

Article

Dual-Targeting Gold Nanoparticles: Simultaneous Decoration with Ligands for Co-Transporters SGLT-1 and B⁰AT1

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Abstract: Sodium–glucose co-transporter 1 (SGLT1) and sodium-dependent neutral amino acid transporter (B⁰AT1) are mainly expressed on the membrane of enterocytes, a type of epithelial cell found in the intestines. In addition to their physiological role in the absorption of nutrients, a protective role in the integrity of the intestinal barrier has been established. The natural ligands of SGLT1 (D-glucose) and of B⁰AT1 (L-glutamine) can trigger a protective anti-inflammatory effect on the intestinal epithelium. The literature suggests the activation of common intracellular pathways upon engagement of the two transporters, whose functional forms are composed of oligomers or clusters. Simultaneous activation of these two co-transporters could lead to a potential multitarget and synergistic anti-inflammatory effect. Therefore, nanoplatforms containing multiple copies of the ligands could represent chemical tools to study the potential simultaneous activation of the two co-transporters. For these reasons, in this study, a set of different gold nanoparticles decorated with derivatives of D-glucose and of L-glutamine were designed and prepared. In particular, the synthesis of suitable sulfur-ending functionalized ligand derivatives, including a C-glucoside derivative, their anchoring to gold nanoparticles and their physical–chemical characterization have been carried out. The obtained nanostructures could represent promising multifunctional platforms for further investigation of the existence of possible multitarget and synergistic effects toward the two co-transporters SGLT1 and B⁰AT1.

Keywords: gold nanoparticles; multivalency; sodium–glucose co-transporter 1 (SGLT1); sodium–glutamine co-transporter 1 (B⁰AT1); glycoderivatives; thiol–ene coupling



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1. Introduction

The key role of the intestine in the maintenance of body homeostasis as well as in the regulation and control of the immune response to pathogens is nowadays an unquestionable scientific fact, supported by many relevant studies in the field of immunology and pathophysiology [1–6]. Several studies published in the last two decades have demonstrated that D-glucose, administered at high concentrations in vitro to intestinal epithelial cells (IECs) and in vivo in mice (oral administration), has a protective effect against cell/tissue damages caused by pathogens expressing lipopolysaccharides (LPSs) [7,8]. These studies propose that the mechanism of the glucose-mediated cytoprotective effect depends on increased glucose uptake by enterocytes, mediated by sodium–glucose co-transporter 1 (SGLT1). The unidirectional translocation of glucose from the intestinal lumen to the cytoplasm of IECs, mediated by this transmembrane protein, represents one of the principal mechanisms for the absorption of glucose occurring on the brush border membrane in the gut [9–11].

More recently, other works have outlined the protective effect of SGLT1 engagement with high oral doses of D-glucose and non-metabolizable 3-O-methyl-D-glucopyranose

(3OMG) against damage induced by Toll-like receptor (TLR) ligands in intestinal epithelial cells in a murine model of septic shock and in liver injury and death induced by LPS or by an overdose of acetaminophen [12,13]. Glucose- and 3OMG-induced downregulation of systemic inflammatory cytokine production and enhanced anti-inflammatory cytokine production were observed. However, the substantial quantities of D-glucose and 3OMG required to elicit anti-inflammatory effects pose a significant limitation for potential therapeutic applications. In a previous study, we identified a glucose derivative, C-glucoside **1** (Figure 1), which exerts anti-inflammatory activity at a molar concentration five orders of magnitude lower than that of D-glucose [14]. Furthermore, we observed a significant protective role for C-glucoside **1** in intestinal mucosa in chemotherapy-induced mucositis [15]. Our previous results suggested that C-glucoside **1** mediates a protective activity, similar to that observed for D-glucose, due to its agonistic interaction with SGLT1. This binding produces an activated form of the transporter, which activates an intracellular signaling pathway that ultimately results in protective and anti-inflammatory effects.

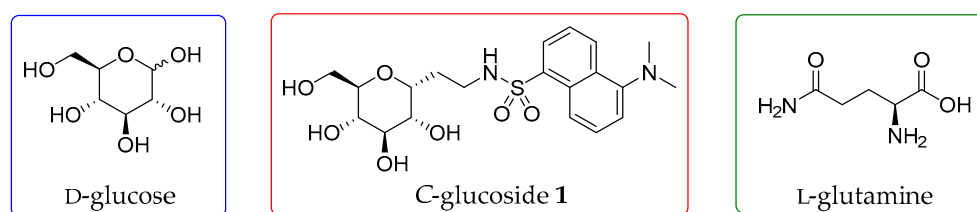


Figure 1. Structure of the three monomeric ligands with known activity toward SGLT1 and B⁰AT1: D-glucose, C-glucoside **1** and L-glutamine.

On the other hand, L-glutamine (Figure 1) is the most abundant amino acid in the total body free amino acid pool, and recently, its role in nutrition is receiving growing interest. L-glutamine is a major energy source for the intestinal epithelium, and it is involved in the maintenance of intestinal epithelial homeostasis. In fact, L-glutamine represents an important supplement in diets within therapeutic supportive care protocols; its enrichment in parenteral hyperalimentation can decrease villus atrophy associated with exclusive parenteral feeding [16–18]. The protective anti-inflammatory effects of glutamine are also well established [19,20]. For example, diet supplementation with glutamine may protect the intestinal tract from radiation and some chemotherapeutic agents. In animal models of gastrointestinal mucositis, oral glutamine treatment mitigates mucosal damage and enhances intestinal recovery after chemotherapy-induced injury [21–24]. For example, studies have shown that orally administering L-glutamine can prevent gut mucosal injuries caused by cisplatin in rats. Additionally, this drug upregulates L-glutamine transport in human intestinal epithelial cells, potentially leading to increased intracellular levels of glutathione (GSH) [25,26].

Similarly to D-glucose, the absorption of L-glutamine by the intestinal epithelium is mediated by several transport systems, among which sodium–glutamine cotransporter 1 (B⁰AT1) is the main transmembrane protein that mediates the absorption of L-glutamine by villus enterocytes [27]. One of the critical events compromising the biological functions of the intestinal epithelium—such as nutrient absorption and the barrier effect against pathogen invasion—is the disruption of the correct permeability due to alterations in the junctional systems of epithelial cells in response to various forms of damage [28,29]. The fundamental link between SGLT1 activation and L-glutamine uptake is the ability to modulate tight junctions activating intracellular signaling pathways involved in junctional open–close mechanisms and remodeling [30–34]. Thus, the simultaneous activation of both receptors could be of great relevance, as it could lead to a multitarget and synergistic protective effect.

Moreover, both SGLT1 and B⁰AT1 co-transporters have been described to be involved in anti-inflammatory and anti-apoptotic signaling in the repair of plasma membrane integrity and tight junction integrity, thus representing potential molecular targets for a

therapeutic approach aimed at the prevention of intestinal barrier damages and/or its recovery [35].

Several reports have suggested that functional SGLT1 is an oligomeric protein resulting from the homodimerization of two identical subunits [36–39]. The evidence also suggests that the B⁰AT1 transporter is mainly assembled as a cluster [40], and the B⁰AT1 transporter localized in the small intestine displays a low-affinity constant for the amino acid substrate [40]. These findings suggest that the study of multitarget and multivalent effects of the ligand C-glycoside **1** or L-glutamine could lead to a significant increase in the activity.

Multivalent binding is a commonly observed process in biological systems, in particular during cellular recognition events and in signal transduction pathways. Multivalent interactions are significantly stronger than the individual binding of an equal amount of monovalent ligands to a multivalent receptor [41,42]. Receptor dimerization/clustering is a well-known and ubiquitous phenomenon.

Gold nanoparticles (AuNPs) are one of the most used nanoplatforms for the study of multivalency phenomena, owing to their good stability, unique optical features, and ease and versatility in decoration/functionalization with multiple copies of thiol-bearing ligands on the gold surface [43–48]. In particular, Glyco-Gold Nanoparticles (GAuNPs), i.e., AuNPs decorated with carbohydrates, are receiving increasing attention thanks to their marked potentialities in biomedicine [49], for the study of carbohydrate-mediated interactions involved in biochemical and physiological processes [50,51], for diagnostics [52,53], for the investigation of multivalency effects of sugars [54,55], including the modulation of enzyme activity [51,56], and as promising platforms for vaccine development [57–59]. Furthermore, the simultaneous introduction of different thiol ligands on the same gold nanoplatform paves the way for “multifunctional” nanosystems [55,57].

Therefore, we reasoned that GAuNPs displaying multiple copies of SGLT1 and B⁰AT1 ligands could be suitable nanotools for modulating the activity of these co-receptors. In the present article, AuNPs decorated with thiol-ending D-glucose derivatives (a β -O-alkylglucose derivative and C-glycoside **1**) and/or L-glutamine derivatives were prepared and fully characterized. These nanosized tools could be used for future studies aimed at evaluating the multivalent and synergistic effects on the target proteins SGLT1 and B⁰AT1 (Figure 2).

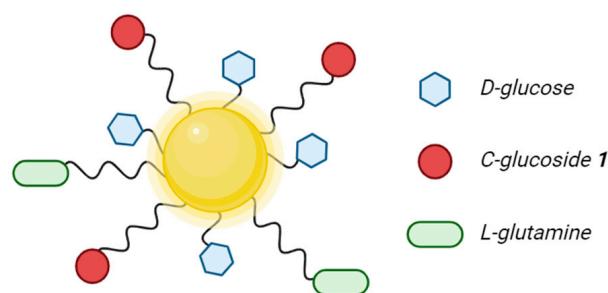


Figure 2. General scheme of the designed nanoplatforms decorated with the ligands of interest of this work (D-glucose, C-glycoside **1** and L-glutamine).

2. Materials and Methods

2.1. General Remarks

All commercial chemicals were purchased from Merck and Alfa Aesar. All chemicals were used without further purification. Thin layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck©, Darmstadt, Germany) with detection under UV light when possible, or by charring with a solution of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (21 g), $\text{Ce}(\text{SO}_4)_2$ (1 g), concentrated H_2SO_4 (31 mL) in water (500 mL) or with an ethanol solution of ninhydrin. Flash-column chromatography was performed on silica gel 230–400 mesh (Merck) or on an Isolera Flash Chromatography System (Biotage Sweden ABTM, Uppsala, Sweden). ¹H and

^{13}C NMR spectra were recorded at 25 °C, unless otherwise stated, with a Varian Mercury 400 MHz instrument (Varian Inc., Palo Alto, CA, USA), on a Bruker DRX-300 spectrometer and on Bruker AvanceTM NEO 400 MHz (Bruker©, Billerica, MA, USA). Chemical shift assignments, reported in parts per million, were referenced to the corresponding solvent peaks. Mass spectra were recorded on an ABSciex 2000 QTRAP LC/MS/MS system with an ESI source (ABSciex©, Framingham, MA, USA). TEM examination was carried out at 200 KeV with a Philips CM200 microscope (Philips©, Amsterdam, The Netherlands). UV spectra were obtained with a UV/vis Perkin-Elmer Lambda 12 spectrophotometer (Perkin-Elmer©, Waltham, MA, USA). Infrared spectroscopy analyses were performed in ATR mode using a Jasco© FT/IR-4600 system (Jasco Europe©, Cremella, Italy).

2.2. Synthesis of Ligands

2.2.1. Synthesis of C-Glucoside Derivative 2

For compounds **6** and **7**, synthetic procedures and characterizations are already reported in [60,61].

2.2.2. 2,3,4-Tri-O-benzyl-6-O-acetyl- α -C-allyl-glucopyranoside **8**

A mixture of Ac₂O/trifluoroacetic acid 4:1 (70 mL), prepared at 0 °C in argon atmosphere, was added via a double-tip needle to a round-bottomed flask containing 2 g (3.55 mmol) of 2,3,4,6-tetra-O-benzyl- α -D-glucopyranose **7**. The solution was stirred vigorously at 0 °C and the reaction followed by TLC (Hexane/EtOAc 8.5:1.5). After 1.5 h, no more starting compound was present, and the solution was gently poured into ice water and stirred for 10 min. The aqueous solution was extracted with EtOAc (3 \times), the organic phase was then washed once with a sodium hydrogen carbonate saturated solution and twice with distilled water. The organic phase was dried over sodium sulfate, filtered and concentrated. The resulting crude was purified by flash chromatography (silica gel, eluent: Hexane/EtOAc 9:1), affording compound **8** (1.61 g, 3.12 mmol, 88% yield).

^1H NMR (400 MHz, CDCl₃) δ 7.43–7.24 (m, 15H, CH Ar), 5.79 (ddt, J = 17.2, 10.2, 6.9 Hz, 1H, H2'), 5.18–5.05 (m, 2H, H3'a,b), 4.98 (d, J = 10.8 Hz, 1H, CH₂Ph), 4.89 (d, J = 10.7 Hz, 1H, CH₂Ph), 4.83 (d, J = 10.8 Hz, 1H, CH₂Ph), 4.72 (d, J = 11.6 Hz, 1H, CH₂Ph), 4.65 (d, J = 11.6 Hz, 1H, CH₂Ph), 4.57 (d, J = 10.8 Hz, 1H, CH₂Ph), 4.24 (d, J = 3.5 Hz, 2H, H6a,b), 4.11 (dt, J = 9.3, 5.9 Hz, 1H, H1, H1), 3.85 (t, J = 9.0 Hz, 1H, H3), 3.76 (dd, J = 9.4, 5.8 Hz, 1H, H2), 3.70 (dt, J = 9.8, 3.5 Hz, 1H, H5), 3.48 (dd, J = 9.8, 8.7 Hz, 1H, H4), 2.50 (t, J = 8.2 Hz, 2H, H1'a,b) and 2.04 (s, 3H, CH₃CO).

^{13}C NMR (101 MHz, CDCl₃) δ 170.92 (CH₃CO), 138.59 (Cq Ar), 138.15 (Cq Ar), 137.83 (Cq Ar), 134.40 (C2'), 128.80, 128.65, 128.59, 128.58, 128.49, 128.29, 128.11, 128.05, 128.00, 127.95 and 127.85 (C Ar \times 15), 117.24 (C3'), 82.37 (C3), 80.04 (C2), 77.89 (C4), 75.65 and 75.21 (CH₂Ph), 73.68 (C1), 73.23 (CH₂Ph), 69.68 (C5), 63.62 (C6), 29.89 (C1') and 21.00 (CH₃CO).

MS: m/z = 517.6 [M+H]⁺.

2.2.3. 2-(2,3,4-Tri-O-benzyl-6-acetyl- α -D-glucopyranosyl)-ethanal **9**

To a solution of **8** (3.53 g, 6.84 mmol) in CH₂Cl₂ (100 mL, 0.07 M) at –78 °C, O₃ was bubbled until a pale blue color appeared. The reaction was followed by TLC (Hexane/EtOAc 8:2). At the end of the reaction, the excess of O₃ was removed by purging the mixture with a stream of argon at –78 °C and then Ph₃P (17.1 mmol, 2.5 eq) was added. The mixture was stirred for 24 h at room temperature and then concentrated by rotary evaporation. The product was purified from the crude reaction by flash chromatography (silica gel, eluent: Hexane/EtOAc 8:2 to 7:3), affording aldehyde **9** (2.92 g, 5.63 mmol, 82% yield).

^1H NMR (400 MHz, CDCl₃) δ 9.72 (s, 1H, H2' (CHO)), 7.43–7.24 (m, 15H, CH Ar), 4.93 (d, J = 10.9 Hz, 1H, CH₂Ph), 4.87 (d, J = 10.8 Hz, 1H, CH₂Ph), 4.83 (d, J = 10.9 Hz, 1H, CH₂Ph), 4.75–4.66 (m, 2H, CH₂Ph, H1), 4.63 (d, J = 11.5 Hz, 1H, CH₂Ph), 4.58 (d, J = 10.8 Hz, 1H, CH₂Ph), 4.30–4.18 (m, 2H, H6), 3.82–3.74 (m, 2H, H2, H3), 3.74–3.63 (m, 1H, H5),

3.53–3.43 (m, 1H, H4), 2.89 (dd, $J = 15.8, 5.0$ Hz, 1H, H1'a), 2.74 (ddd, $J = 16.2, 8.6, 2.8$ Hz, 1H, H1'b) and 2.05 (s, 3H, CH₃CO).

¹³C NMR (101 MHz, CDCl₃) δ 199.49 (C2' (CHO)), 170.83 (CH₃CO), 138.29, 137.67 and 137.65 (Cq Ar), 128.66, 128.64, 128.61, 128.20, 128.18, 128.14, 127.99 and 127.94 (C Ar), 81.90 (C2), 78.88 (C3), 77.30 (C4), 75.54 (CH₂Ph), 75.07 (CH₂Ph), 73.67 (CH₂Ph), 70.95 (C5), 69.58 (C1), 63.20 (C6), 41.26 (C1') and 20.95 (CH₃CO).

MS: $m/z = 519.6$ [M+H]⁺.

2.2.4. 2-(2,3,4-Tri-O-benzyl-6-acetyl- α -D-glucopyranosyl)-ethanol **10**

Compound **9** (2800 mg, 5.39 mmol) was dissolved in dry MeOH under argon. The solution was then cooled to 0 °C and NaBH₄ (2.16 mmol) was added in three portions. The reaction was stirred at 0 °C and followed by TLC (Hexane/EtOAc 6:4). After 4 h, glacial acetic acid was added at 0 °C until pH = 3. The reaction was concentrated, and the residue was diluted in dichloromethane, then washed with a HCl 1 N solution (1 \times) and twice with brine (2 \times). The organic phase was dried over Na₂SO₄, filtered and evaporated. The crude was purified by flash chromatography (silica gel, eluent: Hexane/EtOAc 5.5:4.5 + 1% MeOH), yielding compound **10** (2672 mg, 5.14 mmol, 95% yield).

¹H NMR (400 MHz, CDCl₃) δ 7.40–7.26 (m, 15H, m, CH Ar), 4.95 (d, $J = 10.9$ Hz, 1H, CH₂Ph), 4.88 (d, $J = 10.9$ Hz, 1H, CH₂Ph), 4.81 (d, $J = 10.9$ Hz, 1H, CH₂Ph), 4.73 (d, $J = 11.6$ Hz, 1H, CH₂Ph), 4.64 (d, $J = 11.6$ Hz, 1H, CH₂Ph), 4.59 (d, $J = 10.9$ Hz, 1H, CH₂Ph), 4.29 (dd, $J = 11.7, 2.0$ Hz, 1H, H6a), 4.26–4.14 (m, 2H, H1, H2), 3.87–3.75 (m, 4H, H2'a,b, H3, H5), 3.72 (dd, $J = 9.2, 5.8$ Hz, 1H, H6b), 3.43 (t, $J = 9.0$ Hz, 1H, H4), 2.44 (s, 1H, OH), 2.10–1.98 (m, 4H, CH₃CO, H1'a) and 1.98–1.86 (m, 1H, H1'b).

¹³C NMR (101 MHz, CDCl₃) δ 170.86 (CH₃CO), 138.41, 137.92 and 137.74 (Cq Ar), 128.59, 128.57, 128.55, 128.13, 128.06, 128.03, 128.00, 127.98 and 127.85 (C Ar), 81.87 (C2), 79.46 (C3), 77.82 (C4), 75.49 (CH₂Ph), 75.00 (CH₂Ph), 73.37 (C5), 73.33 (CH₂Ph), 70.30 (C1), 63.77 (C6), 60.81 (C2'), 27.88 (C1') and 20.91 (CH₃CO).

MS: $m/z = 521.4$ [M+H]⁺, 543.5 [M+Na]⁺.

2.2.5. 2-(2,3,4-Tri-O-benzyl-6-acetyl- α -D-glucopyranosyl)-1-azidoethane **11**

Alcohol **10** (2598 mg, 5 mmol) was dissolved in dry THF under argon. Ph₃P (15 mmol) was added and the solution cooled to 0 °C. Diisopropyl azodicarboxylate (DIAD, 15 mmol) and, after 10 min, diphenylphosphoryl azide ((PhO)₂PON₃, 16 mmol) were added dropwise; the reaction was then raised and stirred at room temperature. The disappearance of the starting compound was followed by TLC (Hexane/EtOAc 7:3); after 2 h, the crude was concentrated and purified by flash chromatography (silica gel, eluent: Hexane/EtOAc 9:1). A total of 2446 mg (4.48 mmol, 90% yield) of azide **11** was obtained.

¹H NMR (400 MHz, CDCl₃) δ 7.38–7.26 (m, 15H, CH Ar), 4.94 (d, $J = 10.9$ Hz, 1H, CH₂Ph), 4.86 (d, $J = 10.8$ Hz, 1H, CH₂Ph), 4.80 (d, $J = 10.8$ Hz, 1H, CH₂Ph), 4.71 (d, $J = 11.6$ Hz, 1H, CH₂Ph), 4.62 (d, $J = 11.6$ Hz, 1H, CH₂Ph), 4.56 (d, $J = 10.9$ Hz, 1H, CH₂Ph), 4.23 (d, $J = 3.4$ Hz, 2H, H6a,b), 4.16–4.07 (m, 1H, H1), 3.82–3.70 (m, 2H, H2, H3), 3.68–3.60 (m, 1H, H5), 3.50–3.27 (m, 3H, H4, H2'a,b), 2.05 (s, 3H, CH₃CO) and 1.96 (dd, $J = 13.7, 6.5$ Hz, 2H, H1'a,b).

¹³C NMR (101 MHz, CDCl₃) δ 170.90 (CH₃CO), 138.45, 137.95 and 137.76 (Cq Ar), 128.66, 128.61, 128.23, 128.13, 128.06 and 127.92 (C Ar), 82.07 (C3), 79.48 (C2), 77.71 (C4), 75.60 (CH₂Ph), 75.17 (CH₂Ph), 73.38 (CH₂Ph), 71.34 (C1), 70.19 (C5), 63.64 (C6), 47.96 (C2'), 24.64 (C1') and 21.93 (CH₃CO).

MS: $m/z = 546.5$ = [M+H]⁺.

2.2.6. 2-(2,3,4-Tri-O-benzyl- α -D-glucopyranosyl)-1-azidoethane **12**

Pure azido derivative **11** (2420 mg, 4.44 mmol) was dissolved in MeOH (25 mL, ≈ 0.2 M) and 2 mL of a methanolic solution of sodium methoxide (2.2 M) was added. The reaction was stirred at room temperature for 12 h. After the disappearance of the starting compound, checked by TLC (Hexane/EtOAc 7:3), an amount of acidic resin (Amberlite

IRA-120 H⁺) was added to the reaction. The resin was filtered off and the solution was evaporated to dryness, affording compound **12** (2116 mg, 4.21 mmol, 95% yield), which did not require further purification.

¹H NMR (400 MHz, CDCl₃) δ 7.41–7.27 (m, 15H, CH Ar), 4.94 (d, J = 10.9 Hz, 1H, CH₂Ph), 4.88 (d, J = 10.9 Hz, 1H, CH₂Ph), 4.82 (d, J = 10.9 Hz, 1H, CH₂Ph), 4.72 (d, J = 11.6 Hz, 1H, CH₂Ph), 4.67–4.60 (m, 2H, CH₂Ph), 4.11 (dd, J = 13.2, 7.2 Hz, 1H, H1), 3.83–3.74 (m, 2H, H6a, H3), 3.74–3.64 (m, 2H, H2, H6b), 3.56–3.45 (m, 2H, H4, H5), 3.45–3.37 (m, 1H, H2'a), 3.37–3.27 (m, 1H, H2'b), 1.98 (dd, J = 13.8, 7.0 Hz, 2H, H1'a,b) and 1.87 (s, 1H, OH).

¹³C NMR (101 MHz, CDCl₃) δ 138.60, 138.05, 138.02 (Cq Ar), 128.65, 128.57, 128.17, 128.09, 128.04, 128.01 and 127.84 (C Ar), 82.11 (C3), 79.69 (C2), 77.85 (C4), 75.55 (CH₂Ph), 75.22 (CH₂Ph), 73.44 (CH₂Ph), 72.14 (C5), 71.38 (C1), 62.34 (C6), 48.04 (C2') and 24.71 (C1').

MS: *m/z* = 504.4 [M+H]⁺, 526.3 [M+Na]⁺.

2.2.7. 2-(2-(2-(Pent-4-en-1-yloxy)ethoxy)ethoxy)ethan-1-ol **13**

Compound **13** was prepared according to a procedure described in [62].

A solution of 1006 mL of NaOH 50% (12.6 mmol) was added to 10 g of triethyleneglycol at 100 °C. The reaction was stirred for 30 min at 100 °C and 5-bromo-1-pentene (12.6 mmol) was added and the reaction stirred at the same temperature for 24 h. The reaction was followed by TLC (CH₂Cl₂/MeOH 9:1). After 24 h, the reaction was diluted with water and extracted six times with EtOAc. The organic phases were combined, dried over Na₂SO₄ and evaporated; the crude was purified by flash chromatography (silica gel, eluent CH₂Cl₂/MeOH 11:1). A total of 2039 g (9.34 mmol, 74% yield) of monopentenylated triethyleneglycol **13** was obtained.

¹H NMR (400 MHz, CDCl₃) δ 5.75 (ddt, J = 16.9, 10.1, 6.6 Hz, 1H, H4), 4.96 (dd, J = 17.2, 1.6 Hz, 1H, H5a), 4.90 (d, J = 10.2 Hz, 1H, H5b), 4.08 (t, J = 5.1 Hz, 1H, OH), 3.66 (d, J = 3.7 Hz, 2H, -CH₂CH₂O), 3.63–3.57 (m, 6H, -CH₂CH₂O), 3.57–3.50 (m, 4H, -CH₂CH₂O), 3.42 (t, J = 6.7 Hz, 2H, H1a,b), 2.05 (q, J = 7.3 Hz, 2H, H3a,b) and 1.68–1.57 (m, 2H, H2a,b).

¹³C NMR (101 MHz, CDCl₃) δ 138.15 (C4), 114.84 (C5), 72.73, 70.80, 70.53, 70.44, 70.12 and 69.94 (-CH₂CH₂O), 61.49 (C1), 30.15 (C3) and 28.61 (C2).

MS: *m/z* = 219.2 [M+H]⁺.

2.2.8. 2-(2-(2-(Pent-4-en-1-yloxy)ethoxy)ethoxy)ethyl-4-methylbenzenesulfonate **14**

To a solution of compound **13** (1.62 g, 7421 μmol) and DMAP (0.148 mmol) in CH₂Cl₂ (40 mL, ≈0.2 M), *p*-toluenesulphonyl chloride (14.84 mmol) and Et₃N (18.55 mmol) were added. The reaction was stirred at r.t. and followed by TLC (CH₂Cl₂/MeOH 9.5:0.5). After 10 h, TLC indicated the disappearance of the starting material, and 40 mL of water was added. The organic phase was separated and the aqueous phase was extracted thrice with CH₂Cl₂. The combined organic phases were dried with Na₂SO₄ and evaporated. The crude was purified by flash chromatography (silica gel, eluent CH₂Cl₂/MeOH 9.9:0.1), affording tosylate **14** (1976 μg, 5.3 μmol, 72% yield).

¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, J = 8.2 Hz, 2H, CH Ar), 7.33 (d, J = 8.0 Hz, 2H, CH Ar), 5.80 (ddt, J = 16.8, 10.1, 6.6 Hz, 1H, H4), 5.00 (dd, J = 17.1, 1.5 Hz, 1H, H5a), 4.94 (d, J = 9.4 Hz, 1H, H5b), 4.20–4.09 (m, 2H, -CH₂CH₂OTs), 3.72–3.64 (m, 2H, CH₂CH₂OTs), 3.64–3.52 (m, 8H, -CH₂CH₂O-), 3.45 (t, J = 6.7 Hz, 2H, H1a,b), 2.44 (s, 3H, CH₃PhSO₃), 2.10 (q, J = 7.2 Hz, 2H, H3a,b) and 1.72–1.60 (m, 2H, H2a,b).

¹³C NMR (101 MHz, CDCl₃) δ 144.90 (Cq Ar), 138.36 (C4), 133.04 (Cq Ar), 129.92 and 128.08 (C Ar), 114.82 (C5), 70.86, 70.81, 70.74, 70.64, 70.17, 69.36 and 68.77 (CH₂CH₂O-), 30.32 (C3), 28.84 (C2) and 21.77 (CH₃PhSO₃).

MS: *m/z* = 373.4 [M+H]⁺.

2.2.9. 2-(2,3,4-Tri-O-benzyl-6-(2-(2-(2-(pent-4-en-1-yloxy)ethoxy)ethoxy)ethyl)- α -D-glucopyranosyl)-1-azidoethane **15**

Compound **12** (1024 mg, 2036 mmol) was dissolved in dry DMF (20 mL, 0.1 M) under argon and 3054 mmol of NaH (60% dispersion in mineral oil) was added at room temperature; after 10 min, a solution of tosylate **14** (3.9 mmol) in 5 mL of dry DMF was added dropwise. The reaction was stirred at r.t. under argon atmosphere and followed by TLC (Hexane/EtOAc 7:3). After 12 h, the reaction was quenched by adding some drops of MeOH; the mixture was diluted with water and extracted three times with EtOAc. The organic phase was dried over sodium sulfate, filtered and evaporated and the crude purified by flash chromatography (silica gel, eluent Hexane/EtOAc 7:3), affording 1065 g (1.7 mmol, 83% yield) of compound **15**.

^1H NMR (400 MHz, CDCl_3) δ 7.35–7.22 (m, 15H, CH Ar), 5.79 (ddt, $J = 16.9, 10.1, 6.6$ Hz, 1H, H10), 4.99 (dd, $J = 17.1, 1.6$ Hz, 1H, H11a), 4.93 (d, $J = 12.0$ Hz, 1H, H11b), 4.90 (d, $J = 11.1$ Hz, 1H, CH_2Ph), 4.84 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.79 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.69 (d, $J = 11.6$ Hz, 1H, CH_2Ph), 4.63 (d, $J = 11.0$ Hz, 1H, CH_2Ph), 4.59 (d, $J = 11.6$ Hz, 1H, CH_2Ph), 4.15–4.05 (m, 1H, H1), 3.75–3.70 (m, 2H, H2, H3), 3.68–3.54 (m, 13H, H4, $-\text{CH}_2\text{CH}_2\text{O}-$), 3.54–3.47 (m, 3H, H5, H6a,b), 3.42 (t, $J = 6.7$ Hz, 2H, H7a,b), 3.39–3.25 (m, 2H, H2'a,b), 2.08 (dd, $J = 14.2, 7.3$ Hz, 2H, H9a,b), 1.99–1.89 (m, 2H, H1'a,b) and 1.70–1.60 (m, 2H, H8a,b).

^{13}C NMR (101 MHz, CDCl_3) δ 138.70 (Cq Ar), 138.39 (C10), 138.34 (Cq Ar), 138.09 (Cq Ar), 128.58, 128.55, 128.50, 128.04, 128.01, 127.98, 127.88 and 127.74 (C Ar), 114.80 (C11), 82.31 (C3), 79.63 (C2), 77.98 (C4), 75.59 (CH_2Ph), 75.16 (CH_2Ph), 73.37 (CH_2Ph), 71.67 (C1), 71.43 (C5), 71.10, 70.78, 70.73, 70.67, 70.59, 70.29 and 70.15 (C6, $\text{CH}_2\text{CH}_2\text{O}-$), 48.02 (C2'), 30.32 (C9), 28.84 (C8) and 24.47 (C1').

MS: $m/z = 704.7$ $[\text{M}+\text{H}]^+$.

2.2.10. 2-(2,3,4-Tri-O-benzyl-6-(1-thioacetylpentyl-(5-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl))) α -D-glucopyranosyl)-1-azidoethane **16**

Compound **15** (384 mg, 0.545 mmol) was dissolved in CH_2Cl_2 (5.4 mL, 0.1 M) and the solution transferred into a sealed glass vial; thioacetic acid (0.656 mmol) and DPAP (0.055 mmol) were added and the reaction was stirred at r.t. under UV light exposure at 365 nm (using a UVA lamp of 4 W located 2 cm away from the glass vial). The progress of the reaction was followed by TLC (Hexane/EtOAc 7:3). After 2 h, TLC indicated the disappearance of the starting material; the crude was directly subjected to flash chromatography (silica gel, eluent Hexane/EtOAc 7:3), which afforded pure thioester **16** (357 mg, 0.46 mmol, 85% yield).

^1H NMR (400 MHz, CDCl_3) δ 7.36–7.27 (m, 15H, CH Ar), 4.91 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.85 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.81 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.70 (d, $J = 11.6$ Hz, 1H, CH_2Ph), 4.67–4.58 (m, 2H, CH_2Ph), 4.16–4.07 (m, 1H, H1), 3.75–3.71 (m, 2H, H2, H3), 3.69–3.47 (m, 16H, H4, H5, H6a,b, $\text{CH}_2\text{CH}_2\text{O}-$), 3.44–3.27 (m, 4H, H7a,b, H2'a,b), 2.85 (t, $J = 7.3$ Hz, 2H, H11a,b), 2.31 (s, 3H, $\text{CH}_3\text{C}(\text{O})\text{S}$), 2.00–1.90 (m, 2H, H1'a,b), 1.62–1.52 (m, 4H, H10a,b, H8a,b) and 1.46–1.34 (m, 2H, H9a,b).

^{13}C NMR (101 MHz, CDCl_3) δ 196.10 ($\text{CH}_3\text{C}(\text{O})\text{S}$), 138.73, 138.37 and 138.12 (Cq Ar), 128.61, 128.58, 128.52, 128.07, 128.03, 128.01, 127.91 and 127.76 (C Ar), 82.34 (C3), 79.67 (C2), 78.02 (C4), 75.61 (CH_2Ph), 75.19 (CH_2Ph), 73.40 (CH_2Ph), 71.71 (C1), 71.46 (C5), 71.22, 71.12, 70.75, 70.70, 70.61, 70.34 and 70.18 (C6 sugar, $\text{CH}_2\text{CH}_2\text{O}-$), 48.06 (C2'), 30.79 ($\text{CH}_3\text{C}(\text{O})\text{S}$), 29.49 (C8), 29.23 (C10), 29.16 (C11), 25.49 (C9) and 24.51 (C1').

MS: $m/z = 780.6$ $[\text{M}+\text{H}]^+$, 752.6 $[\text{M}-\text{N}_2+\text{H}]^+$, 802.6 $[\text{M}+\text{Na}]^+$, 818.6 $[\text{M}+\text{K}]^+$.

2.2.11. 2-(2,3,4-Tri-O-benzyl-6-(1-thio-(5-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl))) α -D-glucopyranosyl)-1-azidoethane (dimer) **17**

Pure thioester **16** (38 mg, 0.049 mmol) was dissolved in MeOH (1 mL, 0.05 M) and 1.3 mg (0.024 mmol) of solid sodium methylate was added. The reaction was stirred at r.t. whilst exposed to air to promote the oxidation of the thiol group into a disulfide bond. After 24 h, TLC (Hexane/EtOAc 6.5:3.5) indicated the completion of the reaction. The reaction

mixture was concentrated to dryness; $^1\text{H-NMR}$ analysis of the crude sample indicated the complete removal of the acetyl group and the formation of a disulfide bond (triplet signal at 2.65 ppm). The residue was re-suspended in CH_2Cl_2 , and the undissolved material was filtered off and discarded; the filtrate was concentrated to dryness and directly used for the next reaction.

$^1\text{H NMR}$ (crude sample) (500 MHz, CDCl_3) δ 7.37–7.24 (m, 15H, CH Ar), 4.94–4.90 (m, 1H, CH_2Ph), 4.85 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.81 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.72–4.68 (m, 1H, CH_2Ph), 4.65–4.59 (m, 2H, CH_2Ph), 4.12 (dt, $J = 9.8, 5.0$ Hz, 1H, H1), 3.73 (dd, $J = 8.5, 6.0$ Hz, 2H, H2, H3), 3.70–3.49 (m, 16H, $\text{CH}_2\text{CH}_2\text{O-}$, H4, H5, H6a,b), 3.44–3.29 (m, 4H, H7a,b, H2'a,b), 2.71–2.62 (t, 2H, $J = 7.3$ Hz, H11a,b), 2.01–1.89 (m, 2H, H1'a,b), 1.68 (dt, $J = 15.0, 7.5$ Hz, 2H, H10a,b), 1.62–1.52 (m, 2H, H8a,b) and 1.47–1.37 (m, 2H, H9a,b).

2.2.12. 2-(6-(1-Thio-(5-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)))- α -D-glucopyranosyl)-1-aminoethane (dimer) **18**

Compound **18** was prepared following a Birch reduction protocol on protected derivative **17**, according to a procedure described in [63], with slight modifications. Some drops of liquid ammonia were collected in a two-necked round-bottomed flask cooled to -78°C and a small piece of sodium (approx. 20–30 mg) was added. The solution turned blue immediately, and after 10 min, a dry THF solution (1,4 mL) of the crude compound **17** was added. The reaction was stirred at -78°C for 15 min, then powdered NH_4Cl (≈ 200 mg) was added and the reaction was warmed to r.t. The solvent was evaporated and the crude was subjected to reverse phase C18 chromatography (gradient eluent: $\text{H}_2\text{O-MeOH}$); a total of 14 mg (0.032 mmol as monomer, 65% yield over two steps) of **18** was obtained.

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ 4.11 (ddd, $J = 11.6, 5.1, 2.7$ Hz, 1H, H1), 3.79–3.58 (m, 18H, H2, H3, H4, H5, H6a,b, $\text{CH}_2\text{CH}_2\text{O-}$), 3.51 (t, $J = 6.6$ Hz, 2H, H7a,b), 3.23–3.18 (m, 1H, H2'a), 3.17–3.10 (m, 1H, H2'b), 2.74–2.67 (m, 2H, H11a,b), 2.15–2.05 (m, 1H, H1'a), 1.99–1.90 (m, 1H, H1'b), 1.76–1.68 (m, 2H, H10a,b), 1.66–1.57 (m, 2H, H8a,b) and 1.51–1.43 (m, 2H, H9a,b).

$^{13}\text{C NMR}$ (101 MHz, CD_3OD) δ 76.10 (C1), 74.44, 71.87, 71.23, 70.73, 70.63, 70.60, 70.32 and 70.17 (C3, C2, C4, C5, C6, C7, $\text{CH}_2\text{CH}_2\text{O-} \times 6$), 39.63 (C11), 38.06 (C2'), 29.45 (C8), 29.21 (C10), 25.63 (C9) and 24.20 (C1').

MS: $m/z = 882.2$ $[\text{M}+\text{H}]^+$.

2.2.13. 5-(N,N-Dimethylamino)-N-(2-(6-(1-thio-(5-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)))- α -D-glucopyranosyl)-naphthalene-1-sulfonamide (dimer) **2**

Compound **18** (10 mg, 0.023 mmol as monomer) was dissolved in MeOH; Et_3N (10 μL) and dansyl chloride (16 mg, 0.06 mmol) were added and the reaction was stirred at r.t. The reaction was followed by TLC (EtOAc/MeOH 9:1 and $\text{EtOAc/MeOH/H}_2\text{O/AcOH}$ 5:5:1:1). After 10 h, the crude was purified by normal phase flash chromatography (silica gel, eluent: EtOAc/MeOH 8:2) and then with reverse phase C18 Cartridge, affording 6 mg (0.009 mmol, 39% yield) of compound **2**.

$^1\text{H NMR}$ (500 MHz, CD_3OD) δ 8.54 (t, $J = 14.1$ Hz, 1H, CH Ar), 8.36 (d, $J = 8.7$ Hz, 1H, CH Ar), 8.21 (dd, $J = 7.3, 1.1$ Hz, 1H, CH Ar), 7.59 (t, $J = 7.7$ Hz, 2H, CH Ar), 7.27 (d, $J = 7.3$ Hz, 1H, CH Ar), 3.86–3.77 (m, 1H, H1), 3.68–3.63 (m, 2H, H6a, $\text{CH}_2\text{CH}_2\text{O-}$), 3.62–3.55 (m, 10H, $\text{CH}_2\text{CH}_2\text{O-}$), 3.55–3.51 (m, 2H, H6b, $\text{CH}_2\text{CH}_2\text{O-}$), 3.49–3.42 (m, 3H, H2, H7a,b), 3.39–3.34 (m, 2H, H3, H5), 3.18–3.11 (m, 1H, H4), 3.00–2.91 (m, 2H, H2'a,b), 2.88 (s, 6H, $(\text{CH}_3)_2\text{N-}$), 2.65 (t, $J = 7.2$ Hz, 2H, H11a,b), 1.77–1.71 (m, 2H, H1'a,b), 1.69–1.62 (m, 2H, H10a,b), 1.60–1.51 (m, 2H, H8a,b) and 1.47–1.39 (m, 4H, H9a,b).

$^{13}\text{C NMR}$ (101 MHz, CD_3OD) δ 152.4 (CqAr), 135.2 (CqAr), 131.18 (CH Ar), 130.9 (CqAr), 130.32 (CH Ar), 129.12 (CH Ar), 124.39 (CH Ar), 120.60 (CH Ar), 116.43 (CH Ar), 114.2 (CqAr), 75.23, 75.05, 73.52, 72.56 and 72.17 (C1, C2, C3, C4, C5), 72.93, 72.13, 71.92, 71.52, 71.50 and 71.30 (C7, $\text{CH}_2\text{CH}_2\text{O-} \times 6$), 57.46 (C6), 45.85 $(\text{CH}_3)_2\text{N-}$, 39.82 (C11), 41.30 (C2'), 29.78 (C8), 29.45 (C10), 26.12 (C1') and 25.23 (C9).

MS: $m/z = 1348.8$ $[\text{M}+\text{H}]^+$, 1370.7 $[\text{M}+\text{Na}]^+$.

2.3. Synthesis of L-Glutamine Derivative 4 and 5

2.3.1. 5-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)pent-1-ene 19

To a solution of tosylate **14** (1976 mg, 5.3 mmol) in dry DMF (35 mL), 15.9 mmol of NaN_3 was added. The reaction was stirred at r.t. for 2 h and then at 80 °C for 10 h. The reaction was followed by TLC (Hexane/EtOAc 8:2). At the end of the reaction, the suspension was diluted with water and extracted with EtOAc (3×). The organic phases were combined, dried over sodium sulphate and evaporated. The residue was purified by flash chromatography (silica gel, eluent: Hexane/EtOAc 8:2), affording azide **19** (1012 mg, 4.16 mmol, 78% yield).

^1H NMR (400 MHz, CDCl_3) δ 5.80 (ddt, $J = 16.9, 10.2, 6.7$ Hz, 1H, H4), 5.01 (dd, $J = 17.1, 1.6$ Hz, 1H, H5a), 4.95 (d, $J = 10.2$ Hz, 1H, H5b), 3.72–3.61 (m, 8H, $-\text{OCH}_2\text{CH}_2-$), 3.61–3.54 (m, 2H, $-\text{OCH}_2\text{CH}_2-$), 3.46 (t, $J = 6.7$ Hz, 2H, H1a,b), 3.38 (t, $J = 5.0$ Hz, 2H, $-\text{OCH}_2\text{CH}_2\text{N}_3$), 2.10 (q, $J = 7.3$ Hz, 2H, H3a,b) and 1.73–1.61 (m, 2H, H2a,b).

^{13}C NMR (101 MHz, CDCl_3) δ 138.40 (C4), 114.80 (C5), 70.82, 70.81, 70.77, 70.21 and 70.15 ($-\text{OCH}_2\text{CH}_2-$), 50.78 ($-\text{OCH}_2\text{CH}_2\text{N}_3$), 30.34 (C3) and 28.86 (C2).

MS: $m/z = 244.2$ $[\text{M}+\text{H}]^+$.

2.3.2. 2-(2-(2-(Pent-4-en-1-yloxy)ethoxy)ethoxy)ethan-1-amine 20

Compound **19** (1153 mg, 4.73 mmol) was dissolved in THF (30 mL) and Ph_3P (5.68 mmol) was added. The reaction was stirred at r.t. for 2 h, then 5.67 mmol of distilled water was added and the reaction was heated to 60 °C and stirred for 30 min, following the disappearance of the starting material observed by TLC (Hexane/EtOAc 8:2 and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1). The solvent was then evaporated and the crude purified by flash chromatography (silica gel, eluent: $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1 to $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ (aq) 7:3:1), affording amine **20** (0.951 g, 4.4 mmol, 93% yield).

^1H NMR (400 MHz, CD_3OD) δ 5.83 (ddt, $J = 13.5, 10.1, 6.7$ Hz, 1H, H4), 5.02 (dd, $J = 17.1, 1.1$ Hz, 1H, H5a), 4.95 (d, $J = 10.1$ Hz, 1H, H5b), 3.69–3.53 (m, 12H, $-\text{OCH}_2\text{CH}_2-$), 3.52–3.45 (m, 2H, $-\text{OCH}_2\text{CH}_2\text{NH}_2$), 2.12 (dd, $J = 14.4, 7.2$ Hz, 2H, H3a,b) and 1.72–1.58 (m, 2H, H2a,b).

^{13}C NMR (101 MHz, CD_3OD) δ 139.44 (C4), 115.23 (C5), 73.67, 71.56, 71.50, 71.26, 71.12 and 70.39 (C1, $-\text{OCH}_2\text{CH}_2-$), 41.42 ($-\text{OCH}_2\text{CH}_2\text{NH}_2$), 31.33 (C3) and 30.03 (C2).

MS: $m/z = 218.3$ $[\text{M}+\text{H}]^+$.

2.3.3. (9H-Fluoren-9-yl)methyl-(S)-(3,7-dioxo-1,1,1-triphenyl-11,14,17-trioxa-2,8-diazadocos-21-en-6-yl) carbamate 21

Compound **20** (133 mg, 0.612 mmol) was dissolved in dry CH_2Cl_2 (6 mL, 0.1 M) in argon atmosphere. The solution was cooled to 0 °C and Fmoc-L-Gln(Trt)-OH (0.64 mmol), HOBt (0.918 mmol) and EDC (0.918 mmol) were added. The reaction was warmed to r.t. and stirred under argon. After 15 h, TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9.5:0.5 and $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ (aq) 9:1:0.1) indicated the absence of the starting compound; the reaction was diluted with CH_2Cl_2 and washed with water (3×). The organic phase was collected, dried over sodium sulphate, filtered and evaporated. The crude was purified by flash chromatography on Biotage System (silica gel, gradient CH_2Cl_2 100%– $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9.3:0.7). A total of 390 mg (0.48 mmol) of compound **21** was obtained (79% yield).

^1H NMR (500 MHz, CDCl_3) δ 7.80 (d, $J = 7.5$ Hz, 2H, CH Ar Fmoc), 7.63 (d, $J = 7.4$ Hz, 2H CH Ar Fmoc), 7.43 (t, $J = 7.4$ Hz, 2H CH Ar Fmoc), 7.38–7.20 (m, 17H, CH Ar Fmoc, CH Ar Trt), 7.11 (s, 1H, CONHTrt), 6.84 (s, 1H, $-\text{NHCH}_2\text{CH}_2\text{O}-$), 6.04 (d, $J = 6.7$ Hz, 1H, CONH Fmoc), 5.83 (ddt, $J = 16.9, 10.2, 6.6$ Hz, 1H, H15), 5.04 (ddd, $J = 17.1, 3.3, 1.5$ Hz, 1H, H16a), 5.01–4.96 (m, 1H, H16b), 4.41 (d, $J = 7.1$ Hz, 2H, $\text{NHCOCH}_2\text{-Fmoc}$), 4.24 (t, $J = 7.0$ Hz, 1H, CH Fmoc), 4.19–4.10 (m, 1H, H4), 3.64–3.51 (m, 10H, H7, H8, H9, H10, H11), 3.49–3.40 (m, 4H, H12a,b, H6a,b), 2.58–2.49 (m, 1H, H2a), 2.49–2.39 (m, 1H, H2b), 2.18–2.07 (m, 3H, H14a,b, H3a), 2.03–1.95 (m, 1H, H3b) and 1.73–1.65 (m, 2H, H13a,b).

^{13}C NMR (101 MHz, CDCl_3) δ 171.80 (C5), 171.32 (C1), 156.23 (CONH Fmoc), 144.48 (Cq Ar Trt), 143.80 (Cq Ar Fmoc), 143.68 (Cq Ar Fmoc), 141.17 (Cq Ar Fmoc), 141.14 (Cq

Ar Fmoc), 138.11 (C15), 128.64 (C Ar Trt, C Ar Fmoc), 127.82 (C Ar Trt, C Ar Fmoc), 127.62 (C Ar Trt, C Ar Fmoc), 127.01 (C Ar Trt, C Ar Fmoc), 126.88 (C Ar Trt, C Ar Fmoc), 125.10 (C Ar Fmoc), 119.87 (C Ar Fmoc), 114.73 (C16), 70.56, 70.48, 70.37, 70.34, 70.07 and 69.90 (C7, C8, C9, C10, C11, Cq Trt), 69.30 (C12), 66.76 (NHCOCH₂-Fmoc), 54.06 (C4), 47.06 (CH Fmoc), 39.20 (C6), 33.17 (C2), 30.11 (C14), 29.33 (C3) and 28.59 (C13).

MS: $m/z = 810.9 [M+H]^+$.

2.3.4. (S)-2-amino-N1-(2-(2-(2-(pent-4-en-1-yloxy)ethoxy)ethoxy)ethyl)pentanediamide **22**

Compound **21** (148 mg, 0.18 mmol) was dissolved in 1.8 mL of CH₂Cl₂ (0.1 M) and 0.4 mL of TFA was added. After 6 h, TLC (CH₂Cl₂/MeOH 9.5:0.5) showed no more starting compound (Trt deprotection) and the reaction was neutralized to pH 7 with NH₃(aq). The solvent was evaporated and the residue was dissolved in DMF (8 mL). Piperidine was added (2 mL) and the reaction was stirred at r.t. After 12 h, TLC showed the formation of a more polar compound (Fmoc removal); the reaction was then concentrated and the residue purified by flash chromatography (silica gel, eluent CH₂Cl₂/MeOH/NH₃ (aq) 8.5:1.5:0.1). A total of 60 mg (0.17 mmol) of deprotected compound **22** was obtained (96% yield).

¹H NMR (500 MHz, CD₃OD) δ 5.83 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H, H15), 5.02 (ddd, J = 17.1, 3.5, 1.6 Hz, 1H, H16a), 4.95 (ddt, J = 10.2, 2.2, 1.2 Hz, 1H, H16b), 3.67–3.60 (m, 6H, -CH₂CH₂O-), 3.60–3.54 (m, 4H, -CH₂CH₂O-), 3.48 (t, J = 6.5 Hz, 2H, H12a,b), 3.46–3.33 (m, 3H, H4, H6a,b), 2.28 (t, J = 7.8 Hz, 2H, H2a,b), 2.15–2.09 (m, 2H, H14a,b), 1.99–1.90 (m, 1H, H3a), 1.88–1.79 (m, 1H, H3b) and 1.70–1.62 (m, 2H, H13a,b).

¹³C NMR (101 MHz, CD₃OD) δ 173.20 (C5), 172.90 (C1), 138.78 (C15), 115.91 (C16), 70.65, 70.62, 70.38, 70.34, 70.12 and 69.85 (C7, C8, C9, C10, C11), 69.45 (C12), 54.06 (C4), 40.20 (C6), 32.17 (C2), 30.53 (C14), 29.26 (C3) and 28.87 (C13).

MS: $m/z = 346.4 [M+H]^+$, 368.4 [M+Na]⁺.

2.3.5. (S)-S-(1,4-Diamino-1,5-dioxo-9,12,15-trioxa-6-azaicosan-20-yl)-ethanethioate **23**

Compound **22** (60 mg, 0.174 mmol) was dissolved in MeOH (1.7 mL, 0.1 M). Thioacetic acid (0.23 mmol) and DPAP (0.035 mmol) were added to this solution and the reaction was stirred at r.t. under UV light exposure at 365 nm. After 2 h, TLC (CH₂Cl₂/MeOH/NH₃ (aq) 7:3:0.2) indicated the complete conversion of the starting compound; the reaction was concentrated and the residue was purified by flash chromatography (silica gel, eluent CH₂Cl₂/MeOH/NH₃ (aq) 8.5:1.5:0.1). A total of 29 mg (0.07 mmol) of thioester **23** was obtained (47% yield).

¹H NMR (500 MHz, CD₃OD) δ 4.32 (dd, J = 8.4, 5.7 Hz, 1H, H4), 3.66–3.60 (m, 6H, -CH₂CH₂O-), 3.60–3.53 (m, 4H, -CH₂CH₂O-), 3.47 (t, J = 6.5 Hz, 2H, H12a,b), 3.40 (t, J = 5.4 Hz, 1H, H6a), 3.36 (t, J = 5.5 Hz, 1H, H6b), 2.87 (t, J = 7.3 Hz, 2H, H16a,b), 2.34–2.26 (m, 5H, CH₃COSCH₂, H2a,b), 2.11–2.01 (m, 1H, H3a), 1.95–1.86 (m, 1H, H3b), 1.63–1.55 (m, 4H, H13a,b, H15a,b) and 1.47–1.39 (m, 2H, H14a,b).

¹³C NMR (101 MHz, CD₃OD) δ 197.53 (CH₃C(O)S-), 173.91 (C5), 173.30 (C1), 72.04, 71.62, 71.56, 71.28, 71.14 and 70.44 (-CH₂CH₂O-), 54.38 (C4), 40.39 (C6), 32.56 (C2), 30.62 (C13), 30.52 (CH₃C(O)S-), 30.17 (C15), 29.77 (C16), 29.10 (C3) and 26.39 (C14).

MS: $m/z = 422.6 [M+H]^+$, 444.5 [M+Na]⁺.

2.3.6. (S)-2-Amino-N1-(2-(2-(2-((5-mercaptopentyl)oxy)ethoxy)ethoxy)ethyl)pentanediamide (dimer) **4**

Compound **23** (10 mg, 0.0237 mmol) was dissolved in 0.6 mL of MeOH (previously degassed under a stream of argon) and 0.06 mmol of sodium methoxide was added. The reaction was stirred at r.t., and after 24 h, a sample of the crude was analyzed by ¹H-NMR to check the complete thioester deprotection. The spectrum clearly indicated the disappearance of the triplet at 2.9 ppm (-CH₂-SAc) and of the singlet at 2.3 ppm (CH₃C(O)S-). A new triplet at 2.65 ppm was present, indicating the formation of a disulfide bond. The reaction was then acidified with HCl 1 N (final pH = 6) and the solvent evaporated. The crude was directly used for the preparation of the nanoparticles.

^1H NMR (500 MHz, CD_3OD) δ 4.31 (dd, $J = 8.6, 5.6$ Hz, 1H, H4), 3.68–3.58 (m, 8H, $-\text{CH}_2\text{CH}_2\text{O}-$), 3.56 (t, $J = 5.5$ Hz, 2H, $-\text{CH}_2\text{CH}_2\text{O}-$), 3.49 (t, $J = 6.5$ Hz, 2H, H12a,b), 3.44–3.33 (m, 2H, H6a,b), 2.70 (t, $J = 7.2$ Hz, 2H, H16a,b), 2.30 (t, $J = 7.6$ Hz, 2H, H2a,b), 2.11–2.03 (m, 1H, H3a), 1.96–1.90 (m, 1H, 3b), 1.71 (dt, $J = 14.7, 7.3$ Hz, 2H, H15a,b), 1.65–1.56 (m, 2H, H13a,b) and 1.54–1.43 (m, 2H, H14a,b).

^{13}C NMR (101 MHz, CDCl_3) δ 174.01 (C5), 173.33 (C1), 72.13, 71.50, 71.47, 71.24, 71.08 and 70.55 ($-\text{CH}_2\text{CH}_2\text{O}-$), 54.49 (C4), 40.33 (C6), 39.54 (C16), 32.55 (C2), 30.26 (C13), 30.00 (C15), 29.01 (C3) and 26.03 (C14).

MS: $m/z = 758.1$ $[\text{M}+\text{H}]^+$.

2.3.7. Tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-5-oxo-9,12,15-trioxa-6-azaicos-19-enoate **24**

Boc-L-glutamic acid 1-tert-butyl ester (149 mg, 0.49 mmol) and compound **20** (0.58 mmol) were dissolved in CH_2Cl_2 (4.9 mL, 0.1 M) under argon and 0.98 mmol of Et_3N was added; the solution was stirred at r.t. for 15 min. The temperature was set to 0°C and HOBt (0.73 mmol) and DCC (0.73 mmol) were added. The reaction was stirred at r.t. until TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9.5:0.5) indicated the disappearance of the starting material. The reaction was recovered as described for compound **22** and the product was purified by flash chromatography (silica gel, eluent $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9.8:0.2), affording compound **24** (207 mg, 0.47 mmol, 95% yield).

^1H NMR (400 MHz, CDCl_3) δ 6.44 (s, 1H, $-\text{NHCH}_2\text{CH}_2\text{O}-$), 5.80 (td, $J = 16.9, 6.7$ Hz, 1H, H15), 5.26 (d, $J = 7.6$ Hz, 1H, NHBoc), 5.01 (d, $J = 17.2$ Hz, 1H, H16a), 4.95 (d, $J = 10.2$ Hz, 1H, H16b), 4.14 (s, 1H, H2), 3.67–3.52 (m, 10H, $-\text{CH}_2\text{CH}_2\text{O}-$), 3.51–3.36 (m, 4H, H12a,b, H6a,b), 2.30–2.20 (m, 2H, H4a,b), 2.18–2.00 (m, 3H, H14a,b, H3a), 1.95–1.81 (m, 1H, H3b), 1.74–1.62 (m, 2H, H13a,b) and 1.50–1.37 (m, 18H, $(\text{CH}_3)_3-\text{Boc}$, $-\text{tBu}$).

^{13}C NMR (101 MHz, CDCl_3) δ 172.20 (C5), 171.57 (C1), 155.81 (CONH Boc), 138.25 (C15), 114.87 (C16), 82.14 (Cq OtBu), 79.78 (Cq Boc), 70.77, 70.59, 70.33, 70.12 and 69.87 (C7–C12), 53.71 (C2), 39.37 (C6), 32.70 (C4), 30.27 (C14), 29.14 (C3), 28.78 (C13), and 28.40 and 28.05 ($(\text{CH}_3)_3$ OtBu, Boc).

MS: $m/z = 503.4$ $[\text{M}+\text{H}]^+$.

2.3.8. (S)-2-Amino-5-oxo-9,12,15-trioxa-6-azaicos-19-enoic acid **25**

Protected L-glutamine derivative **24** (246 mg, 0.49 mmol) was dissolved in a mixture of CH_2Cl_2 /trifluoroacetic acid (12 mL/3 mL) and stirred at r.t. The reaction was followed by TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ (aq) 7:3:0.3), and after 2 h, the solution was neutralized with NH_3 (aq). The crude was concentrated and the product purified by flash chromatography (silica gel, eluent: $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ (aq) 7:3:0.2), yielding 52 mg (0.15 mmol, 30% yield) of compound **25**.

^1H NMR (400 MHz, CD_3OD) δ 5.83 (ddt, $J = 17.0, 10.1, 6.8$ Hz, 1H, H15), 5.07–4.96 (m, 2H, H16a,b), 3.67–3.52 (m, 11H, H2, H7a,b, H-8a,b, H9a,b, H10a,b, H11a,b), 3.49 (t, $J = 6.5$ Hz, 2H, H12a,b), 3.37 (t, $J = 5.3$ Hz, 2H, H6a,b), 2.46 (t, $J = 7.1$ Hz, 2H, H4a,b), 2.20–2.03 (m, 4H, H14a,b, H3a,b) and 1.72–1.62 (m, 2H, H13a,b).

^{13}C NMR (101 MHz, CD_3OD) δ 174.87 (C1), 173.28 (C5), 139.43 (C15), 115.24 (C16), 71.57, 71.51, 71.24, 71.08 and 70.47 (C7–C12), 55.10 (C2), 40.47 (C6), 32.91 (C4), 31.34 (C14), 29.98 (C13) and 27.79 (C3).

MS: $m/z = 347.3$ $[\text{M}+\text{H}]^+$.

2.3.9. (S)-22-Amino-2,19-dioxo-9,12,15-trioxa-3-thia-18-azatricosan-23-oic acid **26**

Compound **25** (50 mg, 0.144 mmol) was dissolved in MeOH (1.5 mL, 0.1 M). Thioacetic acid (0.19 mmol) and DPAP (0.03 mmol) were added to this solution and the reaction was stirred at r.t. under UV light exposure at 365 nm. After 2 h, TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ (aq) 7:3:0.2) indicated the complete conversion of the starting compound; the reaction was concentrated and the residue was purified by flash chromatography (silica gel, eluent

$\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3$ (aq) 7:3:0.2). A total of 32 mg (0.075 mmol) of thioester **26** was obtained (53% yield).

^1H NMR (500 MHz, CD_3OD) δ 3.66–3.60 (m, 7H, $-\text{CH}_2\text{CH}_2\text{O}-$, H2), 3.60–3.57 (m, 2H, $-\text{CH}_2\text{CH}_2\text{O}-$), 3.55 (t, $J = 5.4$ Hz, 2H, H7), 3.48 (t, $J = 6.5$ Hz, 2H, H12), 3.37 (t, $J = 5.5$ Hz, 2H, H6), 2.87 (t, $J = 7.3$ Hz, 2H, H16a,b), 2.45 (t, $J = 7.1$ Hz, 2H, H4a,b), 2.30 (s, 3H, $\text{CH}_3\text{C}(\text{O})\text{S}$), 2.18–2.04 (m, 2H, H3a,b), 1.64–1.55 (m, 4H, H15a,b, H13a,b) and 1.48–1.40 (m, 2H, H14a,b).

^{13}C NMR (101 MHz, CD_3OD) δ 197.51 ($\text{CH}_3\text{C}(\text{O})\text{S}$), 190.57 and 175.09 (C1), 173.52 (C5), 72.02, 71.57, 71.52, 71.25, 71.09 and 70.50 ($-\text{CH}_2\text{CH}_2\text{O}-$), 55.63 (C2), 40.47 (C6), 33.07 (C4), 30.60 (C13), 30.53 ($\text{CH}_3\text{C}(\text{O})\text{S}$), 30.14 (C15), 29.76 (C16), 27.97 (C3) and 26.36 (C14).

MS: $m/z = 423.3$ [$\text{M}+\text{H}$] $^+$.

2.4. General Procedure for the Preparation of Ligand-Loaded Gold Nanoparticles

The thiol-ending ligand (3 eq. overall) was dissolved in MeOH at a final concentration of 12 mM. For nanoparticles decorated with different ligands, a suitable methanolic mixture was prepared, with a total final concentration of the ligands set to 12 mM (3 eq. overall). To this solution, 1 eq of a 25 mM aqueous solution of HAuCl_4 (hydrogen tetrachloroaurate) was added. After the addition, the reaction was stirred for 5 min at room temperature. During this step, Au(I) polymers were formed, and the reaction became turbid, with a pale-yellow color. Then, a 1 M water solution of NaBH_4 (27–33 eq) was added in four portions, ensuring vigorous stirring during the addition. The formation of a black precipitate indicated the generation of Au nanoparticles. The reaction was left for 2 h at 25 °C with 180 r.p.m. stirring. The black precipitate (AuNPs) was washed several times with MeOH, with three cycles of vortexing and centrifugation (12,000 rpm, 2 min), removing the supernatant and adding fresh MeOH. In this step, the excess of dissolved ligands, not attached to the NPs, was removed. The washed residue containing AuNPs was diluted in HPLC-grade water and then purified by dialysis (Spectrum™ Spectra/Por™ Float-A-Lyzer™, 10,000 MWCO, 1–5 mL volume). The water of the dialysis bath was replaced with fresh HPLC-grade water thrice per day for three days. The final water dispersion of the functionalized AuNPs was freeze-dried, obtaining a dark-black powder. The prepared AuNPs were stored at 4 °C and then subjected to ^1H NMR, UV–Vis spectroscopy and TEM analysis. The ratio between the thiol ligands attached to the AuNPs was assessed, recording ^1H -NMR spectra of the initial mixture and of the methanolic supernatants after AuNP formation.

2.4.1. Preparation of AuNP1

A solution of 4.97 mg of **GlcC₅SH** (17.6 μmol) in 1.47 mL of HPLC-grade MeOH, 234.7 μL of a 25 mM aqueous solution of HAuCl_4 and 197 μL of a 1 M aqueous solution of NaBH_4 were used to obtain 1.21 mg of AuNP1.

2.4.2. Preparation of AuNP2

A 2.3:1 mixture solution of **GlcC₅SH** (1.954 mg, 6.92 μmol) and compound **2** (2 mg, 2.97 μmol) was mixed with 132 μL of a 25 mM aqueous solution of HAuCl_4 and with 99 μL of a 1 M aqueous solution of NaBH_4 , affording 1.12 mg of AuNP2.

2.4.3. Preparation of AuNP3

A 1:1 mixture solution of **GlcC₅SH** (0.75 mg, 2.642 μmol) and L-glutamine derivative **4** (1 mg, 2.642 μmol) was mixed with 70.5 μL of a 25 mM aqueous solution of HAuCl_4 and with 58 μL of a 1 M aqueous solution of NaBH_4 , affording 0.49 mg of AuNP3.

2.4.4. Preparation of AuNP4

A 1.4:1:1.6 mixture solution of **GlcC₅SH** (0.55 mg, 1.947 μmol), L-glutamine derivative **4** (0.526 mg, 1.391 μmol) and compound **2** (1.5 mg, 2.226 μmol) was mixed with 74.18 μL of a 25 mM aqueous solution of HAuCl_4 and with 65 μL of a 1 M aqueous solution of NaBH_4 , affording 0.23 mg of AuNP4.

3. Results and Discussion

In the first phase of the present work, we designed a set of sulfur-functionalized derivatives of D-glucose, C-glucoside **1** and L-glutamine (Figure 3) in order to have the suitable compounds for the preparation of the corresponding gold nanoparticles (Figure 4) [54,64].

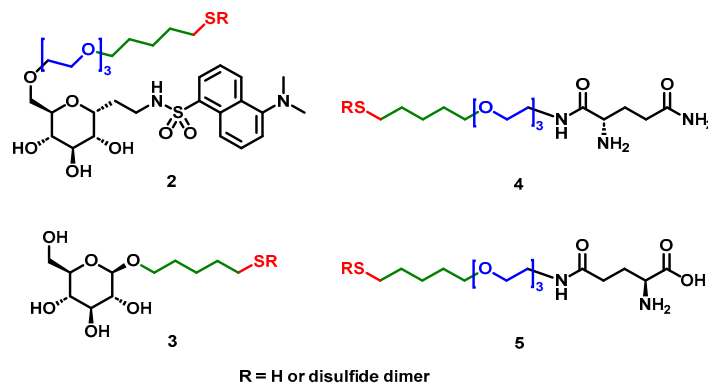


Figure 3. Sulfur-functionalized ligands for the preparation of the AuNPs.

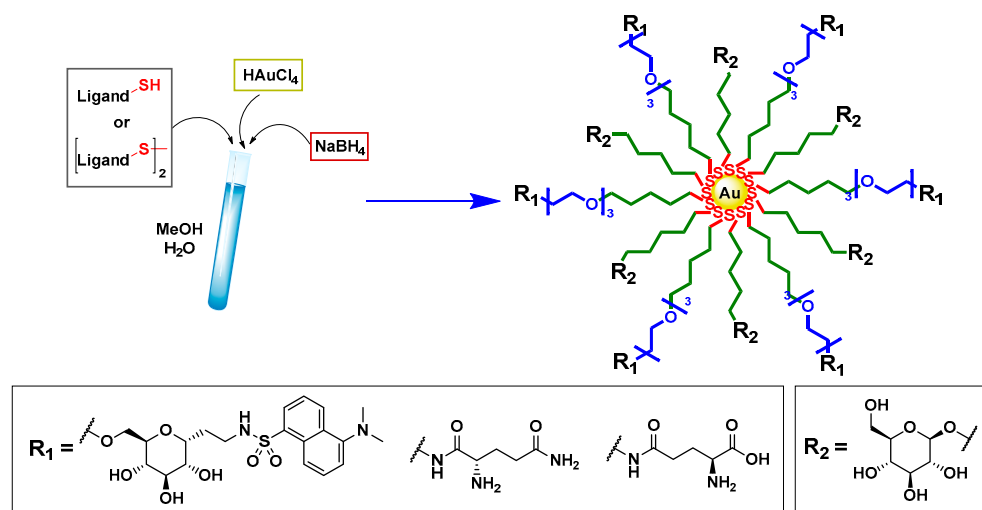
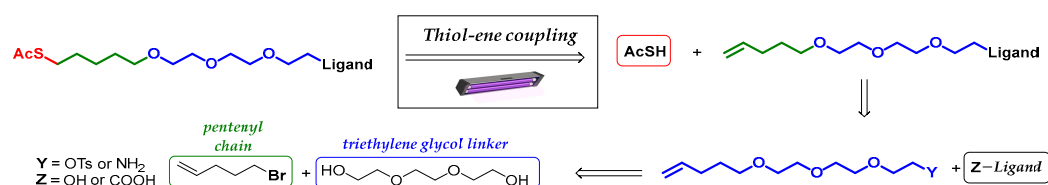


Figure 4. General structure of the designed nanoparticles: AuNPs decorated with the “active components” C-glucoside **2** and/or L-Gln **4** or **5**. The density of the “active components” can be modulated by varying their molar equivalents with respect to the “inner component” (GlcC₅SH).

The role of the D-glucose derivative *O*-(5'-thiopentyl)-β-D-glucopyranoside (compound **3**, GlcC₅SH), synthesized following a well-established protocol [65,66], was to improve the water dispersibility of the nanoparticles, since compound **1** showed low water solubility (approximately 0.5 mg/mL). Furthermore, GlcC₅SH was also selected as an “inner component” [51,66]. It should be noted that while ligands **2**, **4** and **5** have a long and amphiphilic linker to impart flexibility and assist in the water dispersibility of the final AuNPs, GlcC₅SH has a short and hydrophobic linker to ensure the rigidity of the nanoparticle and allow the above-mentioned ligands to protrude from the gold surface. GlcC₅SH should thus assist the multivalent effect of the other thiol-ending ligands, rather than interfering with their biological effects, since SGLT1-mediated anti-inflammatory effects are observed only with huge amounts of orally administered D-glucose.

Firstly, we designed the synthetic route of the derivatives considering the attachment of a suitable spacer/linker bearing a terminal thiol group. This linker ensured the correct spatial separation between the core of the gold nanoparticle and the “active component” (C-glucoside **1** or L-glutamine), which is necessary for the interaction of the bioactive molecules with the target receptor. The linker that we chose was composed

of a tri(ethyleneglycol) (TEG) chain and a five-carbon atom (C₅) spacer. Ethylene glycol polymers are extensively used in the pharmaceutical industry for their low toxicity, excellent solubility in aqueous media, resistance to non-specific protein adsorption and potential limited immunogenic and allergenic effects [67,68]. PEGylation is an important and emerging aspect in the drug delivery field; the conjugation of PEG to drugs has a positive impact on the pharmacokinetic and pharmacodynamic profile of the molecule to which it is conjugated. Moreover, polyethylene glycol (PEG) represents an inert polymer and is not metabolized. From a synthetic point of view, the insertion of a PEG chain on small molecules or drugs as well as biomacromolecules is now a relatively simple chemical transformation owing to numerous differently functionalized and commercially available PEG chains, and thanks to the development, over the last few decades, of many chemical (bio)orthogonal ligation strategies. For example, in a previous work, we developed a synthetic strategy to obtain heterobifunctionalized (poly)ethylene glycol chains for use as chemical tools in the modification of bioactive molecules and bio(macro)molecules [69]. The monofunctionalization of the triethyleneglycol linker with a pentenyl chain allowed for the introduction of a terminal double bond, which was exploited for the insertion of a sulfur-containing group, through a thiol–ene coupling (TEC) reaction [70]. Several synthetic procedures allow for the conversion of a terminal alkene into a thioether linkage, for example, using a Michael addition reaction between a thiol compound and a α,β -unsaturated carbonyl compound or exploiting a radical mechanism, as in the case of AIBN-catalyzed reactions. We decided to rely on a radical photoinitiator, called 2,2-dimethoxy-2-acetophenone (DPAP), which allows the thiol–ene reaction to occur in a faster and simpler fashion. This method permits the use of non-degassed solvents, and tolerates air atmosphere, moisture and even aqueous solvents. The reaction proceeded smoothly at room temperature; only a short (generally 30 min–1 h) UV light exposure at 365 nm was required (Scheme 1).



Scheme 1. General retrosynthetic scheme for the preparation of sulfur-functionalized ligands.

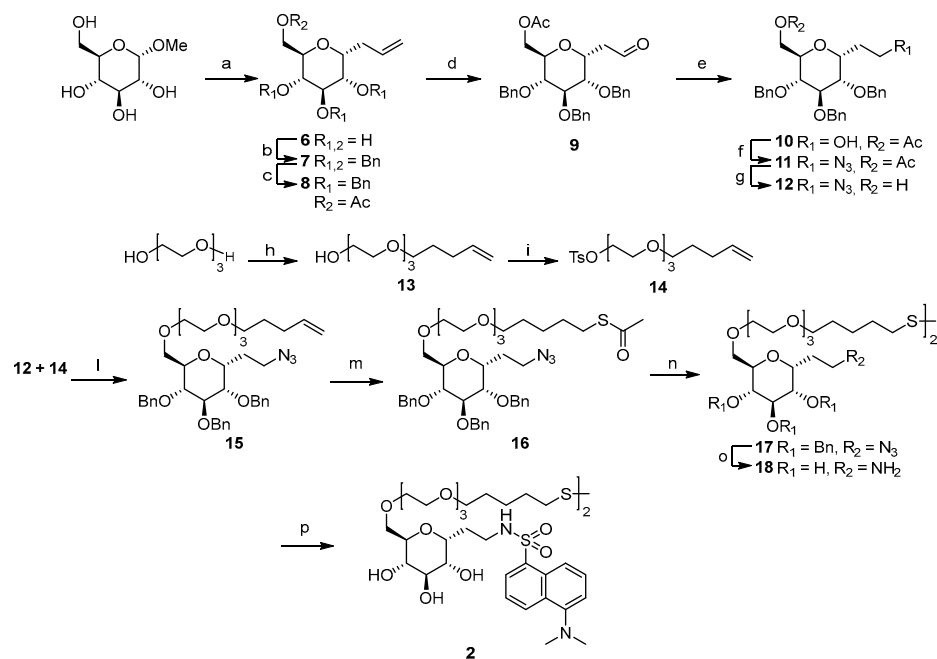
Concerning the compound **1** derivative, we decided to connect the linker to the hydroxyl group on the C-6 position of the pyranose ring for two main reasons: firstly, several works suggest that the C6 OH group of D-glucose is not crucial for binding to the SGLT1 interaction and translocation sites [71]; secondly, the primary hydroxyl group is the most easily functionalized among the hydroxyl groups of compound **1**. Hence, to the best of our knowledge, the presence of a linear chain should not affect the biological activity of the molecule. For L-glutamine ligands, we designed two different derivatives bearing, respectively, the TEG-C5 chain on the nitrogen atom of the amide of lateral chain or connecting it directly to the C α position of the amino acid, with the formation of a new amide group. We decided to synthesize two L-glutamine derivatives with TEG chains linked on two different positions of the aminoacidic structure, lacking information about the binding mode between L-glutamine and B⁰AT1 (i.e., which groups are involved in the binding). The preparation of both C-glucoside **1** and L-Gln derivatives proceeded to adopt a multistep synthesis, starting from commercially available O-Me- α -D-glucopyranoside and protected L-glutamine compounds, as described in Section 3.1.

Once the target ligand compounds were prepared, the experiment consisted of the preparation, purification and characterization of gold nanoparticles (Figure 4) loaded with compound **2**, D-glucose derivative (GlcC₅SH) and L-glutamine derivatives **4** and **5**, as described in Section 3.2.

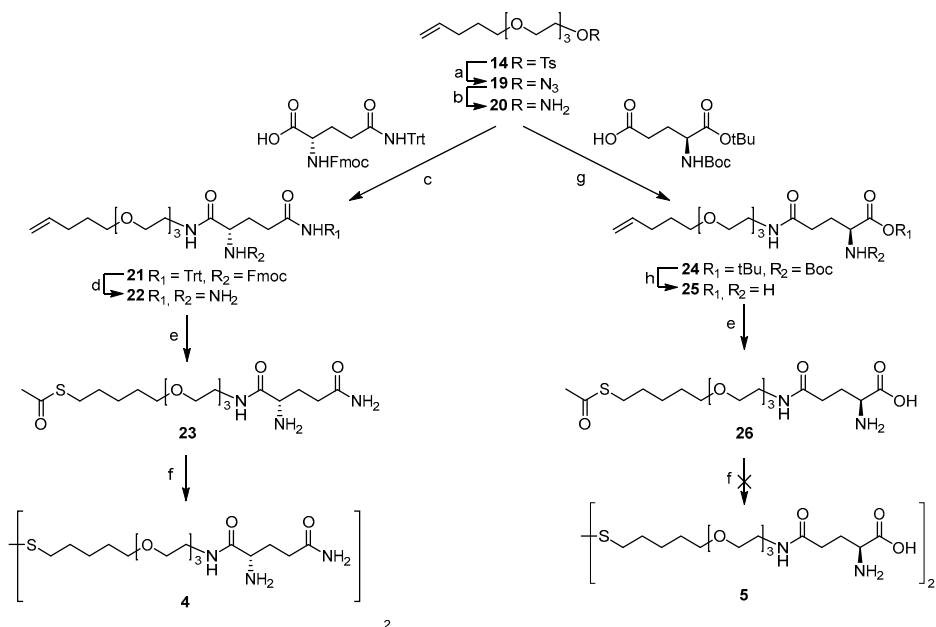
3.1. Preparation of Ligands

Concerning the synthesis of compound **2** (i.e., the C-glucoside **1** bearing a suitable sulfur-ending linker), the following synthetic steps were carried out (see Scheme 2 which also includes the reaction conditions and yields of each step). Commercially available *O*-Me- α -D-glucopyranoside was subjected to a chemoselective protecting-group-free C-allylation according to the procedure described in [60] (compound **6**). After perbenzylation (compound **7**), a successive acetolysis reaction, using a mixture of trifluoroacetic acid and acetic anhydride, allowed for the regioselective replacement of the benzyl protecting group on the C-6 position with an acetyl ester (compound **8**). The allyl handle was then manipulated by firstly oxidizing it into aldehyde **9**, then reducing it to alcohol **10**, and finally converting it into azidoethyl derivative **11** through a Mitsunobu-like reaction. The acetyl group was successively removed from the C6-OH position (compound **12**), which was in turn alkylated with the electrophilic TEG-C5 chain bearing a *p*-toluenesulphonate function as leaving group (compound **14**). The tosylate linker **14** spacer was synthesized starting from triethyleneglycol, then monoalkylated with a 4-pentenyl chain (compound **13**) and finally reacted with tosyl chloride. In our first planned synthesis, we subjected compound **15** to BCl_3 -promoted benzyl ether deprotection, but we observed the simultaneous cleavage of the polyethyleneglycol chain. Hence, we decided firstly to subject the TEGylated sugar derivative to a radical-mediated reaction. Upon UV exposure at 365 nm and adding DPAP, a radical photoinitiator, the allyl group was converted into thioester **16**. Successively, we attempted to use both catalytic hydrogenolysis and Birch conditions for the azide reduction and benzyl group cleavage, but surprisingly, in both cases, complete removal of the thioester group, generating a pentyl-ending chain, occurred. Indeed, the desulfurization of thioester by SET (single electron transfer) processes is described and rationalized in the literature [72]. Therefore, a change in strategy was needed, so we proceeded with deprotecting the thioester group and promoting its transformation into a disulfide bond to obtain a kind of “protected” thiol derivative (compound **17**). Birch reduction of this compound led to the desired fully deprotected dimer **18**, the two amine functionalities of which were transformed into dansyl-sulfonamides, affording the final dimer **2**. This compound was used directly for the generation of the nanoparticles; it is possible to use both thiol-free and disulfide-bearing molecules, since the addition of an excess of NaBH_4 leads to the disulfide bridge reduction.

The synthesis of the L-glutamine derivatives **4** and **5** proceeded with the following steps (Scheme 3). The tosyl group on the C5-TEG chain of compound **14** was converted into the azide functionality (compound **19**), which was reduced to amine derivative **20**. A condensation between this compound and Fmoc-L-Gln-Trt-OH afforded the TEGylated derivative **21**, which was subjected to protecting-group cleavage both in basic and acid conditions, affording the deprotected amino acid derivative **22**. The thiol-ene coupling (TEC) with thioacetic acid gave thioester **23**, which was then converted into the respective dimer **4** (the results was obtained from NMR analysis). The obtained disulfide was directly used for nanoparticle preparation. The condensation of amine **20** with Boc-L-Glu-O-tBu (Boc-L-glutamic acid 1-tert-butyl ester) gave intermediate **24**, which was deprotected by acidic conditions. The successive TEC with thioacetic acid afforded thioester **26**. Then, thioester deprotection with the methanolysis conditions used for compound **4** led to a complex mixture of side products, probably due to the competing deprotonation reaction of the α -carbon on the glutamine residue; this process did not occur for the first aminoacidic derivative since the racemizable carbon atom of the glutamine was located on the α position of the newly formed amide, and not on a carboxylic acid group as in **26**. Hence, we decided to perform the preparation of the nanoparticles using the L-glutamine derivative **4**.



Scheme 2. Reagents and conditions: (a) i. BSTFA, CH_3CN , reflux, 3 h; ii. allyl trimethylsilane, TMSOTf, 0°C to r.t., 12 h [60]; (b) BnBr, NaH, DMF dry, r.t., 3 h [61]; (c) Ac_2O , TFA, 0°C , 1 h, 88%; (d) i. O_3 , CH_2Cl_2 , -78°C , 1 h; ii. Ph_3P , CH_2Cl_2 , -78°C to r.t., 24 h, 82%; (e) NaBH_4 , MeOH, 0°C , 2 h, 95%; (f) DIAD, Ph_3P , $(\text{PhO})_2\text{PON}_3$, THF dry, 0°C to r.t., 2 h, 90%; (g) MeONa, MeOH dry, r.t., 1 h, 95%; (h) 5-Bromo-1-pentene, NaOH, neat, 100°C , 14 h, 74%; (i) TsCl, Et_3N , DIPEA, CH_2Cl_2 , r.t., 3 h, 72%; (l) NaH, DMF dry, r.t., 12 h, 83%; (m) AcSH, DPAP, CH_2Cl_2 , r.t., UV light (365 nm), 1 h, 85%; (n) MeONa, MeOH, r.t., 16 h; (o) Na, $\text{NH}_3(\text{l})$, THF dry, -78°C , 15 min, then NH_4Cl , r.t., 65%; (p) dansyl chloride, Et_3N , MeOH, r.t., 5 h, 39%.



Scheme 3. Reagents and conditions: (a) NaN_3 , DMF dry, 80°C , 48 h, 78%; (b) i. Ph_3P , THF, reflux, 3 h; ii. H_2O , r.t., 12 h, 93%; (c) EDC, HOBT, Et_3N , DMF dry, 0°C to r.t., 12 h, 79%; (d) i. CH_2Cl_2 , TFA, r.t., 2 h; ii. piperidine, DMF, r.t., 12 h, 96%; (e) AcSH, DPAP, MeOH, r.t., UV light (365 nm), 1 h, 47% (for 20) and 53% (for 24); (f) MeONa, MeOH, r.t., 2 h; (g) EDC, HOBT, Et_3N , DMF dry, 0°C to r.t., 12 h, 95%; (h) CH_2Cl_2 , TFA, r.t., 2 h, 30%.

3.2. Preparation of the Au Nanoparticles

After completing the synthesis of the ligands of interest, the preparation of four different gold nanoparticles (AuNP1–AuNP4, Figure 5) was carried out by using the disulfide ligands **2** and **4** and the D-glucose derivative *O*-(5'-thiopentyl)- β -D-glucopyranoside (GlcC₅SH, compound **3**), synthesized according to [65,66].

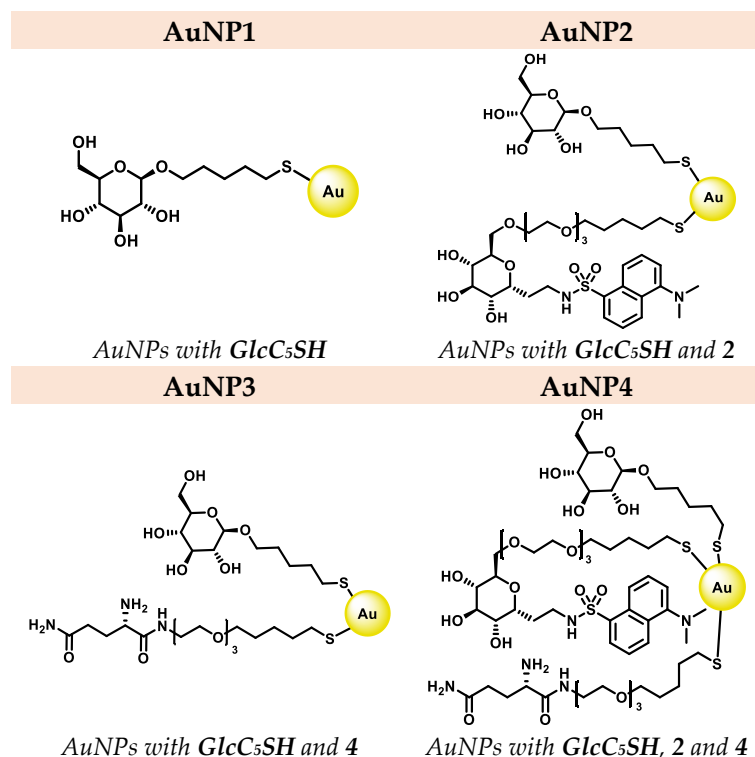


Figure 5. Structure of the four multivalent nanoparticles prepared in this work.

The preparation of the gold nanoparticles involved a one-pot process, inspired by the methods developed in 1994 by Brust and Schiffrin [73], and successively optimized by Penades and coworkers for the generation of GAuNPs [54]. The thiol-ending ligands were dissolved in MeOH, then added with a gold (III) salt, hydrogen tetrachloroaurate (HAuCl₄), to generate a kind of Au(I) polymer on which thiol compounds were grafted. The successive addition of a reducing agent, NaBH₄, provoked the further reduction from Au(I) to Au⁰, with the generation of the gold nanostructure decorated on its surface with the ligands of interest [64,66]. Experimental details and procedures for the preparation of the gold nanoparticles are described in the Materials and Methods section.

3.3. Nanoparticle Characterization

After dialysis and freeze-drying, the AuNPs were obtained as black powder and could be easily re-dispersed in water without flocculation.

The obtainment of decorated AuNPs with the synthesized ligands was preliminary assessed with infrared spectroscopy. In particular, FT-IR/ATR analyses on solid samples of AuNPs 1–4 were performed (Figure 6). The registered IR spectra on AuNP1–4 showed the presence of several bands associated with the functional groups of the decorating ligands, for example the stretching bands of sugar OH groups around 3300 cm⁻¹, the stretching bands of CH₂ of pentenyl chains at 2900 cm⁻¹, the C–O stretching band of ethylene glycol chains and of sugar rings in the region of 1015 cm⁻¹, as well as bands around 1650 cm⁻¹ (stretching of aromatics) and 1350 cm⁻¹ (OH bending). The IR spectra of AuNP3 and AuNP4 present a narrow band around 830 cm⁻¹ that can be associated with the bending vibrations of the amino group of the glutamine derivative grafted on AuNP 3 and 4.

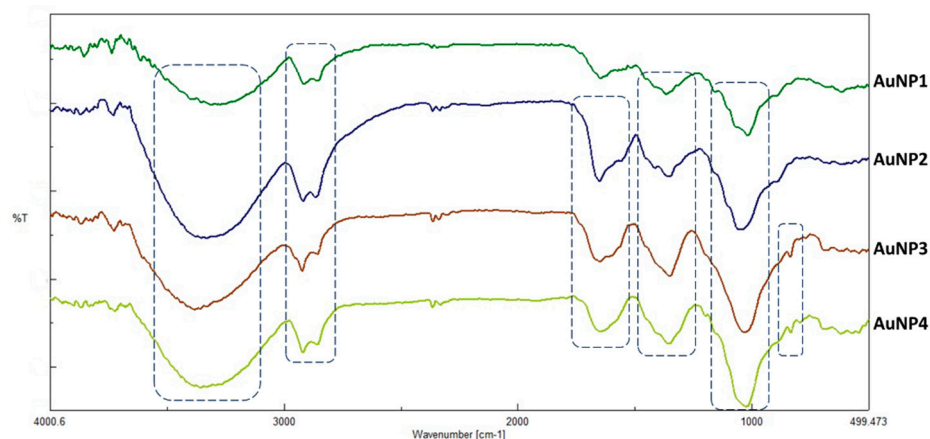


Figure 6. FT-IR/ATR spectra of prepared AuNPs 1–4. Some diagnostic stretching and bending vibrations are circled. Individual IR spectra of AuNP1–4 are reported in the Supporting Information.

Morphological characterization of AuNP1–4 was carried out by TEM (transmission electron microscopy) analysis. The measurement of the AuNPs' size and their distribution was accomplished using *ImageJ* (version 1.54 bundled 64-bit) and *Origin* (version Pro 2023 64-bit SR1) software, adopting the procedure and the processing steps described recently by Zhang and Wang [74]. Except for the AuNP4 sample, for which we counted a little bit less than 100 nanoparticles, in AuNP1, 2 and 3, we counted more than 100 nanoparticles. The four analyzed dispersions revealed the presence of nanoparticles ranging between 2 nm and 6 nm in diameter (gold core), with those of 2 nm being the most representative one, as indicated in the diameter distribution histograms of Table 1.

In order to further confirm the mean size of the AuNPs, the AuNP2 sample was chosen as a model and was subjected to UV–VIS spectroscopy analysis (Figure 7). The nanoparticles were dissolved in water at two different dilutions (0.5 mg/mL and 0.25 mg/mL), and only at the higher concentration was it possible to notice a weak plasmon absorption band around 520 nm, which is correlated to the presence of few nanoparticles with a mean bigger core diameter.

According to the obtained results, AuNP2 and AuNP3 showed the best features in terms of final mass yield and size distribution. Moreover, AuNP2 and AuNP3 were the most representative model nanoparticles among the prepared ones, since they exposed the C-glucoside derivative 2 (AuNP2) or the L-glutamine derivative 4 (AuNP3), the two active anti-inflammatory ligands of interest of this work, and the D-glucose GlcC_5SH derivative, used for the modulation of the dispersibility of the nanoparticles.

Table 1. TEM micrographs and size (diameter) distribution of the prepared nanoparticles.

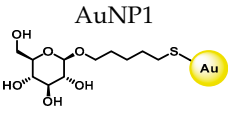
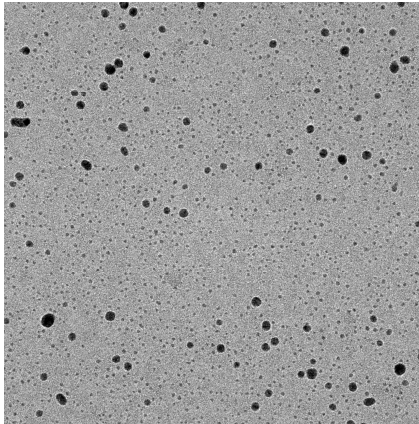
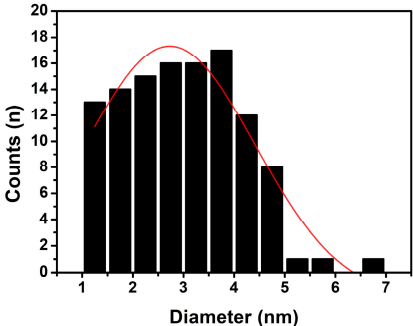
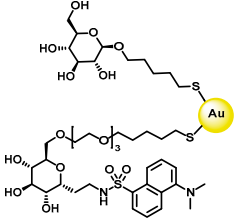
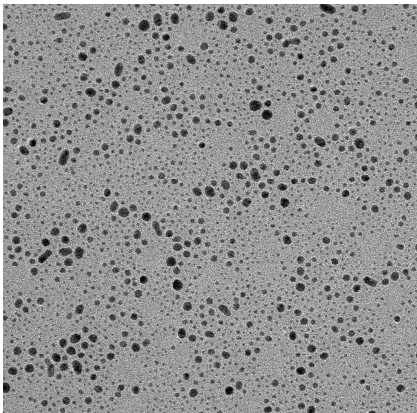
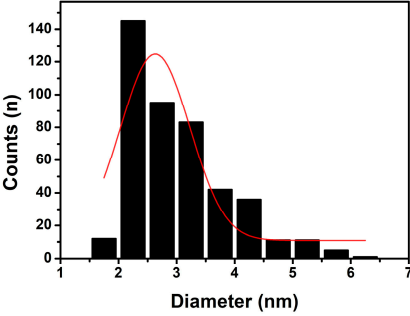
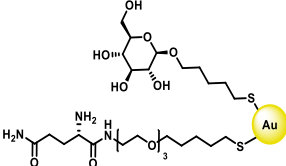
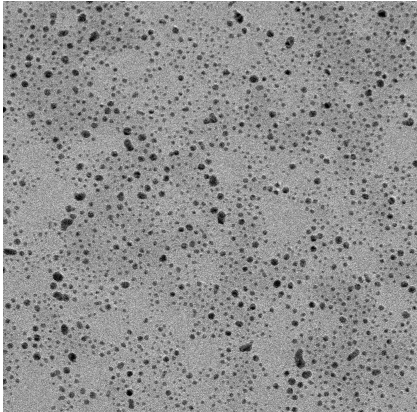
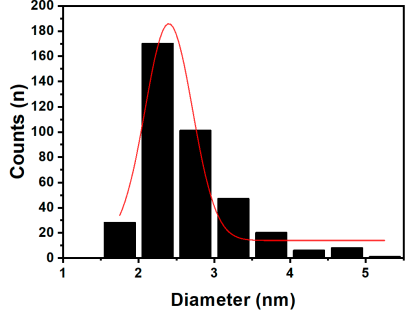
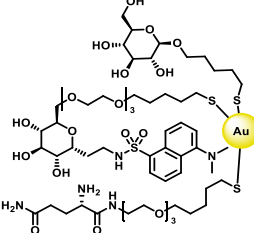
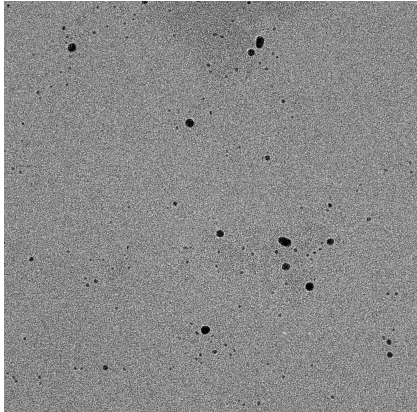
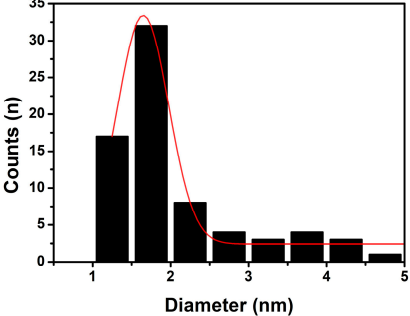
Nanoparticle	TEM Micrograph	Diameter Distribution Histogram
 <p>AuNP1</p>		

Table 1. Cont.

Nanoparticle	TEM Micrograph	Diameter Distribution Histogram
<p data-bbox="244 432 325 459">AuNP2</p> 	 <p data-bbox="518 757 571 779">50 nm</p>	
<p data-bbox="244 913 325 940">AuNP3</p> 	 <p data-bbox="518 1211 571 1234">50 nm</p>	
<p data-bbox="244 1339 325 1366">AuNP4</p> 	 <p data-bbox="518 1666 571 1688">50 nm</p>	

For these reasons, we decided to deepen the physical–chemical characterization of AuNP2–3 by means of $^1\text{H-NMR}$ analysis, which allowed us to obtain more detailed structural information about the loading of the ligands on the AuNPs. The $^1\text{H-NMR}$ experiments were performed by dissolving the powder comprised of AuNP2 and AuNP3 in deuterium oxide; the obtained spectra show broad signals of the ligands, typical for this kind of nanosystem [64,66]. In particular, signals of D-glucose derivative **GlcC₅SH**, C-glucoside derivative **2** (in AuNP2) and L-glutamine derivative **4** (in AuNP3) grafted onto a nano-sized entity are clearly visible in the respective $^1\text{H-NMR}$ spectra (Figure 8A). Before the preparation of the nanoparticles, an $^1\text{H-NMR}$ spectrum of a ligand-containing mixture

was recorded to check the ligands' ratio (Figure 8B); this was compared with the $^1\text{H-NMR}$ spectra recorded for the respective AuNPs, allowing for qualitative determination of the loading of the ligands on the NPs.

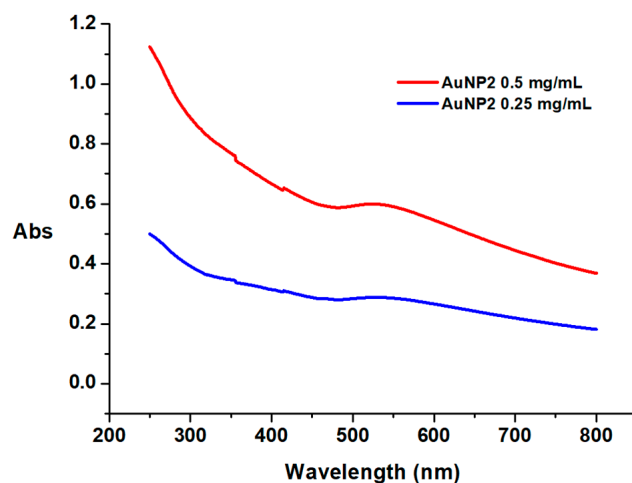


Figure 7. UV–VIS spectrum of AuNP2 dissolved in water. Red line: dilution of 0.5 mg/mL; Blue line: dilution of 0.25 mg/mL.

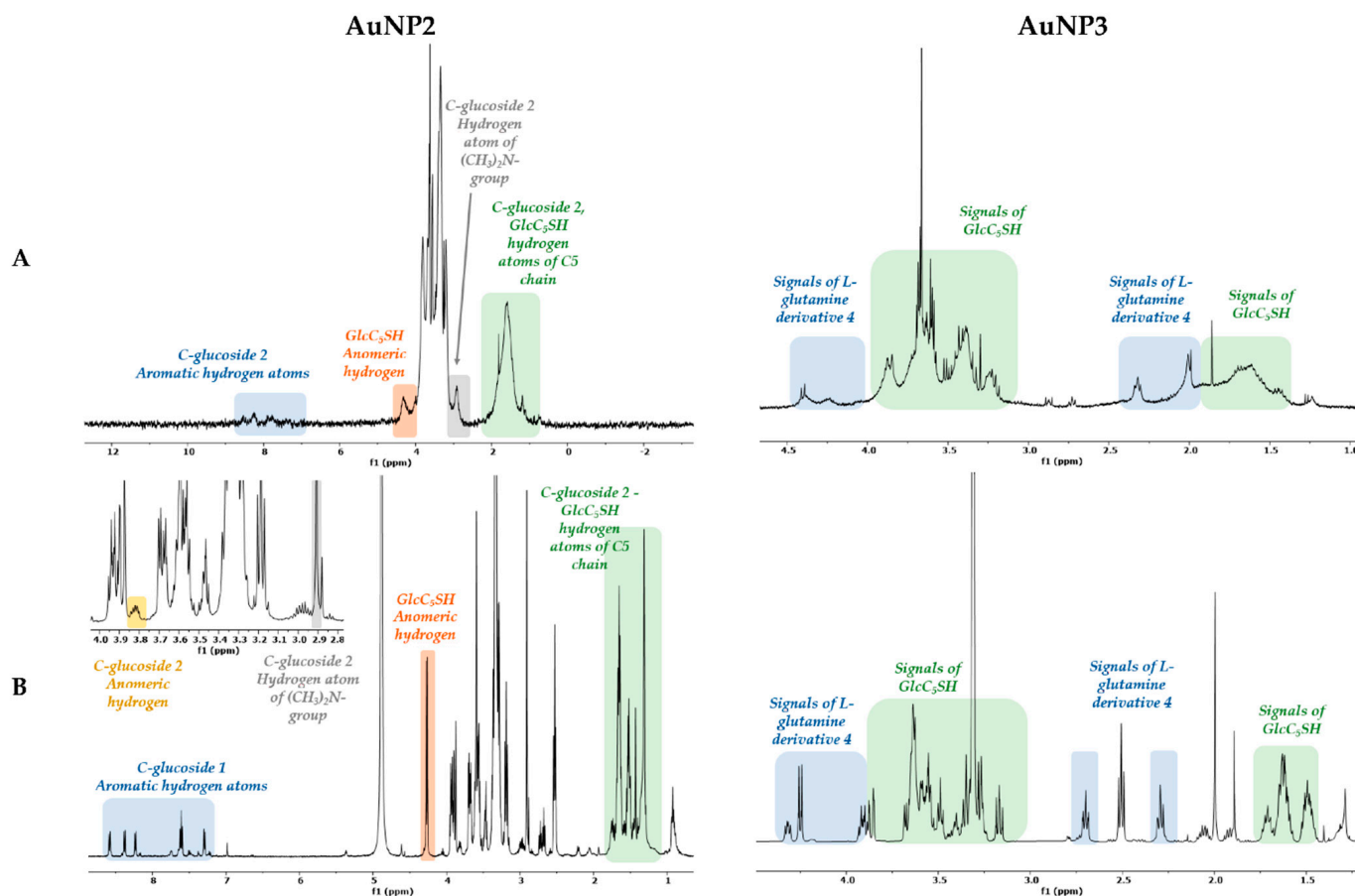


Figure 8. (A) $^1\text{H-NMR}$ spectra (with water suppression signal sequence) of the final AuNP2 (on the left) and AuNP3 (on the right). (B) $^1\text{H-NMR}$ spectrum of the solution of GlcC₅SH **3** and C-glycoside derivative **2** (in CD₃OD) before AuNP2 preparation (on the left); $^1\text{H-NMR}$ spectrum of the solution of GlcC₅SH **3** and L-glutamine derivative **4** (in CD₃OD) before AuNP3 preparation (on the right). Some diagnostic signals are highlighted with different colors.

4. Conclusions

In this work, we presented the design, synthesis and characterization of gold nanoparticles decorated with different compounds, derivatives of D-glucose, C-glycoside 1 and L-glutamine. The last two represent active ligands of the co-transporters SGLT1 and B⁰AT1, with a protective role against inflammatory agents. The two active compounds were functionalized with a tri(ethylene glycol) chain and a five-carbon spacer with a terminal thiol group to ensure their attachment to the gold nanoparticles, exploiting the affinity between the gold atoms and the thiol groups, and to provide an adequate distance of these molecules from the core of each gold nanoparticle. A glucose derivative, with a pentyl chain bearing a terminal thiol group, was also included to improve the water solubility of the NPs. The obtained AuNPs represent a useful tool to assess the multivalent/synergic activities of the ligands and to unveil the biochemical and physiological mechanisms at the basis of the anti-inflammatory activity correlated with the two co-transporters SGLT1 and B⁰AT1.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app14062248/s1>.

Author Contributions: Conceptualization, G.D., B.L.F. and M.M.; methodology, G.D., B.L.F. and M.M.; writing—original draft preparation, G.D.; writing—review and editing, G.D., B.L.F. and M.M.; supervision, B.L.F. and M.M. All authors have read and agreed to the published version of the manuscript.

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