.8872 cP. The results are reported as number mean and polidispersity index (PDI) values are shown.

The electrophoretic light scattering of samples, performed with the same instruments, was used to derive the ζ-potential values. ζ-potential values were calculated by the Zetasizer Software basing on electrophoretic mobility and considering a viscosity of 0.8872 cP and a dielectric constant of 78.5. Samples were prepared with an average concentration of 200 µg/mL in ultrapure water and they were allowed to equilibrate at 25 °C for 30 seconds before the analysis.

[1] *Chem. Soc. Rev.,* 2013, **42**, 1147

Morphology

Transmission electron microscopy (TEM) images of nanoparticles were obtained on a "FEI Tecnai G"' Spirit BioTWIN microscope (Hilsboro, OR) operating at 120 kV. The samples were prepared by evaporating 2 μL of nanoparticles (0.15 mg/mL for IRG-007 and VC-009 and 1 mg/mL for IRG-023 and IRG-028) onto carbon-coated copper grid (200 mesh) and allowing it to dry on the air. Nanoparticles were positively stained with 2% uranyl acetate in phosphate saline buffer (PBS). The average particle diameter were obtained by measuring about 100 particles by using imageJ software (National Institute of Health, USA).

Concentration. Nanoparticle tracking analysis (NTA) was performed using a Nano-sight LM10 instrument (Malvern, Worcestershire, UK), working with a monochromatic laser beam at λ=532 nm. Typically a nanoparticle dispersion was diluted 1000 fold with ultrafiltered and autoclaved water (Sigma Aldrich) and then illuminated by the instrument laser. A video of 90 seconds duration was recorded with a mean frame rate of 30 frames/s. Nanoparticles were tracked by their Brownian motion and analyzed by the NTA software (version 3.0, NanoSight). The velocity of particle movement was used to calculate particle size (nm) and concentration (number of particles per mL).

Cell cultures

HeLa (human cervix adenocarcinoma) and HepG2 (human hepatocellular carcinoma) cell lines were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.) and MEM (Sigma Chemical Co.), respectively. 10% Heat-inactivated fetal calf serum (Biowest), 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B (Sigma Chemical Co.) were added to both media. The cells were cultured at 37 °C in a moist atmosphere of 5% carbon dioxide in air.

Stock solutions of the compounds were made in dimethylsulfoxide and then diluted with complete medium for the antiproliferative assays in such a way that the final volume of solvent did not exceed 0.5%.

Inhibition growth assay

HeLa and HepG2 (3×10^4) cells were seeded into each well of a 24-well cell culture plate. After incubation for 24 h and various concentrations of the test agents were added. The cells were then incubated in standard conditions for a further 72 h. A Trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as GI_{50} values, that is, the concentration of the test agent inducing 50% reduction in cell number compared with control cultures.

Nanoparticle characterization

Table S1: comparison of size values (Hd: hydrodynamic diameter) obtained using different techniques. PDI and ζ-potential values are also reported.

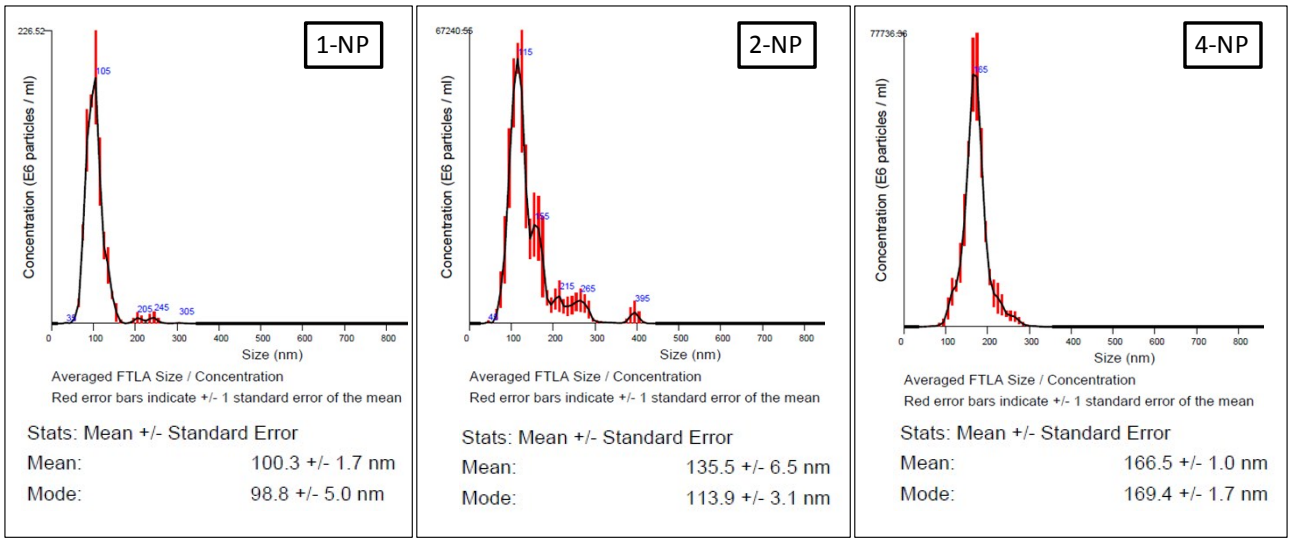
Nanoformulation	Hd (nm) by DLS[a]	Hd (nm) by NanoSight	Mean Diameter (nm) by TEM	PDI by DLS	ζ-potential (mV)
1-NP	85.93 ± 2.76	100.3 ± 1.7	69.30 ± 8.05	0.087 ± 0.019	-23.9 ± 0.36
2-NP	105.2 ± 10.5	135.5 ± 6.5	75.72 ± 9.50	0.094 ± 0.021	-23.0 ± 0.50
4-NP	133.4 ± 9.1	166.5 ± 1.0	114.29 ± 23.4	0.178 ± 0.007	-29.8 ± 0.32

[a]Hd values are reported as number mean gave by DLS

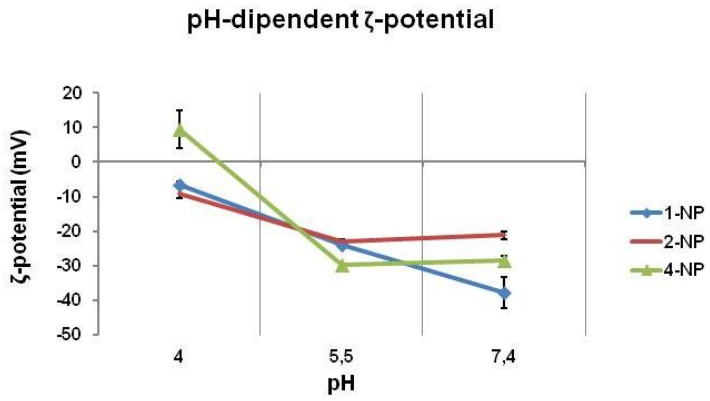
Table S2: Complete data obtained by DLS

Nanoformulation	Number mean (nm)	Intensity mean (nm)	z-average (nm)
1-NP	85.93 ± 2.76	132.7 ± 1.49	120.2 ± 0.23
2-NP	105.2 ± 10.5	158.5 ± 2.19	143.0 ± 5.30
4-NP	133.4 ± 9.1	288.1 ± 51.74	195.4 ± 1.27

Figure S3: NanoSight profiles of the nanoparticle suspension showing the concentration of each size population detected by the instrument.

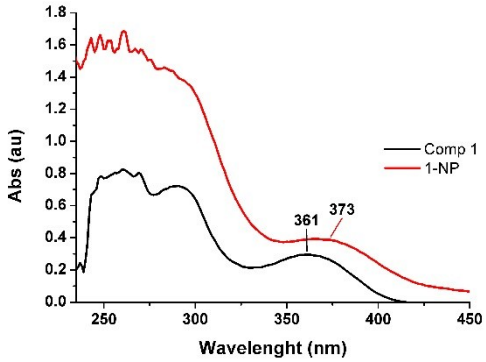


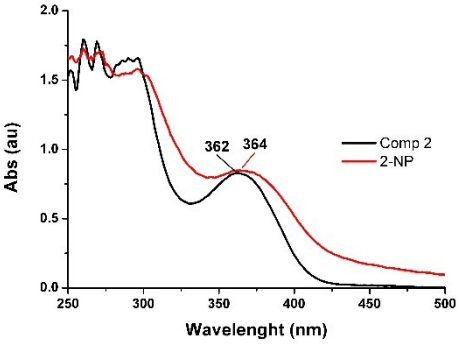
pH-dipendent ζ-potential

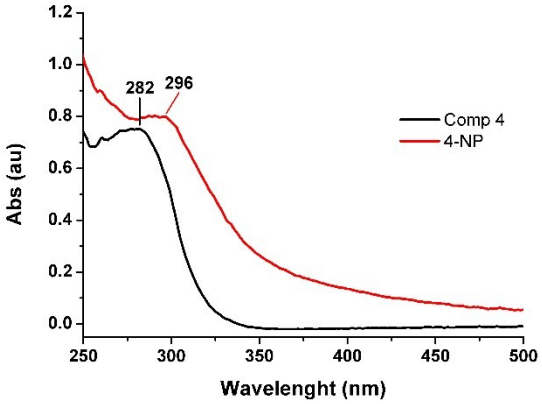


ζ-potential at different pH: in Hepes buffer 1 mM at pH 7.4 and in potassium hydrogen phtalate 1 mM at pH 4. The samples were prepared with an average concentration of 200 µg/mL in ultrapure water, and they were allowed to equilibrate at 25 °C for 30 seconds before the analysis.

UV measurements







The samples were prepared with an average concentration of 10-30 µg/mL in $CHCl_3$ for compounds **1**, **2** and **4** and in milliQ water for the corresponding NPs.