The well-known cluster glycoside effect has spawned the construction of myriad multivalent glycopolymers for the study of carbohydrate–protein interactions. Integrated nanoparticle–biomolecule multifunctional systems constitute useful tools to mimic the behavior of biomolecules in cells, thus helping to explore the mechanisms of biological process with a variety of potential applications. Gold nanoclusters functionalized with carbohydrates provide a well-defined chemical composition to intervene in the cell–cell adhesion and recognition processes where carbohydrates are involved. However, assembly of glyconanoparticles from authentic oligosaccharides are rather difficult due to the insufficient quantities and methodology limitations, while the syntheses of pseudo-oligosaccharides or oligosaccharide mimics in which certain glycosidic bonds are substituted are involved. However, assembly of glyconanoparticles from authentic oligosaccharides are complex and often feature low overall yields. The mission may be considerably simplified if target structures are represented by pseudo-oligosaccharides or oligosaccharide mimics in which certain glycosidic bonds are substituted with non-glycosidic motifs. Among these efforts, 1,2,3-triazole chemistry because of their facile and efficient method of synthesis, has been successfully applied for the synthesis of various glycoconjugates including multivalent glycopolymers for the study of the behavior of biomolecules in cells, thus helping to explore the mechanisms of biological process with a variety of potential applications.
with NaBH₄ and HAuC₄, according to a reported procedure, furnished glyconanoparticle 2, which was characterized by ¹H NMR, and FTIR spectroscopy, and transmission electron microscopy (TEM, Fig. 2). The mean diameter of the generated particles is 3.4 nm, corresponding to an average number of 976 gold atmos. The bioactivity of compounds 1 and 2 is currently under investigation by our collaborators, and the results will be disclosed in due course.

1. Experimental

1.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer model 241-Mc automatic polarimeter; [α]₀⁻values are in units of 10⁻¹ deg cm² g⁻¹. ¹H NMR and ¹³C NMR spectra were recorded with a Bruker ARX 400 spectrometer for the solutions in CDCl₃, DMSO-d₆, CD₂OD, or D₂O. The chemical shifts are given in ppm downfield from internal Me₄Si. Mass spectra were measured using a MALDI-TOF mass spectrometer with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. FTIR spectra were recorded using a Perkin–Elmer 1720X spectrophotometer by the standard KBr technique. TLC was performed on silica gel HF₂₅₄ with detection by charring with 30% (v/v) H₂SO₄ in MeOH or in some cases by a UV lamp. Column chromatography was conducted by elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. The solutions were concentrated at <60 °C under reduced pressure. For TEM examinations, a single drop of the aq solution (ca. 0.1 mg/mL) of the gold glyconanoparticles was placed onto a copper grid coated with a carbon film. The grid was left to dry in air for several hours at room temperature.

Scheme 1. Reagents and conditions: (a) MeONa, MeOH, rt; (b) (i) MeOH, Bu₂SnO, reflux, (ii) propargyl bromide, Bu₄NI, toluene, 60 °C, 80%; (c) Pyr, BzCl, DMAP, 92%; (d) (i) CAN, 4:1 CH₃CN–H₂O; (ii) Cl₃CCN, DBU, CH₂Cl₂, 90% from 6; (e) 11-thioacetylundecanol, TMSOTf, CH₂Cl₂, 0 °C, 95%; (f) 6-azido-1-hexanol, NIS, TMSOTf, 1:1 CH₂Cl₂–CH₃CN, –40 °C, 70%.
TEM analysis was carried out in a Hitachi H-7500 microscope working at 200 kV.

1.2. p-Methoxyphenyl 3-0-propargyl-β-D-galactopyranosidase (5)

To a solution of 3 (10.5 g, 23.1 mmol) in anhyd MeOH (100 mL) was added 1 N NaOMe in MeOH until pH 9.5. The mixture was stirred at rt for 5 h, then neutralized with Amberlite IR-120 (H+) and filtered. The filtrate was concentrated to dryness, and the resulting compound 4 was used without further purification. After refluxing of 4 (5.521 g, 23.1 mmol) and dibutyltin oxide (5.299 g, 21.26 mmol) in anhyd MeOH (85 mL) under a nitrogen protection for 4 h, the reaction mixture was concentrated under vacuum. The residue was suspended in toluene (136 mL) under nitrogen atmosphere, and propargyl bromide (4.6 mL, 23.19 mmol) and tetrabutylammonium iodide (7.11 g, 19.28 mmol) were added to a solution of 5 (10.5 g, 23.1 mmol) in anhyd MeOH (100 mL) and sodium ascorbate, 1:1 THF–H2O, 92%; (b) NaOMe, MeOH; O2, 24 h at rt; then H2O, 80%; (c) NaBH4, HAuC4, Milli-Q water.

To a solution of 5 (1.81 g, 5.58 mmol) in pyridine (10 mL) were added BzCl (2.32 mL, 19.9 mmol) and DMAP (30 mg) at 0 °C. The reaction mixture was stirred at rt for 4 h, then quenched by adding MeOH (1 mL), and the mixture was stirred at rt for 4 h, then quenched by adding MeOH (1 mL), and the resulting mixture was concentrated under diminished pressure. The residue was redissolved in CH2Cl2 and was washed successively with 1 N HCl (aq), aq NaHCO3, and brine. The organic phase was dried over Na2SO4 and concentrated. Purification of the residue by column chromatography on silica gel (1:3 EtOAc–petroleum ether) afforded 6 (3.26 g, 92%) as an amorphous solid: [α]25D + 2 (c 06, CHCl3): 1H NMR (400 MHz, CDCl3): δ 2.24 (t, 1H, J 2.3 Hz, CH); 3.71 (s, 3H, OMe), 4.20–4.27 (m, 4H, H-3, H-5, and OC\textsuperscript{Me}); 4.52 (dd, 1H, J 11.4, 7.0 Hz, H-6b), 4.55 (dd, 1H, J 11.4, 7.0 Hz, H-6b), 5.15 (d, 1H, J 8.0 Hz, H-1), 5.74 (dd, 1H, J 9.9, 8.0 Hz, H-2), 6.87 (br d, 1H, J 2.8 Hz, H-4), 6.65, 6.95 (2d, 2 × 2H, CH2), 7.44–8.18 (m, 15H, 3Ph). Anal. Calcd for C37H32O10: C, 69.80; H, 5.07. MALDI-TOFMS: calcd for C37H32O10: 636.20 [M]+; found: 659.27 [M+Na]+.

1.3. p-Methoxyphenyl 2,4,6-tri-O-benzoyl-3-O-propargyl-β-D-galactopyranosidase (6)

To a solution of 6 (637 mg, 1 mmol) in CH2CN (16 mL) and H2O (4 mL) was added ammonium cerium nitrate (1.64 g, 3.0 mmol) at rt. After stirring for 1.5 h, the reaction mixture was diluted with EtOAc and H2O. The organic layer was washed with brine, dried over Na2SO4, and concentrated. Further purification was carried out by silica gel column chromatography (1:2 EtOAc–petroleum ether) to give a yellowish amorphous solid. To a solution of the above solid (516 mg) in CH2Cl2 (3 mL) were added trichloroacetonitrile (0.3 mL, 3 mmol) and DBU (30 μL) at rt. After stirring for 3 h, the reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (1:4 EtOAc–petroleum ether) to give 7 (594 mg, 90%) as an amorphous white solid: [α]25D + 11 (c 0.75, CHCl3): 1H NMR (400 MHz, CDCl3): δ 1.245 (t, 1H, J 2.4 Hz, CH); 4.27 (dd, 1H, J 16.4, 2.3 Hz, OCH\textsubscript{2}); 4.48 (dd, 1H, J 16.4, 2.3 Hz, OCH\textsubscript{2}); 4.52 (dd, 1H, J 11.4, 5.5 Hz, H-6a), 4.55 (dd, 1H, J 11.4, 7.0 Hz, H-6b), 4.66 (dd, 1H, J 10.3, 3.3 Hz, H-3), 4.70 (dd, 1H, J 5.5, 7.0 Hz, H-5), 5.67 (dd, 1H, J 10.3, 3.7 Hz, H-2), 6.02 (d, 1H, J 2.6 Hz, H-4), 6.84 (d, 1H, J 3.7 Hz, H-1), 7.41–8.14 (m, 15H, 3Ph), 8.57 (s, 1H, NH). MALDI-TOFMS: calcd for C33H26Cl3NO7: 673.07 [M]+; found: 695.16 [M+Na]+.

1.4. 2,4,6-Tri-O-benzoyl-3-O-propargyl-β-D-galactopyranosyl trichloroacetimidate (7)

To a solution of 7 (637 mg, 1 mmol) in CH2Cl2 (16 mL) and H2O (4 mL) was added ammonium cerium nitrate (1.64 g, 3.0 mmol) at rt. After stirring for 1.5 h, the reaction mixture was diluted with EtOAc and H2O. The organic layer was washed with brine, dried over Na2SO4, and concentrated. Further purification was carried out by silica gel column chromatography (1:2 EtOAc–petroleum ether) to give a yellowish amorphous solid. To a solution of the above solid (516 mg) in CH2Cl2 (3 mL) were added trichloroacetonitrile (0.3 mL, 3 mmol) and DBU (30 μL) at rt. After stirring for 3 h, the reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (1:4 EtOAc–petroleum ether) to give 7 (594 mg, 90%) as an amorphous white solid: [α]25D + 11 (c 0.75, CHCl3): 1H NMR (400 MHz, CDCl3): δ 2.45 (t, 1H, J 2.4 Hz, CH); 4.27 (dd, 1H, J 16.4, 2.3 Hz, OCH\textsubscript{2}); 4.48 (dd, 1H, J 16.4, 2.3 Hz, OCH\textsubscript{2}); 4.52 (dd, 1H, J 11.4, 5.5 Hz, H-6a), 4.55 (dd, 1H, J 11.4, 7.0 Hz, H-6b), 4.66 (dd, 1H, J 10.3, 3.3 Hz, H-3), 4.70 (dd, 1H, J 5.5, 7.0 Hz, H-5), 5.67 (dd, 1H, J 10.3, 3.7 Hz, H-2), 6.02 (d, 1H, J 2.6 Hz, H-4), 6.84 (d, 1H, J 3.7 Hz, H-1), 7.41–8.14 (m, 15H, 3Ph), 8.57 (s, 1H, NH). MALDI-TOFMS: calcd for C33H26Cl3NO7: 673.07 [M]+; found: 695.16 [M+Na]+.
1.5. 11-Thioacetylundecyl 2,4,6-tri-O-benzoyl-3-O-propargyl-β-D-galactopyranoside (8)

To a mixture of 7 (290 mg, 0.438 mmol), 11-thioacetylundecanol (90.14 mg, 0.366 mmol), and 4 Å molecular sieves (100 mg) in anhyd CH₂Cl₂ (5 mL) was added TMSOTf (7.94 μL, 0.044 mmol) at 0 °C under an N₂ atmosphere. The reaction mixture was stirred under these conditions for 1.5 h, then neutralized with Et₃N and filtered. The filtrate mixture was concentrated to dryness. The residue was purified by silica gel column chromatography (1:3 EtOAc–petroleum ether) to give 8 (594 mg, 95%) as an amorphous solid: [α]D²⁵ +127 (c 1, CHCl₃); [M]¹⁺ +H 184.5 (92%); [M+H] + 432 (100%); [M+2H]²⁺ 216.2 (70%); [M+3H]³⁺ 144.1 (50%). MALDI-TOFMS: calcd for C₂₈H₄₀N₄O₁₃: 616.26 [M] +; found: 639.37 [M+Na] +.

1.6. Methyl (6-azidohexyl 5-acetamido-4,7,8,9-tetrahydropyranoside (11)

To a stirred mixture of 9 (250 mg, 0.4284 mmol), 6-azido-1-hexanol (55.7 mg, 0.43 mmol), and 4 Å molecular sieves (30 mg) in dry 1:1 CH₂Cl₂–CH₃CN (5 mL) were added NIS (131 mg, 0.64 mmol) and TMSOTf (7 μL, 0.043 mmol) at −40 °C. The reaction mixture was stirred under these conditions for 6 h until all starting materials were consumed. The mixture was then neutralized with Et₃N and filtered, and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography (2:1 EtOAc–petroleum ether) to give 10 (186 mg, 70%) as an amorphous solid: [α]D²⁵ +12 (c 1, CHCl₃); [M]¹⁺ +H 184.5 (92%); [M+H] + 432 (100%); [M+2H]²⁺ 216.2 (70%); [M+3H]³⁺ 144.1 (50%). MALDI-TOFMS: calcd for C₂₆H₄₀N₄O₁₃: 616.26 [M] +; found: 639.37 [M+Na] +.

1.7. 11-Thioacetylundecyl 3-O-[(methyl 5-acetamido-4,7,8,9-tetrahydro-2-α-D-glycero-β-D-galacto-2-nonulopyranosylhexanoyl)triazol-1-methyl]-β-D-galactopyranoside (11)

To a suspension of 10 (266 mg, 0.434 mmol) and 8 (272 mg 0.4313 mmol) in 1:1 H₂O–THF (40 mL) were added sodium ascorbate (34.18 mg, 0.17 mmol) and CuSO₄·5H₂O (21.4 mg, 0.086 mmol) under vigorous stirring. The mixture was stirred in a dark room at 50–60 °C until complete consumption of the reactants was indicated by TLC analysis. The solvent was evaporated, and the residue was diluted with EtOAc, and washed with H₂O and brine. The aq layer was extracted with EtOAc, and the combined organic layers were dried over Na₂SO₄ and concentrated. Further purification was carried out by silica gel column chromatography (3:1 EtOAc–petroleum ether) to give 11 (545 mg, 92%) as a white amorphous solid: [α]D²⁵ +70 (c 0.6, CHCl₃); [M]¹⁺ +H 184.5 (92%); [M+H] + 432 (100%); [M+2H]²⁺ 216.2 (70%); [M+3H]³⁺ 144.1 (50%). MALDI-TOFMS: calcd for C₂₆H₄₀N₄O₁₃: [M] + 616.26; found: 639.37 [M+Na] +.

1.8. 11,11′-Dithiobi(undecyl [3-O-[(5-acetamido-3,5-dideoxy-2-α-D-glycero-β-D-galacto-2-nonulopyranosylhexanoyl)triazol-1-methyl]-β-D-galactopyranoside] (1)

To a solution of 11 (100 mg, 0.07 mmol) in anhyd MeOH (20 mL) was added 1 N NaOMe until pH 9.5. The reaction mixture was stirred at rt for 40 h, then a gentle stream of oxygen was bubbled through the solution, and stirring was continued for approximately 24 h, at the end of which time H₂O (3 mL) was added. After stirring for another 12 h, the reaction mixture was cooled to 0 °C, neutralized with amberlite IR-120 (H⁺), and then filtered, and the filtrate was evaporated. The residue was purified with Sephadex LH-20 and the desired fractions were lyophilized to give 1 (487.8 mg, 80%) as a white amorphous solid: [α]D²⁵ +90 (c 0.5, H₂O); [M]¹⁺ +H 184.5 (92%); [M+H] + 432 (100%); [M+2H]²⁺ 216.2 (70%); [M+3H]³⁺ 144.1 (50%). MALDI-TOFMS: calcd for C₂₆H₄₀N₄O₁₃: 616.26 [M] +; found: 639.37 [M+Na] +.

1.9. Synthesis of GNP (2)

To a solution of disulfide 1 (30 mg, 0.018 mmol) in Milli-Q H₂O (4 mL) was added HAuCl₄·4H₂O (22.11 mg). The solution was stirred at rt for 10 min, kept at 0 °C for 15 min, then NaBH₄ (3.585 mg, 0.0087 mmol, in 0.5 mL H₂O) was added in small portions under vigorous stirring conditions. The reaction mixture turned immediately to deep brown. The mixture was kept under these conditions for another 15 min, then warmed up to rt and stirred for further 3 h. At the end of this time, the solvent was removed by centrifugation, and the black residue was redissolved in H₂O (12 mL) and purified by centrifugal filtering (40 min, 14,000 rpm). The process was repeated three times. Finally, the H₂O phase was lyophilized to afford the gold glyconanoparticles 2 (10 mg) as a dark brown powder. Average diameter and number of gold atoms: 3.4 nm, 976. [M]¹⁺ +H 184.5 (92%); [M+H] + 432 (100%); [M+2H]²⁺ 216.2 (70%); [M+3H]³⁺ 144.1 (50%). MALDI-TOFMS: calcd for C₂₆H₄₀N₄O₁₃: 616.26 [M] +; found: 639.37 [M+Na] +.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.06.039.

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