Electronic Supporting Information

On the chiroptical properties of Au(I)-thiolate glycoconjugate precursors and their influence in sugar-protected gold nanoparticles (glyconanoparticles)

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1. Materials and methods

All chemicals were purchased as reagent grade from Sigma-Aldrich, except chloroauric acid (Strem Chemicals), and used without further purification. L-Glucose, L-mannose and L-galactose were obtained from Carbosynth. Water HPLC gradient grade was supplied by Fischer Chemical.

Proton nuclear magnetic resonance (^{1}H-NMR): All the measurements were performed on a Bruker Advance (500 MHz) spectrometer.

*Transmission Electron Microscopy (*TEM*)*: For the TEM characterization, aqueous solutions of 0.1 mg/mL were prepared. A drop of 5 μL of this solution was deposited on an ultrathin carbon film/holey carbon 400 mesh copper grid (supplied by Ted Pella Inc.) and dried overnight inside a plastic small box. The characterization was carried out indistinctly in two different microscopes: JEOL JEM-2100F-UHR, operated at 200kV and JEOL JEM-1400PLUS-HC, operated at 120kV. Size was determined as the average of 250 measurements.

Optical measurements: UV-vis absorption, Circular Dichroism (CD), Photoluminescence (PL) spectra were recorded in a Beckman DU-800 spectrophotometer, a JASCO J-815 spectropolarimeter and a Perkin Elmer LS 55 fluorimeter, respectively. The optical rotation was measured in a Perkin Elmer Model 341 polarimeter. An aqueous solution (0.25 mg/mL) of the gold compound was prepared for the UV-vis, CD and PL characterization. The concentrations of the glucose glycoconjugate (thiol or disulphide) for the CD were 0.05 to 50 mM. The sample concentration for the optical rotation measurements was 1 mg/mL in water. The characterization was performed in a 1 mm path cuvette of 0.25 mL, at 589 nm and 20ºC.

X-ray photoelectron spectroscopy (XPS) measurements: The measurements were performed in a SPECS Sage HR 100 spectrometer with a non-monochromatic X-ray source (Mg Kα line of 1253.6 eV energy and 250 W), placed perpendicular to the analyzer axis and calibrated using the $3d_{5/2}$ line of Ag with a full width at half maximum (FWHM) of 1.1 eV. The sample was solved in HPLC water at a concentration of 2.5 mg/mL. 10 μL of this solution were placed on a microscope slide of 2.5 × 2.5 cm and dried overnight. The selected resolution for the spectra was 30 eV of Pass Energy and 0.5 eV/step for the general spectra and 15 eV of Pass Energy and 0.1 eV/step for the detailed spectra of the different spectra of the different elements. All measurements were made in an ultra high vacuum (UHV) chamber at pressure around 10^{-8} mbar.

Mass spectrometry experiments: Mass spectra were recorded using Matrix Assisted Laser Desorption/Ionization (MALDI-TOF) technique by means of a Bruker UltrafleXtreme III. Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) was used as matrix. An aqueous solution of the samples at a concentration of 0.25 mg/mL was used for this characterization.

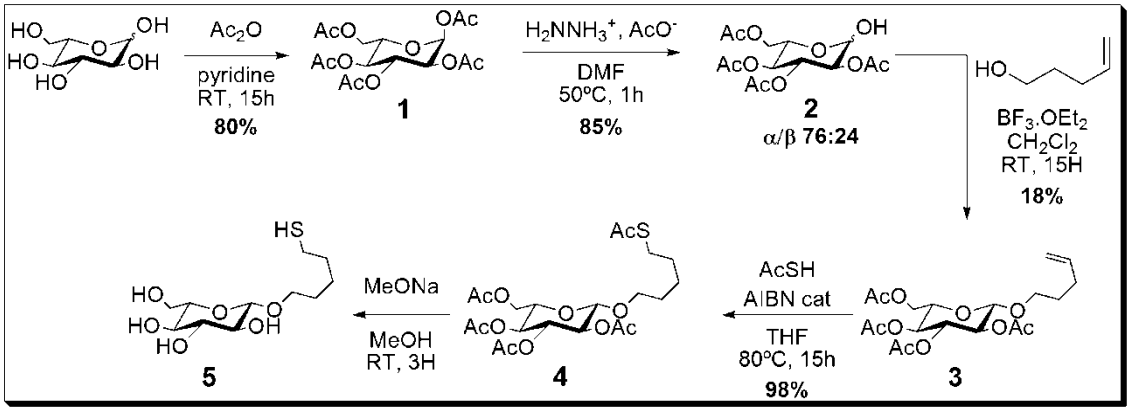
Size Exclusion Chromatography (SEC): For SEC experiments, the samples were solved in HPLC water at a concentration of 1 mg/mL. Approximately 1 mL of the solution was injected and water was used as eluent. A HiLoad 16/600 Superdex 200 preparation grade (General Electric Healthcare Life Sciences) was used for the chromatography coupled to a Bio-Rad Biologic DuoFlow system.

X-ray diffraction (XRD) experiments: The measurements were carried out at room temperature on a Bruker D8 Advance diffractometer operating at 30kV and 20mA equipped with Cu Kα source and a Vantec-1 PSD detector. Approximately, 1 mg of sample (as solid) was necessary to carry out the characterization. The experiments were performed by the X-ray Molecules and Surfaces Unit of the General Research Service (SGIker), University of the Basque Country.

2. Synthesis of glycoconjugates

The 5-mercaptopentyl β-D-glucopyranoside, β-D-galactopyranoside, and α-D-mannopyranoside were synthesized according to literature.[1] 23-mercapto-3,6,9,12-tetraoxatricosyl β-D-glucopyranoside ($GlcTEGC_{11}SH$) was synthesized according to literature.[1a]

The 5-mercaptopentyl β-L-glucopyranoside, β-L-galactopyranoside, and α-L-mannopyranoside were synthesized as described for the 5-mercaptopentyl β-L-glucopyranoside (5) in the following Scheme:



β-L-Glucopyranoside pentaacetate (1). Acetic anhydride (44 mmol, 8 eq.) was added dropwise to a solution of L-glucopyranose (5.5 mmol) in dry pyridine (15 mL) at 0ºC. The mixture was stirred overnight at room temperature under argon atmosphere and pyridine was evaporated. The residue was dissolved in CH_2Cl_2, washed three times with saturated $NaHCO_3$ solution. Organic fraction was dried over $MgSO_4$, concentrated to dryness and remaining pyridine was coevaporated with toluene. Compound **1** was precipitated and recristallized in a mixture of hexane/AcOEt 9:1, to afford a white powder (4.4 mmol, 80%). Rf=0.37 (Hexane/AcOEt 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.33 (d, *J* = 3.7 Hz, 1H, H_1), 5.47 (t, *J* = 9.9 Hz, 1H, H_3), 5.14 (t, *J* = 9.9 Hz, 1H, H_4), 5.10 (dd, *J* = 10.3 Hz, 3.7 Hz, 1H, H_2), 4.27 (dd, *J* = 12.7 Hz, 4.2 Hz, 1H, H_6), 4.18-4.02 (m, 2H, $H_{6'}$+H_5), 2.18 (s, 3H, CH_3 (OAc)), 2.09 (s, 3H, CH_3 (OAc)), 2.04 (s, 3H, CH_3 (OAc)), 2.03 (s, 3H, CH_3 (OAc)), 2.02 (s, 3H, CH_3 (OAc)); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7-168.8 (5s, CO (OAc)), 89.1 (C_1), 69.8 (C_3+C_5), 69.2 (C_2), 67.9 (C_4), 61.5 (C_6), 20.9-20.5 (5s, CH_3 (OAc)); HRMS ESI (+) *m/z* calcd for $C_{17}H_{22}O_{11}NH_4$ $(M+NH_4)^+$ 408.1506, found 408.0842.

2,3,4,6-Tetra-*O*-acetyl-β-L-glucopyranoside (2). To a solution of β-L-glucopyranoside pentaacetate **1** (2.6 mmol) in dry DMF (10 mL) at 50 ºC was added hydrazine acetate (3.3 mmol, 1.3 eq.). The mixture was stirred 45 min at 50ºC, then cooled down to room temperature, diluted with AcOEt and washed three times with brine. Organic fraction was dried over $MgSO_4$ and concentrated to dryness. The crude was purified by flash chromatography (AcOEt in hexane 12% to 100%) to afford a syrup (2.2 mmol, 85%). Rf=0.26 (Hexane/AcOEt 1:1); HRMS ESI (+) *m/z* calcd for $C_{14}H_{20}O_{10}Na$ $(M+Na)^+$ 371.0954, found 371.0917.

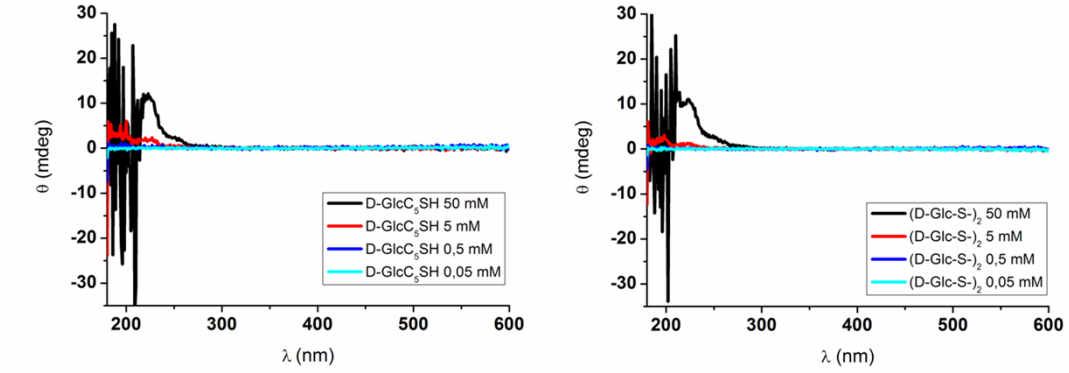
4-Pentenyl 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranoside (3). To a solution of 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranoside **2** (2.2 mmol) in dry CH_2Cl_2 (10 mL) at 0ºC were added 4-penten-1-ol (13 mmol, 6 eq.) and $BF_3.OEt_2$ (22 mol, 10 eq.). The mixture was stirred overnight at room temperature under argon atmosphere and monitored by TLC. After night, since it remained compound **2**, $BF_3.OEt_2$ (3.7 eq.) was added one more time and the mixture was stirred 4 hr more, before dilution with CH_2Cl_2. Organic fraction was washed three times with saturated $NaHCO_3$ solution, dried over $MgSO_4$, concentrated to dryness and remaining 4-penten-1-ol was coevaporated with toluene. The crude was purified by flash chromatography (AcOEt in hexane 12% to 100%) to afford a colourless oil (0.40 mmol, 18%). Rf=0.61 (Hexane/AcOEt 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.75 (ddt, *J* = 16.9 Hz, 10.2 Hz, 6.7 Hz, 1H, -C***H***=CH_2), 5.18 (t, *J* = 9.5 Hz, 1H, H_3), 5.05 (t, *J* = 9.7 Hz, 1H, H_4), 5.02-4.91 (m, 3H, H_2+-CH=C***H***$_2$), 4.47 (d, *J* = 8.0 Hz, 1H, H_1), 4.24 (dd, *J* = 12.3 Hz, 4.7 Hz, 1H, H_6), 4.11 (dd, *J* = 12.3 Hz, 2.2 Hz, 1H, $H_{6'}$), 3.85 (dt, *J* = 9.7 Hz, 6.2 Hz, 1H, -O-CH_2- (alkyl)), 3.67 (ddd, *J* = 9.9 Hz, 4.6Hz , 2.4 Hz, 1H, H_5), 3.47 (dt, *J* = 9.6 Hz, 6.7 Hz, 1H, -O-CH_2'- (alkyl)), 2.12-1.95 (m, 14H, CH_3(OAc)+-C***H***$_2$-CH=CH_2), 1.64 (qt, *J* = 14.2 Hz, 7.2 Hz, 2H, -C***H***$_2$-CH_2-O-); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6-169.2 (4s, CO (OAc)), 137.8 (-***C***H=CH_2), 115.1 (-CH=***C***H_2), 100.8 (C_1), 72.8 (C_3), 71.7 (C_5), 71.3 (C_2), 69.3 (-CH_2-O-), 68.4 (C_4), 62.0 (C_6), 29.8 (-***C***H_2-CH=CH_2), 28.5 (-***C***H_2-CH_2-O-), 20.7-20.6 (3s, CH_3 (OAc)); HRMS ESI (+) *m/z* calcd for $C_{19}H_{28}O_{10}Na$ $(M+Na)^+$ 439.1580, found 439.1575.

5-Thioacetylpentyl 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranoside (4).Thioacetic acid (1.6 mmol, 4 eq.) and AIBN (catalytic amount) were added to a solution of 4-pentenyl-2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranoside **3** (0.4 mmol) in THF (5 mL). The mixture was stirred overnight at reflux, then cooled down to room temperature and THF was evaporated. The residue was dissolved in AcOEt, washed three times with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated to dryness. The crude was purified by flash chromatography (AcOEt in hexane 12% to 100%) to afford a yellow oil (0.39 mmol, 98%). Rf=0.51 (Hexane/AcOEt 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.22 (t, J = 9.5 Hz, 1H, H_3), 5.11 (t, J = 9.7 Hz, 1H, H_4), 5.00 (dd, J = 9.5 Hz, 8.1 Hz, 1H, H_2), 4.51 (d, J = 8.0 Hz, 1H, H_1), 4.28 (dd, J = 12.3 Hz, 4.7 Hz, 1H, H_6), 4.16 (dd, J = 12.3 Hz, 2.3 Hz, 1H, $H_{6'}$), 3.89 (dt, J = 9.6 Hz, 6.2 Hz, 1H, -O-CH_2- (alkyl)), 3.71 (ddd, J = 9.9 Hz, 4.6 Hz, 2.4 Hz, 1H, H_5), 3.50 (dt, J = 9.6 Hz, 6.7 Hz, 1H, -O-CH_2'- (alkyl)), 2.87 (t, J = 7.3 Hz, 2H, -CH_2-S-), 2.34 (s, 3H, CH_3 (SAc)), 2.11-2.03 (4s, 12H,

CH_3 (OAc)), 1.66-1.57 (m, 4H, -C***H***$_2$-CH_2-O- + -C***H***$_2$-CH_2-S-), 1.43 (m, 2H, -C***H***$_2$-CH_2-CH_2-O-); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7-169.3 (4s, CO (OAc)), 100.8 (C_1), 72.9 (C_3), 71.8 (C_5), 71.3 (C_2), 69.8 (-CH_2-O-), 68.5 (C_4), 62.0 (C_6), 30.6 (CH_3 (SAc)), 29.2-28.9 (3s, -***C***H_2-CH_2-O- +-***C***H_2-***C***H_2-S-), 25.0 (-***C***H_2-CH_2-CH_2-O-), 20.8-20.6 (4s, CH_3 (OAc)); HRMS ESI (+) *m/z* calcd for $C_{21}H_{32}O_{11}SNa$ $(M+Na)^+$ 515.1563, found 515.1669.

5-mercaptopentyl β-L-glucopyranoside (5). To a solution of 5-thioacetylpentenyl-2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranoside **4** (0.35 mmol) in degassed MeOH (2 mL) was added MeONa (0.35 mmol, 1 eq.). The mixture was stirred 3 hr at room temperature under argon atmosphere. Amberlite was then added to neutralize MeONa, the mixture was filtered and MeOH was evaporated to afford a colourless oil (0.32 mmol, 92 %) containing a mixture thiol/disulfide 9:1. Rf=0.51 ($MeOH/CH_2Cl_2$ 1:3); ^{1}H NMR (500 MHz, D_2O) δ 4.38 (d, J = 8.0 Hz, 1H, H_1), 3.92-3.80 (m, 2H, H_6 +-O-CH_2- (alkyl)), 3.69-3.55 (m, 2H, $H_{6'}$+ -O-CH_2'- (alkyl)), 3.41 (t, J = 9.2, 1H, H_3), 3.38-3.35 (m, 1H, H_5), 3.30 (t, J = 9.4, 1H, H_4), 3.18 (t, J = 8.7, 1H, H_2), 2.70 (t, J = 7.2, 0.35H, -CH_2-S-S- (disulfide)), 2.49 (t, J = 7.1, 1.60 H, -CH_2-SH (thiol)), 1.71-1.50 (m, 4H, -C***H***$_2$-CH_2-O- + -C***H***$_2$-CH_2-S-), 1.38 (m, 2H, -C***H***$_2$-CH_2-CH_2-O-); ^{13}C NMR (126 MHz, D_2O) δ 102.2 (C_1), 75.9-75.8 (2s, C_3+C_5), 73.1 (C_2), 70.4 (-CH_2-O-), 69.7 (C_4), 60.8 (C_6), 32.7 (-***C***H_2-CH_2-S-), 28.2 (-***C***H_2-CH_2-O-), 23.8 (-***C***H_2-CH_2-CH_2-O-), 23.6 (-CH_2-S-). HRMS ESI (+) *m/z* calcd for $C_{11}H_{22}O_6S$ $(M+Na)^+$ 305.1035, found 305.1041; $C_{22}H_{42}O_{12}S_2$ $(M+Na)^+$ 585.2015, found 585.2029. $[\alpha]^{20}_D$= +22 (c=1 in H_2O).

The circular dichroism (CD) of 5-mercaptopentyl β-D-glucopyranoside (β-D-GlcC_5SH) and its disulfide derivative is showed below:



3. Synthesis of the original gold glyconanoparticles

A 12 mM solution of thiol-glycoconjugate (12.6 mg, 3.7mL, 45 μmol) in MeOH/H_2O (1:1) was prepared and aliquots (813 μL, 2.5 eq.) of the solution were added to 3 eppendorfs. To each eppendorf, 156 μL of a 25 mM aqueous solution of $HAuCl_4$ (3.9 μmol, 1 eq.) and 86 μL (22 eq.) of 1 M aqueous solution of $NaBH_4$ were added. The mixture was shaken at room temperature for 2 h (1000 rpm) before evaporation of MeOH and water. The residue was washed 3 times with MeOH by centrifugation, dissolved in water, dialyzed against water and freeze-dried. The routine characterization of our gold glyconanoparticles involves TEM measurements, for the core size determination, the size was determined as the average of 250 measurements; ^{1}H NMR spectroscopy, to confirm the presence of glycoconjugate, and UV-vis spectroscopy. The optical rotation of GNPs were also measured (Table S1).

Table S1. Average size, optical rotation, UV-vis, and CD of some of the original GNPs

Sample	Size (nm)[a]	α_D[b]	UV (nm)[c]	CD min (nm)[c]	CD max (nm)[c]
D-Glc-C_5-Au NP	1,67	+100-200º	230; 295; 329	187; 292	238; 327
L-Glc-C_5-Au NP	1,65	-100-200º	220; 300; 520	238; 326	189; 298
D-Gal-C_5-Au NP	1,57	+8-12º	230; 520	185; 289	236; 322
D-Man-C_5-Au NP	1,67	+50-125º	235; 300; 530	188; 295	237; 326

[a] Determined by TEM. [b] Performed in a 1 mm path cuvet at 589 nm, 20ºC, 1 mg/mL in water. The measure is an average since the samples are colloidal solution of nanoparticules. [c] Concentration 0.25 mg/mL in water

4. Preparation of extensively washed glyconanoparticles and isolation of the "byproducts"

To a solution of thiol-glycoconjugate in MeOH (9.76 mM, 6.039 mL, 58.9 μmol, 2.5 eq.), an aqueous solution of $HAuCl_4$ (25 mM, 942 μL, 23.55 μmol, 1 eq.) were added. The mixture was shaken 2 min at 500 rpm, and then an aqueous solution of $NaBH_4$ (1 M, 528 μL, 22 eq.) was added in 4 times. After 2 h shaking at 1000 rpm, a dark precipitate was obtained. The nanoparticles were separated from the supernatant by centrifugation (2 min at 5000 rpm) and the precipitate was washed 10 times with MeOH

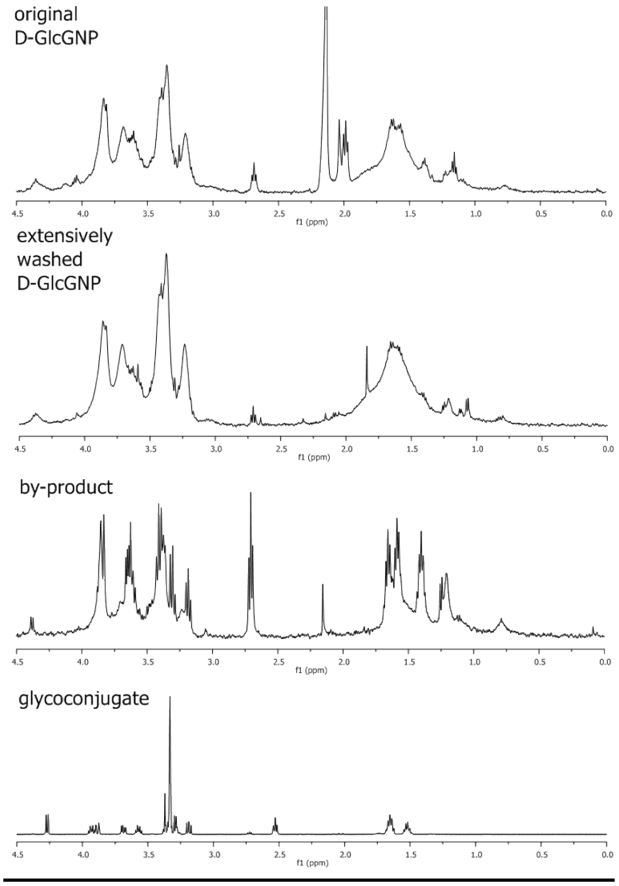
(10 mL each) by centrifugation. The pellet was dissolved in water, dialyzed against water (three changes of water during three days) and freeze-dried and characterized. The methanol washings were collected and evaporated. The residue was dissolved in water, filtered in Amicon 5 kDa and the residue was washed with water. This operation was repeated 2 times more, then the residue was re-dissolved in water and freeze dried. A luminescent white cotton-like compound was obtained (0.75 mg, yield 17% from the gold salt). Increasing the equivalents of $NaBH_4$ (27-28 eq.), lower yield of "byproduct" (~6%) was obtained.

5. Synthesis of gold(I)-glycoconjugate polymers

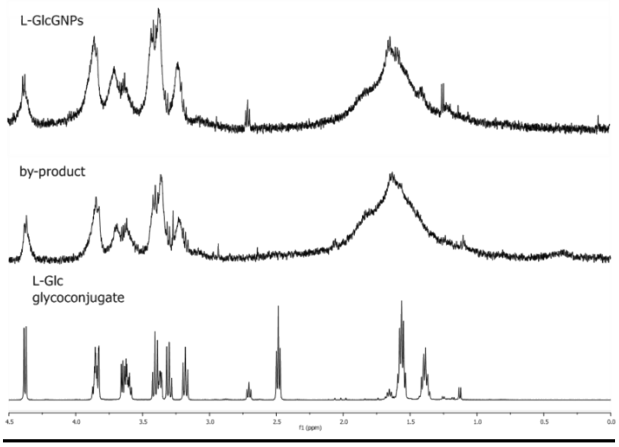
To a 9.76 mM solution of thiol-glycoconjugate in MeOH (1000 μL, 2.5 eq.), an aqueous solution of $HAuCl_4$ (25 mM, 156 μL, 3.9 μmol) were added. The mixture was shaken 2 min at 500 rpm, then a solution of NaOH (80 μL, 0.2 M) was added in 4 times to adjust the pH to 8. After 2 h shaking at 1000 rpm, a white precipitate was obtained by centrifugation. The white solid was dissolved in water, filtered in Amicon 5 kDa and washed with water (1 mL×4). The solid was re-dissolved in water and freeze-dried. A white cotton-like compound with strong photoluminescence was obtained (80% yield from the gold salt).

6. Selected ^{1}H-NMR spectra in D_2O of 5-mercaptopentyl glycoconjugates, GNPs, "byproducts" and Au(I)-thiolate polymers

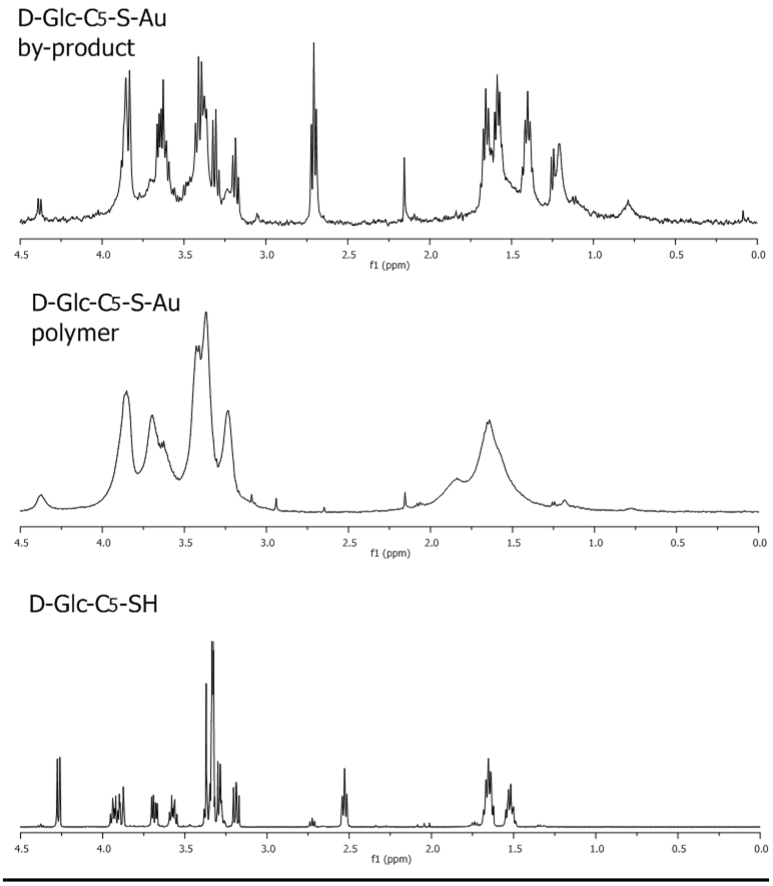
a) ^{1}H-NMR spectra in D_2O at 500 MHz of the original D-GlcGNP, extensively washed D-GlcGNP, the byproduct, and the glycoconjugate β-D-GlcC_5SH.



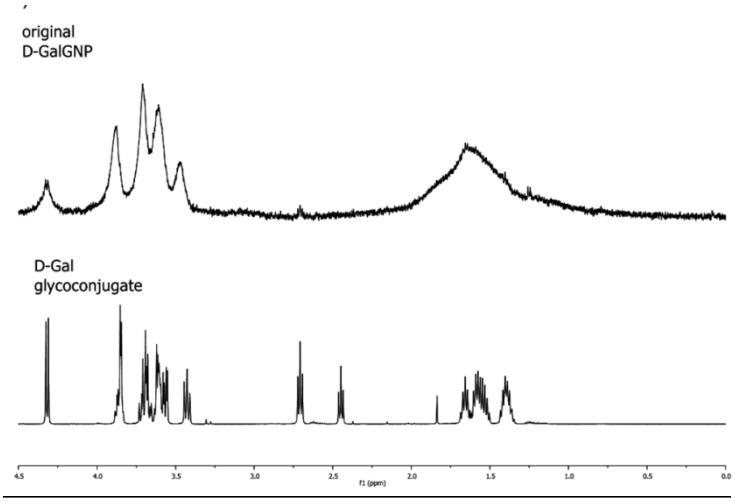
b) ^{1}H-NMR spectra in D_2O at 500 MHz of L-GlcGNP, byproduct and β- L-GlcC_5SH glycoconjugate.



c) ^{1}H NMR spectra of D-Glc byproduct, $[GlcC_5S\text{-}Au(I)]_n$ polymer and of the β-D-$GlcC_5SH$ glycoconjugate. The well-resolved signals in the byproduct spectrum correspond to the $(\beta\text{-D-}GlcC_5S)_2$ disulfide



d) ^{1}H-NMR in D_2O at 500 MHz of the original D-GalGNP, and the β-D $GalC_5SH$ glycoconjugate.



7. Selected data for the original GNPs, extensively washed D/L GNPs and byproducts

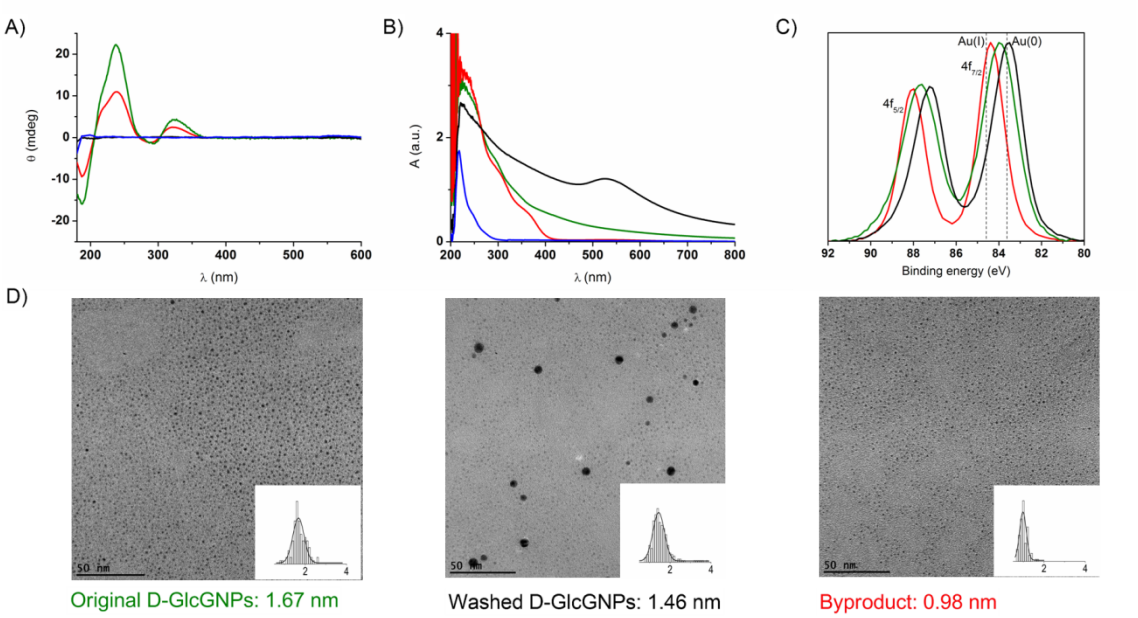


Fig. S1 CD (A), UV-vis (B), XPS (C) spectra and TEM micrographs (D) of original (green), extensively washed D-GlcGNP (black), and "byproduct" (red). The blue CD and UV spectra correspond to the glycoconjugate β-D-GlcC_5SH. As yet described in literature, Au(I) complex can produced AuNPs under beam irradiation.[2]

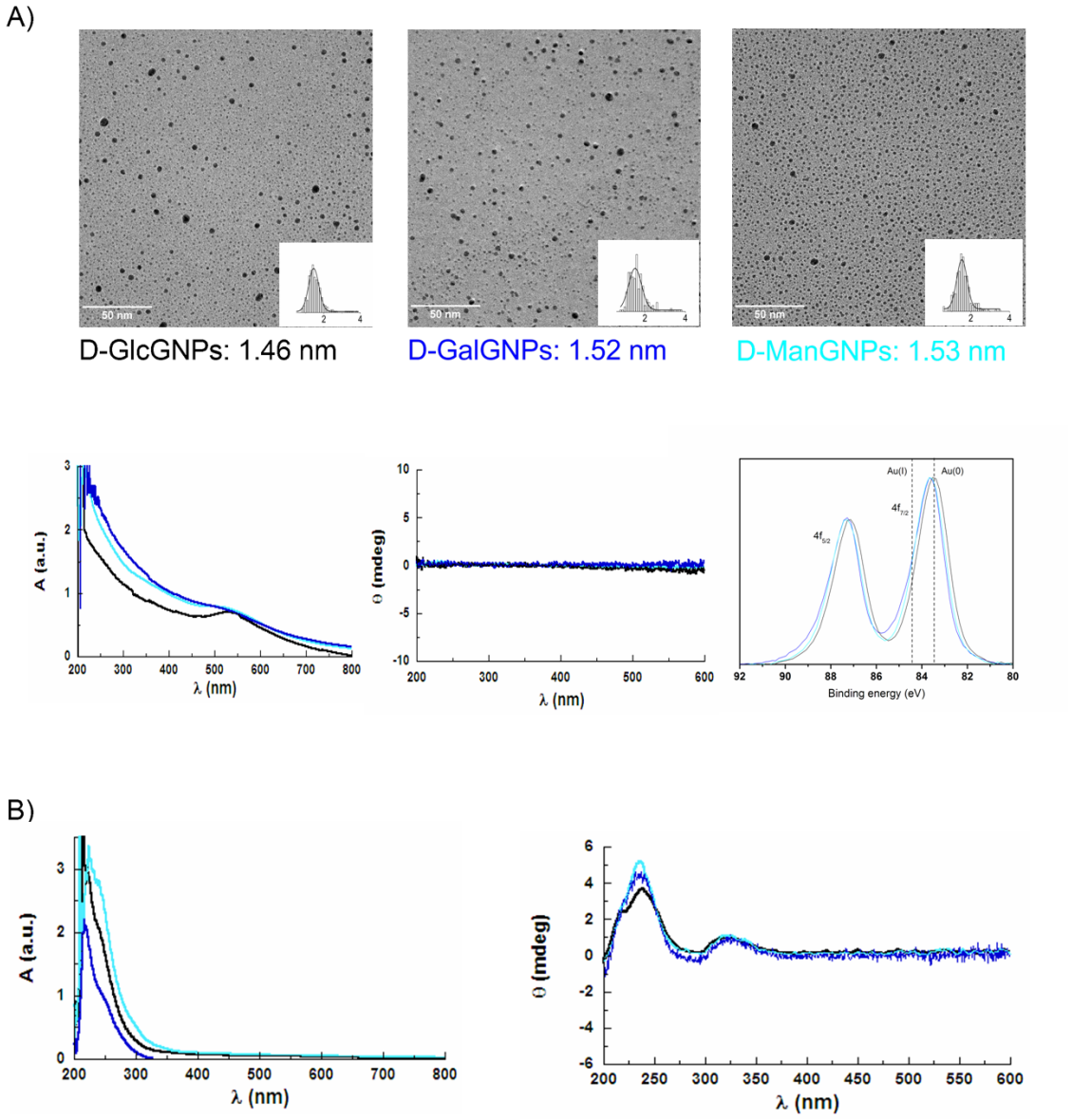


Fig. S2 (A) TEM micrographs, UV-vis, CD and XPS spectra of the extensively washed D-GlcGNP (black), D-GalGNP (blue), and D-ManGNP (cyan). (B) UV-vis and CD spectra of the corresponding byproducts obtained after extensively washing with methanol.

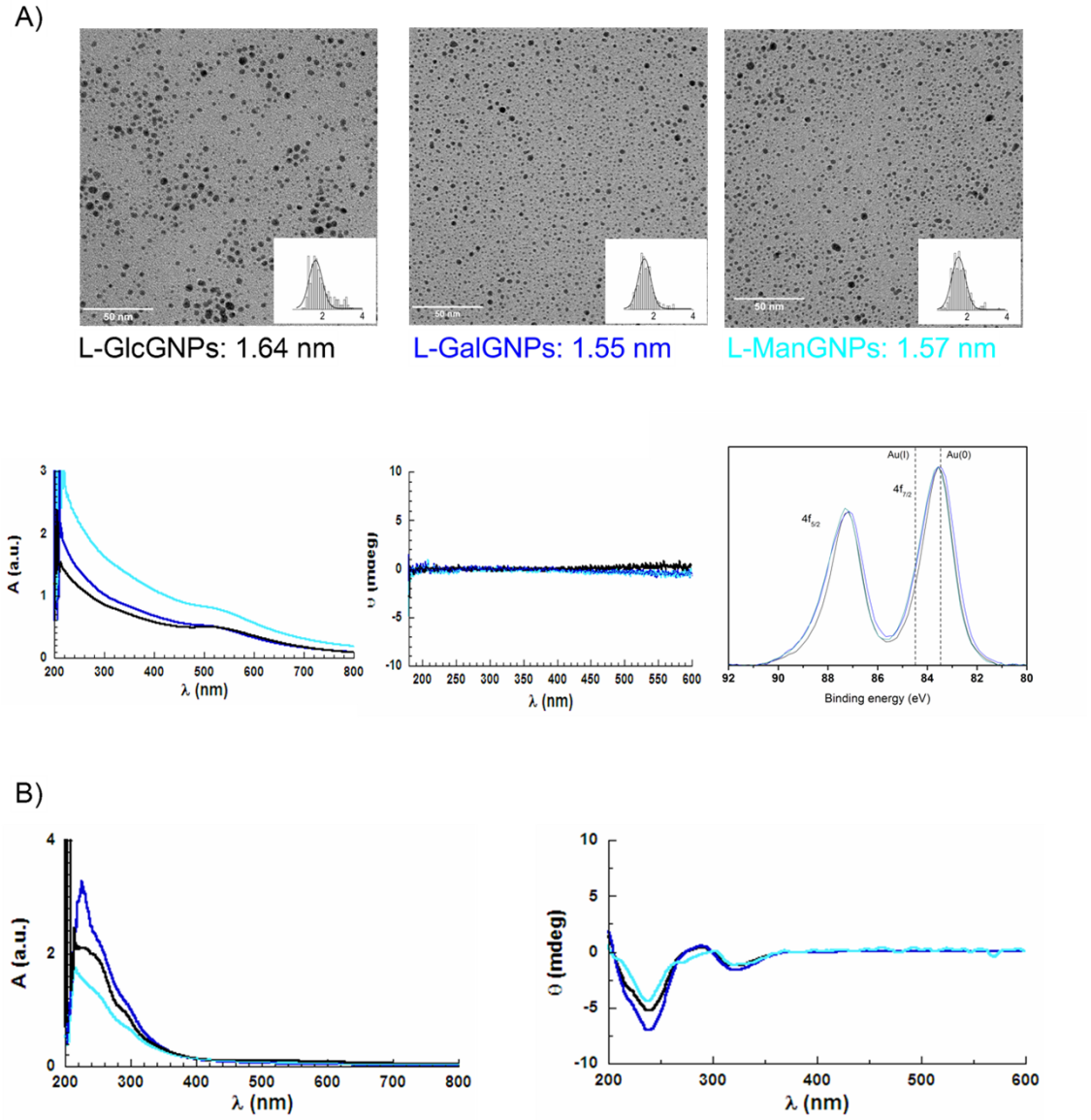


Fig. S3 (A) TEM micrographs, UV-vis, CD, and XPS spectra of the extensively washed L-GlcGNP (black), L-GalGNP (blue), and L-ManGNP (cyan). (B) UV-vis and CD of the corresponding byproducts obtained after extensively washing with methanol.

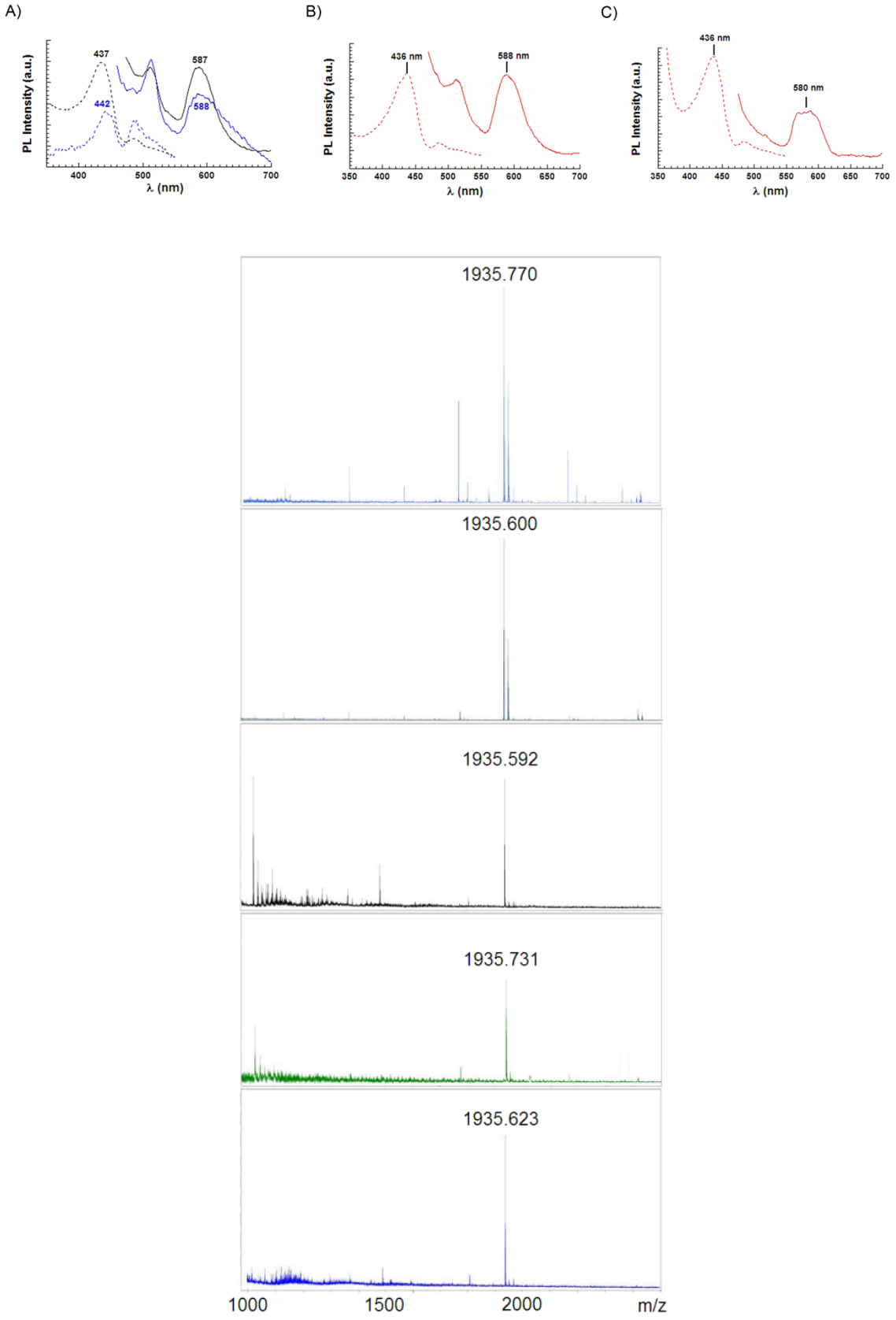


Fig. S4 Photoluminescence, excitation (dotted lines) and emission (solid lines), spectra of spectra of (A) D-GlcC_5SAu(I), (B) L-GlcC_5SAu(I), and (C) D-GlcTEGC_{11}SAu(I) polymers and mass spectra (from top to bottom) of L-Glc, D-Gal, L-Gal, D-Man and L-Man byproducts.

8. Characterization of D-GlcTEGC$_{11}$SAu(I) polymer and D-GlcTEGC$_{11}$-GNPs

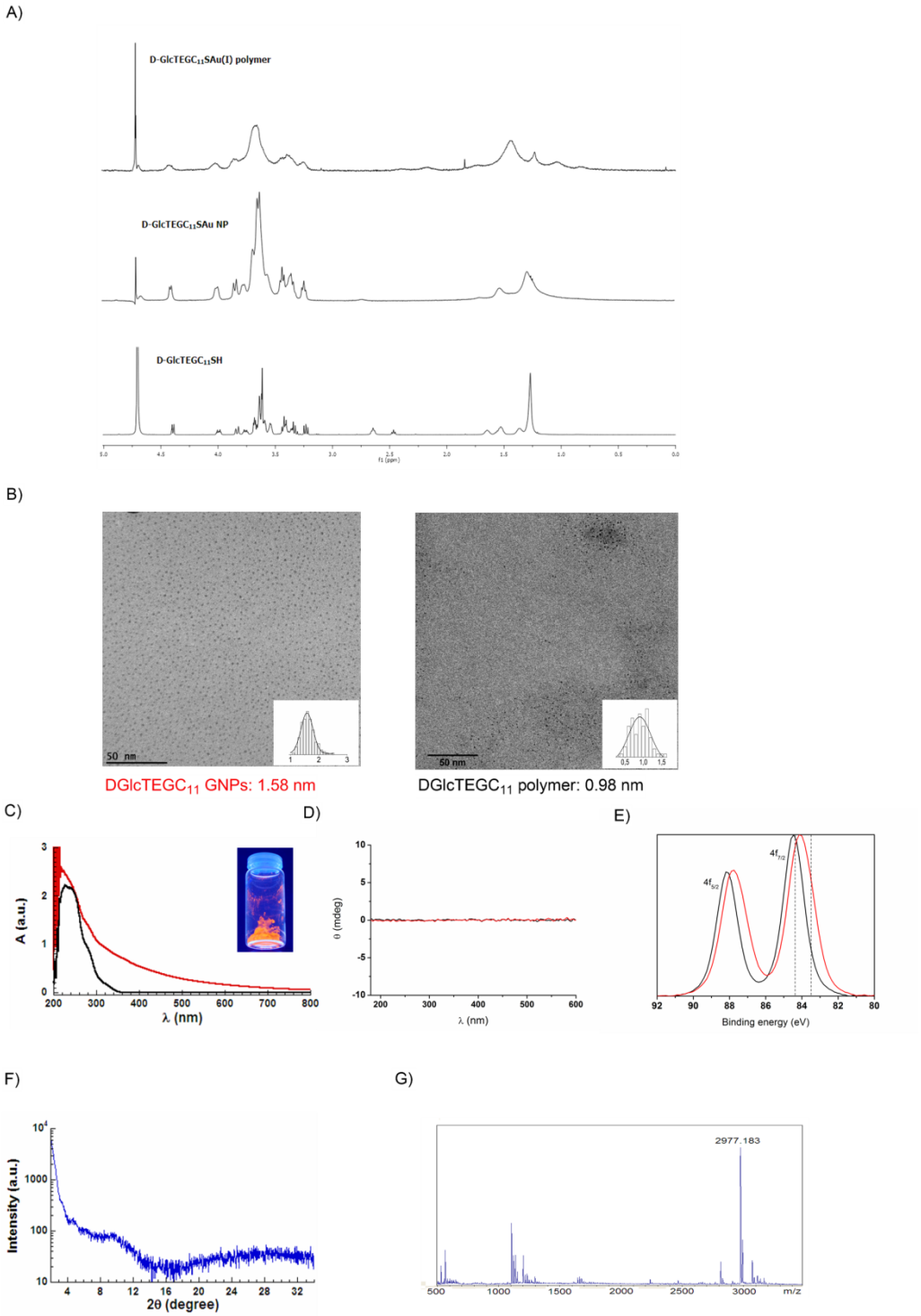
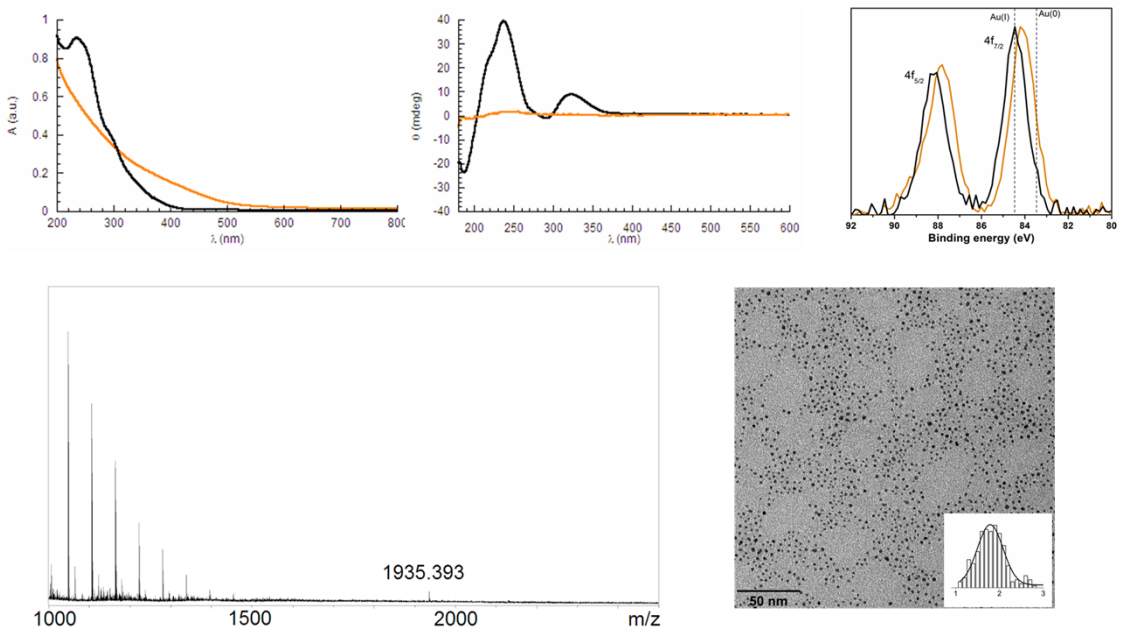


Fig. S5 (A) ^{1}H NMR spectra of D-GlcTEGC$_{11}$SAu(I) polymer, D-GlcTEGC$_{11}$SGNPs and the D-GlcTEGC$_{11}$SH glycoconjugate. (B) TEM micrographs of original D-GlcTEGC$_{11}$SGNPs (1.58 nm), left, and D-GlcTEGC$_{11}$SAu(I) polymer (0.98 nm), right. (C) UV-vis (inset: Luminescence under λ=300nm), (D) CD and (E) XPS spectra of the original D-GlcTEGC$_{11}$SGNPs (red) and the D-GlcTEGC$_{11}$SAu(I) polymer (black). (F) Small-angle XRD and G) mass spectra of D-GlcTEGC$_{11}$SAu(I) polymer. The peak at 2977 corresponds to the open tetramer.

9. Evolution of D-GlcC$_5$SAu(I) polymer and d D-GlcTEGC$_{11}$SAu(I) polymer under different conditions

A)



B)

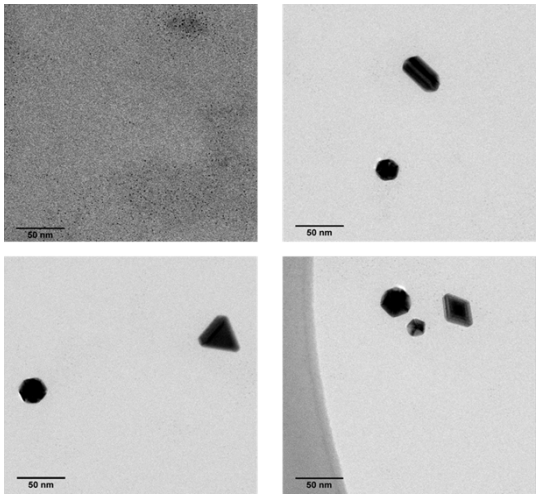


Fig. S6 (A) Evolution of an aqueous solution of D-GlcC$_5$SAu(I) polymer after 6 weeks under sunlight; (B) TEM micrographs taken from different regions of the carbon grid of an aqueous solution of GlcTEGC$_{11}$SAu(I) polymer (0.1 mg/mL) after 20 days at room temperature. In the top left image the mean diameter of the polymers is 0.96 ± 0.25nm (from 250 measurements).

[1] (a) Barrientos, A.G.; de la Fuente, J.M.; Rojas, T.C.; Fernández, A.; Penadés, S. *Chem. Eur. J.* **2003**, *9*, 1909-1921; (b) Martínez-Ávila, O.; Hijazi, K.; Marradi, M.; Clavel, C.; Campion, C.; Kelly, C.; Penadés, S. *Chem. Eur. J.* **2009**, *15*, 9874-9888

[2] Kim, J. U.; Cha, S.H.; Shin, K.; Young Jho, J.; Lee, J.C.; *J. Am. Chem. Soc.*, 2005, 127, 9962-9963