Electronic Supplementary Material (ESI) for Dalton Transactions.
This journal is © The Royal Society of Chemistry 2021

Electronic Supplementary Information

Design, physico-chemical characterization and *in vitro* biological activity of organogold(III) glycoconjugates

Andrea Pettenuzzo,[a] Keti Vezzù,[b] Maria Luisa Di Paolo,[c] Eirini Fotopoulou,[a] Luciano Marchiò,[d] Lisa Dalla Via*[e] and Luca Ronconi*[a]

[a] National University of Ireland Galway, School of Chemistry, University Road, H91 TK33 Galway, Co. Galway, Ireland.

[b] University of Padova, Department of Industrial Engineering, Via F. Marzolo 8, 35131 Padova, Italy.

[c] University of Padova, Department of Molecular Medicine, Via G. Colombo 3, 35131 Padova, Italy.

[d] University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parco Area delle Scienze 11/a, 43124 Parma, Italy.

[e] University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Via F. Marzolo 5, 35131 Padova, Italy.

Table of Contents

Materials and methods

Materials

(α,β)-D-glucosamine hydrochloride, *N*-acetyl-(α,β)-D-glucosamine, PPh_3, *N*-bromosuccinimide (NBS), boron trifluoride diethyletherate, ethyl isonipecotate, isonipecotamide, isonipecotic acid (TCI), Et_3N, NaN_3, NaOMe, BaO, Pd/C 10%$_w$, Amberlite IR120 (H^+ form) resin, *N*-(benzyloxycarbonyloxy)succinimide (Cbz-OSu), ethanolamine, CS_2, $ZnCl_2$, $[Zn(OAc)_2]\cdot 2H_2O$, *N*,*N*-diisopropylethylamine (DIPEA), 2-benzylpyridine (2-Bnpy), KPF_6, *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin), 4,6-*O*-ethylidene-α-D-glucose (EDG) (Merck), 1,2,3,4,6-pentaacetate-β-D-glucopyranose, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) (Carbosynth), $Na[AuCl_4]\cdot 2H_2O$ (Alfa Aesar), and all deuterated solvents for NMR analysis (Deutero) were of reagent grade or comparable purity and were used as supplied. Anhydrous DMF was obtained by passing the solvent over a column of alumina and subsequently stored over 4 Å activated molecular sieves under an inert atmosphere of nitrogen. Anhydrous DCM was obtained by distillation over CaH_2 and subsequently stored over 4 Å activated molecular sieves under an inert atmosphere of nitrogen. All other reagents and solvents were used as purchased without any further purification. Phosphate buffered saline (PBS) solution (pH 7.4, 0.01 M phosphate buffer, 0.0027 M KCl, 0.137 M NaCl) was obtained by dissolving one tablet (Sigma-Aldrich) in 200 mL of Milli-Q water at room temperature.

Instrumentation

Thin layer chromatography (TLC) was performed on silica gel Merck $60F_{254}$ pre-coated aluminum sheets. Spots were visualized by direct UV irradiation at 254 nm or developed by exposure to either *p*-anisaldehyde or potassium permanganate staining solutions as appropriate.

Flash column chromatography was performed on Merck 60 Å silica gel (40-63 μm, 230-400 mesh) as stationary phase using the appropriate eluent.

Melting points were recorded on a Stuart SMP10 digital melting point apparatus and are uncorrected.

Elemental analyses (carbon, hydrogen and nitrogen) were performed with a Perkin Elmer 2400 CHNS/O Series II analyzer.

FT-IR spectra of the carbohydrate precursors were recorded from thin films at room temperature on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer equipped with a UATR accessory in the range 4000-650 cm^{-1}. FT-IR spectra of the metal complexes were recorded from CsI disks at room temperature on a Perkin Elmer Frontier FT-MIR/FIR spectrophotometer in the range 4000-200 cm^{-1}. Data processing was carried out using OMNIC version 5.1 (Nicolet Instrument Corporation).

NMR spectra were acquired in the specified deuterated solvent at room temperature on a Jeol 400 MHz spectrometer equipped with *z*-field gradients. ^{1}H and ^{13}C chemical shifts were referenced to TMS at 0.00 ppm *via* internal referencing to the residual peak of the deuterated solvent employed. ^{1}H and $^{13}C\{^{1}H\}$ signals were assigned with the aid of [^{1}H,^{1}H] COSY, ^{13}C DEPT, [^{1}H,^{13}C] HSQC and [^{1}H,^{13}C] HMBC experiments. Data processing was carried out using MestReNova version 12.0 (Mestrelab Research S.L.).

UV-Vis spectra were acquired in the specified solvent at room temperature (unless otherwise stated) on a Thermo Scientific NanoDrop 2000C Spectrophotometer in the range 190-840 nm. Data processing was carried out using Spectragryph optical spectroscopy software version 1.2 (Dr. Friedrich Menges, http://www.effemm2.de/spectragryph/).

ESI-MS were obtained in acetonitrile/water (1:1) on an Agilent G6540B Q-TOF mass spectrometer equipped with a Dual AJS ESI ion source interface operated in positive ion mode over a mass range of *m/z* 100-1200.

A summary of data collection and structure refinement for **Au1**, **Au2** and **Au2·CH$_3$CN** is reported in **Figure S1** (see below). Single crystal X-ray diffraction data were collected using a Bruker D8 Photon II instrument (Mo-K_α radiation: λ = 0.71073 Å). Data collection was performed to cover the sphere of reciprocal space.[1] Absorption correction was applied using the software SADABS.[2] The crystal structures were solved with the ShelXT structure solution program[3] using intrinsic phasing, and refined with the ShelXL refinement package[4] using least squares minimization. Graphical material was prepared using Mercury version 3.9.[5] CCDC 2058611 (**Au1**), 2058613 (**Au2**) and 2058612 (**Au2·CH$_3$CN**) contain the supplementary crystallographic data for this paper.

Measurement of lipophilicity (Log$D_{7.4}$)

The *n*-octanol/water partition coefficients at pH 7.4 (Log$D_{7.4}$) were determined using the shake-flask method.[6] 100 mL of PBS solution (pH 7.4, 0.01 M phosphate buffer, 0.0027 M KCl, 0.137 M NaCl) and 100 mL of analytical grade *n*-octanol were mutually saturated and mechanically shaken for 72 h using a Stuart STR4 Rotator Drive unit. A calculated amount of a freshly prepared 5 mM DMSO solution of each individual gold(III) compound was diluted with the appropriate volume of an *n*-octanol/PBS-saturated solution to obtain a final stock 25 μM solution of the metal complex (the final DMSO content in solution being 0.5% v/v). 5 mL of this stock solution was then added to 5 mL of a PBS/*n*-octanol-saturated solution and the resulting mixture was mechanically shaken for 1 h. The biphasic mixture was centrifuged at 3000 rpm for 5 min allow full separation of the two phases. Eventually, the concentration of each metal derivative in the various phases was measured by UV-Vis spectrophotometry. Measurements were carried out in triplicate for each sample.

Due to its greater hydrophobicity, absorbance of compound **Au1** was measured by UV-Vis spectrophotometry at 265 nm in *n*-octanol before and after partitioning, whereas for all the other complexes **Au2-Au6** absorbance was measured at 261 nm in PBS before and after partitioning.

For complexes **Au2-Au6**, Log$D_{7.4}$ was calculated using the following equation:

$$LogD_{7.4} = Log\left(\frac{C_{oct}}{C_{PBS}}\right) \cong Log\left(\frac{A_{oct}}{A_{PBS}}\right) = Log\left(\frac{A_0 - A_{PBS}}{A_{PBS}}\right)$$

where:

- C_{oct} and C_{PBS} are the molar concentrations of each individual metal complex in the octanolic and PBS fractions, respectively, after partitioning;
- A_{oct} and A_{PBS} are the absorbances of each individual metal complex in the octanolic and PBS fractions, respectively, after partitioning;
- A_0 is the absorbance of each individual metal complex in the 25 μM *n*-octanol/PBS-saturated stock solution.

For complex **Au1** the following modified equation was used, accounting for the different volumes of *n*-octanol/PBS saturated and PBS/*n*-octanol-saturated solutions employed:

$$LogD_{7.4} = Log\left(\frac{C_{oct}}{C_{PBS}}\right) \cong Log\left(\frac{A_{oct}}{A_{PBS}} \times \frac{V_{PBS}}{V_{oct}}\right) = Log\left(\frac{A_{oct}}{A_0 - A_{oct}} \times \frac{V_{PBS}}{V_{oct}}\right)$$

where V_{PBS} and V_{oct} are, respectively, the volumes of *n*-octanol/PBS-saturated and PBS/*n*-octanol-saturated solutions used.[7]

β-Glucosidase activity assay

The activity of β-glucosidase towards the β-C[1]-functionalized gold(III)-dithiocarbamato glycoconjugate **Au6** was assessed by ^{1}H NMR spectroscopy following a modified protocol previously reported in the literature.[8] Compound **Au6** (5 mg) was dissolved with 1 mL of acetate buffer at pH 5 in D_2O (prepared by mixing 714 μL of a 0.1 M D_2O solution of sodium acetate with 1286 μL of a 0.1 M D_2O solution of acetic acid). The solution was split into two equivalent (500 μL) aliquots labeled as sample A (reference control) and sample B. The latter was treated with 50 μL of a 5 U mL^{-1} solution of β-glucosidase (Merck) in acetate buffer at pH 5 in D_2O. Both samples were incubated at 37°C and ^{1}H NMR spectra were recorded over a three-hour period. Sample B was than treated with further 50 μL of the β-glucosidase solution and additional ^{1}H NMR spectra were recorded at regular time intervals. 24 hours after the first addition of β-glucosidase to sample B, 30 μL of DMSO-D_6 were added as internal reference and the final ^{1}H NMR spectra were recorded.

Cell lines, culture conditions and samples preparation

Human tumor cell lines were purchased from the European Collection of Authenticated Cell Cultures (ECACC).

Human cervix adenocarcinoma HeLa cells were grown in Ham's F12 Nutrient Mixture (Merck N6760). Human colorectal carcinoma HT29 cells and human ovarian carcinoma A2780 cells were grown in RPMI 1640 medium (Merck R6504). The parent human ovarian carcinoma cisplatin-resistant A2780cis cells were obtained by addition to the RPMI 1640 medium of a sub-lethal dose of cisplatin (1 μM) every 2-3 passages. Cells were cultured at 37°C in 5% CO_2 and moisture-enriched atmosphere in the respective medium supplemented with 1.5 g L^{-1} $NaHCO_3$ (Merck), 10% heat-inactivated fetal bovine serum (Biowest S181H), 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 0.25 μg mL^{-1} amphotericin B (Merck A5955).

Stock solutions of the gold(III) complexes **Au1**-**Au6** in DMSO (20 mM) were freshly prepared and subsequently diluted with the appropriate complete cell culture medium in such a way that the final amount of organic solvent did not exceed 0.5% v/v. A stock 4 mM solution of cisplatin was prepared in physiological saline solution (NaCl 0.9% w/v).

Cell growth inhibition assay

Cells (5×10^4 per well) were seeded in 24-well cell culture plates in complete medium and incubated at 37°C in 5% CO_2 humidified atmosphere. After 24 h, the cells were treated with the test compounds at different concentrations (0.1-20 μM, containing 30 mM EDG where appropriate) and incubated for further 48 h at 37°C. The medium was then discarded, cells were washed with fresh PBS solution and harvested, and a Trypan blue assay was performed to determine cell viability. The cell suspension was diluted with 0.1% w/v Trypan blue (Merck) solution and viable (unstained) cells were counted in a Bürker chamber (Blaubrand) within 3-5 minutes. Antiproliferative data are expressed as GI_{50} values, that is, the concentration of the test agent inhibiting cell growth by 50% reduction compared with control cultures. Results were calculated as the mean values of at least five independent experiments.

Cell cycle distribution analysis

A2780 cells (3×10^5) were seeded in TC P60 dishes (Sartsedt) in complete medium and incubated at 37°C in 5% CO_2 humidified atmosphere. After 24 h, the cells were treated with the test compounds at the indicated concentrations and incubated for further 40 h at 37°C. Cells were then harvested, centrifuged, treated with ice-cold 70% w/v ethanol at 4°C for 20 min, washed twice with PBS solution (8 mM $Na_2HPO_4{\cdot}2H_2O$, 1.5 mM KH_2PO_4, 2 mM KCl, 0.1 M NaCl), and then incubated in PBS solution containing 0.1 mg mL^{-1} RNAse (Merck R6513) and 36 μg mL^{-1} propidium iodide

(Merck P4170). The analysis of DNA content was performed on a FACSCanto II flow cytometer (Becton-Dickinson) at λ_{ex} = 488 nm/λ_{em} = 620 nm.

Topoisomerase I and II relaxation assay

Supercoiled pBR322 plasmid DNA (0.25 μg, Fermentas Life Sciences), 1U topoisomerase I (Inspiralis) or topoisomerase II (human topoisomerase II alpha, Inspiralis) and the test complexes at the indicated concentrations were incubated at 37°C for 60 min in a final volume of 20 μL of the supplied assay buffer (Inspiralis). Reactions were stopped by incubating for 30 min at 37°C with 4 μL stop buffer (5% sodium dodecyl sulfate, 0.125% bromophenol blue, 25% glycerol) and 50 μg mL^{-1} proteinase K (Merck). Samples were analyzed by electrophoresis on 1% agarose gel in TAE buffer (0.04 M tris-acetate, 0.001 M EDTA, pH 8) at room temperature for 90 min. Gels were then stained with 1 μg mL^{-1} ethidium bromide in TAE and visualized by a Gel Doc XR apparatus (Bio-Rad).

Cell uptake

A2780 cells (2.5×10^6) were seeded into 6-well cell culture plates (Greiner Bio-One) in complete medium and incubated at 37°C in 5% CO_2 humidified atmosphere. After 24 h, the cells were treated with the test compounds at 100 μM concentration. Cells were then harvested at fixed time intervals (1-3 h incubation), washed twice with physiological saline solution (NaCl 0.9% w/v), and the cell pellets were mineralized upon treatment with 195 μL of HNO_3 65% w/v followed by heating at 90°C for 1 h. The samples were then diluted with 3 volumes of HCl 37% w/v and, eventually, milli-Q water was added up to 5 mL.

Gold content was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) at the emission line λ(Au) = 242.795 nm using a Spectroflame Modula sequential and simultaneous ICP-spectrometer (ICP SPECTRO Arcos with EndOnPlasma torch) equipped with a capillary cyclonic nebulizer (Spectro Analytical). Analytical determinations were performed using a plasma power of 1.4 kW, a radiofrequency generator of 27.12 MHz and an argon gas flow with nebulizer, auxiliary, and coolant set at 1, 0.5 and 14 L min^{-1}, respectively. Calibration was carried out by preparing five standard solutions containing Au in the concentration range 0-1 mg L^{-1} (ppm). Standard solutions were prepared by diluting Au and P stock solutions of 1,000 mg L^{-1} (Spectrascan standards from Teknolab) with HCl 1% v/v. Results were calculated as the mean values of four independent experiments.

Determination of mitochondrial transmembrane potential

The mitochondrial transmembrane potential was assayed in A2780 cells by using the BD™ MitoScreen Kit (BD Pharmigen). Cells (3×10^5) were seeded in TC P60 dishes (Sartsedt) in complete medium and allowed to grow at 37°C in 5% CO_2 humidified atmosphere over the next 24 h. Cells were treated with the test compounds at the indicated concentrations and incubated for further 40 h at 37°C. Cells were then harvested, centrifuged, gently resuspended in JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide) Working Solution and incubated for 30 min at 37°C in the dark. Following incubation, cells were washed twice in Assay Buffer, gently resuspended and immediately analyzed at λ_{ex} = 488 nm/λ_{em} = 525 and 575 nm by flow cytometry using a FACSCanto II flow cytometer (Becton-Dickinson). Ten thousand events per sample were analyzed.

Determination of Reactive Oxygen Species (ROS)

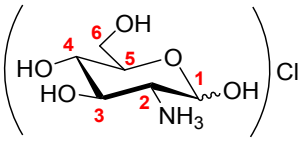
A2780 cells (1×10^4) were seeded into a P96 cell culture plate in complete growth medium and incubated at 37°C in 5% CO_2 humidified atmosphere. After 24 h, the medium was removed and cells were washed with PBS solution and incubated with 10 μM 2',7'-dichlorofluorescin diacetate (Merck) at 37°C in the dark for 20 min. Cells were washed with PBS solution, incubated in PBS solution containing the test compounds at the indicated concentrations and incubated for further 120 min at 37°C. Fluorescence intensity was measured using a Victor X3 Multilabel plate reader (Perkin Elmer) at λ_{ex} = 485 nm/λ_{em} = 527 nm. Results were calculated as the mean values of three independent experiments performed with eight replicates.

Statistical Analysis

Results from cellular accumulation and ROS production experiments are reported as means ± SD of at least four experiments. Student's *t*-test was used to determine statistical significance, and values of $p < 0.05$ were considered statistically significant. Statistical analyses were carried out using SigmaPlot version 10.0 (Jandel Scientific). Significant differences between control and treated conditions are marked with an asterisk (*).

Synthesis and spectroscopic characterization of the amino-sugar precursors (GlcN1, GlcN2, GlcN3 and GlcN4)

2-Amino-2-deoxy-(α,β)-D-glucose (aka (α,β)-D-glucosamine, GlcN1) hydrochloride



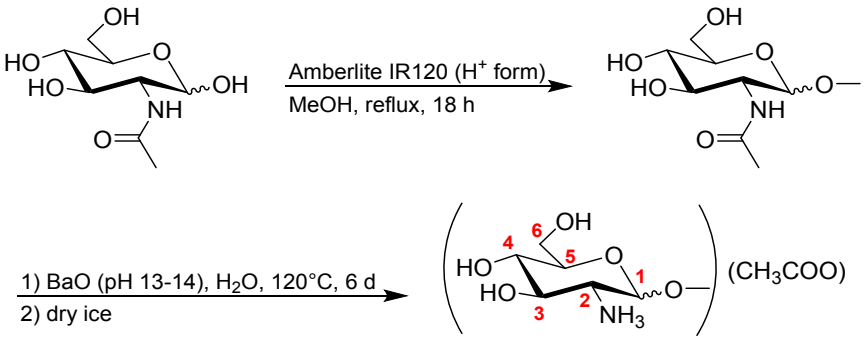
GlcN1.HCl

Commercially available. FT-IR (ATR; 298 K): $\tilde{\nu}_{max}$ $3392_{br}/3282_{br}/3244_{br}$ (ν, OH), 3093/3035 (ν_a, NH_3^+), 2844 (ν_s, NH_3^+), 1614/1580/1538 ($\delta_{a,s}$, NH_3^+), 1031/1003 (ν, C–OH) cm^{-1}. ^1H NMR (400 MHz; DMSO-D_6; 298 K): δ 8.01 (3 H, br s, N***H***$_3^+$), 7.16 (1 H, d, C^1O***H***), 5.67 (1 H, d, C^3O***H***), 5.30 (1 H, d, C^4O***H***), 5.23 (1 H, br dd, C^1***H***), 4.57 (1 H, br s, C^6O***H***), 3.63-3.45 (4 H, m, C^3***H*** + C^5***H*** + C^6***H***$_2$ overlapped), 3.17 (1 H, td, C^4***H***), 2.82 (1 H, dd, C^2***H***, $^3J_{1,2}$ = 3.2 Hz) ppm. $^{13}C\{^1H\}$ NMR (100 MHz; DMSO-D_6; 298 K): δ 88.9 (***C***^{1}H), 72.3 (***C***^{5}H), 70.2 (***C***^{4}H), 69.8 (***C***^{3}H), 60.6 (***C***$^6H_2$), 54.5 (***C***^{2}H) ppm.

The NMR signals refer to the α anomer and are consistent with literature data,[9] including the $^3J_{1,2}$ value within the 1-4 Hz range.[10] Although the substance is sold as a mixture of α and β anomers, the NMR signals of the latter are hardly detectable and/or overlapped with those of the former. Therefore, the amount of β anomer cannot be quantified based on the ^1H NMR spectrum only.

1-*O*-methyl-2-amino-2-deoxy-(α,β)-D-glucopyranoside (GlcN2) acetic acid

This amino-sugar precursor was prepared as previously described.[11]



GlcN2.CH_3COOH

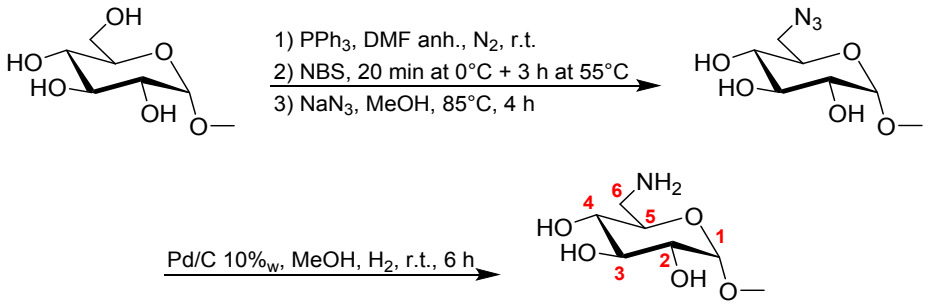
Yield final step: 72%. FT-IR (ATR; 298 K): $\tilde{\nu}_{max}$ 3205_{br} (ν, OH + NH_3^+ overlapped), 2844 (ν_s, NH_3^+), 1628 (δ_a, NH_3^+), 1546 (ν_a, COO^-), 1039 (ν, C–OH + C^1–O–CH_3 overlapped) cm^{-1}. ^1H NMR (400 MHz; DMSO-D_6; 298 K): δ 4.55 (1 H, d, C^1***H*** α, $^3J_{1,2}$ = 3.5 Hz), 4.07 (3.6 H, br s, C^3O***H*** + C^4O***H*** + C^6O***H*** α and β overlapped), 3.98 (0.3 H, d, C^1***H*** β, $^3J_{1,2}$ = 7.9 Hz), 3.68-3.40 (~2.5 H, m, C^6***H***$_2$ α and β overlapped), 3.38 (0.6 H, s, OC***H***$_3$ β), 3.32-3.28 (1 H, m, C^5***H*** α), 3.26 (3 H, s, OC***H***$_3$

α), 3.22 (1 H, dd, C^3***H*** α), 3.07-3.00 (1.7 H, m, C^4***H*** α + C^3***H*** + C^4***H*** + C^5***H*** β overlapped), 2.45 (1 H, dd, C^2***H*** α), 2.40-2.36 (0.2 H, m, C^2***H*** β), 1.79 (3.2 H, s, C***H***$_3$COO$^-$) ppm. ^{13}C{^{1}H} NMR (100 MHz; DMSO-D$_6$; 298 K): δ 174.4 (CH$_3$***C***OO$^-$), 104.5 (***C***^{1}H β), 99.6 (***C***^{1}H α), 77.2 (***C***^{3}H β), 76.1 (***C***^{5}H β), 74.4 (***C***^{3}H α), 73.2 (***C***^{5}H α), 70.4 (***C***^{4}H α), 70.1 (***C***^{4}H β), 61.1 (***C***^{6}H$_2$ β), 61.0 (***C***^{6}H$_2$ α), 57.3 (***C***^{2}H β), 56.1 (O***C***H$_3$ β), 56.0 (***C***^{2}H α), 54.5 (O***C***H$_3$ α), 23.1 (***C***H$_3$COO$^-$) ppm.

Experimental data are consistent with those reported in the literature.[12] Solution α:β anomers ratio ≈ 5:1 (based on the ^{1}H NMR spectrum).

1-*O*-methyl-6-amino-6-deoxy-α-D-glucopyranoside (GlcN3)

This amino-sugar precursor was prepared as previously described.[11]



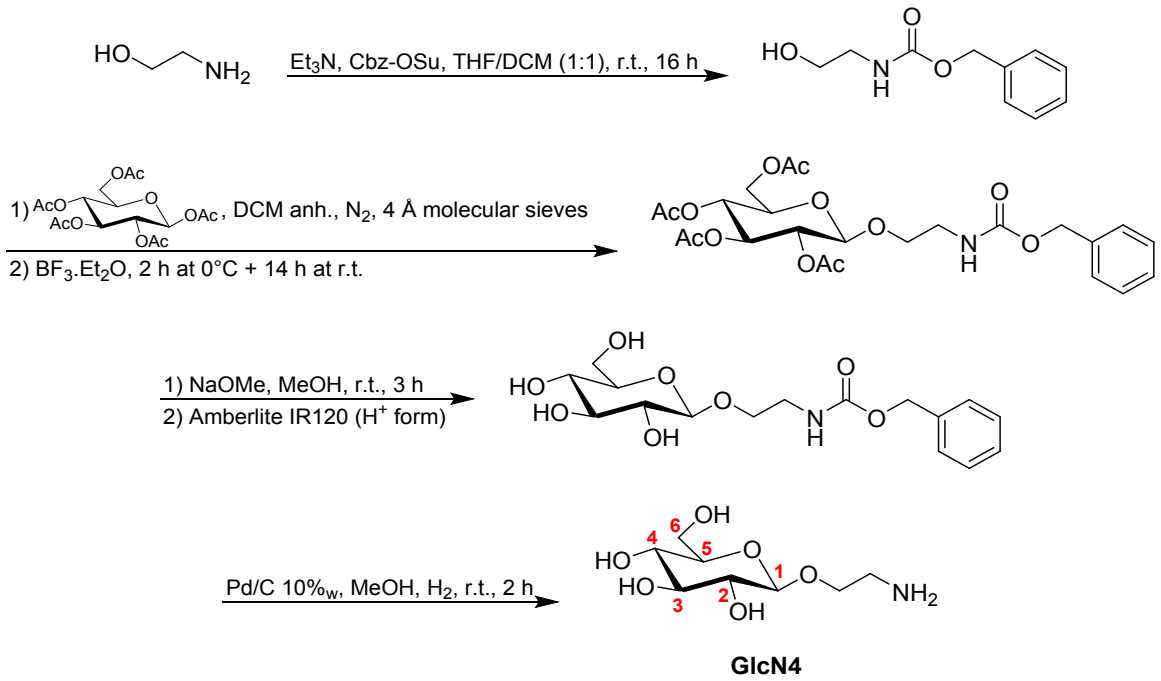
GlcN3

Yield final step: 89%. FT-IR (ATR; 298 K): $\tilde{\nu}_{max}$ 3349$_{br}$/3260$_{br}$ (ν, OH + NH$_2$ overlapped), 1598 (δ, NH$_2$), 1033/1007 (ν, C–OH + C^1–O–CH$_3$ overlapped) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D$_6$; 298 K): δ 4.75 (~3 H, br s, C^{2}O***H*** + C^{3}O***H*** + C^{4}O***H*** overlapped), 4.51 (1 H, d, C^1***H***, $^3J_{1,2}$ = 3.7 Hz), 3.34 (1 H, dd, C^3***H***), 3.26 (3 H, s, OC***H***$_3$), 3.24-3.22 (1 H, m, C^5***H***), 3.18 (1 H, dd, C^2***H***), 2.99 (1 H, dd, C^4***H***), 2.83-2.55 (2 H, m, C^6***H***$_2$). ^{13}C{^{1}H} NMR (100 MHz; DMSO-D$_6$; 298 K): δ 99.7 (***C***^{1}H), 73.2 (***C***^{3}H), 72.4 (***C***^{5}H), 72.1 (***C***^{4}H), 72.0 (***C***^{2}H), 54.3 (O***C***H$_3$), 43.2 (***C***^{6}H$_2$) ppm.

Experimental data are consistent with those reported in the literature and confirm the presence of the α anomer only,[13] including the $^3J_{1,2}$ value within the 1-4 Hz range.[10]

1-*O*-(2'-aminoethyl)-β-D-glucopyranoside (GlcN4)

This amino-sugar precursor was prepared according to a modified literature procedure.[14]



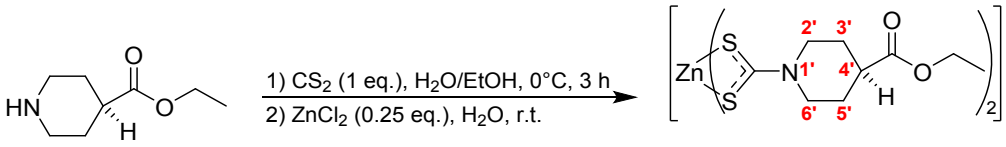
GlcN4

Yield final step: 94%. FT-IR (ATR; 298 K): $\tilde{\nu}_{max}$ 3294_{br} (ν, OH + NH_2 overlapped), 1591 (δ, NH_2), 1072/1015 (ν, C–OH + C^1–O–CH_2 overlapped) cm^{-1}. ^1H NMR (400 MHz; CD_3OD; 298 K): δ 4.27 (1 H, d, $C^1\boldsymbol{H}$, $^3J_{1,2}$ = 7.8 Hz), 3.93 (1 H, ddd, OC***H'***), 3.86 (1 H, dd, $C^6\boldsymbol{H'}$), 3.68-3.60 (2 H, m, $C^6\boldsymbol{H''}$ + OC***H''*** overlapped), 3.35 (1 H, m, $C^3\boldsymbol{H}$), 3.27 (2 H, m, $C^4\boldsymbol{H}$ + $C^5\boldsymbol{H}$ overlapped), 3.20 (1 H, dd, $C^2\boldsymbol{H}$), 2.85-2.82 (2 H, m, $NC\boldsymbol{H}_2$) ppm. $^{13}C\{^1H\}$ NMR (100 MHz; CD_3OD; 298 K): δ 104.5 ($\boldsymbol{C}^1$H), 77.99 ($\boldsymbol{C}^4$H), 77.95 ($\boldsymbol{C}^3$H), 75.2 ($\boldsymbol{C}^2$H), 72.1 ($\boldsymbol{C}^5$H), 71.6 ($O\boldsymbol{C}H_2$), 62.7 ($\boldsymbol{C}^6H_2$), 42.2 ($N\boldsymbol{C}H_2$) ppm. Experimental data are consistent with those reported in the literature and confirm the presence of the β anomer only, including the $^3J_{1,2}$ value within the 7-8 Hz range.[14]

Synthesis and spectroscopic characterization of the zinc(II)-dithiocarbamato intermediates (Zn1, Zn2, Zn3, Zn4 and Zn5)

$[Zn^{II}(SSC\text{-}Inp\text{-}OEt)_2]$ (Zn1)

This zinc(II)-dithiocarbamato intermediate was prepared as previously described.[11]

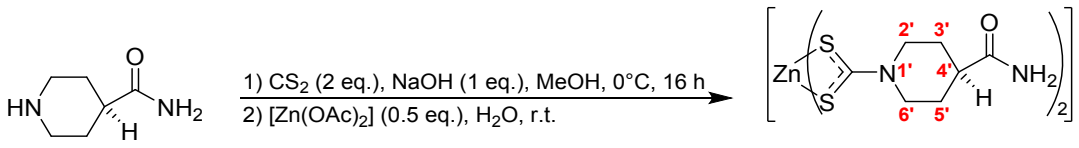


$[Zn^{II}(SSC\text{-}Inp\text{-}OEt)_2]$ (**Zn1**)

Yield: 72%. M.p. 200-202°C. Anal. (%) calcd. for $C_{18}H_{28}N_2O_4S_4Zn$ (MM = 530.05 g mol^{-1}): C, 40.79; H, 5.32; N, 5.29; found: C, 40.57; H, 5.39; N, 5.10. FT-IR (CsI disk; 298 K): $\tilde{\nu}_{max}$ 1733 (ν, C=O), 1494 (ν, N–CSS), 1176 (ν, C–OEt), 1042 (ν, O–Et), 1007 (ν_a, SCS), 568 (ν_s, SCS), 398 (ν_a, ZnS_4) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D_6; 298 K): δ 4.72 (4 H, m, $C^{2',6'}\boldsymbol{H}_{eq}$), 4.08 (4 H, q, $OC\boldsymbol{H}_2$), 3.39 (4 H, m, $C^{2',6'}\boldsymbol{H}_{ax}$), 2.72-2.60 (2 H, m, $C^{4'}\boldsymbol{H}$), 1.95 (4 H, dd, $C^{3',5'}\boldsymbol{H}_{eq}$), 1.57 (4 H, m, $C^{3',5'}\boldsymbol{H}_{ax}$), 1.19 (6 H, t, $C\boldsymbol{H}_3$) ppm. ^{13}C{^{1}H} NMR (100 MHz; DMSO-D_6; 298 K): δ 202.6 (N***C***SS), 173.6 (***C***=O), 60.2 (O***C***H_2), 50.4 (***C***$^{2',6'}H_2$), 38.9 (***C***$^{4'}$H), 27.8 (***C***$^{3',5'}H_2$), 14.1 (***C***H_3) ppm.

$[Zn^{II}(SSC\text{-}Inp\text{-}NH_2)_2]$ (Zn2)

This zinc(II)-dithiocarbamato intermediate was prepared as previously described.[11]

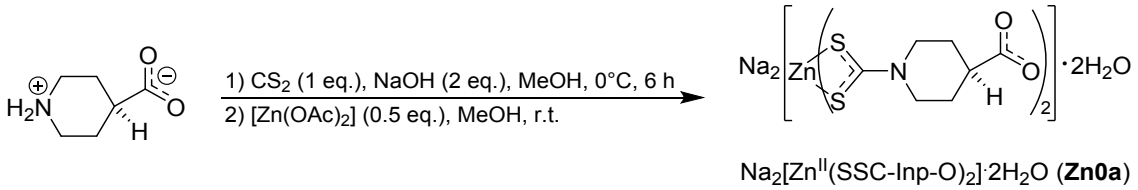


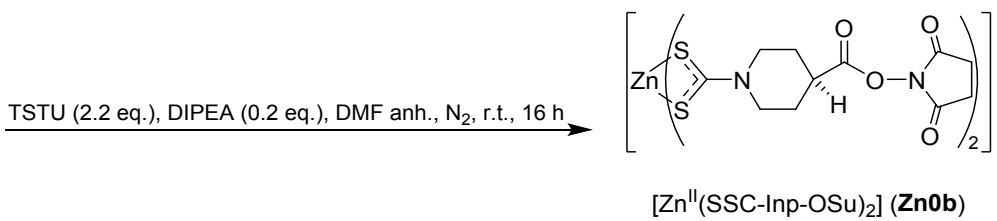
$[Zn^{II}(SSC\text{-}Inp\text{-}NH_2)_2]$ (**Zn2**)

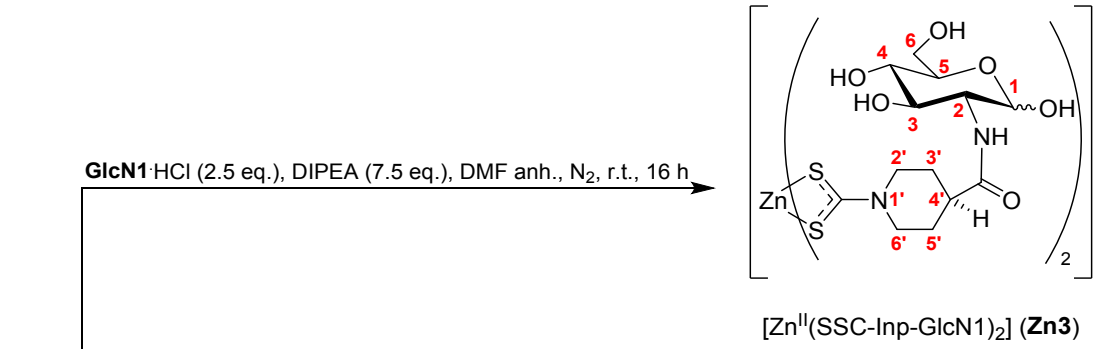
Yield: 96%. M.p. 293-294°C (dec.). Anal. (%) calcd. for $C_{14}H_{22}N_4O_2S_4Zn$ (MM = 471.98 g mol^{-1}): C, 35.63; H, 4.70; N, 11.87; found: C, 35.74; H, 4.89; N, 11.76. FT-IR (CsI disk; 298 K): $\tilde{\nu}_{max}$ 3439/3208 ($\nu_{a,s}$, NH_2), 1667 (ν, C=O (amide I)), 1649 (δ_{ip}, CNH_2 (amide II)), 1492 (ν, N–CSS), 1007 (ν_a, SCS), 561 (ν_s, SCS), 392 (ν_a, ZnS_4) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D_6; 298 K): δ 7.36 (2 H, br s, N$\boldsymbol{H}_{cis}$), 6.87 (2 H, br s, N$\boldsymbol{H}_{trans}$), 4.82 (4 H, br d, $C^{2',6'}\boldsymbol{H}_{eq}$), 3.26 (4 H, m, $C^{2',6'}\boldsymbol{H}_{ax}$), 2.40 (2 H, m, $C^{4'}\boldsymbol{H}$), 1.82 (4 H, dd, $C^{3',5'}\boldsymbol{H}_{eq}$), 1.54 (4 H, m, $C^{3',5'}\boldsymbol{H}_{ax}$) ppm. ^{13}C{^{1}H} NMR (100 MHz; DMSO-D_6; 298 K): δ 202.3 (N***C***SS), 175.6 (***C***=O), 50.7 (***C***$^{2',6'}H_2$), 40.1 (***C***$^{4'}$H), 28.4 (***C***$^{3',5'}H_2$) ppm.

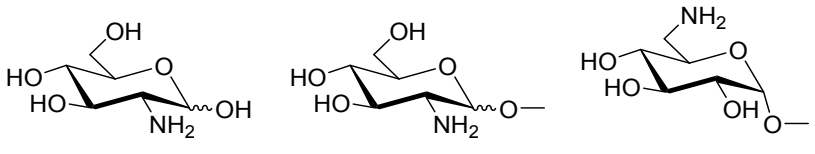
Zinc(II)-bis-dithiocarbamato glycoconjugates

These zinc(II)-dithiocarbamato intermediates were prepared as previously described.[11]









[Zn^{II}(SSC-Inp-GlcN1)$_2$] (Zn3). Yield: 61%. M.p. 200-203°C (dec.). Anal. (%) calcd. for $C_{26}H_{42}N_4O_{12}S_4Zn$ (MM = 796.26 g mol^{-1}): C, 39.22; H, 5.32; N, 7.04; found: C, 39.18; H, 5.19; N, 6.91. FT-IR (CsI disk; 298 K): $\tilde{\nu}_{max}$ 3293_{br} (ν, OH + NH overlapped), 1638 (ν, C=O (amide I)), 1547 (δ_{ip}, CNH (amide II)), 1487 (ν, N–CSS), 1071_{br}/1060_{br} (ν, C–OH), 1005 (ν_a, SCS), 570 (ν_s, SCS), 373 (ν_a, ZnS_4) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D_6; 298 K): δ 7.70 (0.5 H, d, N***H*** β), 7.67 (2 H, d, N***H*** α), 6.49 (0.5 H, d, C^{1}O***H*** β), 6.41 (2 H, d, C^{1}O***H*** α), 4.94-4.90 (4 H, m, C^{3}O***H*** + C^1***H*** α overlapped), 4.85-4.78 (5 H, br m, C$^{2',6'}$***H***$_{eq}$ α and β overlapped), 4.61 (2 H, d, C^{4}O***H*** α), 4.53 (0.5 H, dd, C^{6}O***H*** β), 4.45-4.42 (2.5 H, br m, C^{6}O***H*** α + C^1***H*** β overlapped), 3.69-3.39 (11.5 H , m, C^2***H*** α + C^4***H*** α and β + C^5***H*** α + C^6***H***$_2$ α and β overlapped), 3.31-3.22 (6 H, m, C$^{2',6'}$***H***$_{ax}$ α and β + C^2***H*** β + C^3***H*** β overlapped), 3.18-3.02 (2.5 H, m, C^3***H*** α + C^5***H*** β overlapped), 2.57-2.52 (2.5 H, m, C$^{4'}$***H*** α and β overlapped), 1.81-1.75 (5 H, m, C$^{3',5'}$***H***$_{eq}$ α and β overlapped), 1.62-1.56 (5 H, br m, C$^{3',5'}$***H***$_{ax}$ α and β overlapped) ppm. C^{3}O***H*** β and C^{4}O***H*** β could not be undoubtedly assigned: those peaks are probably overlapped with other peaks in the 4.92-4.42 ppm range. ^{13}C{^{1}H} NMR (100 MHz; DMSO-D_6; 298 K): δ 202.2 (N***C***SS), 173.8 (***C***=O), 95.4 (***C***^{1}H β), 90.5 (***C***^{1}H α), 76.9 (***C***^{5}H β), 74.2 (***C***^{3}H β), 72.1 (***C***^{5}H α), 71.1 (***C***^{3}H α), 70.9 (***C***^{4}H β), 70.4 (***C***^{4}H α), 61.2 (***C***^{6}H_2 β), 61.1 (***C***^{6}H_2 α), 57.1 (***C***^{2}H β), 54.3 (***C***^{2}H α), 50.8 (***C***$^{2',6'}$$H_2$), 40.1 (***C***$^{4'}$H), 28.5 (***C***$^{3',5'}$$H_2$) ppm. No differentiation of the ^{13}C signals of the isonipecotic moiety due to the presence of both α and β anomers could be observed. Solution α:β anomers ratio ≈ 4:1 (based on the ^{1}H NMR spectrum).

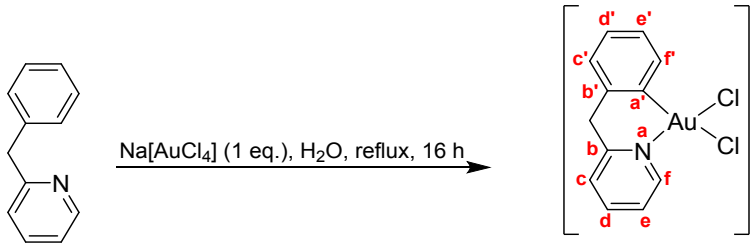
[Zn^{II}(SSC-Inp-GlcN2)$_2$] (Zn4). Yield: 35%. M.p. 268-272°C (dec.). Anal. (%) calcd. for $C_{28}H_{46}N_4O_{12}S_4Zn$ (MM = 824.31 g mol^{-1}): C, 40.80; H, 5.63; N, 6.80; found: C, 40.65; H, 5.74; N, 6.81. FT-IR (CsI disk; 298 K): $\tilde{\nu}_{max}$ 3319_{br} (ν, OH + NH overlapped), 1645 (ν, C=O (amide I)), 1557 (δ_{ip}, CNH (amide II)), 1494 (ν, N–CSS), 1062_{br}/1040_{br} (ν, C–OH + C^1–O–CH_3 overlapped), 1007 (ν_a, SCS), 576 (ν_s, SCS), 382 (ν_a, ZnS_4) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D_6; 298 K): δ 7.80 (2 H, d, N***H***), 5.00 (2 H, d, C^{4}O***H***), 4.82 (4 H, br d, C$^{2',6'}$***H***$_{eq}$), 4.73 (2 H, d, C^{3}O***H***), 4.56-4.52 (4 H, m, C^{6}O***H*** + C^1***H*** overlapped), 3.67-3.42 (8 H, m, C^2***H*** + C^3***H*** + C^6***H***$_2$ overlapped), 3.33-3.24 (6 H, m, C^5***H*** + C$^{2',6'}$***H***$_{ax}$ overlapped), 3.24 (6 H, s, OC***H***$_3$), 3.15-3.09 (2 H, m, C^4***H***), 2.59-2.52 (2 H, m, C$^{4'}$***H***), 1.81-1.76 (4 H, br m, C$^{3',5'}$***H***$_{eq}$), 1.57 (4 H, br m, C$^{3',5'}$***H***$_{ax}$) ppm. The ^{1}H NMR signals refer to the α anomer. Signals related to the β anomer were hardly detectable, and only a very few were observed and could be undoubtedly assigned (such as δ(N***H***) = 7.68, δ(C^{3}O***H***) = 4.91, δ(C^1***H***) = 4.19 ($^3J_{1,2}$ = 8.4 Hz), δ(OC***H***$_3$) = 3.58 ppm). ^{13}C{^{1}H} NMR (100 MHz; DMSO-D_6; 298 K): δ 202.2 (N***C***SS), 173.9 (***C***=O), 97.9 (***C***^{1}H), 72.8 (***C***^{5}H), 70.8 (***C***^{3}H), 70.7 (***C***^{4}H), 60.9 (***C***^{6}H_2), 54.5 (O***C***H_3), 53.8 (***C***^{2}H), 50.8 (***C***$^{2',6'}$$H_2$), 40.0 (***C***$^{4'}$H), 28.5 (***C***$^{3',5'}$$H_2$) ppm. No ^{13}C signals assignable to the β anomer were detected. Solution α:β anomers ratio ≈ 25:1 (based on the ^{1}H NMR spectrum).

[Zn^{II}(SSC-Inp-GlcN3)$_2$] (Zn5). Yield: 76%. M.p. 245-248°C (dec.). Anal. (%) calcd. for $C_{28}H_{46}N_4O_{12}S_4Zn$ (MM = 824.31 g mol^{-1}): C, 40.80; H, 5.63; N, 6.80; found: C, 40.91; H, 5.83; N, 6.76. FT-IR (CsI disk; 298 K): $\tilde{\nu}_{max}$ 3392_{br} (ν, OH + NH overlapped), 1637 (ν, C=O (amide I)), 1543 (δ_{ip}, CNH (amide II)), 1494 (ν, N–CSS), 1050_{br} (ν, C–OH + C^1–O–CH_3 overlapped), 1010 (ν_a, SCS), 564 (ν_s, SCS), 366 (ν_a, ZnS_4) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D_6; 298 K): δ 7.97 (2 H, dd, N***H***), 4.97 (2 H, d, C^4O***H***), 4.83 (4 H, br m, $C^{2',6'}$***H***$_{eq}$), 4.82 (2 H, d, C^3O***H***), 4.75 (2 H, d, C^2O***H***), 4.51 (2 H, d, C^1***H***, $^3J_{1,2}$ = 3.6 Hz), 3.58-3.52 (2 H, m, C^6***H′***), 3.40-3.35 (4 H, m, C^3***H*** + C^5***H*** overlapped), 3.24 (6 H, s, OC***H***$_3$), 3.28-3.19 (6 H, m, C^2***H*** + $C^{2',6'}$***H***$_{ax}$ overlapped), 3.08-2.98 (2 H, m, C^6***H″***), 2.92-2.88 (2 H, m, C^4***H***), 2.57-2.53 (2 H, m, $C^{4'}$***H***), 1.78-1.75 (4 H, m, $C^{3',5'}$***H***$_{eq}$), 1.57 (4 H, br m, $C^{3',5'}$***H***$_{ax}$) ppm. ^{13}C{^{1}H} NMR (100 MHz; DMSO-D_6; 298 K): δ 202.2 (N***C***SS), 173.8 (***C***=O), 99.6 (C^1H), 73.0 (C^3H), 72.1 (C^4H), 72.0 (C^2H), 70.3 (C^5H), 54.3 (O***C***H_3), 50.7 ($C^{2',6'}H_2$), 39.9 ($C^{4'}$H), 39.8 (C^6H_2), 28.4 ($C^{3',5'}H_2$) ppm.

Synthesis and spectroscopic characterization of the gold(III) precursor (Au0)

[Au^{III}(2-Bnpy)Cl_2] (Au0)

This gold(III) precursor was prepared according to a modified literature procedure.[15]



[Au^{III}(2-Bnpy)Cl_2] (**Au0**)

Yield: 78%. M.p. 243-245°C. Anal. (%) calcd. for $C_{12}H_{10}AuCl_2N$ (MM = 436.09 g mol^{-1}): C, 33.05; H, 2.31; N, 3.21; found: C, 33.18; H, 2.33; N, 3.19. FT-IR (CsI disk; 298 K): $\tilde{\nu}_{max}$ 710 (δ_{oop}, ring), 655/617 (δ_{ip}, ring), 450 (δ_{oop}, ring), 360/350 (ν, Au–Cl *trans* to N), 295 (ν, Au–Cl *trans* to C), 237/227 (ν, Au–N and Au–C) cm^{-1}. ^{1}H NMR (400 MHz; DMSO-D_6; 298 K): δ 9.17 (1 H, dd, C^{f}***H***), 8.26 (1 H, m, C^{d}***H***), 7.99 (1 H, br d, C^{c}***H***), 7.71 (1 H, m, C^{e}***H***), 7.41 (1 H, br d, $C^{f'}$***H***), 7.25 (1 H, dd, $C^{c'}$***H***), 7.18 (1 H, m, $C^{d'}$***H***), 7.07 (1 H, m, $C^{e'}$***H***), 4.62/4.35 (2 × 1 H, AB spin system), CH_2) ppm. ^{13}C{^{1}H} NMR (100 MHz; DMSO-D_6; 298 K): δ 155.7 (***C***^{b}), 152.1 (***C***^{f}H), 143.3 (***C***^{d}H), 141.1 (***C***$^{b'}$), 132.7 (***C***$^{f'}$H), 132.0 (***C***$^{a'}$), 128.6 (***C***$^{c'}$H), 128.0 (***C***$^{d'}$H), 127.0 (***C***$^{e'}$H), 126.4 (***C***^{c}H), 124.5 (***C***^{e}H), 46.1 (***C***H_2) ppm. UV-Vis (DMSO, 50 μM, 298 K): λ_{max} (log ε) 263 (3.95)/266 (3.95) (intraligand π*←π), 294 (3.53) (sh, MLCT π*←d) nm.

Crystallographic data of $[Au^{III}(2\text{-Bnpy})(SSC\text{-Inp-OEt})](PF_6)$ (Au1, CCDC 2058611), $[Au^{III}(2\text{-Bnpy})(SSC\text{-Inp-NH}_2)](PF_6)$ (Au2, CCDC 2058613) and $[Au^{III}(2\text{-Bnpy})(SSC\text{-Inp-NH}_2)](PF_6)\cdot CH_3CN$ (Au2·CH_3CN, CCDC 2058612)

Table S1. Crystal data and structure refinement.

	Au1	**Au2**	**Au2·CH_3CN**
Empirical formula	$C_{21}H_{24}AuF_6N_2O_2PS_2$	$C_{19}H_{21}AuF_6N_3OPS_2$	$C_{21}H_{24}AuF_6N_4OPS_2$
Formula weight	742.48	713.44	754.50
Temperature/K	200.0	200.0	200.0
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$
a (Å)	10.6007(5)	14.5240(4)	15.5049(8)
b (Å)	23.271(1)	8.7052(2)	8.7207(4)
c (Å)	11.2065(5)	19.7955(6)	20.512(1)
α (°)	90	90	90
β (°)	115.901(1)	109.332(1)	111.798(2)
γ (°)	90	90	90
Volume ($Å^3$)	2486.9(2)	2361.7(1)	2575.2(2)
Z	4	4	4
ρ_{calc} (g/cm^3)	1.983	2.007	1.946
μ (mm^{-1})	6.216	6.539	6.003
F(000)	1440.0	1376.0	1464.0
Crystal size (mm^3)	0.16 × 0.14 × 0.06	0.21 × 0.2 × 0.16	0.25 × 0.22 × 0.18
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection (°)	4.616 to 51.428	5.946 to 52.784	5.942 to 51.36
Index ranges	$-12 \le h \le 12$, $-28 \le k \le 28$, $-13 \le l \le 13$	$-18 \le h \le 18$, $-10 \le k \le 10$, $-24 \le l \le 24$	$-18 \le h \le 18$, $-10 \le k \le 10$, $-25 \le l \le 25$
Reflections collected	34982	27607	61380
Independent reflections	4713 [R_{int} = 0.0589, R_{sigma} = 0.0349]	4807 [R_{int} = 0.0403, R_{sigma} = 0.0285]	4868 [R_{int} = 0.0506, R_{sigma} = 0.0227]
Data/restraints/parameters	4713/32/368	4807/12/329	4868/0/364
Goodness-of-fit on F^2	1.051	1.055	1.012
Final R indexes [I>=2σ (I)]	R_1 = 0.0419, wR_2 = 0.1009	R_1 = 0.0298, wR_2 = 0.0623	R_1 = 0.0238, wR_2 = 0.0614
Largest diff. peak/hole / e ($Å^{-3}$)	2.22/-1.22	1.27/-1.22	0.99/-1.74

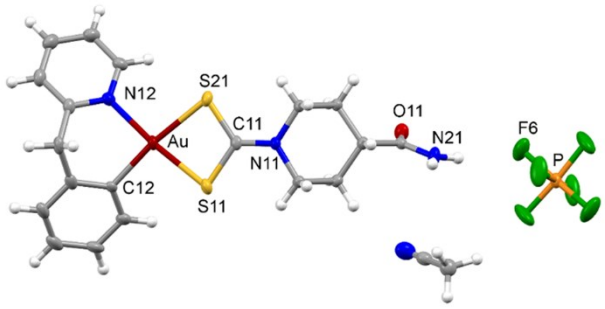
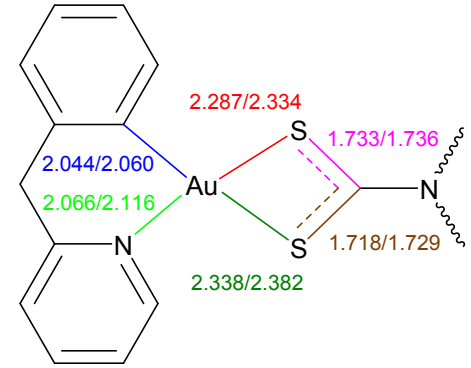


Figure S1. Molecular structure with atom numbering scheme for **Au2·CH_3CN** (CCDC 2058612). Thermal ellipsoids are depicted at the 30% probability level. Disordered fragments are omitted for clarity.



Coordination bond lengths (Å)

Au-C < Au-N << Au-$S_{trans\text{-}N}$ < Au-$S_{trans\text{-}C}$

Figure S2. Schematic representation of the bond lengths pertaining to the coordination environment of **Au1** and **Au2**.

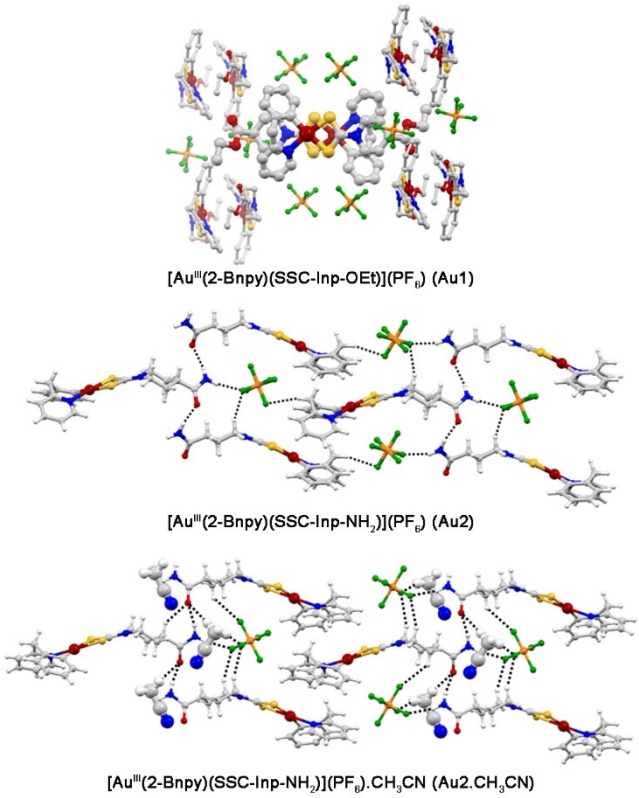


Figure S3. Crystal packing of **Au1**, **Au2** and **Au2·CH$_3$CN**. Disordered fragments are omitted for clarity.

UV-VIS spectra of the gold(III) complexes (Au1, Au2, Au3, Au4, Au5 and Au6)

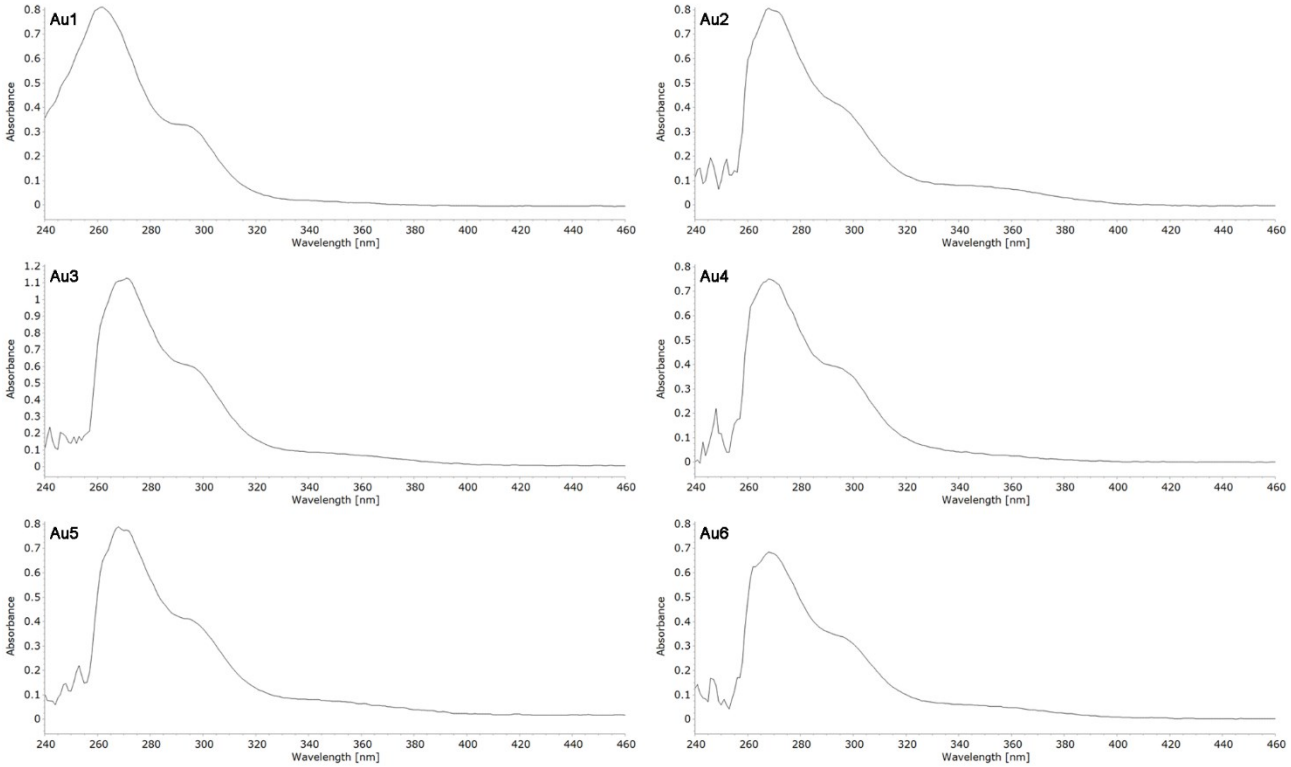


Figure S4. UV-Vis spectra of complexes **Au1-Au6** 25 μM in DMSO at 25°C.

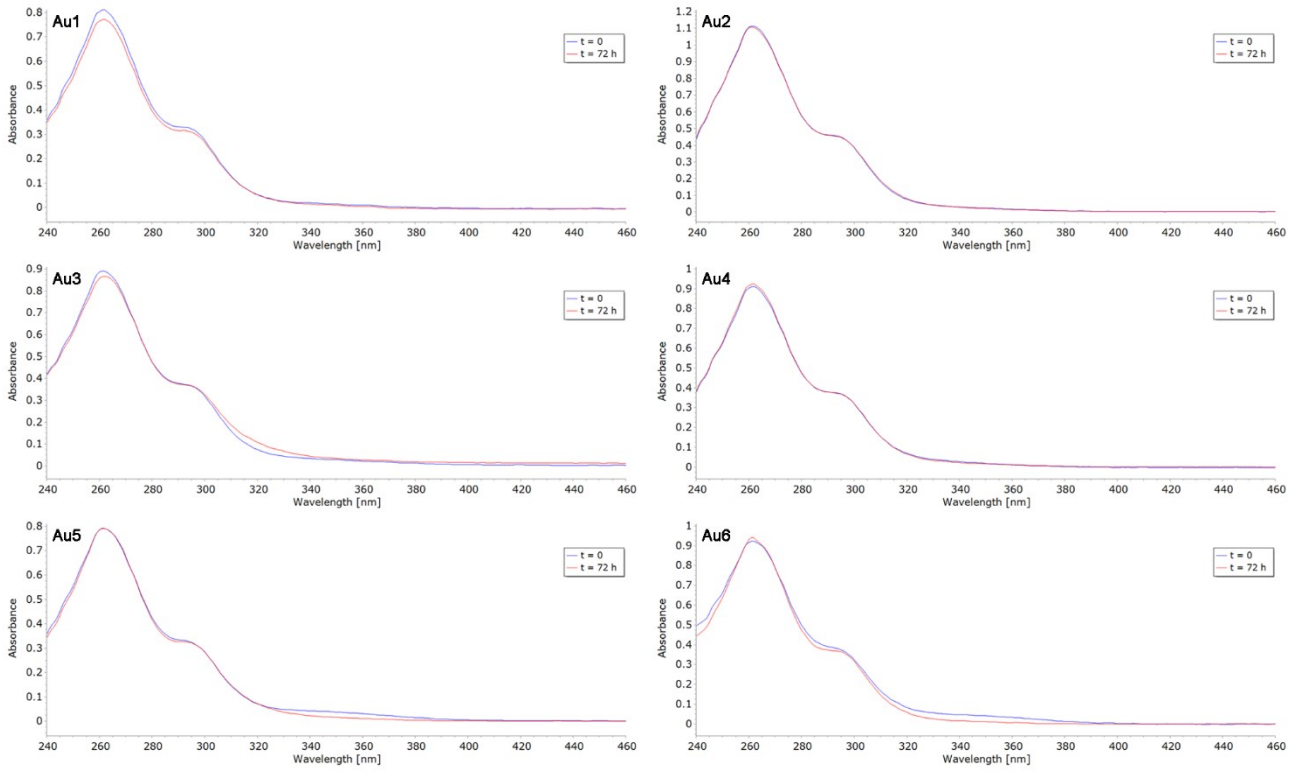


Figure S5. UV-Vis spectra over 72 h of complexes **Au1-Au6** 25 μM in PBS (pH 7.4) at 37°C.

References

1 Bruker. SMART. 2012, Bruker AXS Inc., Madison, Wisconsin, USA.

B. Kang, T. Opatz, K. Landfester and F. R. Wurm, *Chem. Soc. Rev.*, 2015, **44**, 8301.

2 L. Krause, R. Herbst-Irmer, G. M. Sheldrick and D. Stalke, *J. Appl. Cryst.*, 2015, **48**, 3.

3 G. M. Sheldrick, *Acta Cryst.*, 2015, **A71**, 3.

4 G. M. Sheldrick, *Acta Cryst.*, 2015, **C71**, 3.

5 C. F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler and J. van der Streek, *J. Appl. Cryst.*, 2006, **39**, 453.

6 A. Andrés, M. Rosés, C. Ràfols, E. Bosch, S. Espinosa, V. Segarra and J. M. Huerta, *Eur. J. Pharm. Sci*., 2015, **76**, 181.

7 I. V. Tetko, H. P. Varbanov, M. Galanski, M. Talmaciu, J. A. Platts, M. Ravera and E. Gabano, *J. Inorg. Biochem.*, 2016, **156**, 1.

8 G. R. Periyannan, B. A. Lawrence and A. Egan, *J. Chem. Educ.*, 2015, **92**, 1244.

9 W. Moukarzel, J. Fitremann and J.-D. Marty, *Nanoscale*, 2011, **3**, 3285.

10 A. Blasko, C. A. Bunton, S. Bunel, C. Ibarra and E. Moraga, *Carbohydr. Res.*, 1997, **298**, 163.

11 A. Pettenuzzo, D. Montagner, P. McArdle and L. Ronconi, *Dalton Trans.*, 2018, **47**, 10721.

12 X. Hu, W. Zhang, I. Carmichael and A. S. Serianni, *J. Am. Chem. Soc.*, 2010, **132**, 4641.

13 T. Muhizi, S. Grelier and V. Coma, *J. Agric. Food Chem.*, 2009, **57**, 11092.

14 R. Šardzík, G. T. Noble, M. J. Weissenborn, A. Martin, S. J. Webb and S. L. Flitsch, *Beilstein J. Org. Chem.*, 2010, **6**, 699.

15 B. Bertrand, S. Spreckelmeyer, E. Bodio, F. Cocco, M. Picquet, P. Richard, P. Le Gendre, C. Orvig, M. A. Cinellu and A. Casini, *Dalton Trans.*, 2015, **44**, 11911.