Electronic Supporting Information

Gold nanoparticles decorated by clustered multivalent cone-glycocalixarenes actively improve the targeting efficiency toward cancer cells

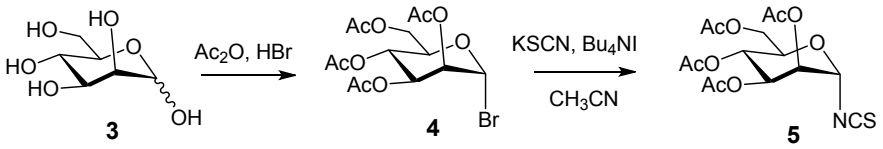
Svetlana Avvakumova, Paola Fezzardi, Laura Pandolfi, Miriam Colombo, Francesco Sansone, Alessandro Casnati, and Davide Prosperi

E-mail: davide.prosperi@unimib.it; alessandro.casnati@unipr.it

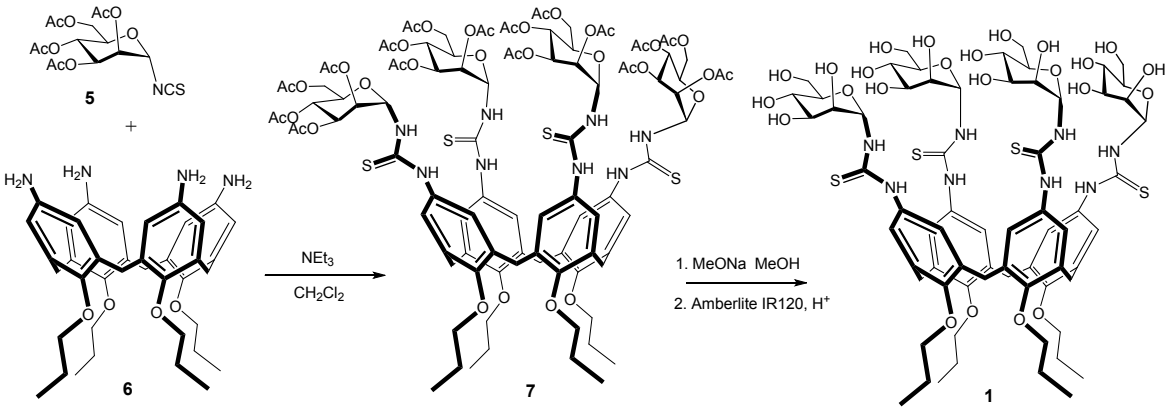
Materials. Tetrachloroauric(III) acid (99.9% w/w), sodium borohydride (98.5% w/w), tetraoctylammonium bromide (98% w/w), and 1-dodecanethiol (98% w/w) were purchased from Sigma-Aldrich. Organic compounds were synthesized and purified following the procedure described below. Water was deionized and ultrafiltered by a MilliQ apparatus from Millipore Corporation (Billerica, MA) before use. Cell culture medium and chemicals for biological experiments were purchased from EuroClone.

Synthesis of Calix-Man and Man-Mon compounds.

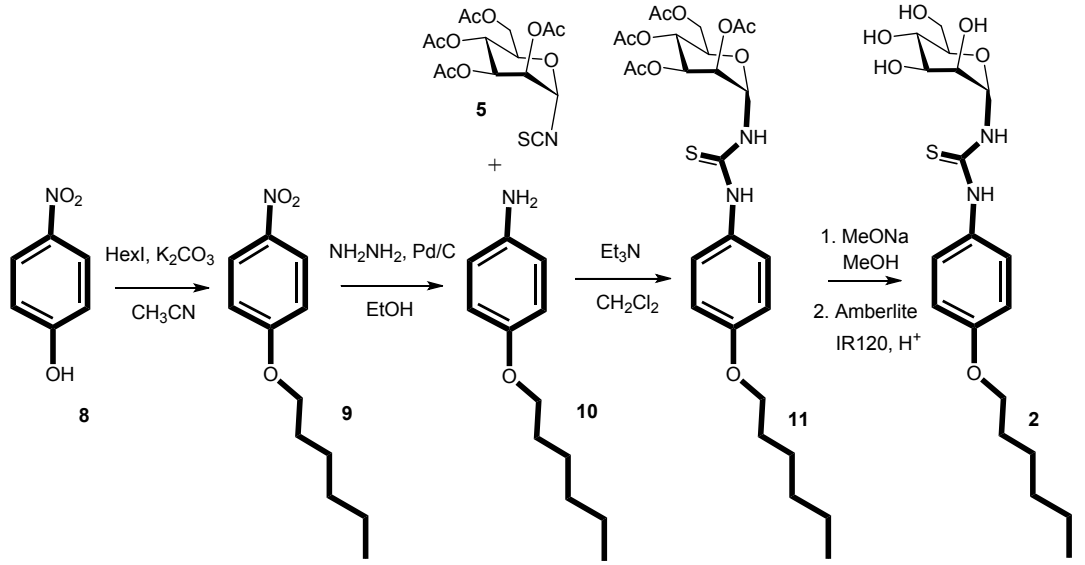
General Information. All moisture sensitive reactions were carried out under nitrogen atmosphere, using previously oven-dried glassware. Dry solvents were prepared according to standard procedures, distilled before use and stored over 3 or 4 Å molecular sieves. Most of the solvents and reagents were obtained from commercial sources and used without further purification. Merck silica gel 60 (70-230 mesh) was used for flash chromatography and for preparative TLC plates. ^{1}H NMR and ^{13}C spectra were recorded on Bruker AV300 and Bruker AV400 spectrometers. Chemical shifts are reported in part per million (ppm) using the residual peak of the deuterated solvent, whose values are referred to tetramethylsilane (TMS, δTMS = 0), as internal standard. ^{13}C NMR spectra were performed with proton decoupling. Electrospray ionization (ESI) mass analyses were performed with a Waters spectrometer. Tetrapropoxy-*p*-aminocalix[4]arene,[1] 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide,[2] and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl isothiocyanate[3] were synthesized according to literature.



Scheme S1. Synthesis of peracetylated mannose-1-α-isothiocyanate **5.**



Scheme S2. Synthesis of Calix-Man **1**.



Scheme S3. Synthesis of Mon-Man **2**.

Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide 4.

In a round bottom flask D-mannose **3** (1.0 g, 5.6 mmol) was dissolved in acetic anhydride (5 mL, 55 mmol) and then 3mL of hydrogen bromide until the sugar is completely dissolved, the other 3 mL (6 mL, 144 mmol). The mixture was stirred at room temperature for 8 h and checked via TLC (eluent: AcOEt/hexane 4:6). When completed, the solvent was removed under reduced pressure to give brownish syrup, that was dissolved in toluene (three times) and then in Et_2O (once) and the solvent always removed. The crude syrup is purified *via* flash column chromatography (eluent hexane/AcOEt 6:4) to give the product as a yellowish syrup with 51% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 6.23 (d, J = 1.4 Hz, 1H, H1), 5.63 (dd, J = 3.3, 10.2 Hz, 1H, H3), 5.36 (dd, J = 1.4, 3.3 Hz, H2), 5.29 (t, J = 9.9 Hz, H4), 4.26 (dd, J = 4.8, 12.3 Hz, 1H, H6), 4.17–4.12 (m, 1H, H5), 4.06 (dd, J = 2.1, 12.3 Hz, 1H, H6'), 2.10, 2.03, 2.00, 1.93 (4s, 12H, CH_3).

Synthesis of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl isothiocyanate 5.

In a round bottom flask under nitrogen KSCN (0.150 g, 1.57 mmol), tBu_4NI (0.285 g, 0.77 mmol) were dissolved in CH_3CN (10 mL) and stirred for 3h with 4Å molecular sieves. Then the sugar **4** (0.318 g, 0.77 mmol) dissolved in CH_3CN (3 mL) is added dropwise. The reaction is refluxed for 24h and monitored with TLC (eluent hexane/AcOEt 6:4). The solution is cooled to room temperature and filtered. The solvent is removed by rotary evaporation and the crude is purified by flash column chromatography (eluent hexane/AcOEt 6:4). The product is obtained as a yellowish oil in a 63% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 5.52 (d, J = 1.8 Hz, 1H, H1), 5.31–5.20 (m, 3H, H3, H2, H4), 4.24 (dd, J = 5.1, 12.5 Hz, 1H, H6), 4.12–4.04 (m, 2H, H5, H6'), 2.12, 2.05, 2.01, 1.96 (4s, 12H, CH_3).

Synthesis of *Cone*-5,11,17,23-tetrakis[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-thioureido]-25,26,27,28-tetrapropoxycalix[4]arene 7.

Isothiocyanate derivative **5** (50 mg, 0.08 mmol) was added to a solution of amino-calix[4]arene **6** (149 mg, 0.38 mmol) in 5 mL of dry CH_2Cl_2. Et_3N (42 μL, 0.30 mmol) was also added and then the mixture was stirred at room temperature overnight under N_2 atmosphere. The reaction progression was checked via TLC (eluent: AcOEt/hexane/CH_3OH 5:5:1). The solvent was then removed under vacuum and the residue purified via column chromatography (eluent: AcOEt/hexane/CH_3OH 5:5:1) to give product **7** as a light yellow solid. Yield: 65%. ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 6.85 (bs, 8H, ArH), 6.75 (bs, 8H, NH), 6.06 (bs, 4H, H1), 5.39–5.32 (m, 8H, H2, H3), 5.23 (t, J = 8.7 Hz, 4H, H4), 4.47 (d, J = 13.2 Hz, 4H, ArCH_2Ar), 4.36 (dd, J = 5.4, 12.2 Hz, 4H, H6), 4.16–4.13 (m, 4H, H6'), 4.09 – 3.95 (m, 4H, H5), 3.89 (t, J = 7.3 Hz, 8H, OCH_2CH$_2$CH$_3$), 3.24 (d, J = 13.2 Hz, 4H, ArCH_2Ar) 2.17, 2.08, 2.05, 2.02 (4s, 48H, COCH_3), 1.96 (q, J = 7.3 Hz, 8H, OCH$_2$CH_2CH$_3$), 1.05 (t, J = 7.3 Hz, 12H, OCH$_2$CH$_2$CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 182.6 (CS), 171.9, 171.2, 170.2, 170.0 (CO), 154.7 (Ar), 135.4 (Ar), 133.2 (Ar), 124.6 (Ar), 79.7 (C4), 76.7 (O*C*H$_2$), 70.4 (C5), 69.1 (C2), 68.9 (C3), 66.5 (C4), 62.0 (C6), 30.3 (Ar*C*H$_2$Ar), 23.1 (OCH$_2$*C*H$_2$), 19.4, 19.2, 19.1 (CO*C*H$_3$), 9.4 (CH$_2$*C*H$_3$)**.** ESI-MS: m/z 1128.2 $(M+2Na)^{2+}$.

Synthesis of *Cone*-5,11,17,23-tetrakis[-α-D-mannopyranosyl-thioureido]-25,26,27,28-tetrapropoxy calix[4]arene 1.

In a round bottom flask compound **7** (102 mg, 0.046 mmol) was dissolved in MeOH. Some drops of a solution 1M of MeONa were added until pH 9. The reaction was stirred for 3h at room temperature and was monitored with TLC (eluent AcOEt/MeOH/H_2O 5:2:1). Amberlite resin IR 120/H^+ was subsequently added for quenching. After neutralization, the resin was filtered off and the solvent removed from the filtrate under vacuum to give pure product in a quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6, 70 °C): δ (ppm) 9.46–9.16 (m, 8H, NH), 7.03–6.63 (m, 8H, Ar), 5.79–5.55 (m, 4H, H1), 4.86–4.50 (m, 12H), 4.37 (d, J = 12.9 Hz, 4H, $ArCH_2Ar$), 4.27–4.19 (m, 4H), 3.84 (t, J = 6.6 Hz, 8H, $OCH_2CH_2CH_3$), 3.70–3.38 (m, 8H), under solvent peak (4H, $ArCH_2Ar$), 1.92 (q, J = 6.6 Hz, 8H, $OCH_2CH_2CH_3$), 0.99 (t, J = 6.6 Hz, 12H, $OCH_2CH_2CH_3$). ^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) 181.1 (CS), 153.6 (Ar), 134.8 (Ar), 129.3 (Ar), 123.9 (Ar), 81.8, 79.1, 77.1, 74.8, 70.3, 66.9, 61.4, 30.7 ($ArCH_2Ar$), 23.2 (OCH_2CH_2), 10.6 (CH_3). ESI-MS: m/z 1560.4 $(M+Na)^+$.

Synthesis of 1-hexyloxy-4-nitrobenzene 9.

In a round bottom flask under nitrogen 4-nitrophenol **8** (300 mg, 2.2 mmol) and K_2CO_3 (600 mg, 4.3 mmol) were dissolved in 30 mL CH_3CN dry. Then iodohexane (640 μL, 4.3 mmol) was added and the reaction was vigorously stirred and refluxed for 24h. The reaction was checked with TLC (eluent: hexane/AcOEt 1:1). The hot solution was filtered and the solvent was removed under reduced pressure. The compound was dissolved in CH_2Cl_2 and filtered again. The solvent was evaporated giving the pure compound as yellow oil in a 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.09 (d, J = 9.3 Hz, 2H, Ar), 6.87 (d, J = 9.3 Hz, 2H, Ar), 3.97 (t, J = 6.3 Hz, 2H, OCH_2), 3.49 (bs, 2H, NH_2), 1.73 (q, J = 6.3 Hz, 2H, OCH_2CH_2), 1.43–1.25 (m, 6H, CH_2), 0.85 (t, J = 6.3 Hz, CH_3). The product shows the same physical-chemical properties reported in the literature.[4]

Synthesis of 1-hexyloxy-4-aminobenzene 10.

In a round bottom flask compound **9** (506 mg, 2.3 mmol) was dissolved in 30 mL EtOH and hydrazine hydrate (2.2 mL, 45 mmol) was added together with a catalytic amount of Pd/C. The reaction was stirred and refluxed for 24h. The solution was filtered and the solvent was removed under reduced pressure the pure product in 86% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.69–6.61 (m, 4H, Ar), 3.80 (t, J = 6.6 Hz, 2H, OCH_2), 1.65 (q, J = 6.6 Hz, 2H, OCH_2CH_2), 1.37–1.17 (m, 6H, CH_2), 0.81 (t, J = 6.6 Hz, 3H, CH_3). The product shows the same physical-chemical properties reported in the literature.[4]

Synthesis of 4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-thioureido)-1-hexyloxybenzene 11.

Isothiocyanate derivative **5** (450 mg, 1.1 mmol) was added to a solution of **10** (150 mg, 0.77 mmol) in 15 mL of dry CH_2Cl_2. Et_3N (160 μL, 1.1 mmol) was also added and then the mixture

was stirred at room temperature overnight under nitrogen atmosphere. Reaction checked via TLC (eluent: AcOEt/hexane 3:7). The solvent was then removed under vacuum and the residue purified via column chromatography (eluent: AcOEt/hexane) to give product **11** as a light yellow solid. Yield: 90%. ^{1}H NMR (300 MHz, DMSO-d_6, rt): δ (ppm) 10.40 (d, J = 9 Hz, 0.3H, NH), 9.44 (s, 1H, NH), 8.83 (d, J = 9 Hz, 0.7H, NH), 7.32 (d, J = 9 Hz, 2H, ArH), 6.87 (d, J = 9 Hz, 2H, ArH), 6.03 (d, J = 6.9 Hz, 0.7H, H1), 5.85 (d, J = 6.9 Hz, 0.3H, H1), 5.40 (dd, J = 3.6, 9.3 Hz, 1H, H3), 5.15–5.07 (m, 2H, H2, H4), 4.22–4.16 (m, 1H, H6), 4.03–3.90 (m, 4H, H6', H5, O*CH$_2$*CH$_2$), 2.02, 1.99, 1.98, 1.97 (4s, 12H, OCH$_3$), 1.67 (m, 2H, OCH$_2$*CH$_2$*), 1.38–1.29 (m, 6H, CH$_2$), 0.95–0.80 (m, 3H, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 182.1 (C=S), 170.5, 170.4, 169.9, 169.8 (C=O), 156.5 (Ar), 132.1 (Ar), 126.0 (*Ar*H), 114.6 (*Ar*H), 79.6 (C1), 69.6 (C3), 68.8 (C2), 68.0 (O*CH$_2$*), 66.6 (C4), 62.5 (C6), 62.3 (C5), 31.4, 29.1, 25.6, 22.5 (CH$_2$), 14.4 (CH$_3$). ESI-MS: m/z 605.3 (M+Na)$^+$, 1187.7 ([M-M]+Na)$^+$.

Synthesis of 4-α-D-Mannopyranosyl-thioureido-1-hexyloxybenzene 2.

In a round bottom flask compound **11** (140 mg, 0.24 mmol) was dissolved in MeOH. Some drops of a solution 1M of MeONa were added until pH 9. The reaction was stirred for 3 h at room temperature and was monitored with TLC (eluent AcOEt/MeOH/H_2O 5:2:1). Amberlite resin IR 120/H^+ was subsequently added for quenching. After neutralization, the resin was filtered off and the solvent removed from the filtrate under vacuum to give pure product in a quantitative yield. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 7.26 (m, 2H, ArH), 6.91 (m, 2H, ArH), 5.86 (bs, 0.3H, H1), 5.69 (bs, 0.7H, H1), 3.97 (t, J = 6.2 Hz, 2H, O*CH$_2$*CH$_2$), 3.87–3.61 (m, 3H, H2, H6, H6'), 3.53–3.45 (m, 2H, H3, H5), under solvent peak (H4), 1.77 (q, J = 6.2 Hz, 2H, OCH$_2$*CH$_2$*), 1.49–1.22 (m, 6H, CH$_2$), 0.93 (t, J = 6.2 Hz, 3H, CH$_3$). ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 181.0 (CS), 157.5, 156.8 (Ar), 126.5 (*Ar*H), 115.1 (*Ar*H), 81.2 (C1), 78.3 (C4), 74.3 (C5), 70.9 (C2), 67.8 (O*CH$_2$*), 61.5 (C6), 31.4, 28.9, 25.4, 22.3 (CH$_2$), 12.9 (CH$_3$)**.** ESI-MS: m/z 437.1 (M+Na)$^+$, 851.4 ([M-M]+Na)$^+$.

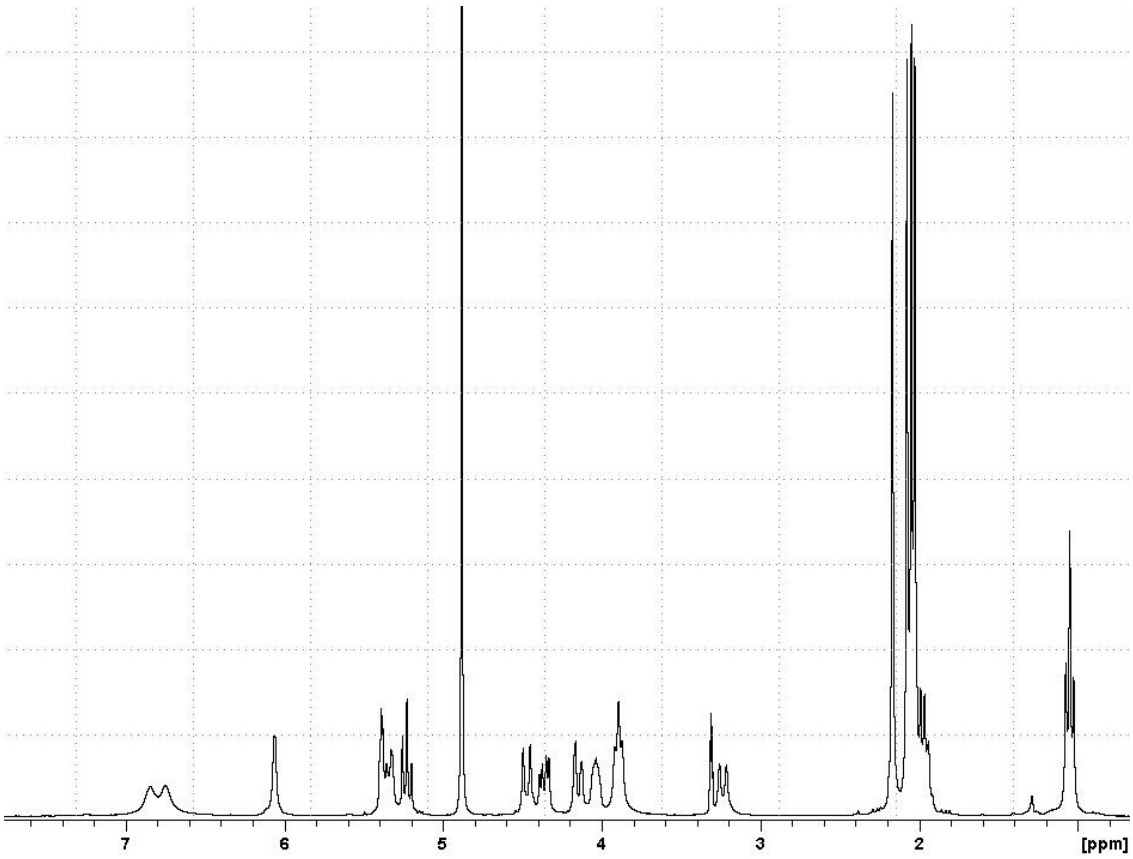


Figure S1 (a): ^{1}H-NMR spectrum (300 MHz, CD_3OD) of compound **7.**

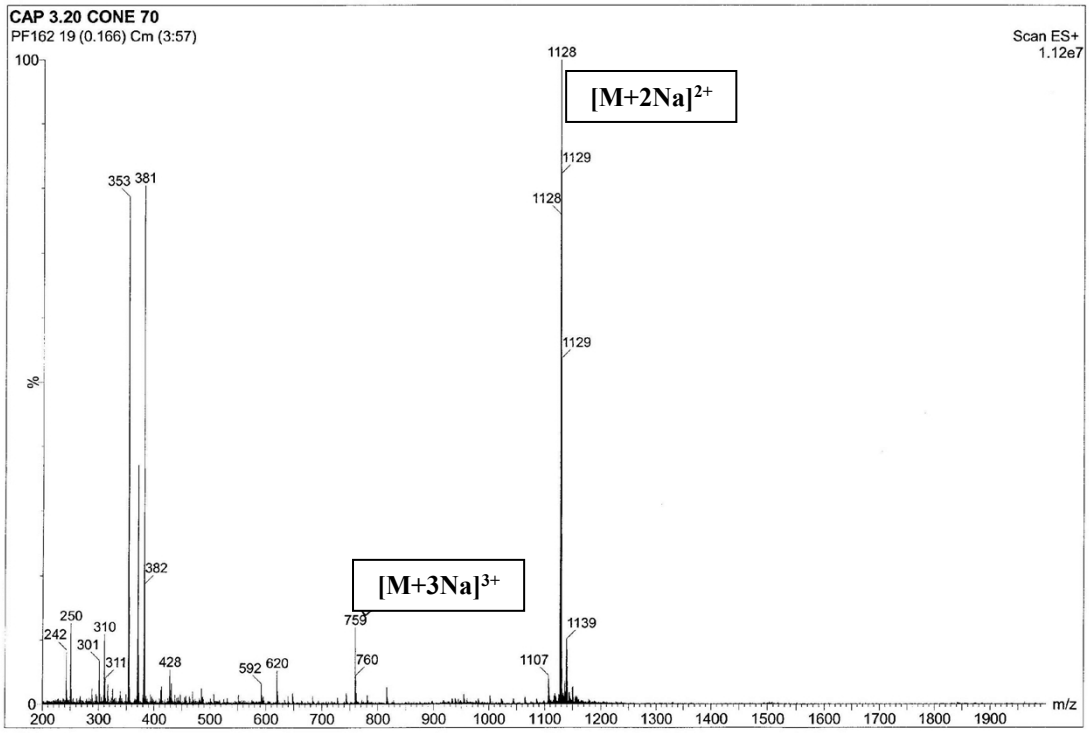


Figure S1 (b): ESI-MS (+) spectrum (MeOH) of compound **7.**

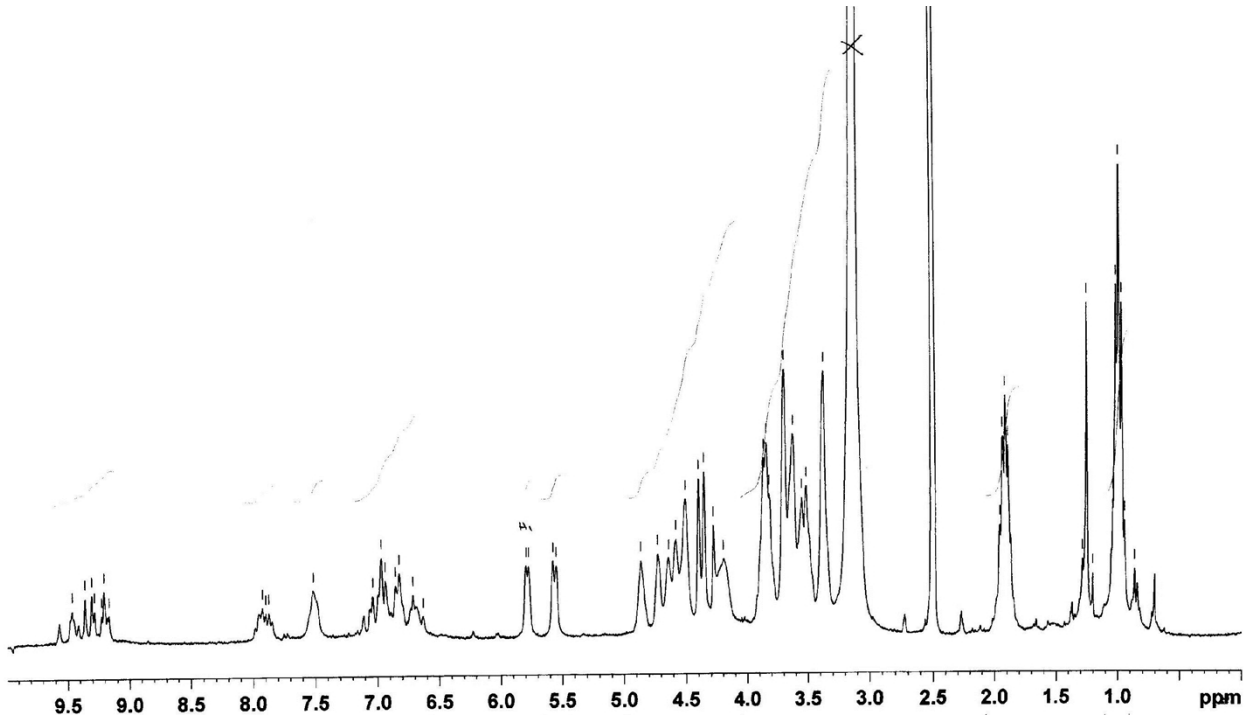


Figure S2 (a): ^{1}H-NMR spectrum (300 MHz, DMSO-d_6, 70 °C) of compound **1.**

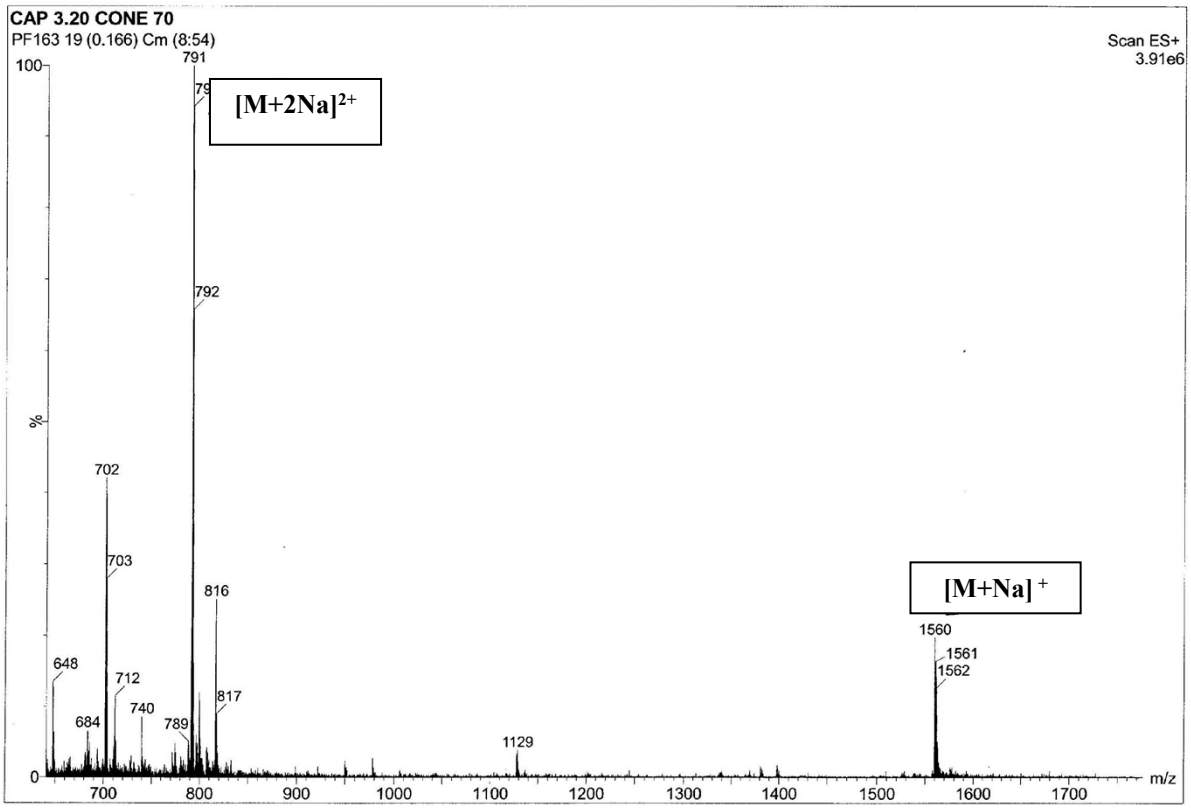


Figure S2 (b): ESI-MS (+) spectrum (MeOH) of compound **1**.

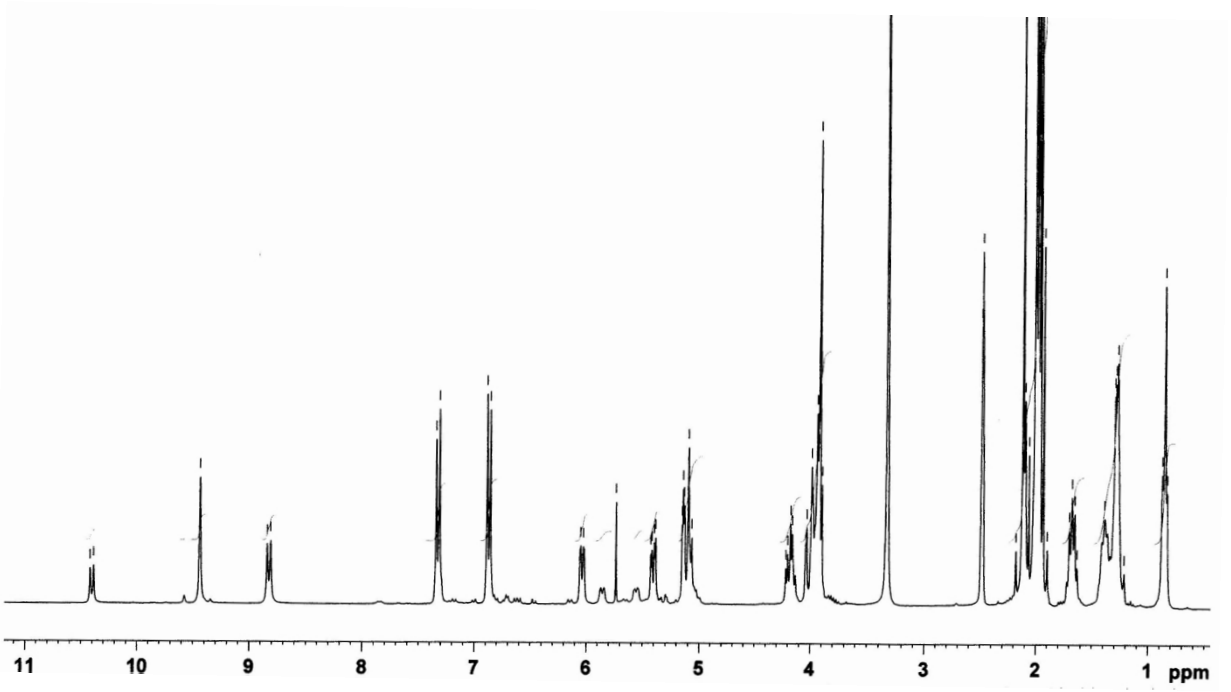


Figure S3 (a). ^{1}H-NMR (300MHz, DMSO-d_6, rt) spectrum of compound **11**.

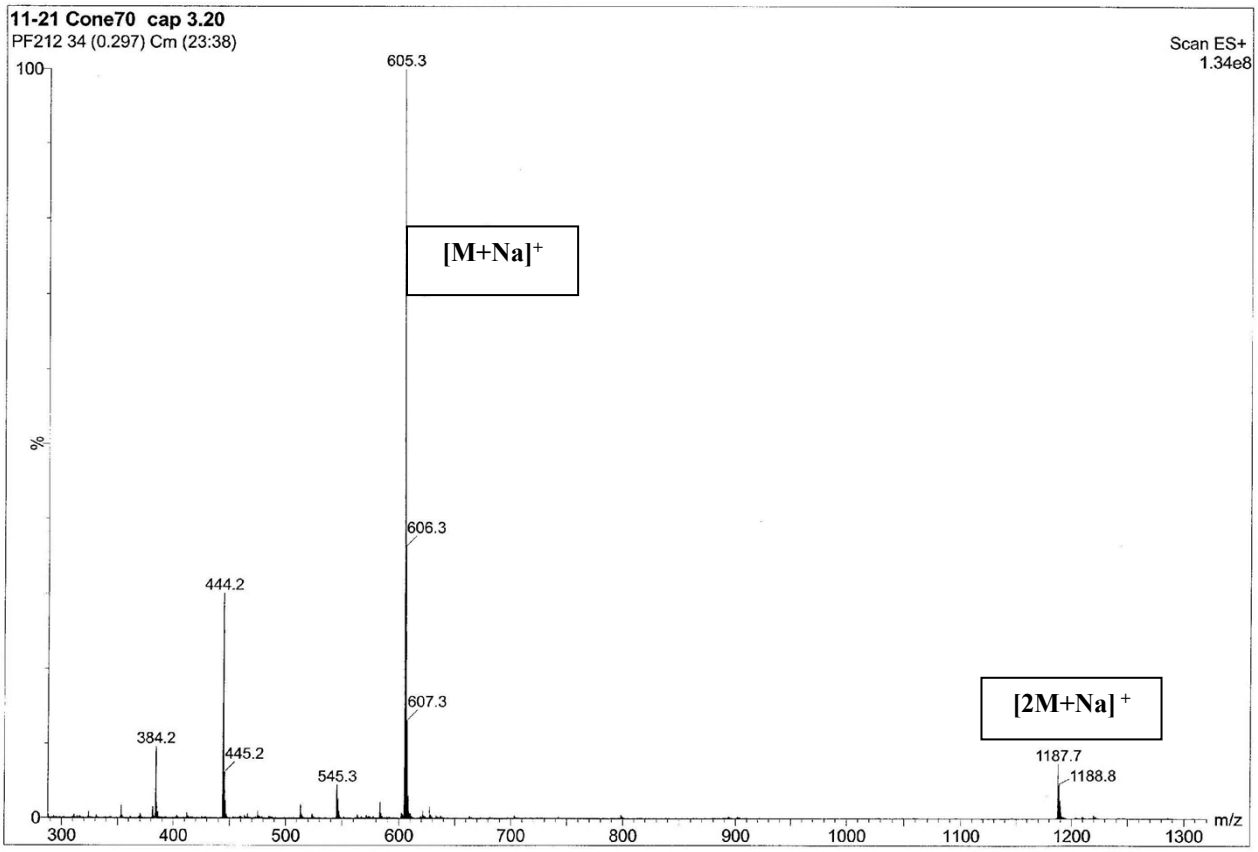


Figure S3 (b): ESI-MS (+) spectrum (MeOH) of compound **11**.

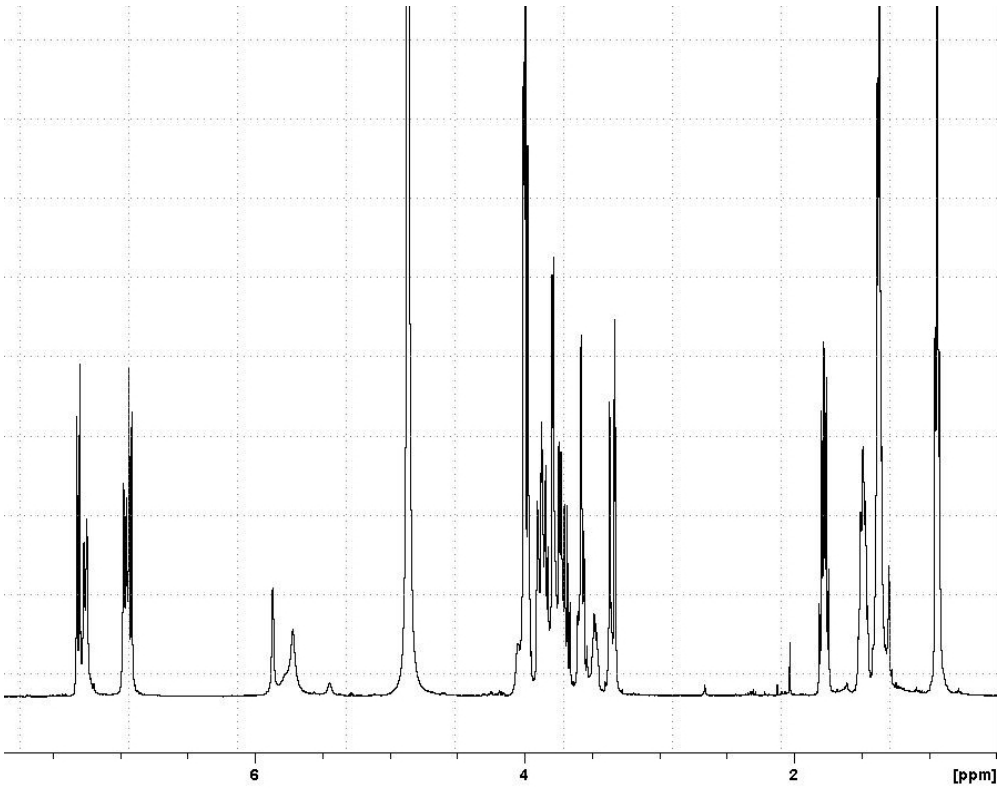


Figure S4 (a). ^{1}H-NMR (400MHz, CD_3OD/D_2O = 9/1) spectrum of compound **2**.

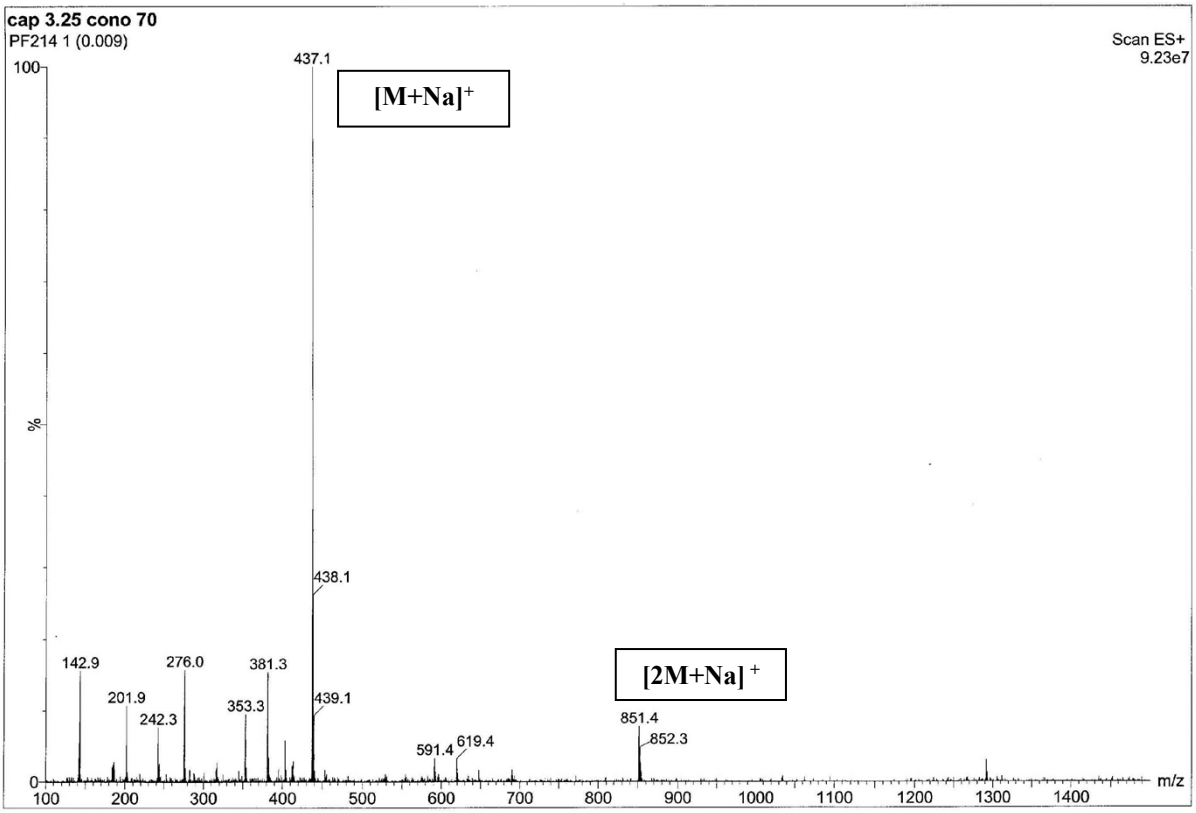
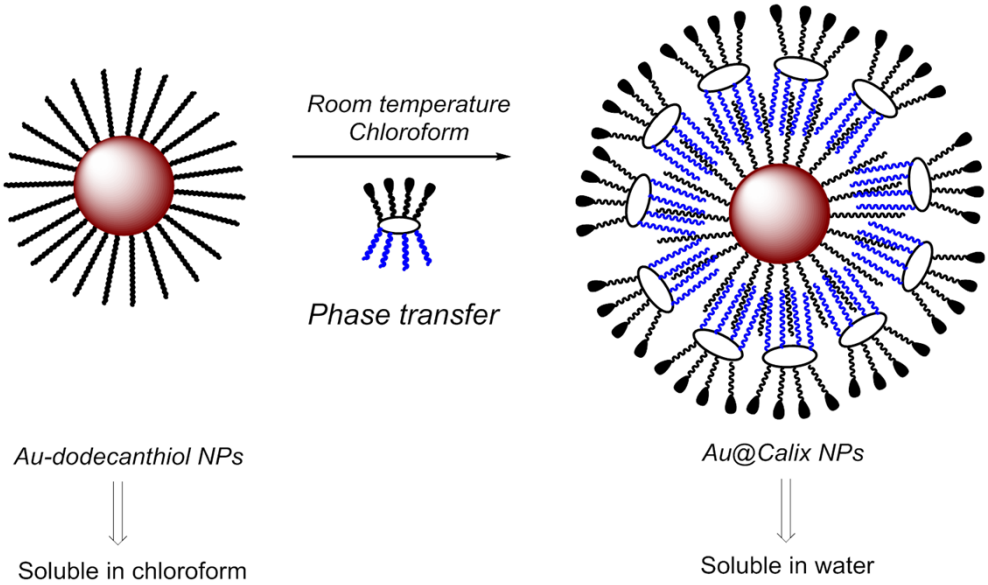


Figure S4 (b): ESI-MS (+) spectrum (MeOH) of compound **2**.

Gold nanoparticle synthesis. Colloidal Au NPs were synthesized in organic solvents according to standard procedures.[5] Transmission electron microscopy (TEM) analysis indicated an inorganic core diameter of 6 ± 1.12 nm. To transfer the NPs into an aqueous solution, amphiphilic compounds (Calix-Man and Mon-Man) were used. In brief, Calix-Man (6.26 μmol, 0.002 M) and Mon-Man (24 μmol, 0.02 M) were added to the hydrophobic AuNPs redispersed in 50 mL of chloroform (4.41 nmol and 4.03 nmol, respectively). The nanoparticles were sonicated for 1 min, followed by the evaporation of organic solvent. Finally, the NPs were re-dispersed in alkaline sodium borate buffer (SBB, pH = 12) and sonicated for 3 min. The NPs were extensively purified by repeated centrifugation using Amicon Ultra centrifugal filters (100 kDa cutoff) leading to pure water-soluble NP suspensions. The final nanoparticles were characterized by UV-vis, TEM, DLS, zeta-potential, and gel-electrophoresis.



Scheme S4. A schematic representation of AuNPs phase transfer procedure: dodecanthiol stabilized hydrophobic AuNPs are reacted with calixarene at room temperature in chloroform, followed by evaporation of the organic solvent and redispersion in water phase.

Physicochemical Characterization. Extinction spectra of hydrophobic and hydrophilic NPs were recorded on a NanoDrop 2000c Spectrophotometer (ThermoScientific) in 1 cm path length quartz cuvettes, using chlorophorm and SBB as media, respectively. The added volume of NP solution was 10 orders of magnitude lower compared to the present volume of medium to maintain the physical conditions of the medium. In each case, the pure medium served as a blank. The maximum absorbance between 220 and 300 nm was recorded for free organic compounds, while the surface plasmon peaks at 518 nm and 516 nm were observed for NPs before and after phase transfer, respectively. In order to determine the concentration of hydrophobic AuNPs, an extinction coefficient of 8.7×10^6 $M^{-1}cm^{-1}$ was used,[6] while the concentration of hydrophilic nanoparticles was calculated using an extinction coefficient of 1.26×10^7 $M^{-1}cm^{-1}$, following the procedure described by Haiss *et al.*[7] Dinamic Light Scattering (DLS) measurements were performed, using a 90 Plus Particle Size Analyzer from Brookhaven Instrument Corporation (Holtsville, NY) operating at 15 mW of a solid-state laser (λ = 661 nm),

using a scattering angle of 90°. A disposable cuvette with 1 cm optical path length was used for the measurements. The cuvettes were cleaned with Milli-Q water and stored dry. The samples were prepared by dilution with Milli-Q water containing 10 mM NaCl, followed by filtration with a 0.45 μm cellulose acetate syringe filter before loading into the cuvette in order to remove large interfering particulate matter. Each sample was allowed to equilibrate for 4 min prior to starting measurement. Three to ten independent measurements of 60 s duration were performed, at 25 °C. The calculations of hydrodynamic diameter were performed using Mie theory, considering absolute viscosity and refractive index values of the medium to be 0.911 cP and 1.334, respectively. The Zeta-potential was determined at 25 °C using a 90 Plus Particle Size Analyzer from Brookhaven Instrument Corporation (Holtsville, NY) equipped with an AQ-809 electrode, operating at applied voltage of 120 V. The samples for measurements were prepared by dilution the concentrated nanoparticles in 10 mM NaCl (pH 7). A minimum of 3 runs and 10 subruns per sample were performed to establish measurement repeatability. The Zeta-potential was automatically calculated from electrophoretic mobility based on the Smoluchowski theory. A viscosity of 0.891 cP, a dielectric constant of 78.6, and Henry function of 1.5 were used for the calculations. TEM micrographs of AuNPs were obtained using a Zeiss EM109 instrument at an accelerating voltage of 80 kV. The samples were prepared by evaporating a drop of nanoparticles onto carbon-coated copper grid and allowing it to dry on the air. The histograms of the particle size distribution and the average particle diameter were obtained by measuring about 150-200 particles by using Measure IT Olympus Software.

Table S1. Molar amounts of hydrophobic Au-dodecanethiol stabilized NPs and Calix-Man and Mon-Man used to transfer the NPs from chloroform to water phase.

Nanoparticle	**AuNPs, mol**	**Calix, mol**	**Calix/Au (Molar ratio)**
Au-Calix-Man	4.41×10^{-9}	6.13×10^{-6}	**1390/1**
Au-Mon-Man	4.04×10^{-9}	17.0×10^{-6}	**4210/1**

Table S2. Physico-chemical characterization of water-soluble functionalized nanoparticles: hydrodynamic diameter measured by dynamic light scattering (DLS), zeta-potential and plasmonic bands found from UV-vis spectra.

Nanoparticle	**Hydrodynamic diameter (DLS), nm**	**Zeta-potential, mV**	**Plasmon band (UV-vis), nm**
Au@Calix-Man	47.2 (PI 0.268)	–41.31±1.79	522
Au@Mon-Man	59.4 (PI 0.223)	–37.65±1.94	527

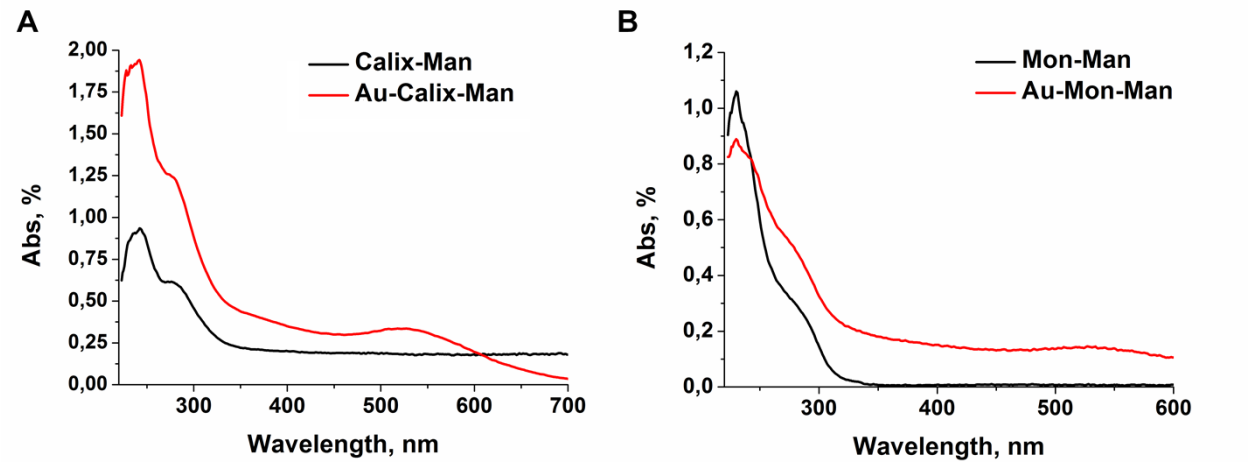


Figure S5 (a). UV-vis spectra of A) Calix-Man (black line) and Au-Calix-Man NPs (red line); B) Mon-Man (black line) and Au-Mon-Man NPs (red line).

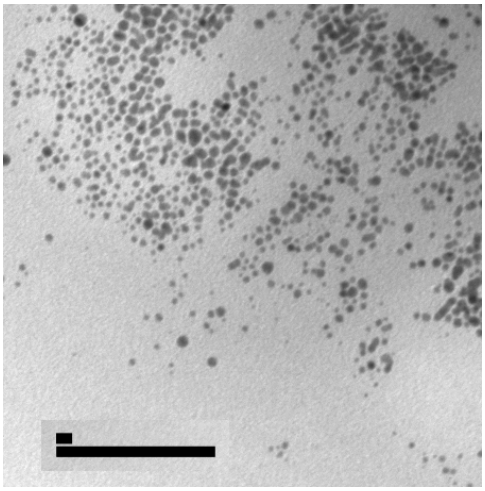


Figure S5 (b). TEM image of Au-Calix-Man NPs (scale bar 10 nm and 100 nm).

Study of nanoparticle stability in DMEM.

As an empirical measurement of the aggregation process, an aggregation parameter, which measures the variation of the integrated absorbance between 600 and 700 nm, was used. The aggregation parameter AP is defined as follows: $AP = (A-A_0)/A_0$, where A is the integrated absorbance between 600 and 700 nm of the sample at a given moment and A_0 is the integrated absorbance between 600 and 700 nm of the initial, fully dispersed solution of nanoparticles [8]. An aliquot of Au-Calix-Man or Au-Mon-Man NPs was re-dispersed in DMEM (lacking phenol-red pH indicator) and incubated for 120 min at room temperature. Subsequently, the absorbance of nanoparticles both at 600 and 700 nm was measured every 30 min by UV-vis spectroscopy, and the values of AP were calculated and plotted as function of incubation time.

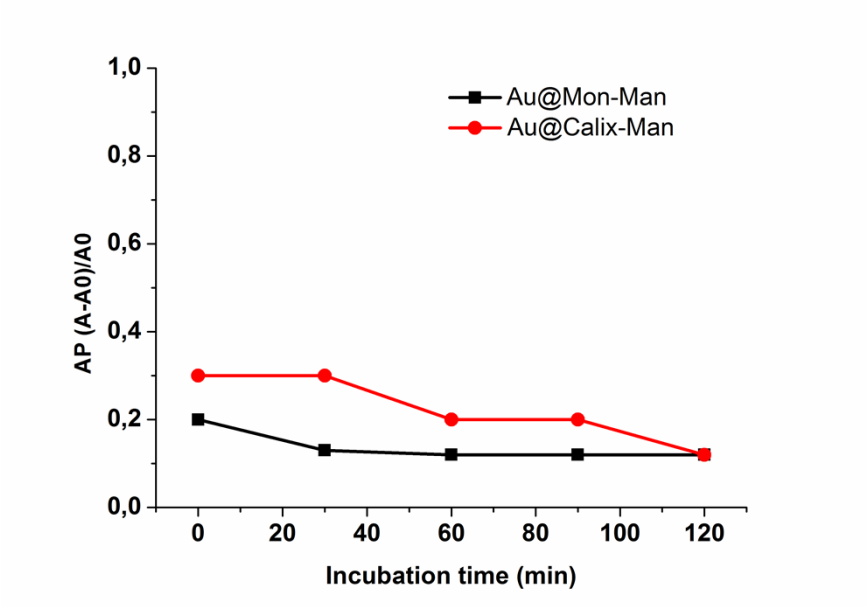


Figure S6. Stability of Au-Calix-Man (red plot) and Au-Mon-Man (black plot) NPs as function of incubation time in DMEM.

Determination of number of Calix-Man and Mon-Man molecules per nanoparticle.

The number of Calix-Man and Mon-Man molecules, and, thus, the number of mannose moieties, present on every nanoparticle was evaluated by UV-vis spectroscopy. In brief, a calibration curve was plotted on the basis of UV-vis absorption of Calix-Man and Mon-Man molecules at 230 nm, as function of concentration. Au-Calix-Man and Au-Mon-Man NPs were thoroughly washed by centrifugation to avoid the presence of free organic compounds, and concentration of Calix-Man and Mon-Man molecules was determined from supernatant using the calibration curves. The number of free Calix-Man and Mon-Man molecules was calculated. From this, the final number of intercalated molecules per nanoparticle was calculated considering AuNPs concentration from UV-vis.

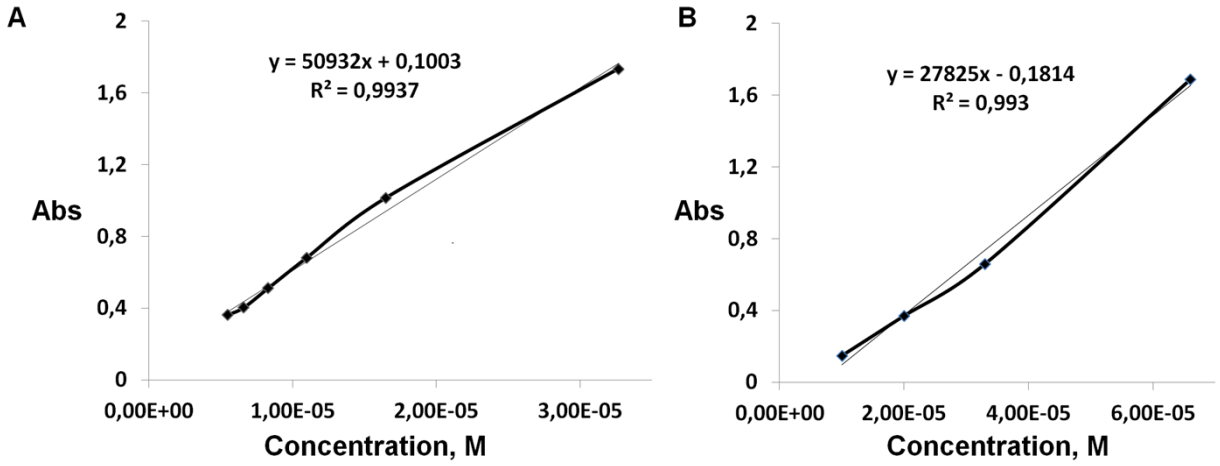


Figure S7. Calibration curves of A) Calix-Man and B) Mon-Man: absorbance at 230 nm as function of concentration.

Investigation of Protein Adsorption. About 10 µL of a dispersion of functionalized AuNPs (~2.4 mg mL^{-1}) was added to 1 mL of phosphate buffer solution (PBS, 5×, pH = 7.4) containing 0.25 mg mL^{-1} of FITC conjugate bovine serum albumin (BSA-FITC). The NPs were incubated for 24 h at room temperature, and purified by centrifugation using Amicon Ultra Centrifugal Filters (100k). An aliquot of NP-free supernatant (10 µL) was diluted 50 times with mQ water and the fluorescence of unbound BSA-FITC was measured using Fluorescence spectra were recorded using a Fluoromax-4P spectrofluorometer from Horiba Scientific (New Jersey, U.S.A.). Samples were excited at a fixed wavelength and spectra were recorded in a wavelength range between 480 and 700 nm. A solution of 0.25 mg mL^{-1} BSA-FITC in PBS was utilized as a reference. The excitation wavelength was set at 488 nm, giving rise to an emission peak around 514 nm. The input/output slit was 2 nm.

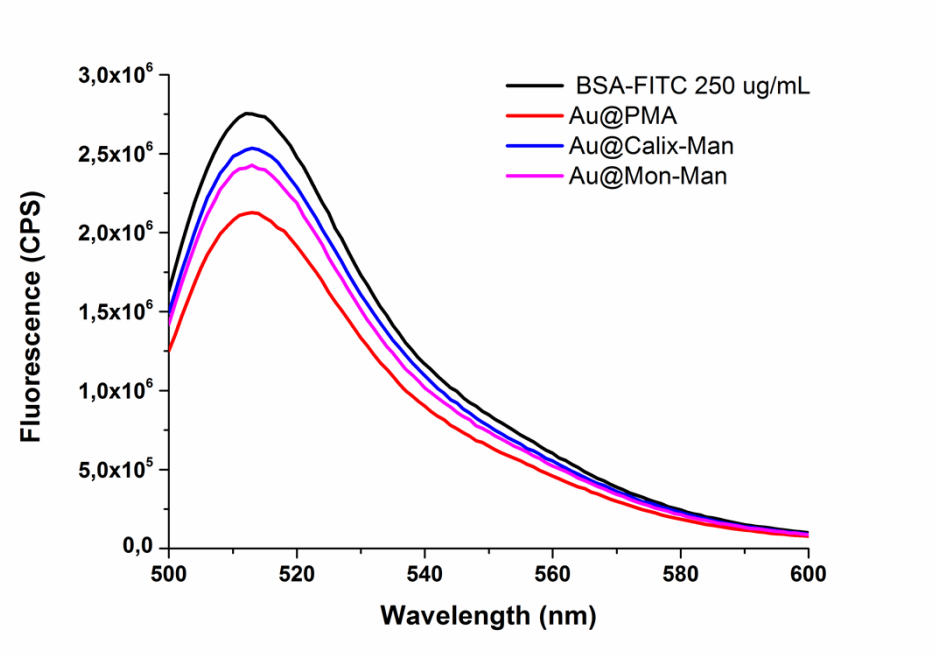


Figure S8. Emission spectra of Au-Mon-Man (fuxia line), Au-Calix-Man (blue line) and Au-PMA (red line) after incubation with 250 µg mL^{-1} BSA-FITC along with emission spectrum of initial BSA-FITC at 250 µg mL^{-1} (black line).

Cell culture. HeLa (human cervical cancer cell line) was used as a cellular model. Cells were cultured in 50% Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (50 UI mL^{-1}) and streptomycin (50 mg mL^{-1}) at 37 °C and 5% CO_2 incubator. Cell culture medium and biological chemicals were purchased from EuroClone.

Nanoparticle uptake experiments. The experiments were carried out at approximately 80% confluence. Before incubation with gold nanoparticles the medium was removed by aspiration and the cells were washed twice with Dulbecco's Phosphate Buffer Saline (PBS) w/o Calcium w/o Magnesium. Then, 1 mL of a 50 nM suspension of gold nanoparticles in fresh medium was

added, and the cells were incubated for 2 h at 37 °C. In order to determine uptake efficiency, gold content was measured by ICP-OES (Optima 7000 DV, Perkin Elmer). For this purpose, the cells were carefully washed with PBS post-incubation in order to remove any excess of gold nanoparticles. The cells were trypsinized, rinsed out with 3 mL of cell culture medium and collected in disposable tube. Afterwards, the cells were centrifuged for 5 min at 2000 rpm and redispersed in 2 mL of PBS. Cells were counted manually using a hemocytometer prior to further procession. The samples were incubated with 5 mL of *aqua regia* (caution: extremely corrosive), and the mixture was left for 72 h, leading to the complete dissolution of all cellular material. Prior to perform the analysis, the samples were diluted with water to a final volume of 16 mL. Standard solution of gold for calibration was purchased from Aldrich.

Inhibition of mannose receptor. For inhibition experiments, HeLa cells were cultured as above and preincubated at 37 °C with 1 mg mL^{-1} dextran (Mw = 60-80 kg/mol) for 45 min before the addition of mannose-functionalized nanoparticles at 50 nM concentration (Formulations Au-Calix-Man and Au-Mon-Man, respectively) suspended in media with 1 mg/mL dextran. The experiment duration was 2 h at 37 °C. At the end of the incubation period, cells were processed as previously described and gold content was analyzed by ICP-OES.

Cell death assay. Cells were cultured at a density of 2×10^5 cells per well in a 12-well plate at the above conditions. One day after plating, cells were incubated at 10 nM and 20 nM concentration of Au-Calix-Man and Au-Mon-Man NPs, for 3 h and 24 h at 37 °C. At the end of the incubation period, floating cells were collected in FACs tubes, while adherent cells were washed twice with PBS and treated according to PE Annexin V Apoptosis Detection Kit I manufacturer's protocol (Becton Dickinson Biosciences) for FACs analysis. Afterwards, cells were analyzed on a FACS Calibur flow cytometer (Becton Dickinson) by acquisition of 20000 events for each analysis, after gating on viable cells. The data were analyzed by CellQuest Pro Software (Becton Dickinson).

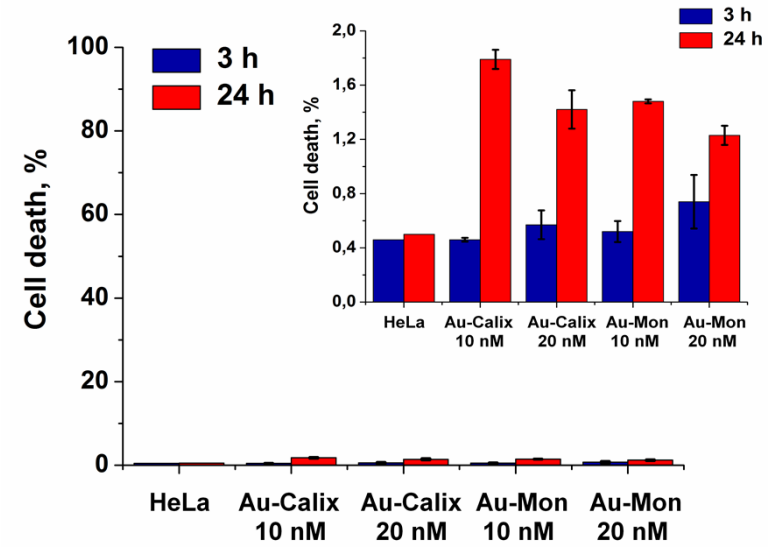


Figure S9. Cell death analysis with Annexin V. Au-Calix-Man and Au-Mon-Man NPs were incubated with HeLa cells for 3 h (blue bar) and 24 h (red bar) at 10 and 20 nM concentration.

References:

[1] F. Sansone, E. Chierici, A. Casnati, R. Ungaro, *Org. Biomol. Chem.,* **2003**, *1*, 1802-1809.
[2] K. P. Ravindranathan Kartha, H. J. Jennings, *J. Carbohydr. Chem.*, **1990**, *9*, 777-781.
[3] J. Camarasa, P. Fernandez-Resa, M. T. Garcia-Lopez, F. G. De Las Heras, P. P. Mendez-Castrillon, A. San Felix, *Synthesis*, **1984**, 509.
[4]. M.J. Jeong, J.H. Park, C. Lee, J.Y. Chang, *Org. Lett.*, **2006**, *8*, 2221-2224.
[5] M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *Chem. Commun.* **1994**, 801–802.
[6] C. Geidel, S. Schmachtel, A. Riedinger, C. Pfeiffer, K. Müllen, M. Klapper, W. J. Parak, *Small*, **2011**, *7* (20), 2929-2934.
[7] W. Haiss, N. T. K. Thanh, J. Aveyard, D. G. Fernig, *Anal. Chem*. **2007**, *79*, 4215-4221.
[8] R. Levy, N. T. K. Thanh, R. C. Doty, I. Hussain, R. J. Nichols, D. J. Schiffrin, M. Brust, D. G. Fernig*, J. Am. Chem. Soc.,* **2004**, *126*, 10076-10084.