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SUPPORTING INFORMATION FOR

Gold nanoparticles are suitable cores for building tunable iminosugar multivalency

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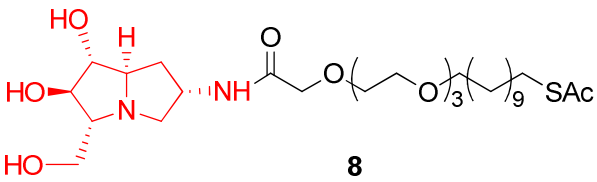
General methods

All chemicals were purchased as reagent grade from Sigma–Aldrich, except chloroauric acid (Strem Chemicals), and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium-backed sheets (Merck) with visualization under UV (254 nm) and/or by staining with *p*-anisaldehyde solution [anisaldehyde (25 mL), H_2SO_4(25 mL), EtOH (450 mL), and CH_3COOH (1 mL)], 10% H_2SO_4 solution in EtOH, ninhydrin solution [ninhydrin (0.25 mL), EtOH (100 mL)] followed by heating at over 200 °C. Size-exclusion column chromatography was performed on Sephadex LH-20 (GE Healthcare). Flash column chromatography (FCC) was performed on silica gel 60 (0.063–0.200 mm; Merck). UV/Vis spectra were measured with Beckman Coulter DU 800 UV/Vis spectrophotometer. Infrared (IR) spectra were recorded from 4000 to 750 cm^{-1} with a Thermo Nicolet 6700 FT-IR model spectrometer; solids were pressed into KBr pellets and oils were subjected to attenuated total reflection (ATR). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz (high resolution) spectrometer. Chemical shifts (δ) are given in ppm relative to the residual signal of the solvent used. Coupling constants (*J*) are reported in Hz. Splitting patterns are described by using the following abbreviations: *br*, broad; s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet. Mass spectra were measured with an Esquire 6000 ESI-Ion Trap spectrometer from Bruker Daltonics. High-resolution mass spectra (HRMS) were obtained using the MALDI technique with a 4700 Proteomics Analyzer (Applied Biosystems) operated in MALDI-TOF-TOF configuration. Optical rotations were determined with a Perkin–Elmer 341 polarimeter. For transmission electron microscopy (TEM) examinations, a single drop (2 µL) of an aqueous solution (ca. 0.05 $mgmL^{-1}$ in Milli-Qwater) of the gold glyconanoparticles (GNPs) was placed on a copper grid coated with a carbon film (Electron Microscopy Sciences). The grid was left to dry in air for several hours at room temperature. TEM analysis was performed with a JEOL JEM-2100Fmicroscope, both operating at 200 kV. The average diameters and numbers of gold atoms of the GNPs were deduced as described in a previous study.[1] Laboratory distilled water was further purified using a Milli-Qreagent grade water system (Millipore).

[1] O. Martínez-Ávila, K. Hijazi, M. Marradi, C. Clevel, C. Campion, C. Kelly, S. Penadés, *Chem. Eur. J*. **2009**, *15*, 9874–9888.

Synthesis of pyrrolizidine alkaloid (PA) derivative 8: A solution of compound **7** (74.5 mg, 171 µmol), 1–hydroxybenzotriazole (HOBt, 34.7 mg, 257 µmol) and *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU, 97.5 mg, 257 µmol) in DMF (2 mL) was left stirring for 30 min and then added to a solution of pyrrolizidine derivative **1** (33.5 mg, 178 µmol) and *N*,*N*-diisopropylethylamine (47 µL, 267 µmol) in DMF (1.8 mL). The reaction mixture was left stirring at room temperature for 15 hours, then diluted with AcOEt (10 mL) and washed with H_2O (5 x 4 mL). The organic layer was then washed with a saturated solution of $NaHCO_3$ (3 x 10mL), water (2 x 10 mL) and brine (1 x 8 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. Purification through gradient column chromatography (DCM/MeOH from 10:1 to 5 : 1) afforded **8** (53 mg, 87 µmol) in a 51% yield. R_f = 0.30 (DCM/MeOH 7 : 1). $[\alpha]_D^{29}$ **=** + 9.6 (c = 0.78, MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 4.54 (quin, *J* = 6.4 Hz, 1H, 6-H), 4.02 (s, 2H, HNCOC*H*$_2$-), 3.84-3.77 (m, 3H, 1-H, 2-H, 8-Ha), 3.72-3.59 (m, 13H, OC*H*$_2$, 8-Hb), 3.53 (q, *J* = 6.5 Hz, 1H, 7a-H), 3.49 (t, *J* = 6.7 Hz, 2H, OC*H*$_2$(CH_2)$_{11}$SAc), 3.22 (dd, *J* = 11.6, 5.9 Hz, 1H, 5-Ha), 3.05 (dd, *J* = 11.6, 6.3 Hz, 1H, 5-Hb), 2.88 (t, *J* = 7.3 Hz, 3H, -C*H*$_2$SAc, 3-H), 2.32 (s, 3H, SAc), 2.22 (dt, *J* = 12.5, 6.0 Hz, 1H, 7-Ha), 2.09 (dt, *J* = 12.5, 7.5 Hz, 1H, 7-Hb), 1.62-1.55 (m, 4H, -C*H*$_2$$CH_2$SAc, -O$CH_2$C*H*$_2$), 1.40-1.29 (m, 14H, -C*H*$_2$-) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 196.3 (s, S*C*OCH_3), 171.3 (s, *C*ONH_2), 80.8 (d, C-1), 77.2 (d, C-2), 70.1 (d, C-3), 70.0-69.8 (t, 8C, O*C*H$_2$), 67.1 (d, C-7a), 61.6 (t, C-8), 58.7 (t, C-5), 47.8 (d, C-6), 35.4 (t, C-7), 29.3-25.8 (11C, t,-*C*H_2-, q, SCO*C*H_3) ppm. IR (KBr): ν = 3351, 2925, 2854, 1691, 1660, 1542, 1465, 1108 cm^{-1}. HRMS (ESI): *m/z* calcd for $C_{29}H_{54}N_2O_9S$: 629.3448. $[M+Na]^+$; found: 629.3485.



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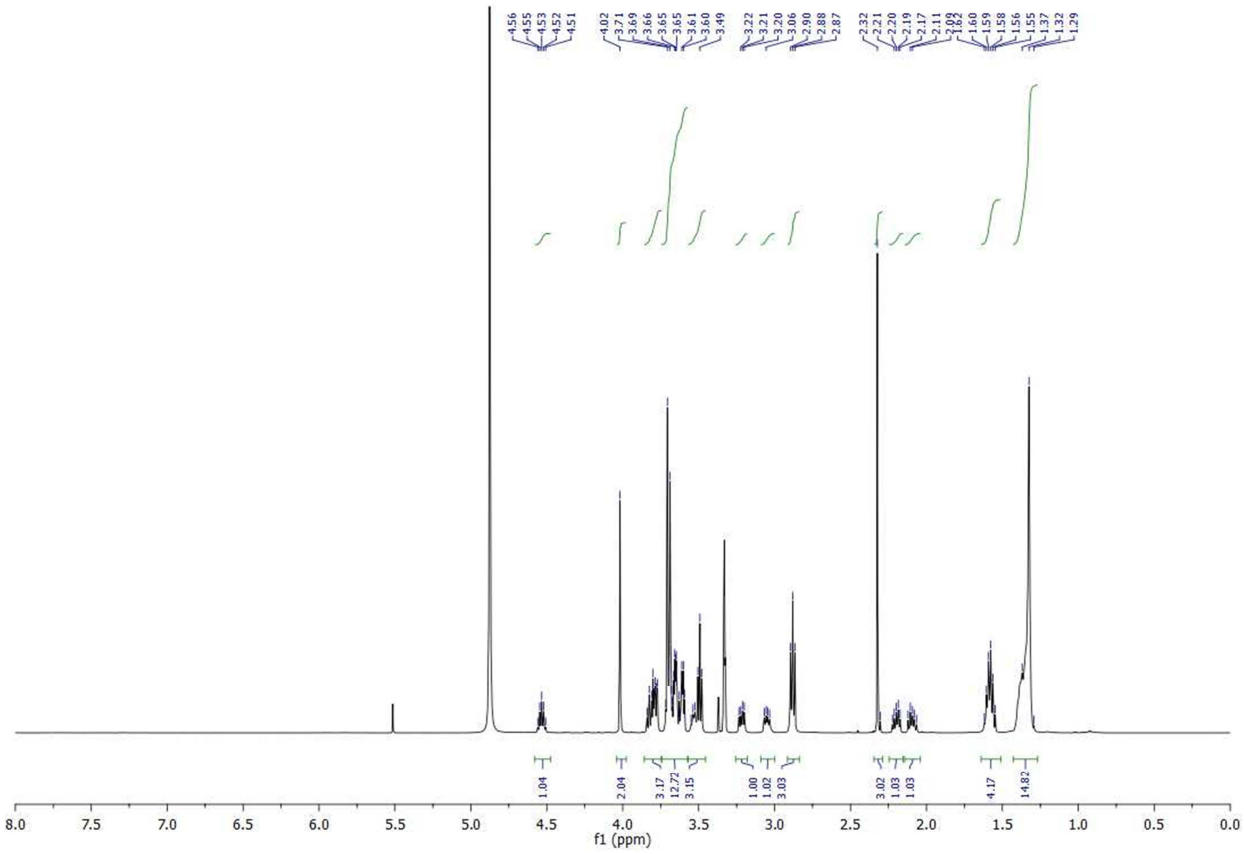
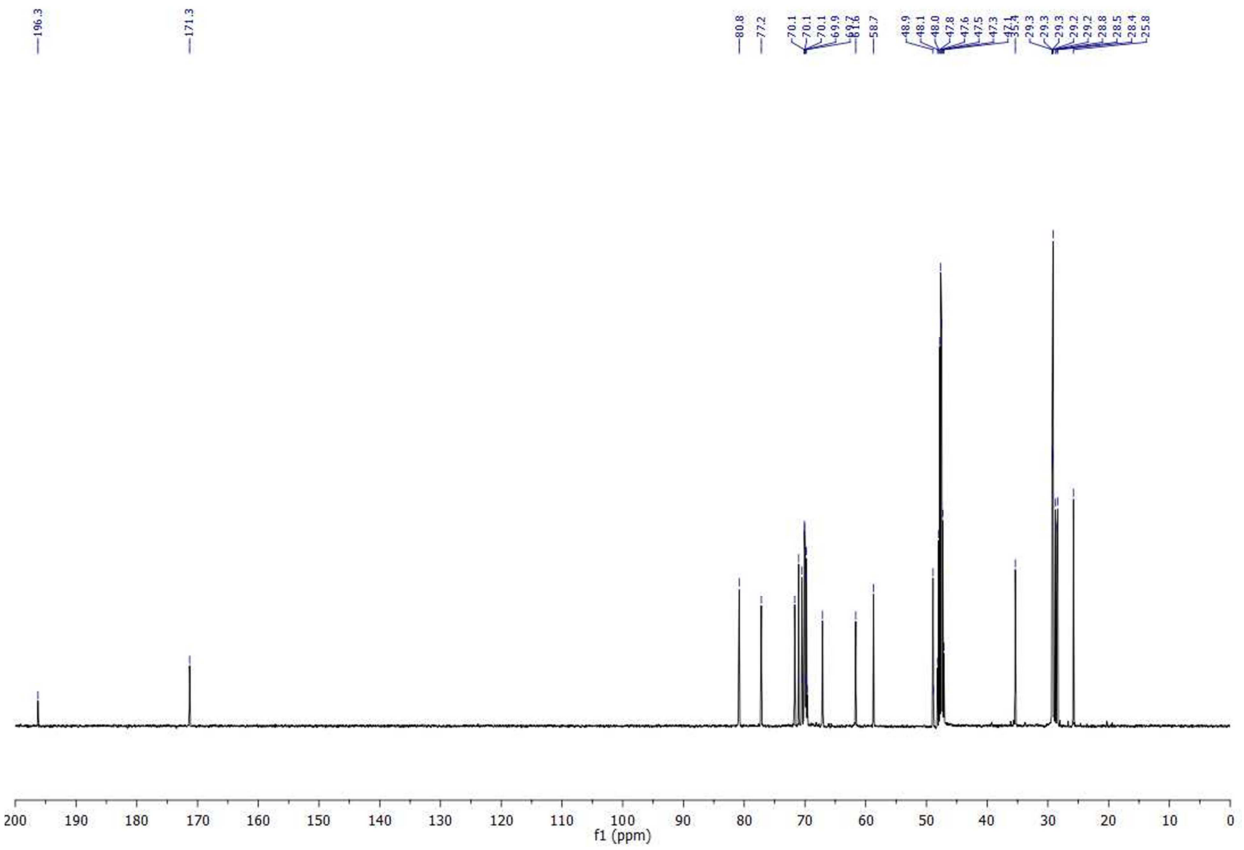
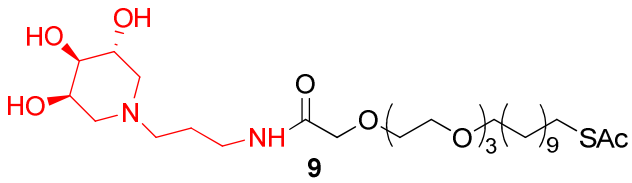


^{1}H NMR of compound **8** (500 MHz, CD_3OD)



^{13}C NMR of compound **8** (125 MHz, CD_3OD)

Synthesis of piperidine alkaloid (PIPA) derivative 9: A solution of EDC·HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride (32.0 mg, 168 µmol), 1–hydroxybenzotriazole (HOBt, 21.0 mg, 158 µmol) and **7** (47.0 mg, 108 µmol) in DMSO (0.4 mL) was left stirring for 10 min. and then added to a solution of piperidine derivative **2** (20.0 mg, 105 µmol) and *N,N*-diisopropylethylamine (33 µL, 189 µmol) in DMSO (0.3 mL). The reaction mixture was left stirring at room temperature, under Ar atmosphere, for 65 hours, then diluted with AcOEt (10 mL) and washed with H_2O (5 x 4 mL). The organic layer was then washed with water (2 x 6 mL) and brine (1 x 4 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude was triturated with *n*-Hexane (5 x 2 mL) and then purified by column chromatography (DCM/MeOH 10:1) affording 56 mg of **9** (92 µmol, 89% yield). R_f = 0.52 (DCM/MeOH 7 : 1). $[\alpha]_D^{29}$ **=** - 12.5 (c = 0.80, MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 4.03 (s, 2H, HNCOCH_2-), 3.99 (*br* s, 1H, 3-H), 3.88 (td, *J* = 7.1, 3.7 Hz, 1H, 5-H), 3.77-3.48 (m, 17H, 1-H, OCH_2, 4-H, 3'-H), 2.97-2.95 (m, 1H, 6-Ha), 2.88 (t, *J* = 7.4 Hz, 3H, -CH_2SAc, 2-Ha), 2.68-2.60 (m, 2H, 1'-H), 2.58-2.48 (m, 1H, 2-Hb), 2.32 (s, 4H, SAc, 6-Hb), 1.82 (quin, 6.8 Hz, 2H, 2'-H), 1.64-1.55 (m, 4H, -CH_2CH$_2$SAc, -OCH$_2$CH_2), 1.43-1.29 (m, 14H, -CH_2-) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 196.2 (s, S*C*OCH$_3$), 171.5 (s, *C*ONH$_2$), 71.1 (d, C-4), 71.0-69.8 (8 C, t, NHCO*C*H$_2$O, *C*H$_2$O), 69.7 (d, C-3), 68.3 (d, C-5), 54.8 (3C, t, C-2, C-6, C-1'), 36.7 (t, C-3'), 29.4-29.1 (7C, t, -*C*H$_2$-), 28.8 (q, SAc), 28.5 (t, -*C*H$_2$-), 25.4 (t, *C*H$_2$SAc), 25.8 (t, -*C*H$_2$-), 25.7 (t, C-2').ppm. IR (KBr): ν = 3319, 2924, 2854, 1691, 1663, 1541, 1456, 1352, 1112 cm^{-1}. HRMS (ESI): *m/z* calcd for $C_{29}H_{56}N_2O_9S$: 610.3863. $[M+2H]^+$, found: 610.3824.



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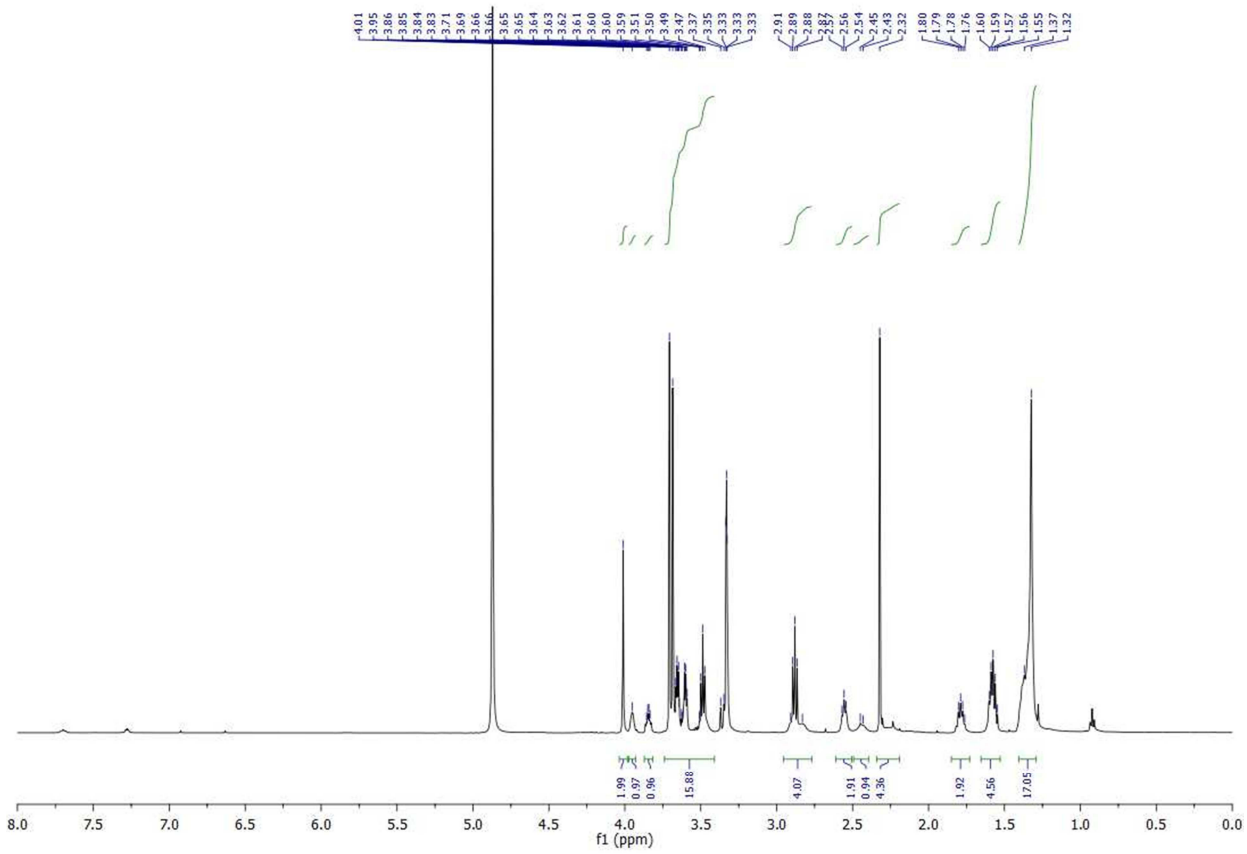
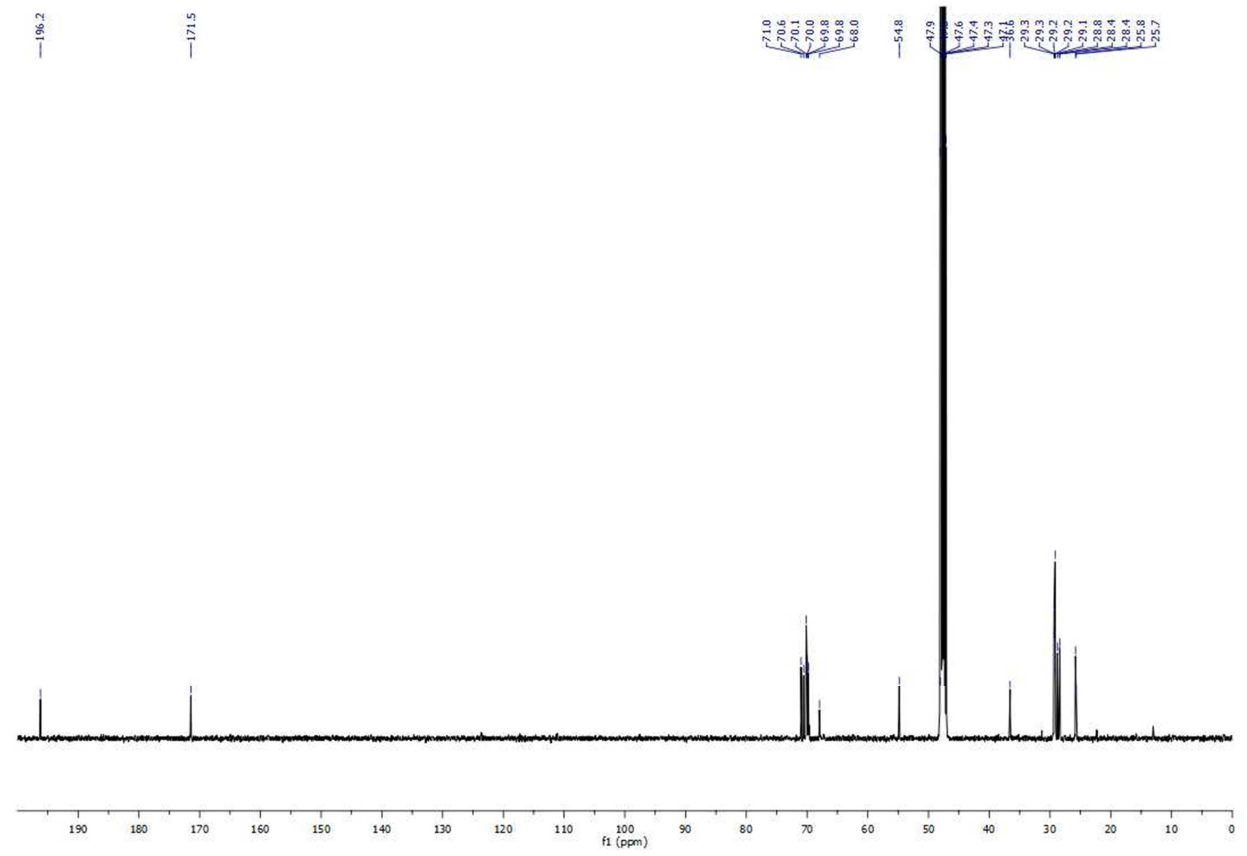


^{1}H NMR of compound **9** (500 MHz, CD_3OD)



^{13}C NMR of compound **9** (125 MHz, CD_3OD)

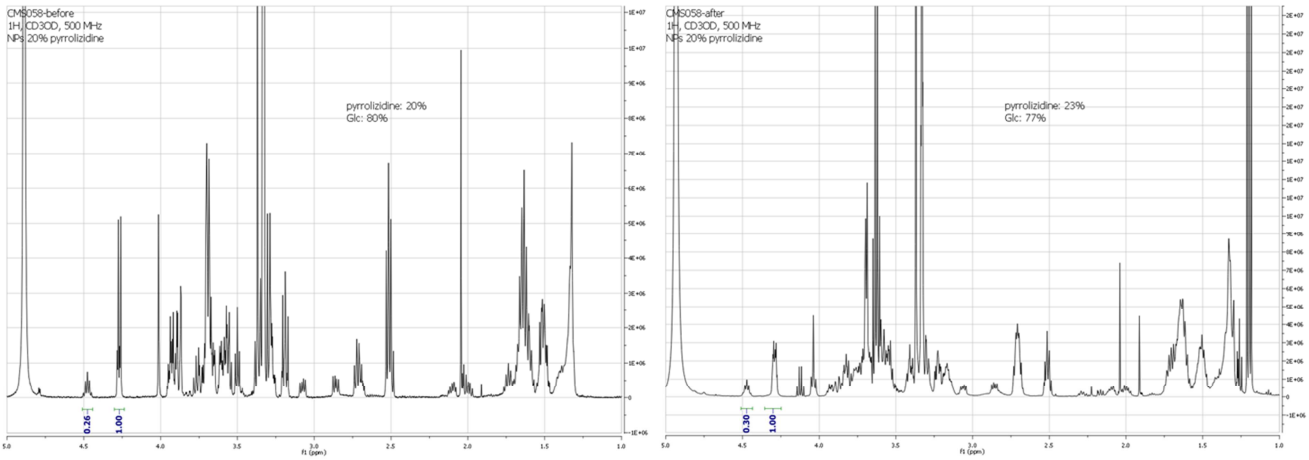
General Procedure for the "in situ" deprotection of *S*-acetyl conjugates 8a and 9a: To a 0.03 M MeOH solution of iminosugar derivative (**8** and **9**) solid CH_3OMe (10 equiv.) was added and the reaction mixture was left stirring for 2 hours at 25 °C under Ar. The complete disappearance of starting material was attested via ^{1}H NMR and the crude was directly used for the preparation of PA-GNPs and PIPA-GNPs.

Preparation and characterization of Au-GNPs: The Au-GNPs coated with iminosugars (PA or PIPA) and simple monosaccharide βGlcC_5S or αManC_5S (PA-Au-GNPs **12-15** and PIPA-Au-GNPs **16-17**) were prepared by reduction of an Au(III) salt using sodium borohydride in the presence of a mixture of thiol-ending iminosugar conjugate (**8a** and **9a**) and βGlcC_5S **10** or αManC_5S **11**, as ligands, in different ratios following a reported procedure.[1] A 4:1 or a 3:2 sugar/iminosugar ligand ratio was used to prepare 20% PA-GNPs and 40% PA-GNPs, respectively. For the analysis of the ratio between the iminosugar ligands and βGlcC_5S (in the case of PA-Au-βGlc or PIPA-Au-βGlc) or αManC_5S (in the case of PA-Au-αMan) ligands, ^{1}H NMR spectra of the initial mixture and of the supernatant after Au-GNPs formation were recorded. The ligands loading on the PA-Au-GNPs and PIPA-Au-GNPs was also evaluated by quantitative NMR (qNMR) using 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid (TSP-*d*$_4$) as an internal standard in the D_2O solution of the PA-Au-GNPs or PIPA-Au-GNPs. The prepared PA-Au-GNPs and PIPA-Au-GNPs were freeze-dried and stored at 4 °C. In these conditions, the Au- GNPs can be stored for months maintaining their biophysical properties. The Au-GNPs coated only with simple monosaccharide **18** and **19** were also prepared as previously described.1

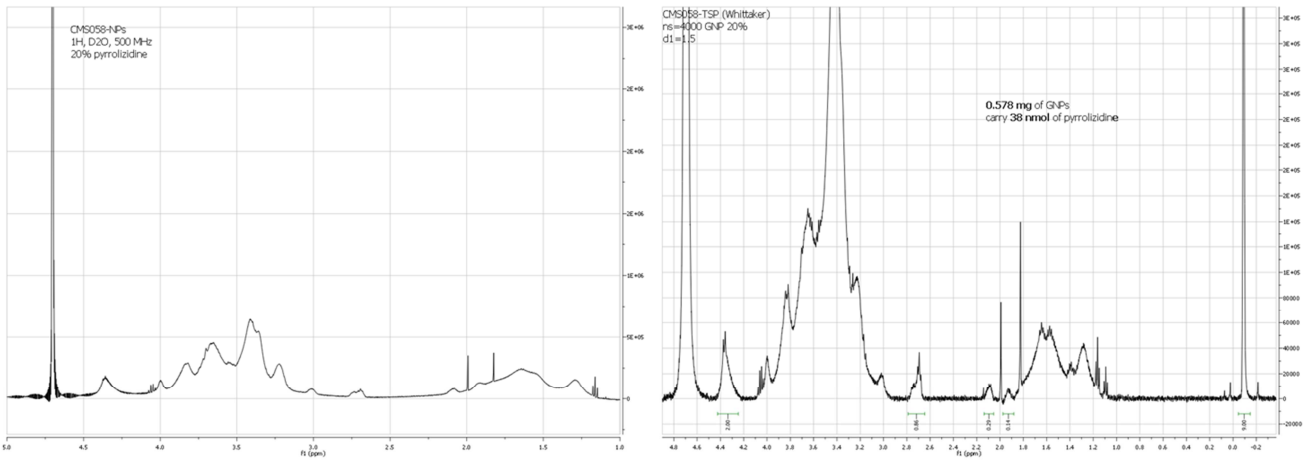
General Procedure for the preparation of Au-GNPs coated with iminosugars: An aqueous solution of $HAuCl_4$ (25 mM, 1 equiv.) was added to a 12 mM methanolic solution of a suitable mixture of thiol-ending sugar and iminosugar conjugates (3 equiv. overall). An aqueous solution of $NaBH_4$ (1 M, 27 equiv.) was then added in four portions, with vigorous shaking. The black suspension formed was shaken for 2 hours at 25 °C. After that, the supernatant was removed and analysed by ^{1}H NMR to study the nanoparticle ligands composition. The residue was washed several times with MeOH. In order to well separate the nanoparticles from the supernatant a centrifugation (4000 xg, 10 °C, 2 min) is required in some cases. The residue was dissolved in a minimal volume of HPLC Gradient grade water and purified by dialysis (SnakeSkin® Pleated Dialysis Tubing, 10,000 MWCO). Iminosugar coated Au-GNPs were obtained as a dark-brown powder after freeze-drying and characterized via ^{1}H NMR, UV-Vis Spectroscopy and TEM analysis. The average number of gold atoms was calculated on the basis of the average diameter obtained by TEM micrographs[2] and molecular formulas of the Au-GNPs were estimated according to previous work.[1]

[2] M. J. Hostetler, J. E. Wingate, C.-J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans, R. W. Murray, *Langmuir* **1998**, *14*, 17– 30.

Preparation of 20% PA-βGlc NPs (12): A 1:4 mixture of thiol-ending PA conjugate **8a** (7.5 mg, 13.2 µmol) and $\beta GlcC_5S$ **10** (14.6 mg, 51.7 µmol) in MeOH (5.4 mL) was used, to afford 5.6 mg of **12** (98% yield in Au). TEM (average diameter): 1.3 ±0.3 nm (main population, >85%). Quantitative ^1H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.58 mg of PA-GNPs were dissolved in 180 µL of D_2O and 40 µL of D_2O containing 0.05 wt.% TSP were added and 38 nmoles of PA conjugate were found.[3] Significant peaks: δ = 4.36 (br s, from $\beta GlcC_5S$), 4.00 (s, NHCOC*H*$_2$- from PA conjugate), 3.84-3.22 (m), 3.05-2.97 (m, from PA conjugate), 2.78-2.66 (m, from PA conjugate), 2.15-1.14 (m); ratio between PA conjugate and $\beta GlcC_5S$ signals ≈1: 4.6. This result is in fair agreement with the molar ratio of conjugates per nanoparticle (20% of PA conjugate and 80% of $\beta GlcC_5S$) as estimated by NMR analysis of the ligand mixture before and after nanoparticles formation. IR (KBr): ν ~ 3388, 2921, 2852, 1635 (amide), 1383, 1077 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): λ =516 nm (gold surface plasmon band). Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_3(C_{11}H_{21}O_6S)_{27}Au_{140}$: ~37.0 KDa.

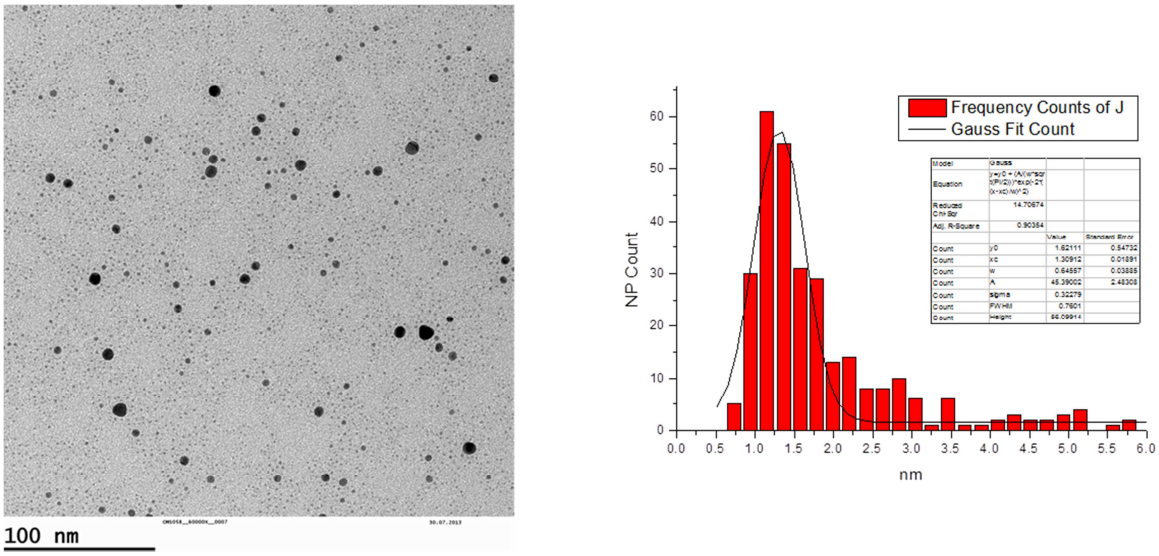


^1H NMR of sugar/iminosugar ligands mixture before and after formation of Au-GNPs **12** (500 MHz, CD_3OD).

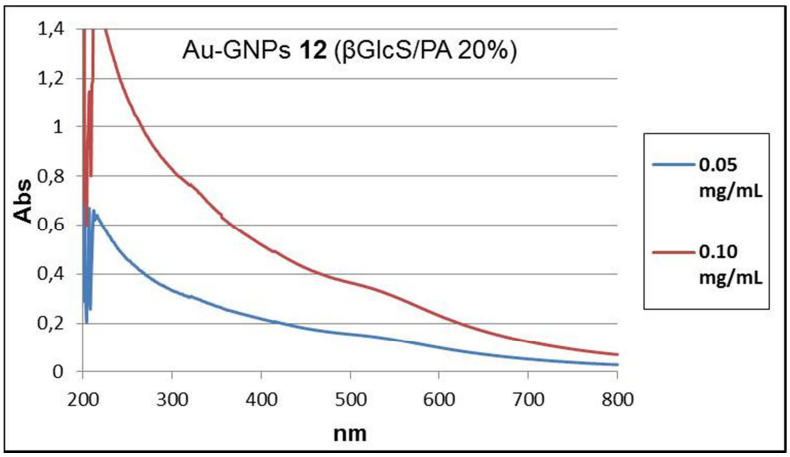


^1H NMR and ^1H qNMR with TSP-d_4 of Au-GNPs **12** (500 MHz, D_2O).

[3] In the quantitative NMR (qNMR) the **7-Ha** proton signal of PA-conjugate was selected for integration as it falls in a spectral region free of other signals.

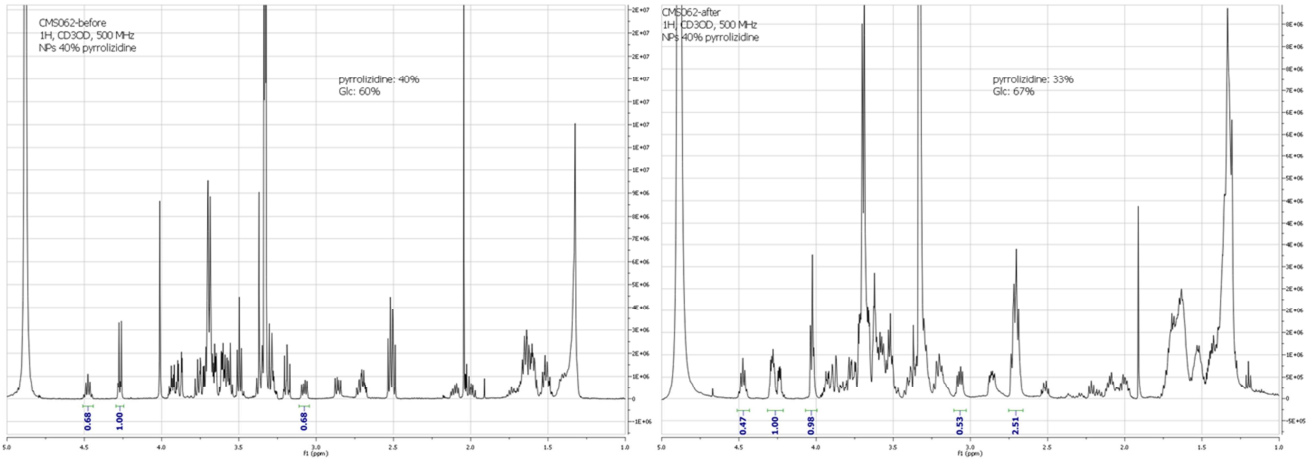


TEM micrograph in H_2O and size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.3 ± 0.3 nm, 13% shows a >3 nm diameter).

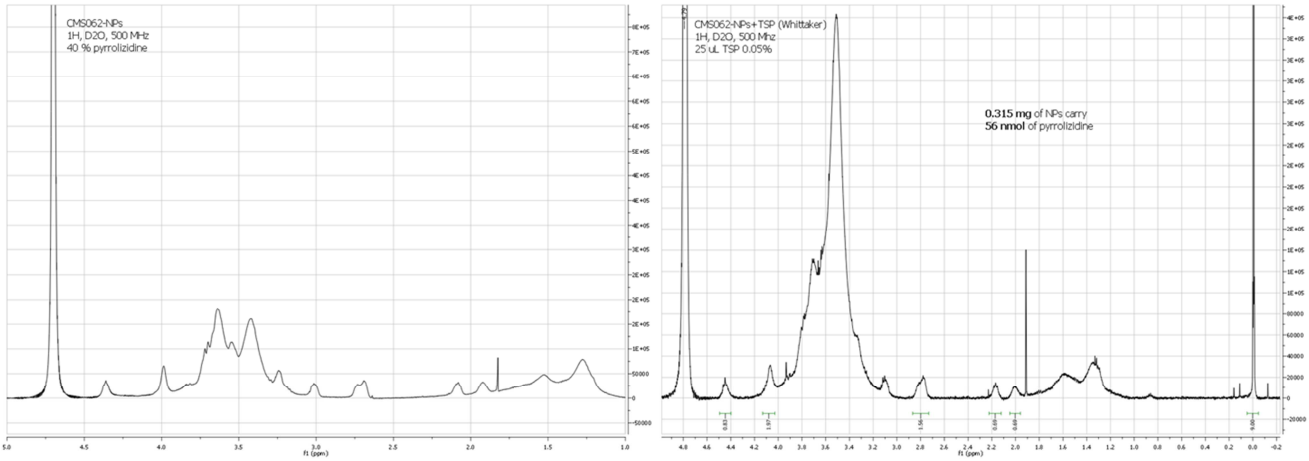


UV/vis spectra of H_2O solution of Au-GNPs **12** recorded at two different concentrations.

Preparation of 40% PA-βGlc NPs (13): A 2:3 mixture of thiol-ending PA conjugate **8a** (3.7 mg, 6.6 µmol) and βGlcC$_5$S **10** (2.8 mg, 9.9 µmol)in MeOH (1.4 mL) was used, to afford 1.1 mg of **13** (77% yield in Au).TEM (average diameter): 1.4±0.4 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.32 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 56 nmoles of PA conjugate were found.[3] Significant peaks: δ = 4.50-4.36 (m, from PA conjugate), 3.99 (s, NHCOC*H*$_2$- from PA conjugate), 3.84-3.14 (m), 3.07-2.97 (m, from PA conjugate), 2.77-2.65 (m, from PA conjugate), 2.15-2.03 (m, from PA conjugate), 1.97-1.87 (m, from PA conjugate), 1.81-1.01 (m); ratio between PA conjugate and βGlcC$_5$S signals is impossible to define since the βGlcC$_5$S anomeric signal disappeared from the spectrum; this phenomenon is in agreement with the literature[1] and it is probably due to the fact that at this densities the long active ligand folds and collapse on the glucose shell hampering a proper proton relaxation.[4] IR (KBr): ν ~3419, 2922, 2853, 1663, 1631, 1600 (amide), 1383, 1108 cm^{-1}. UV/Vis (H_2O, 0.05, 0.10 and 0.20 mg/mL): maximum absorption at 520 nm (gold surface plasmon band) not observed. Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_9(C_{11}H_{21}O_6S)_{14}Au_{140}$: ~36.5 kDa.

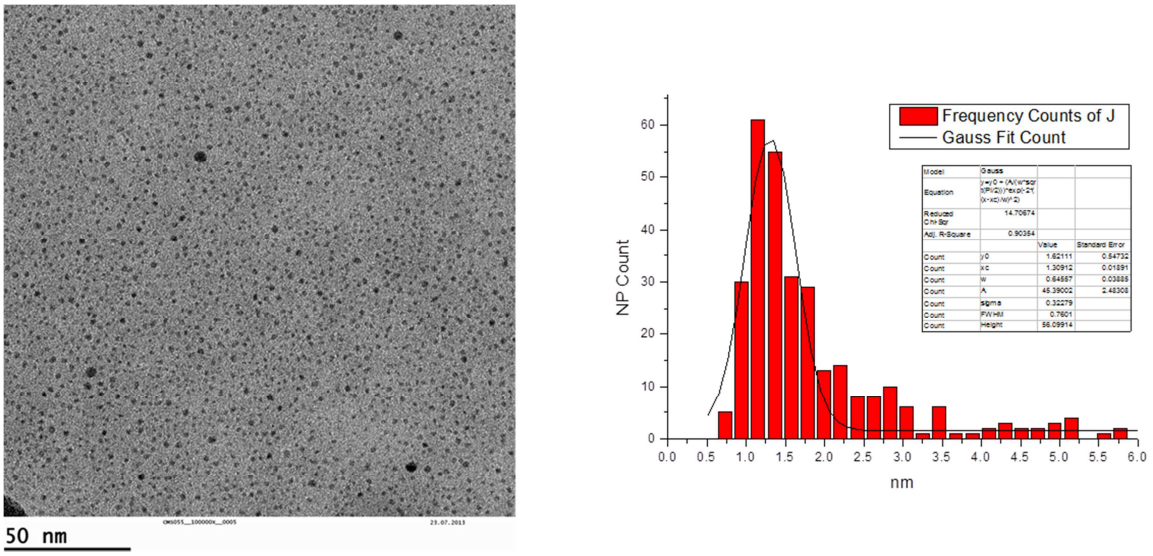


^{1}H NMR of sugar/iminosugar ligands mixture before and after formation of Au-GNPs **13** (500 MHz, CD_3OD).

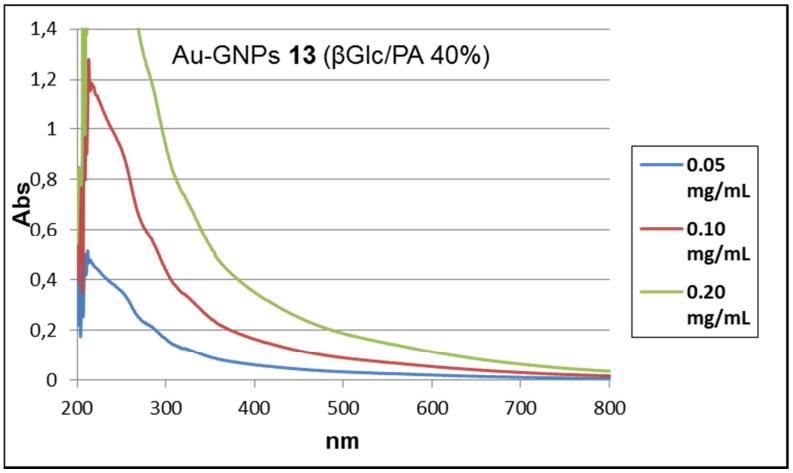


^{1}H NMR and ^{1}H qNMR with TSP-d$_4$ of Au-GNPs **13** (500 MHz, D_2O).

[4] M. Reynolds, M. Marradi, A. Imberty, S. Penades, S. Perez, *Chem. Eur. J.* **2012**, *18*, 4264-4273.

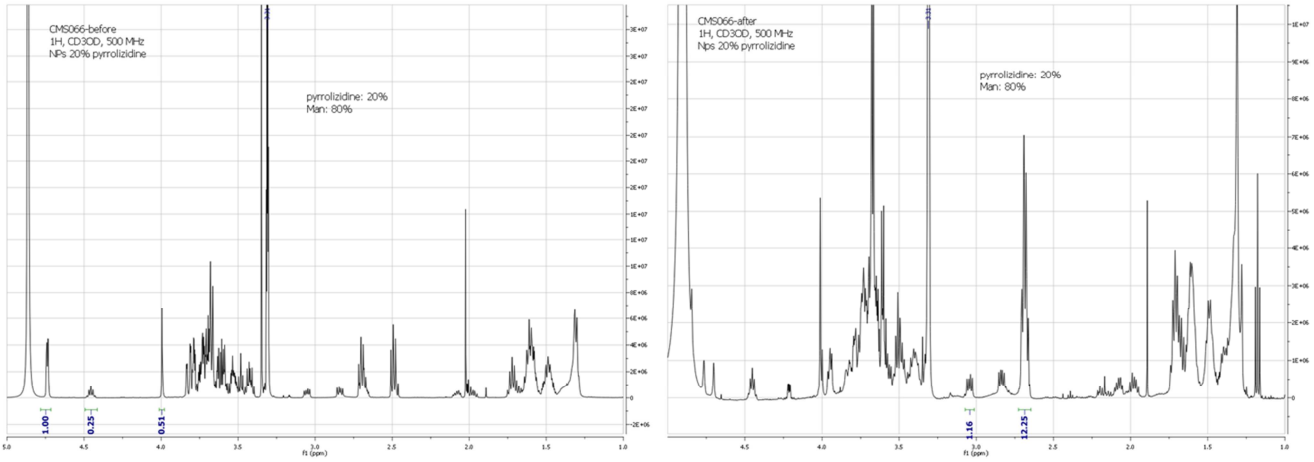


TEM micrograph in H_2O and size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.4 ± 0.4 nm, 2% shows a >3 nm diameter).

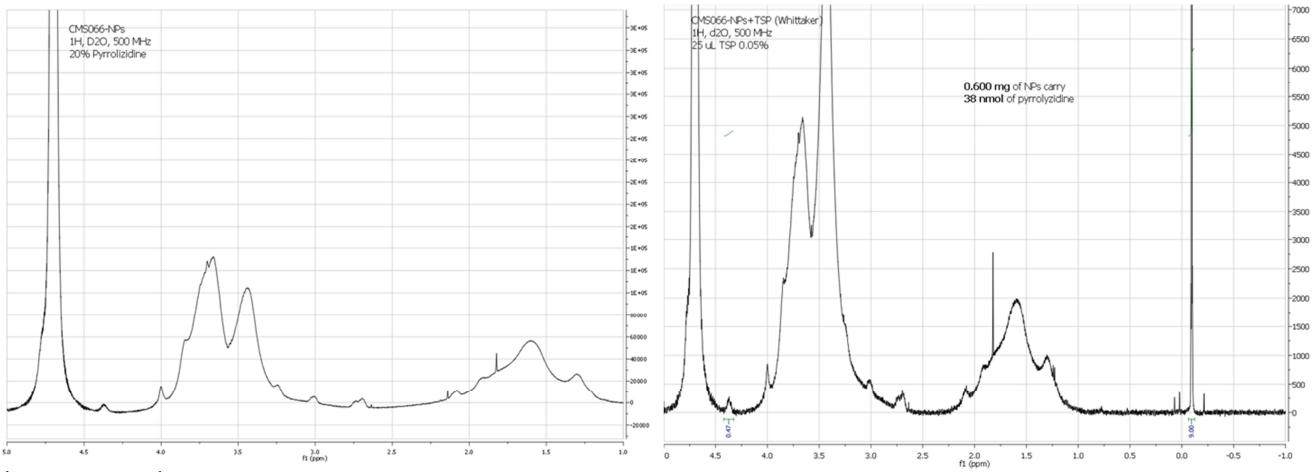


UV/vis spectra of H_2O solution of Au-GNPs **13** recorded at three different concentrations.

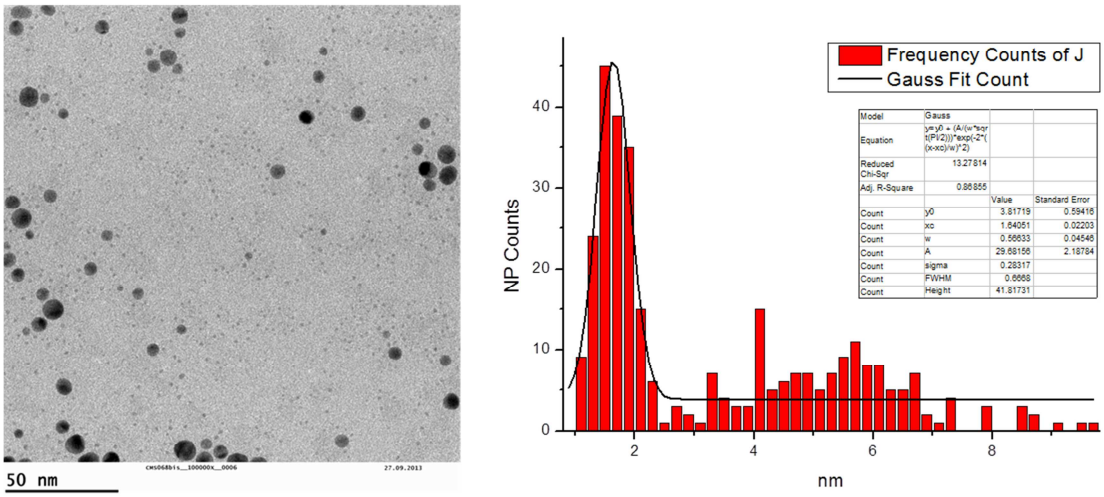
Preparation of 20% PA-αMan NPs (14): A 1:4 mixture of thiol-ending PA conjugate **8a** (4.7 mg, 8.2 µmol) and αManC_5S **11** (9.3 mg, 33.0 µmol) in MeOH (3.4 mL) was used, to afford 4.4 mg of **14** (96% yield in Au). TEM (average diameter): 1.9±0.4 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.60 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 38 nmoles of PA conjugate were found.[3] Significant peaks: δ = 4.41-4.32 (m, from PA conjugate), 4.00 (s, NHCOC*H*$_2$- from PA conjugate), 3.96-3.19 (m), 3.05-2.98 (m, from PA conjugate), 2.78-2.65 (m, from PA conjugate), 2.19-1.06 (m); ratio between PA conjugate and αManC_5S signals is impossible to define since the αManC_5S anomeric signal is covered by the solvent residual peak. IR (KBr): ν ~3368, 2922, 2848, 1675, 1633 (amide), 1447, 1092 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): λ=528 nm (gold surface plasmon band). Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_4(C_{11}H_{21}O_6S)_{19}Au_{140}$**:** ~35 kDa.



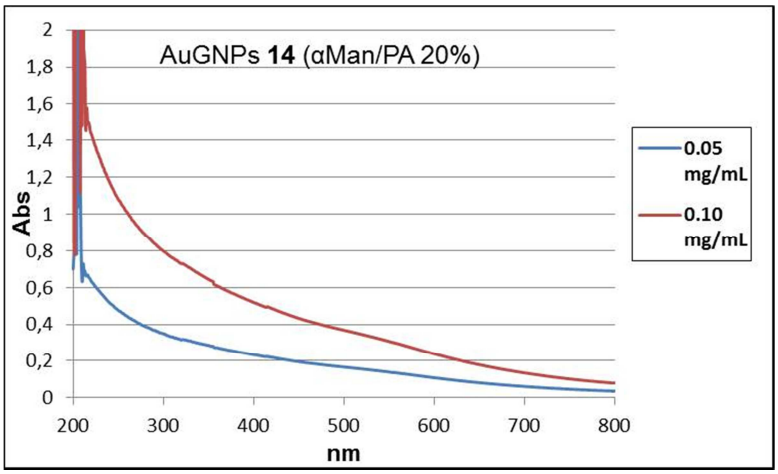
^{1}H NMR of sugar/iminosugar ligands mixture before and after formation of Au-GNPs **14** (500 MHz, CD_3OD).



^{1}H NMR and ^{1}H qNMR with TSP-d_4 of Au-GNPs **14** (500 MHz, D_2O).

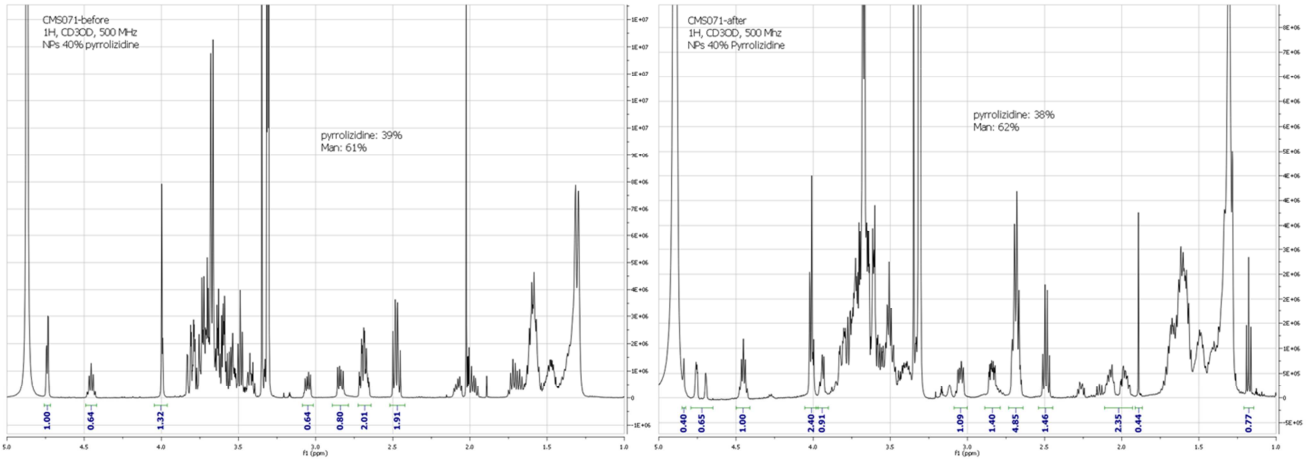


TEM micrograph in H_2O and size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.9 ± 0.4 nm).

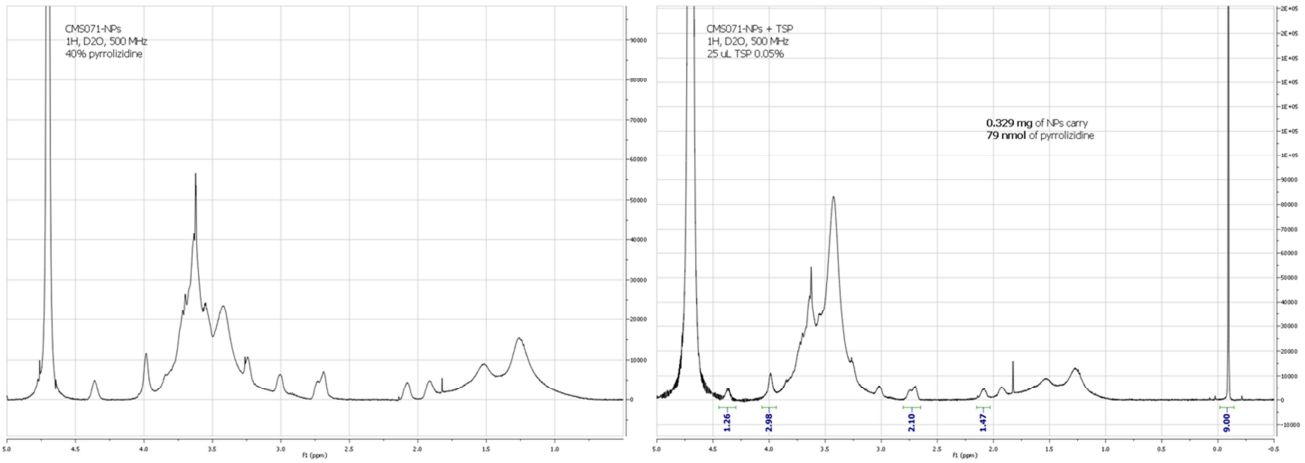


UV/vis spectra of H_2O solution of Au-GNPs **14** recorded at two different concentrations.

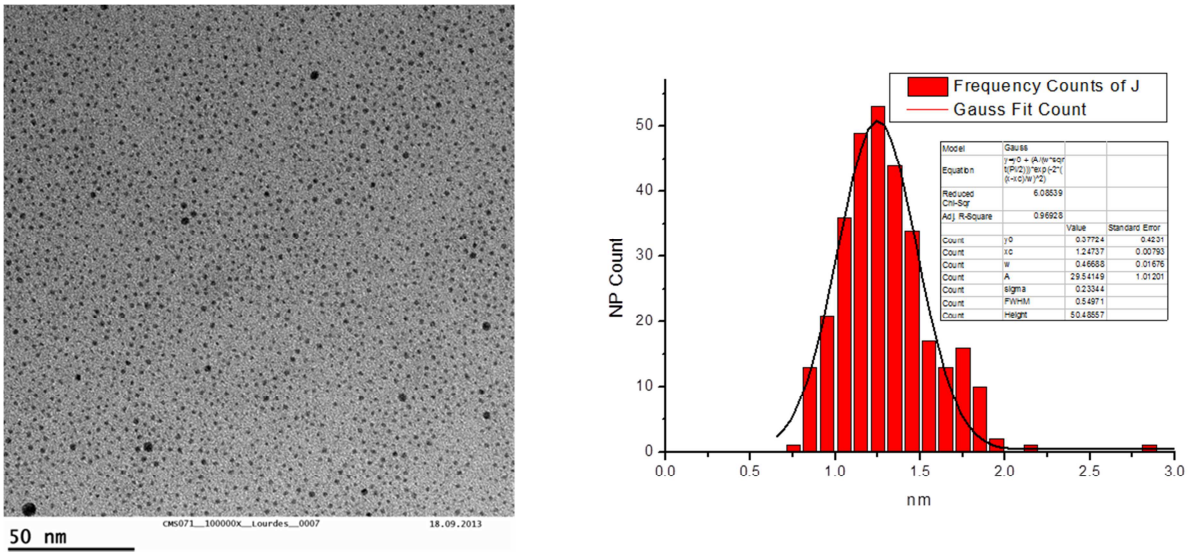
Preparation of 40% PA-αMan NPs (15): A 2:3 mixture of thiol-ending PA conjugate **8a** (6.0 mg, 9.9 µmol) and αManC_5S **11** (4.2 mg, 14.8 µmol) in MeOH (2.0 mL) was used, to afford 1.8 mg of **15** (84% yield in Au). TEM (average diameter): 1.2±0.2 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.33 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 79 nmoles of PA conjugate were found.[3] Significant peaks: δ = 4.43-4.29 (m, from PA conjugate), 3.99 (s, NHCOC*H*$_2$- from PA conjugate), 3.85-3.18 (m), 3.05-2.95 (m, from PA conjugate), 2.78-2.61 (m, from PA conjugate), 2.14-2.02 (m, from PA conjugate), 1.97-1.84 (m, from PA conjugate), 1.72-0.74 (m); ratio between PA conjugate and αManC_5S signals is impossible to define since the αManC_5S anomeric signal is covered by the solvent residual peak. IR (KBr): ν ~ 3418, 2924, 2856, 1660, 1628, 1437, 1383, 1093, 1064 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): maximum absorption at 520 nm (gold surface plasmon band) not observed. Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_9(C_{11}H_{21}O_6S)_{14}Au_{140}$: ~36.5 kDa.



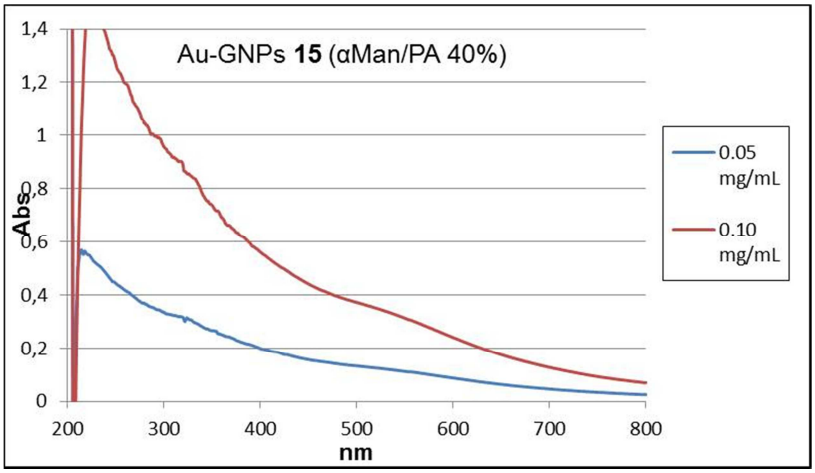
^{1}H NMR of sugar/iminosugar ligands mixture before and after formation of Au-GNPs **15** (500 MHz, CD_3OD).



^{1}H NMR and ^{1}H qNMR with TSP-d_4 of Au-GNPs **15** (500 MHz, D_2O).

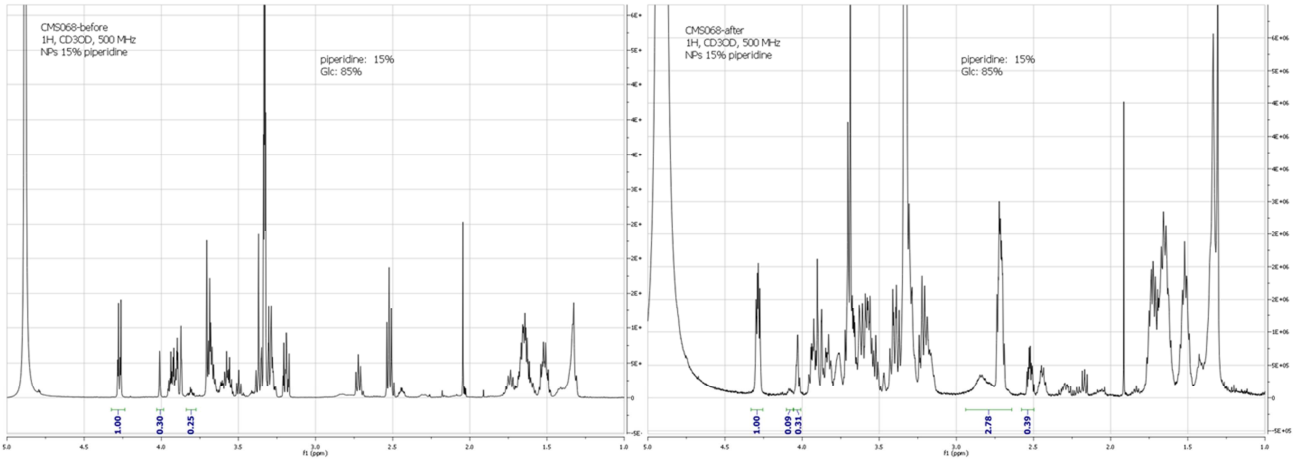


TEM micrograph in H_2O and size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.2± 0.2 nm).

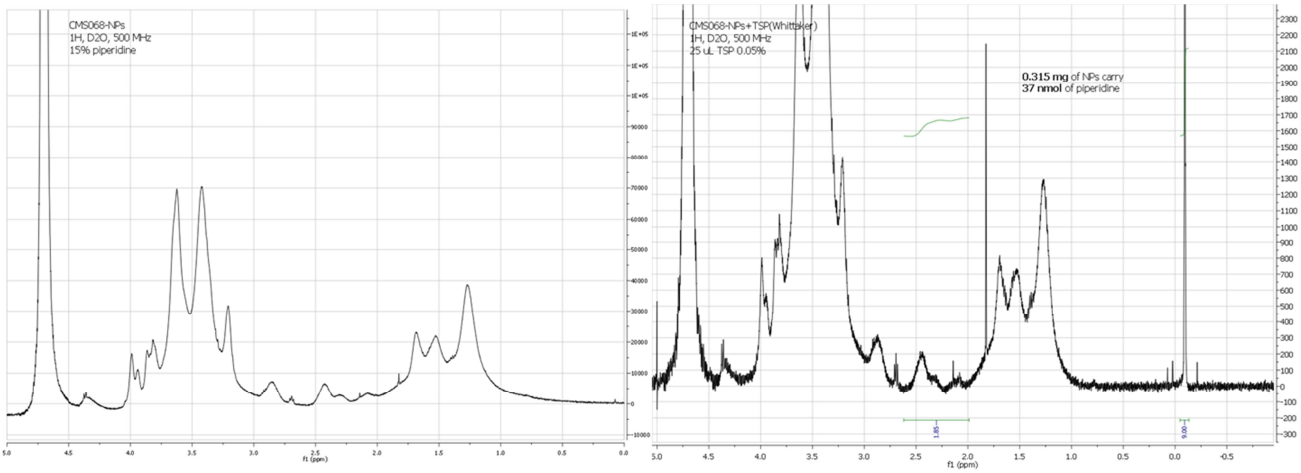


UV/vis spectra of H_2O solution of Au-GNPs **15** recorded at two different concentrations.

Preparation of 20% PIPA-βGlc NPs (16): A 1:4 mixture of thiol-ending PIPA conjugate **9a** (3.1 mg, 5.5 µmol) and $\beta GlcC_5S$ **10** (6.0 mg, 21.3 µmol) in MeOH (5.4 mL) was used, to afford 1.6 mg of **16** (64%% yield in Au). TEM (average diameter): 1.6 ±0.3 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.32 mg of PIPA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 56 nmoles of PA conjugate were found.[5] Significant peaks: δ = 4.34 (br s, from $\beta GlcC_5S$), 3.99 (s, NHCOC*H*$_2$- from PIPA conjugate), 3.94-2.98 (m), 2.94-2.75 (m, from PIPA conjugate), 2.52-2.22 (m, from PIPA conjugate), 2.18-1.99 (m, from PIPA conjugate), 1.82-0.72 (m). IR (KBr): ν ~ 3424, 2920, 2848, 1637 (amide), 1438, 1381, 1076 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): λ =514 nm (gold surface plasmon band). Estimated average molecular weight for $(C_{26}H_{51}N_2O_8S)_7(C_{11}H_{21}O_6S)_{28}Au_{140}$: ~39.0 KDa. In this case a 20% of PIPA conjugate match perfectly with the quantitative analysis, albeit a 15% amount of PIPA conjugate was attested by the NMR analysis of the ligand mixture before and after nanoparticle formation.

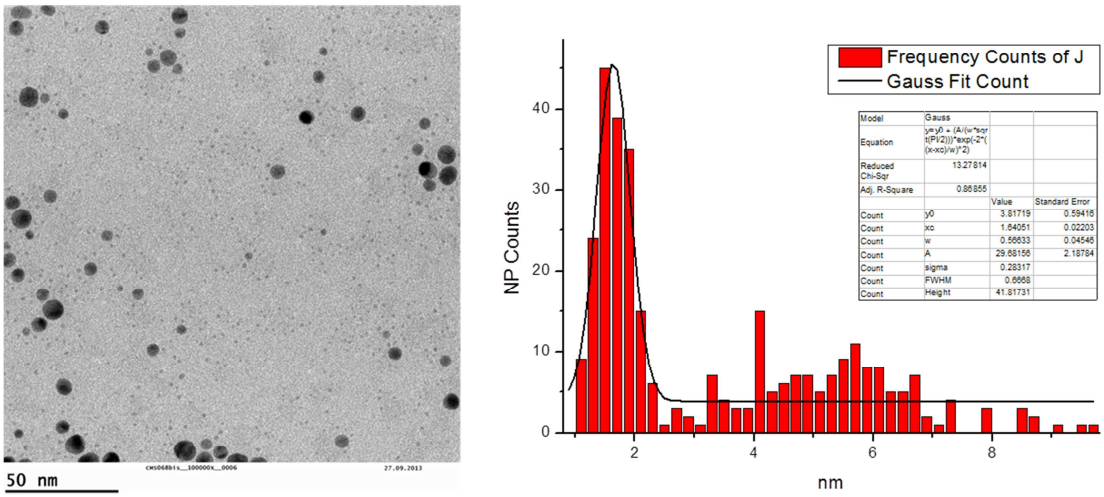


^{1}H NMR of sugar/iminosugar ligands mixture before and after formation of Au-GNPs **16** (500 MHz, CD_3OD).

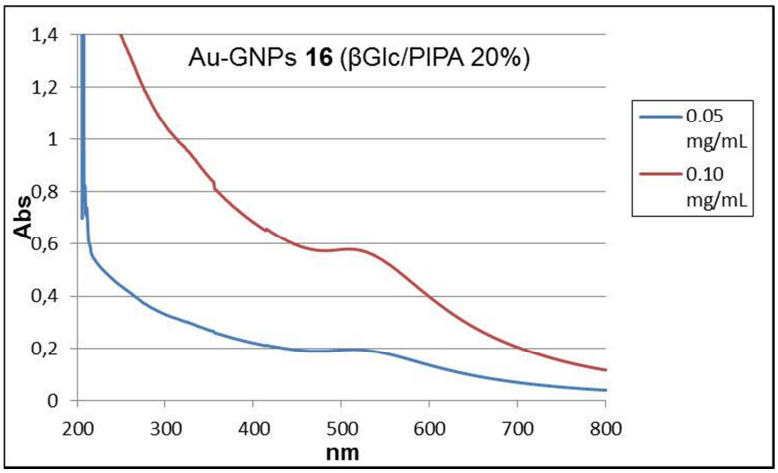


^{1}H NMR and ^{1}H qNMR with TSP-d_4 of Au-GNPs **16** (500 MHz, D_2O).

[5] In the quantitative NMR (qNMR) the multiplet corresponding to **1'-H**, **2-Hb** and **6-Hb** proton signals (*δ* = 2.65-2.02 ppm, 4H) of PIPA-conjugate, was selected for integration as it falls in a spectral region free of other signals.

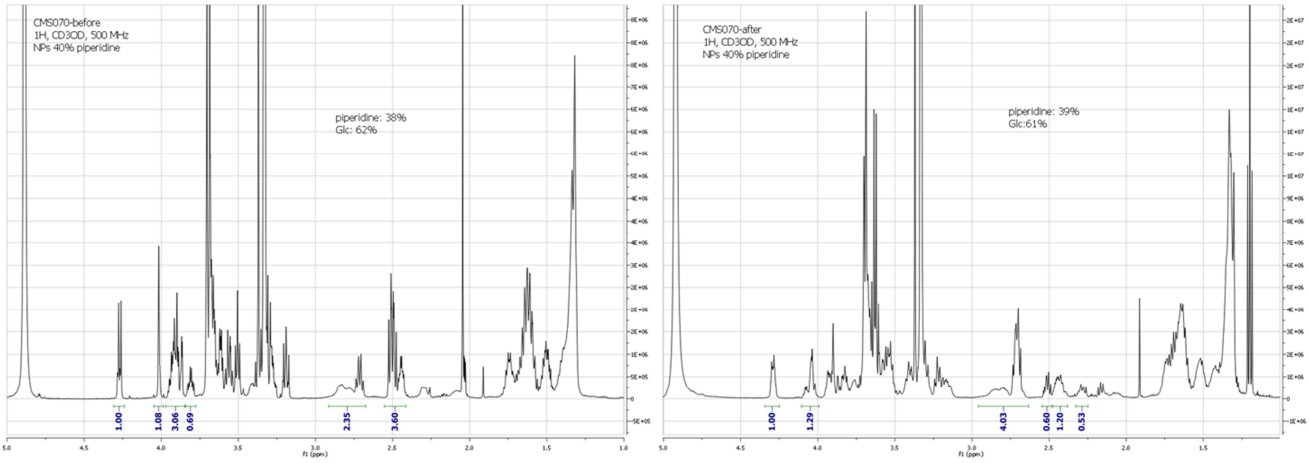


TEM micrograph in H_2O and size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.6± 0.3 nm, 45% shows a >3 nm diameter).

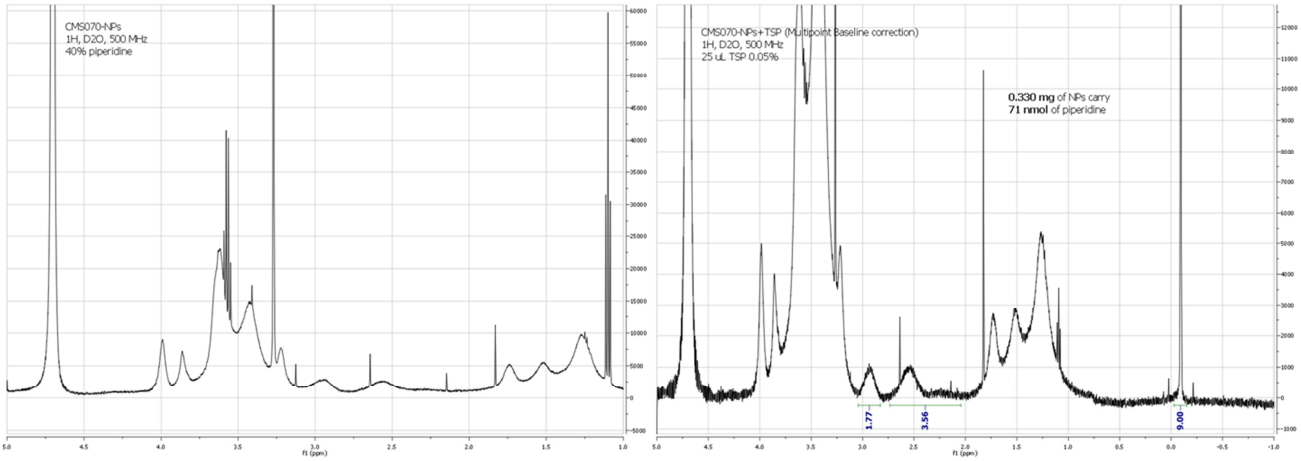


UV/vis spectra of H_2O solution of Au-GNPs **16** recorded at two different concentrations.

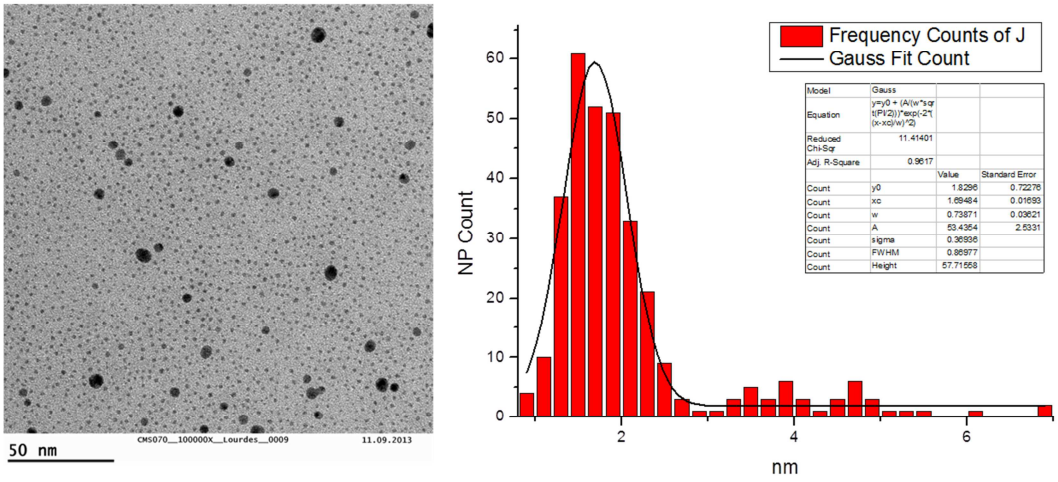
Preparation of 40% PIPA-βGlc NPs (17): A 2:3 mixture of thiol-ending PA conjugate **9a** (3.7 mg, 6.5 µmol) and $\beta GlcC_5S$ **10** (2.8 mg, 9.8 µmol) in MeOH (1.4 mL) was used, to afford 1.1 mg of **17** (77% yield in Au). TEM (average diameter): 1.7±0.4 nm. Quantitative ^1H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.33 mg of PIPA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 71 nmoles of PA conjugate were found.5 Significant peaks: δ = 4.50-4.36 (m, from PA conjugate), 3.99 (s, NHCOC*H*$_2$- from PIPA conjugate), 3.91 (*br* s from PIPA conjugate), 3.80-3.30 (m), 3.56 (q, Et_2O), 3.27 (s, MeOH), 3.25-3.17 (m, from PIPA conjugate), 3.06-2.82 (m, from PIPA conjugate), 2.70-2.45 (m, from PIPA conjugate), 1.82-1.20 (m), 1.16 (t, Et_2O); ratio between PA conjugate and $\beta GlcC_5S$ signals is impossible to define since the $\beta GlcC_5S$ anomeric signal disappeared from the spectrum; see comment on **13**.[4] IR (KBr): ν ~3436, 2920, 2856, 1631 (amide), 1392, 1364, 1033 cm^{-1}. UV/Vis (H_2O, 0.05, 0.10 and 0.20 mg/mL): maximum absorption at 520 nm (gold surface plasmon band) not observed. Estimated average molecular weight for $(C_{26}H_{51}N_2O_8S)_9(C_{11}H_{21}O_6S)_{14}Au_{140}$: ~36.5 kDa.



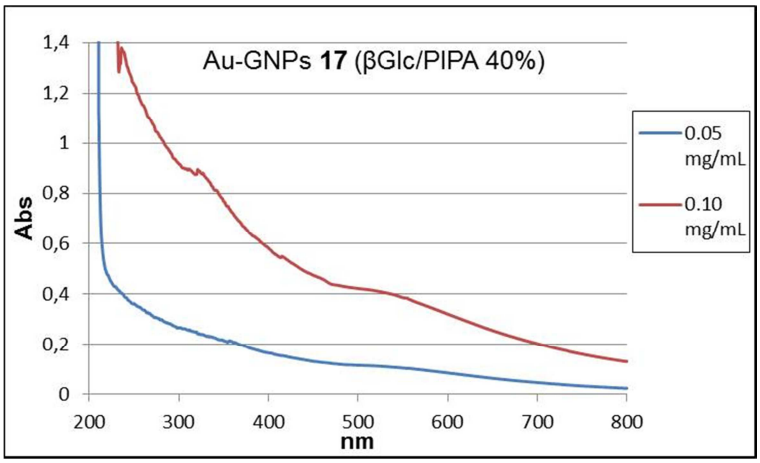
^1H NMR of sugar/iminosugar ligands mixture before and after formation of Au-GNPs **17** (500 MHz, CD_3OD).



^1H NMR and ^1H qNMR with TSP-d_4 of Au-GNPs **17** (500 MHz, D_2O).



TEM micrograph in H_2O and size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.7± 0.4 nm).



UV/vis spectra of H_2O solution of Au-GNPs **17** recorded at two different concentrations.

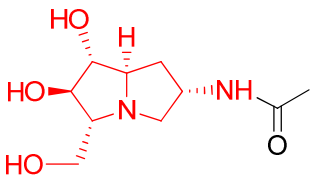
Inhibition data for monovalent reference compound *N*-acetyl 1 and Au-GNPs 12-15, 18 and 19 towards a panel of commercial glycosidases.

The percentage of inhibition towards the corresponding glycosidase was determined in the presence of 1 mM (for ***N*-acetyl 1**) and 0.16 mg/mL (for **Au-GNPs 12-15, 18** and **19**) of the inhibitor on the well (except when other concentration is indicated). Each enzymatic assay (final volume 0.12 mL) contained 0.01 to 0.5 units mL^{-1} of the enzyme and 10 mM aqueous solution of the appropriate *p*-nitrophenyl glycopyranoside substrate buffered to the optimal pH of the enzyme. The enzyme and the inhibitor were preincubated for 5 min at rt, and the reaction started by the addition of the substrate. After 20 min of incubation at 37 °C, the reaction was stopped by the addition of 0.1 mL of sodium borate buffer (pH 9.8). The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 405 nm. Under these conditions, the *p*-nitrophenolate released led to optical densities linear with both reaction time and concentration of the enzyme. For the best inhibitors (% inhibition $\geq$ 80), the IC_{50} value (concentration of inhibitor required for 50% inhibition of enzyme activity) towards the corresponding glycosidase was calculated. IC_{50} values were calculated from plots of percentage of inhibition *versus* inhibitor concentration. Each experiment (%, IC_{50}) was performed in duplicate and the average values were given.

	% Inhibition at 0.16 mg/mL of Au-GNP **IC_{50} μM**[#]					
	***N*-acetyl 1**[§]	**12**	**13**	**14**	**15**	**18**	**19**
α-L-fucosidase bovine kidney	-	-	-	-	-*	nd	nd
α-galactosidase coffee beans	-	-	-	-	-*	nd	nd
β-galactosidase *Escherichia coli*	-	-	-	-	-*	nd	nd
Aspergillus oryzae	-	-	-	-	nd	nd	nd
α-glucosidase yeast	-	-	-	-	nd	nd	nd
rice	-	-	-*	-	nd	nd	nd
amyloglucosidase *Aspergillus niger*	97% **1.7**	83% **4.4**	91% **8.3**	83% **4.0**	90% **13.9**	-	-
β-glucosidase almonds	36%	-	-*	-	nd	nd	nd
α-mannosidase jack beans	-	-	-	-	-*	nd	nd
β-mannosidase snail	-	-	-	-	nd	nd	nd
β-*N*-acetylglucosaminidase jack beans	64%	-	-*	-	nd	nd	nd

[#] : IC_{50} is referred to the μM iminosugar concentration as determined by qNMR of GNPs.
[§] : Percentage of inhibition at 1 mM concentration of inhibitor.
- : no inhibition was detected at 0.16 mg/mL of inhibitor.
-*: no inhibition was detected at 0.033 mg/mL of inhibitor.
nd: not determined

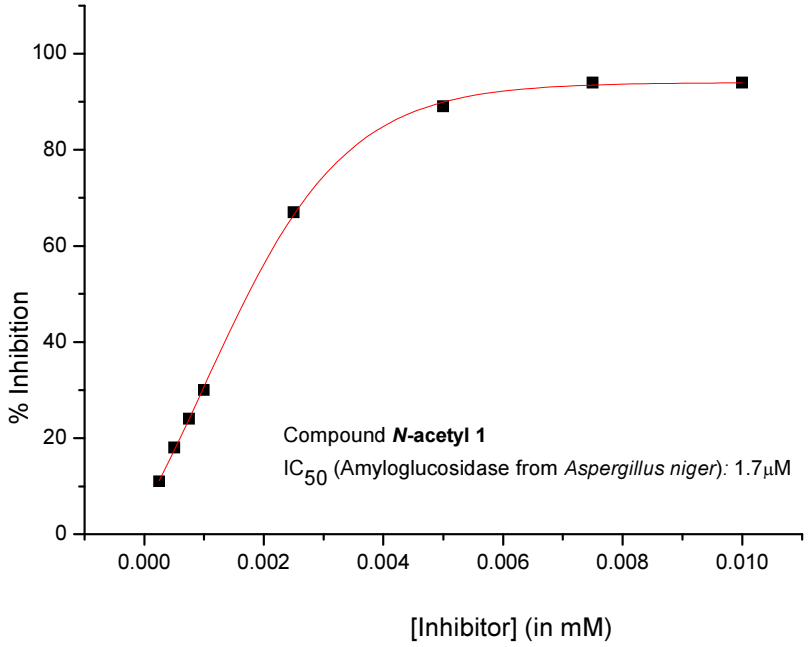
Monovalent compound: ***N*-acetyl 1**, whose synthesis has been previously reported.[6]



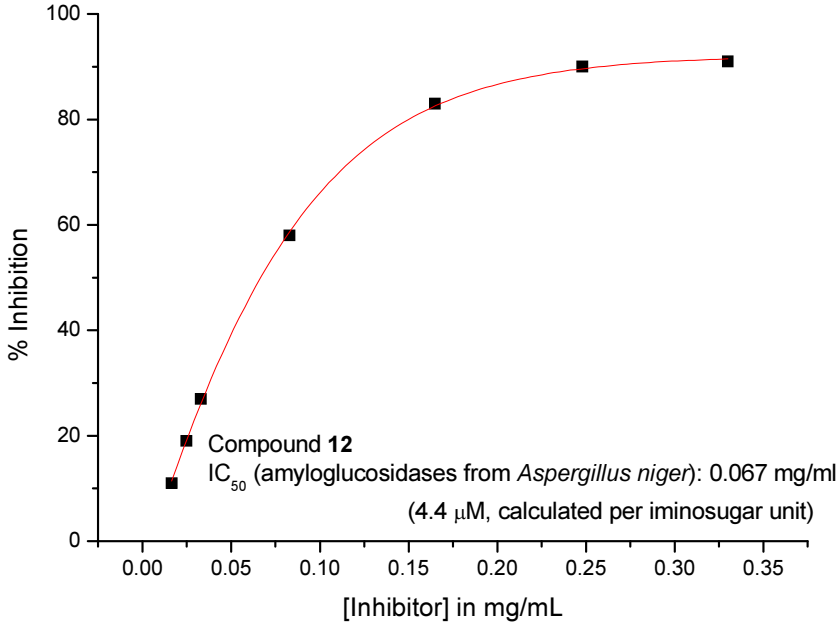
***N*-acetyl 1**

[6] C. Parmeggiani, S. Catarzi, C. Matassini, G. D'Adamio, A. Morrone, A. Goti, P. Paoli, F. Cardona, *ChemBioChem*, 2015, **16**, 2054-2064.

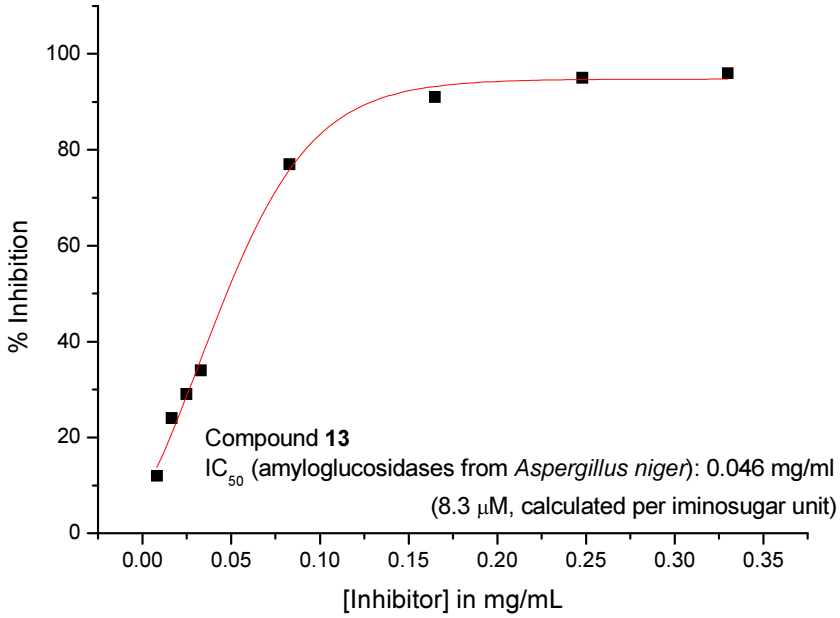
IC_{50} graphic for compound for compound *N*-acetyl 1



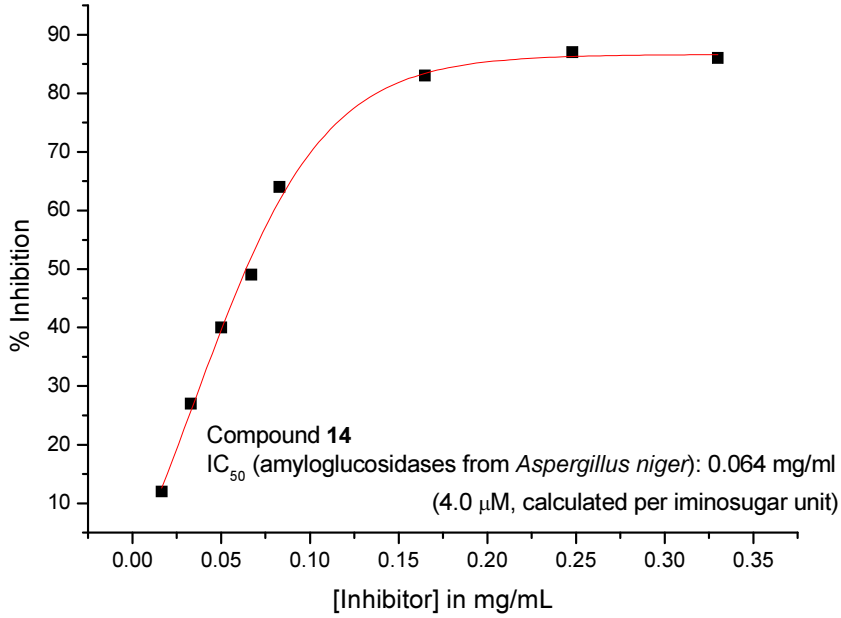
IC_{50} graphic for compound 12:



IC_{50} graphic for compound 13:



IC_{50} graphic for compound 14:



IC_{50} graphic for compound 15: