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Synthesis of a Le^y neoglycoconjugate and Le^y-functionalized gold glyconanoparticles

José-Luis de Paz, Rafael Ojeda, África G. Barrientos, Soledad Penadés and Manuel Martín-Lomas*

Grupo de Carbohidratos, Instituto de Investigaciones Químicas, CSIC, Américo Vespucio s/n, 41092 Sevilla, Spain

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Abstract—The thiol functionalized Le^y neoglycoconjugate **1** has been synthesized and used to prepare the Le^y-functionalized gold glyconanoparticle **2**. The synthesis of **1** has been carried out using a stepwise glycosylation strategy in which a suitably protected D-glucosamine derivative has been sequentially glycosylated at position 3 and then at position 4 with conveniently protected α-L-fucopyranosyl and β-D-galactopyranosyl donors, respectively. The galactosylation step afforded the imidate **16** when the glycosyl acceptor contained a *N*-acetyglucosamine unit and the desired 4-*O*-galactopyranosyl derivative when the amino function was protected as phthalimido group. The gold glyconanoparticle **2** has been prepared from **1** and has been characterized by NMR spectroscopy and transmission electron microscopy (TEM).

1. Introduction

Gold nanoclusters functionalized with carbohydrate antigens (glyconanoparticles[1]) were first described by our laboratory to prove and to evaluate carbohydrate–carbohydrate interactions. These glyconanoparticles provide a polyvalent glycocalix-like globular carbohydrate display with well defined chemical composition, which constitutes a useful tool to study carbohydrate interactions[2–4] and to interfere cell adhesion processes.[5] The carbohydrate functionalized gold nanoclusters were constructed by attaching the carbohydrate antigens to the gold surface through a Au–S covalent bond generated using a modification of the procedure first reported by Brust et al. for the synthesis of monolayer protected gold nanoparticles.[6] The preparation of the glyconanoparticles involved, therefore, the synthesis of neoglycoconjugates of the carbohydrate antigens in which the oligosaccharides were functionalized with a linker endowed with a thiol group, as the first step, and then the reduction of a mixture of the neoglycoconjugate and tetrachloroauric acid to form the carbohydrate functionalized water soluble nanoclusters. A variety of thiol-ended spacers attached to the carbohydrate antigens have been used for the different studies carried out so far as the nature of these linkers influence the behavior of the neoglycoconjugates and also the formation and the properties of the final polyvalent constructs.[1]

In exploring the scope and the limitations of glyconanoparticles to study and to intervene in biological molecular recognition phenomena in which carbohydrates are involved, we have initiated a program on the potential of this technology to develop carbohydrate based anticancer vaccines.[7] This involves the synthesis of neoglycoconjugates of tumor associated antigens containing an appropriate thiol-ended spacer group, which permits an effective presentation of the oligosaccharide epitope, the synthesis of immunostimulant peptides also functionalized with a given thiol-ended linker and the construction of the nanocluster with the concomitant covalent attachment of both, oligosaccharides and peptides, to the gold surface in controlled pre-established proportions. Thus, neoglycoconjugates of a series of tumor associated carbohydrate antigens containing a carefully chosen spacer group had to be synthesized using chemistry, which had to be compatible with the whole preparative process.

As a part of this project, we report herein the synthesis of the Le^y neoglycoconjugate **1** and the preparation and

* Corresponding author. Tel.: +34 954489553; fax: +34 954460565; e-mail: manuel.martin-lomas@iiq.csic.es

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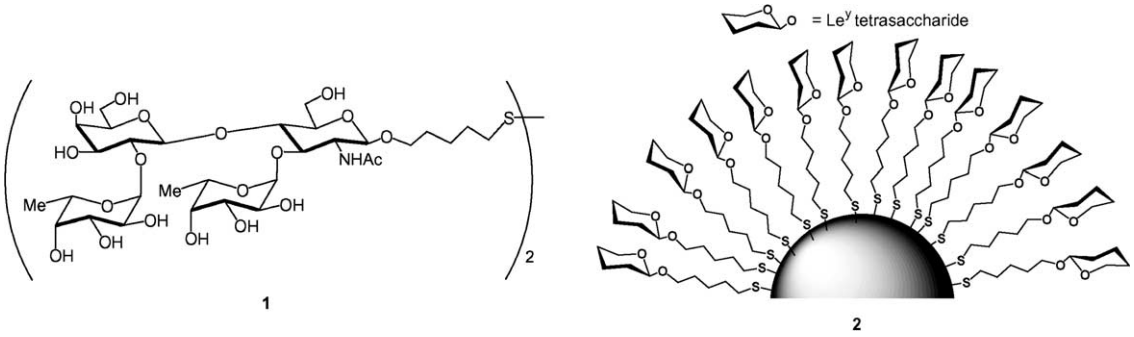
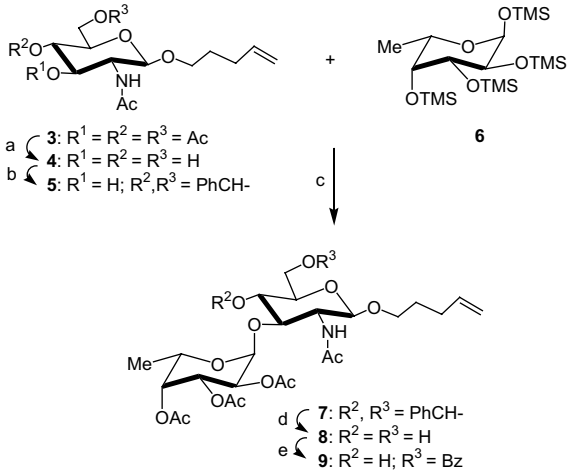


Figure 1. Le^y neoglycoconjugate **1** and Le^y-functionalized gold glyconanoparticles **2**.

characterization of a Le^y-functionalized gold glyconanoparticle **2** (Fig. 1). The Le^y determinant has been identified as an epitope, which elicits antibodies against colon and liver carcinomas[8,9] and is overexpressed in metastatic prostate cancer and in ovarian tumors.[10] It has been previously prepared using different synthetic strategies.[11–16] For the synthesis of **1** we have used a step by step glycosylation strategy, which had been successfully used in our laboratory for the synthesis of a closely related Le^x neoglycoconjugate,[17] subsequently employed in the preparation of Le^x coated gold nanoclusters,[1] rather than a block synthesis approach. In the course of this synthesis we have observed the formation of glycosyl imidate **16** (Scheme 2) when attempting to glycosylate the unreactive 4-OH group of the disaccharide derivative **9**. From neoglycoconjugate **1** the Le^y-functionalized gold glyconanoparticle **2** has been prepared[1] and characterized using NMR spectroscopy and transmission electron microscopy (TEM).

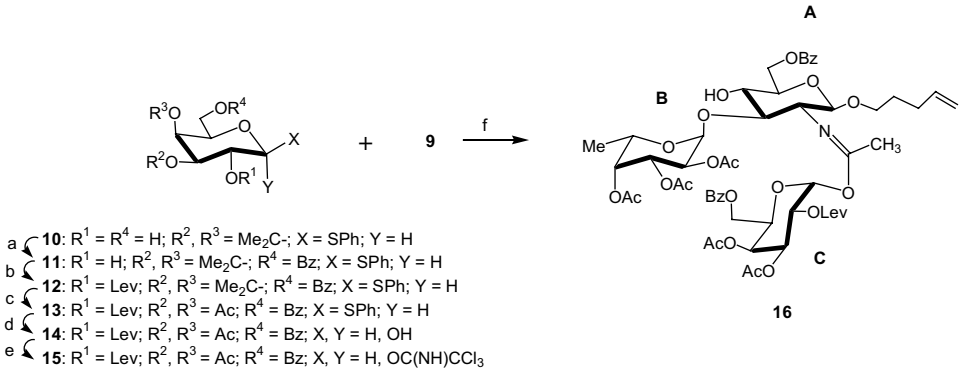


Scheme 1. Reagents and conditions: (a) MeONa/MeOH, 98%; (b) $PhCH(OMe)_2$, CH_3CN, *p*TsOH (cat.), 45 °C, 67%; (c) TMS–I, CH_2Cl_2, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, MeOH, Ac_2O, Py, 65%; (d) TFA, CH_2Cl_2, H_2O, 98%; (e) BzCN, Et_3N (cat.), CH_3CN, −40 °C, 85%.

2. Results and discussion

In order to attain a convenient three dimensional display of the carbohydrate and peptide ligands on the gold nanocluster, it was decided to synthesize the Le^y neoglycoconjugate **1** containing a β oriented thiopentyl spacer group at the reducing end. To this purpose the synthesis was first envisaged from the peracetylated pentenyl glycoside **3**,[18] (Scheme 1) that was readily prepared from peracetylated 2-acetamido-2-deoxy-D-glucopyranose, rather than from a phthalimido thioglycoside as in the synthesis of the Le^x neoglycoconjugate.[17] Deacetylation of **3** gave **4**, which was transformed into the 4,6-*O*-benzylidene derivative **5** (Scheme 1). α-Fucosylation of **5** was then carried out from the pertrimethylsilyl derivative **6**, which in accord with the reported procedure[19] is activated as the corresponding fucosyl iodide, which without isolation is used as glycosylating agent in the presence of 2,6-di-*tert*-butylpyridine. The resulting disaccharide was in situ desilylated and subsequently acetylated to afford disaccharide **7** in 65% overall yield. This is a very simple and convenient α-fucosylation method that we have previously used successfully for the synthesis of the Le^x neoglycoconjugate.[17] Compound **7** was then transformed into glycosyl acceptor **9** after removal of the benzylidene group to give **8** and regioselective benzoylation.

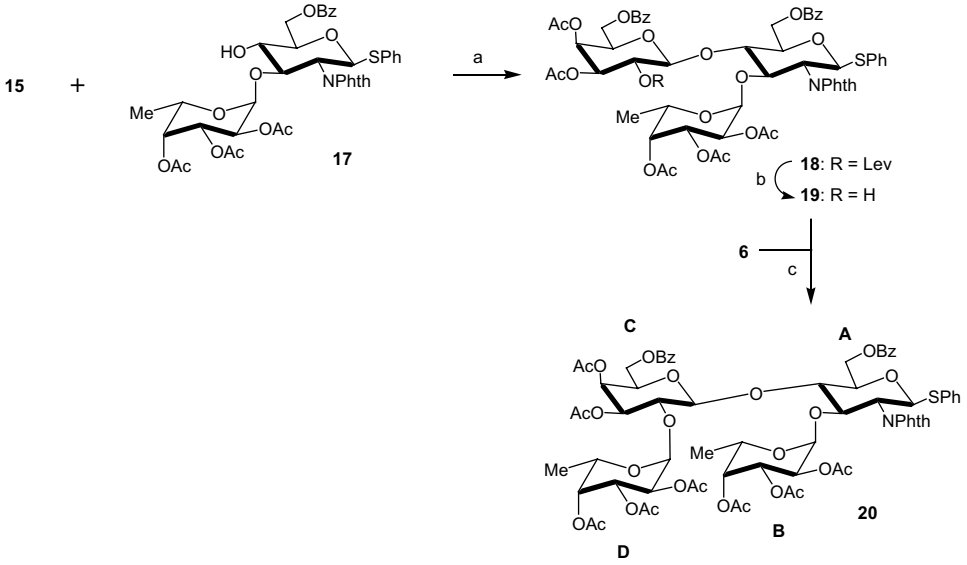
For the β-galactosylation of **9**, trichloroacetimidate **15** was prepared from phenylthio galactoside **10**[20] as depicted in Scheme 2. Compound **10** was regioselectively benzoylated to yield **11** and the 2-OH in **11** was then levulinated to afford **12**. The 3,4-*O*-isopropylidene acetal in **12** was then removed and the resulting diol directly acetylated to give **13**. Removal of the phenylthio group in **13** gave lactol **14** that was transformed in trichloroacetimidate **15**. Attempted glycosylation of **9** with 1.5 equiv of glycosyl donor **15** in the presence of TMSOTf did not lead to the formation of the expected β1→4 glycosidic linkage but to the isolation of a product whose ^1H NMR spectrum showed the absence of the signals corresponding to the acetamido group, the presence of a doublet at δ 3.86 ppm, which was attributed to a free 4-OH group and the presence of a doublet at 6.41 ppm (J = 3.0 Hz), which was assigned to the anomeric proton of a α-D-galactopyranosyl unit (H-1c). These data indicated that the NH group competed with the 4-OH group in **9** in the reaction conditions giving rise to the formation of imidate **16** as the major product. This undesired reaction may occur as a consequence of the well established lack of reactivity of the 4-OH group in *N*-acetyl-D-glucosamine derivatives in glycosylation



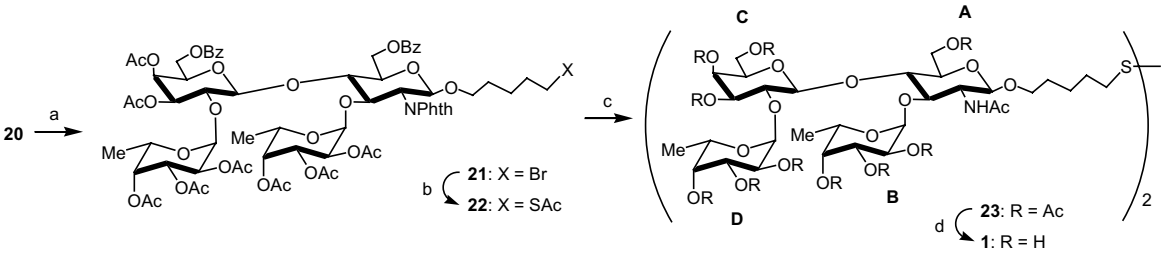
Scheme 2. Reagents and conditions: (a) BzCN, Et_3N (cat.), CH_3CN, −40 °C, 88%; (b) LevOH, DCC, DMAP, CH_2Cl_2, 80%; (c) TFA, CH_2Cl_2, H_2O, Ac_2O, Py, DMAP, 97%; (d) NBS, acetone, H_2O, −20 °C, 70%; (e) Cl_3CCN, DBU, CH_2Cl_2, 83%; (f) TMSOTf, CH_2Cl_2, 48%.

reactions, which has been attributed to steric factors[21] and to the formation of a deactivating hydrogen bond in which the amido group is involved.[22] The formation of glycosyl imidates as by-products in Koenigs–Knorr-type glycosylations has been known for many years[23] and it has also been postulated in halide-ion glycosylations.[12] In a recent paper[24] a similar result has been reported in the attempted glycosylation of the 4-OH group of a protected *N*-acetyl glucosamine derivative with a disarmed α-L-rhamnopyranosyl trichloroacetimidate. In this case the corresponding α-L-rhamnopyranosyl imidate was the major reaction product, which could be isolated in 42% yield from the reaction mixture and fully characterized.[24] This α-L-rhamnopyranosyl imidate rearranged in the glycosylation conditions to give the desired 4-*O*-glycosylated product in 50% yield and the authors have proposed that in the case of *N*-acetylglucosamine glycosyl acceptors these imidates are the kinetic products of the glycosylation reaction, which may or may not rearrange to the thermodynamic glycosides depending on the structure and on the experimental conditions.[24] In our case, product **16** did not rearrange to the desired trisaccharide under prolonged treatment in the glycosylation conditions and the synthetic plan had to be modified.

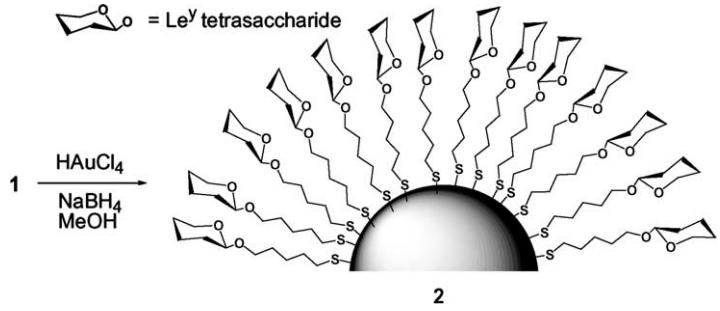
Thus, we decided to use the same synthetic route previously employed in our laboratory for the preparation of a Lewis^x neoglycoconjugate starting from disaccharide **17**, which carries a phthalimido instead of an acetamido group.[17] In this strategy the β-oriented thiopentyl spacer has to be introduced in a later stage of the process. In our synthesis of the Le^x neoglycoconjugate the 4-OH group in **17** reacted sluggishly with peracetylated-D-galactopyranosyl trichloroacetimidate but a final yield of 80% of the 4-*O*-galactosylated derivative could be obtained using a considerable excess (6 equiv) of glycosyl donor. Using 2.5 equiv of the more elaborated donor **15**, trisaccharide **18** was obtained in 37% yield and unreacted **17** could be isolated and reused (Scheme 3). Compound **18** was delevulinated to give **19**, which was again fucosylated with **6**[19] to afford tetrasaccharide **20** in 49%



Scheme 3. Reagents and conditions: (a) TMSOTf, Et_2O, 37% + recovered **17**; (b) hydrazine acetate, CH_2Cl_2, 89%; (c) TMS–I, CH_2Cl_2, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 40 °C, MeOH, Ac_2O, Py, DMAP, 49% + 48% of recovered **19**.



Scheme 4. Reagents and conditions: (a) 5-bromopentanol, TfOH, NIS, CH_2Cl_2, 86%; (b) KSAc, NBu_4I, butanone, 60 °C, 93%; (c) ethylenediamine, 2-butanol, 90 °C, Ac_2O, Py, DMAP, 82%; (d) MeONa, CH_2Cl_2/MeOH, 82%.



Scheme 5. Preparation of Le^y-functionalized gold glyconanoparticles **2**.

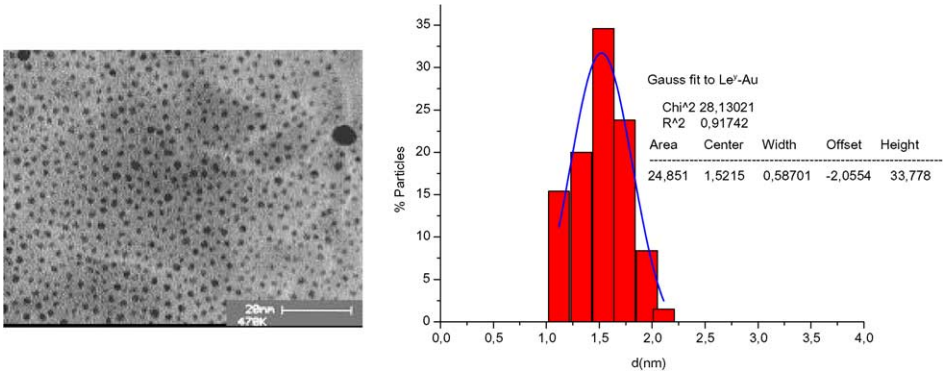


Figure 2. TEM and size distribution histogram of glyconanoparticles **2**.

yield after acetylation. From the glycosylation mixture 48% of the unreacted acceptor **19** could also be isolated and reused. The spacer group was then introduced by reaction of **20** with 5-bromopentanol in the presence of NIS and triflic acid[25] to give **21** in 86% yield (Scheme 4). The bromide group in **21** was replaced by a thioacetate group and the phthalimido and acyl groups in **22** were efficiently removed by treatment with ethylene diamine in 2-butanol.[26] After reacetylation compound **23** was obtained in 82% overall yield. Deacetylation of **23** finally afforded disulfide **1**.

The preparation of the Le^y glyconanoparticles **2** was carried out using a modification[1] of the procedure first reported by Brust et al.[6] by reduction with $NaBH_4$ of a mixture of Le^y neoglycoconjugate **1** and tetrachloroauric acid (Scheme 5). It was purified by centrifugal filtering and characterized by ^1H NMR and TEM (Fig. 2). The mean diameter was 1.52 nm, which corresponds to an average number of 116–140 gold atoms.[27] As previously prepared glyconanoparticles,[1] this material is a water soluble and very stable polyvalent system, which is being used for a series of studies, which will be disclosed in due time.

3. Experimental

3.1. General procedures

Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminum plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9) or with anisaldehyde solution (anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL), and acetic acid (1mL))

followed by heating at over 200 °C. Column chromatography was carried out on silica gel 60 (0.2–0.5, 0.2–0.063, or 0.040–0.015 mm; Merck). Optical rotations were determined with a Perkin–Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were acquired on Bruker DPX-300, DRX-400, and DRX-500 spectrometers and chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal reference or relative to D_2O. Fast atom bombardment (FAB) mass spectra were carried out by the Mass Spectrometry Service, Facultad Química, Seville, with a Kratos MS-80 RFA spectrometer. MALDI-TOF mass spectra were recorded by the Mass Spectrometry Laboratory, SidI, Universidad Autónoma Madrid, with a 4700 Applied Biosystems spectrometer.

3.2. 4-Pentenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside 3

To a solution of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (4.3 g, 11 mmol) in dry CH_2Cl_2 (71 mL), TMSOTf (2.2 mL, 12 mmol) was added and stirred at 40 °C for 72 h. Then 4-pentenol (3.4 mL, 33 mmol) was added and stirred at that temperature for an additional 3 h. The suspension was neutralized with Et_3N, filtered, and concentrated to dryness. The residue was purified by flash chromatography (hexane/AcOEt 1:2) to yield **3** (3.8 g, 83%). TLC 0.25 (hexane/AcOEt 1:2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.75 (m, 1H, –*CH*=CH_2); 5.61 (d, 1H, J = 8.6 Hz, –NH–); 5.27 (t, 1H, J = 9.9 Hz, H-3); 5.03 (t, 1H, J = 9.6 Hz, H-4); 4.98–4.92 (m, 2H, –CH=*CH*$_2$); 4.64 (d, 1H, J = 8.3 Hz, H-1); 4.22 (dd, 1H, J = 4.8, 12.2 Hz, H-6a); 4.09 (dd, 1H, J = 2.3, 12.2 Hz, H-6b); 3.86–3.77 (m, 2H, H-2, –CH_2–O–); 3.67 (m, 1H, H-5); 3.47 (m, 1H, –CH_2–O–); 2.17 (m, 4H, J = 7.5 Hz, –$(CH_2)_2$–); 2.04, 1.99, 1.98, 1.91 (4s, 12H, –$COCH_3$); 2.05–1.59 (m, 4H, –$(CH_2)_2$–). FAB MS: *m*/*z* calcd for $C_{19}H_{30}NO_9$: 416.1921; found: 416.1923 $[M+H]^+$.

3.3. 4-Pentenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 4

To a solution of **3** (3.8 g, 9.1 mmol) in MeOH (10 mL), MeONa (3 mL of a 1 N solution of MeONa in MeOH) was added. After stirring for 3 h at room temperature, the reaction was neutralized with Amberlite IR-120H$^+$. The mixture was filtered and concentrated to yield **4** (2.6 g, 98%). TLC 0.25 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (500 MHz, MeOD): δ 5.78 (m, 1H, –*CH*=CH_2); 4.99–4.83 (m, 2H, –CH=*CH*$_2$); 4.35 (d, 1H, J = 8.4 Hz, H-1); 3.88–3.82 (m, 2H, H-6a, –CH_2–O–); 3.67–3.58 (m, 2H, H-2, H-6b); 3.46–3.42 (m, 2H, H-4, –CH_2–O–); 3.30–3.26 (m, 2H, H-3, H-5); 2.07–1.94 (m, 4H, –$(CH_2)_2$–); 1.94 (s, 3H, –$COCH_3$). FAB MS: *m*/*z* calcd for $C_{13}H_{24}NO_6$: 290.1603; found: 290.1600 $[M+H]^+$.

3.4. 4-Pentenyl 2-acetamido-4,6-di-*O*-benzylidene-2-deoxy-β-D-glucopyranoside 5

To a solution of **4** (2.6 g, 9 mmol) in dry acetonitrile (80 mL), benzaldehyde dimethyl acetal (2.5 mL, 16.7 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added and was stirred at 45 °C for 2 h. Et_3N was added until neutral medium. The solution was concentrated and flash chromatography (toluene/acetone 2:1 → 0:1) of the residue gave **5** (2.2 g, 67%). TLC 0.21 (toluene/acetone 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51–7.34 (m, 5H, Ph); 5.74–5.58 (m, 2H, –*CH*=CH_2, –NH–); 5.55 (s, 1H, Ph–CH–); 5.06–4.98 (m, 2H, –CH=*CH*$_2$); 4.71 (d, 1H, J = 8.2 Hz, H-1); 4.34 (dd, 1H, J = 4.7, 10.5 Hz, H-6a); 4.16 (m, 1H, H-3); 3.93–3.75 (m, 2H, H-6b, –CH_2–O–); 3.59–3.40 (m, 4H, H-2, H-4, H-5, –CH_2–O–); 2.16–2.09 (m, 4H, –$(CH_2)_2$–); 2.04 (s, 3H, –$COCH_3$). FAB MS: *m*/*z* calcd for $C_{20}H_{27}NO_6$: 378.1916; found: 378.1910 $[M+H]^+$.

3.5. 4-Pentenyl *O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzylidene-2-deoxy-β-D-glucopyranoside 7

To a solution of **6**[19] (6.2 g, 13.7 mmol) in dry CH_2Cl_2 (43 mL), iodotrimethylsilane (TMS–I) (2 mL, 13.7 mmol) was added at room temperature and stirred for 20 min. The reaction mixture was then added to a solution of **5** (1.96 g, 5.2 mmol) and 2,6-di-*tert*-butylpyridine (3.1 mL, 13.7 mL) in dry CH_2Cl_2 (86 mL) and stirred for 5 h at room temperature. MeOH (130 mL) was added to the reaction mixture, which was stirred for 1 h. The mixture was neutralized with Et_3N and concentrated to give a syrup. Acetic anhydride (10 mL) and pyridine (20 mL) were added to the residue. The mixture was stirred at room temperature overnight and then diluted with CH_2Cl_2 (300 mL) and washed successively with cold 5% hydrochloric acid and H_2O, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (toluene/acetone 4:1) of the residue yielded **7** (2.2 g, 65%). TLC 0.15 (hexane/AcOEt 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45–7.28 (m, 5H, Ph); 5.76 (m, 2H, –*CH*=CH_2); 5.52 (d, 1H, J = 8.4 Hz, –NH–); 5.48 (s, 1H, Ph–CH–); 5.32–4.90 (m, 6H, H-1′, H-2′, H-3′, H-4′, CH=*CH*$_2$); 4.73 (d, 1H, J = 8.2 Hz, H-1); 4.34–4.26 (m, 3H, H-3, H-6a, H-5′); 3.84–3.41 (m, 5H, H-2, H-4, H-5, H-6b, –CH_2–O–); 2.08, 2.05, 1.94, 1.93 (4s, 12H, –$COCH_3$); 2.10–1.59 (m, 4H, –$(CH_2)_2$–); 0.48 (d, 3H, J = 6.4 Hz, H-6′).

3.6. 4-Pentenyl *O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside 8

To a solution of **7** (1 g, 1.54 mmol) in CH_2Cl_2 (30 mL), a mixture (4:1) of trifluoroacetic acid/H_2O (2.5 mL) was added. After stirring for 1 h at room temperature CH_2Cl_2 (100 mL) was added and the mixture was washed successively with aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography (toluene/acetone 2:1 → 1:1) of the residue yielded **8** (845 mg, 98%). TLC 0.10 (toluene/acetone 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.96 (d, 1H, J = 8.4 Hz, –NH–); 5.81–5.68 (m, 1H, –*CH*=CH_2); 5.38–5.05 (m, 4H, H-1′, H-2′, H-3′, H-4′); 5.04–4.90 (m, 2H, –CH=*CH*$_2$); 4.74 (d, 1H, J = 8.3 Hz, H-1); 4.54 (m, 1H, J = 6.8 Hz, H-5′); 4.27–3.22 (m, 8H, H-2, H-3, H-4, H-5, H-6a, H-6b, –CH_2–O–); 2.13, 2.08, 1.97, 1.94 (4s, 12H, –$COCH_3$); 2.16–1.55 (m, 4H, –$(CH_2)_2$–); 1.10 (d, 3H, J = 6.8 Hz, H-6′). FAB MS: *m*/*z* calcd for $C_{25}H_{40}NO_{13}$: 562.2500; found: 562.2496 $[M+H]^+$.

3.7. 4-Pentenyl *O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside 9

To a solution of **8** (1.1 g, 1.96 mmol) in dry CH_3CN (40 mL) at −40 °C, BzCN (270 mg, 2.06 mmol) and few drops of Et_3N were added. After 30 min, MeOH was added and the mixture was allowed to reach room temperature. The solvent was evaporated and the residue was dissolved in MeOH and concentrated to dryness. The residue was purified by flash chromatography (toluene/acetone 4:1 → 2:1), to yield **9** (1.1 g, 85%). TLC 0.40 (toluene/acetone 2:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.33 (m, 5H, Ph); 5.83–5.66 (m, 2H, –*CH*═CH_2, –NH–); 5.36–5.23 (m, 3H, H-1′, H-3′, H-4′); 5.07 (dd, 1H, J = 3.5 Hz, 11.0 Hz, H-2′); 4.98–4.85 (m, 2H, –CH═*CH*$_2$); 4.75 (d, 1H, J = 8.4 Hz, H-1); 4.63 (dd, 1H, J = 5.0, 12.3 Hz, H-6a); 4.56–4.51 (m, 2H, H-6b, H-5′); 4.13 (t, 1H, J = 9.2 Hz, H-3); 3.96 (m, 1H, OH-4); 3.86 (m, 1H, –CH_2–O–); 3.60 (m, 1H, H-5); 3.52–3.32 (m, 3H, H-2, H-4, –CH_2–O–); 2.11, 2.06, 1.94, 1.93 (4s, 12H, –$COCH_3$); 2.00–1.60 (m, 4H, –$(CH_2)_2$–); 1.08 (d, 3H, J = 6.4 Hz, H-6′). FAB MS: *m*/*z* calcd for $C_{32}H_{44}NO_{14}$: 666.2762; found: 666.2757 $[M+H]^+$.

3.8. Phenyl 6-*O*-benzoyl-3,4-di-*O*-isopropylidene-1-thio-β-D-galactopyranoside 11

To a cooled (−40 °C) solution of phenyl 3,4-di-*O*-isopropylidene-1-thio-β-D-galactopyranoside **10** (4.20 g, 13.4 mmol) in dry CH_3CN (110 mL), BzCN (1.85 g, 14.1 mmol) and catalytic Et_3N were added. After 5 h, MeOH was added and the mixture was allowed to reach room temperature. The solvent was evaporated and the residue was dissolved in MeOH and concentrated to dryness. The purification was carried out by flash chromatography (1:1 hexane/AcOEt) to afford **11** (4.91 g, 88%). $[\alpha]_D^{20} = -29.4$ (c 0.7, $CHCl_3$). TLC 0.75 (1:3 hexane/AcOEt). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08–7.11 (m, 10H, Ph); 4.69–4.56 (m, 2H, H-6a, H-6b); 4.49 (d, 1H, J = 10.2 Hz, H-1); 4.28–4.11 (m, 3H, H-3, H-4, H-5); 3.63 (m, 1H, H-2); 2.51 (d, 1H, J = 2.4 Hz, O*H*); 1.48–1.36 (2s, 6H, $C(CH_3)_2$). FAB MS: *m*/*z* 439 $[M+Na]^+$.

3.9. Phenyl 6-*O*-benzoyl-3,4-di-*O*-isopropylidene-2-*O*-levulinyl-1-thio-β-D-galactopyranoside 12

A mixture of **11** (4.91 g, 11.8 mmol), levulinic acid (6.1 mL, 59.0 mmol), 1,3-dicyclohexylcarbodiimide (3.65 g, 17.7 mmol), and DMAP (100 mg) in CH_2Cl_2 (50 mL) was stirred overnight from −20 °C to room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ solution, brine, and water. The organic layer was dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel (3:1 hexane/AcOEt) to give product **12** (4.86 g, 80%). $[\alpha]_D^{20} = +18.2$ (c 0.85, $CHCl_3$). TLC 0.78 (1:3 hexane/AcOEt). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12–7.06 (m, 10H, Ph); 5.08 (dd, 1H, J = 10.0, 6.8 Hz, H-2); 4.72–4.55 (m, 3H, H-1, H-6a, H-6b); 4.30–4.11 (m, 3H, H-3, H-4, H-5); 2.85–2.66 (m, 4H, OCO(CH_2)$_2$); 2.20 (m, 3H, $COCH_3$); 1.55–1.36 (2s, 6H, $C(CH_3)_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 206.2, 171.6, 166.3, 133.7–127.7 (Ph), 111.0, 86.0, 74.4, 73.6, 71.6, 64.1, 38.0, 30.0, 28.1, 27.6, 26.4. MALDI-TOF MS: *m*/*z* calcd for $C_{27}H_{30}SO_8Na$: 537.1554; found: 537.1574 $[M+Na]^+$.

3.10. Phenyl 3,4-di-*O*-acetyl-6-*O*-benzoyl-2-*O*-levulinyl-1-thio-β-D-galactopyranoside 13

To a solution of **12** (4.86 g, 9.4 mmol) in CH_2Cl_2 (160 mL), trifluoroacetic acid (13.9 mL, 0.18 mol), and water (3.2 mL, 0.18 mol) were added. After stirring for 1 h, the mixture was neutralized with solid $NaHCO_3$, diluted with CH_2Cl_2 (250 mL), washed with H_2O (250 mL), dried ($MgSO_4$), and concentrated to dryness. The residue was dissolved in Py (8 mL) at 0 °C. Acetic anhydride (4 mL) and DMAP (50 mg) were added and the mixture was stirred for 2 h at room temperature. The solution was diluted with CH_2Cl_2 (150 mL) and washed with 1 M HCl solution and H_2O. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to afford **13** (5.10 g, 97%): $[\alpha]_D^{20} = +1.6$ (c 1.45, $CHCl_3$). TLC 0.68 (1:3 hexane/AcOEt). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01–7.16 (m, 10H, Ph); 5.52 (br d, 1H, H-4); 5.30 (dd, 1H, H-2); 5.13 (dd, 1H, J = 9.9, 3.3 Hz, H-3); 4.77 (d, 1H, J = 9.9 Hz, H-1); 4.50 (dd, 1H, J = 7.5, 11.4 Hz, H-6a); 4.33 (dd, 1H, J = 5.4 Hz, H-6b); 4.09 (m, 1H, H-5); 2.91–2.50 (m, 4H, OCO(CH_2)$_2$); 2.18–2.04 (3s, 9H, OCOCH_3 and $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.9, 171.4, 170.3, 170.2, 166.0, 133.4–128.1 (Ph), 86.8, 74.7, 71.7, 67.6, 67.5, 62.3, 37.7, 29.7, 27.9, 20.7, 20.6. MALDI-TOF MS: *m*/*z* calcd for $C_{28}H_{30}SO_{10}Na$: 581.1452; found: 581.1443 $[M+Na]^+$.

3.11. 3,4-Di-*O*-acetyl-6-*O*-benzoyl-2-*O*-levulinyl-α,β-D-galactopyranose 14

To a solution of **13** (4.31 g, 7.72 mmol) in acetone (170 mL), NBS (1.84 g, 10.3 mmol), and water (185 μL, 10.3 mmol) were added at −20 °C. The reaction mixture was stirred under exclusion of light for 2 h, then quenched with saturated $NaHCO_3$ solution, diluted with AcOEt, and washed with water. The organic layer was dried over $MgSO_4$ and concentrated to dryness. The residue was purified by column chromatography on silica gel (1:1 toluene/AcOEt) to give product **14** (2.53 g, 70%). TLC 0.53 (1:3 hexane/AcOEt). ^{1}H NMR for the α anomer (300 MHz, $CDCl_3$): δ 8.00–7.39 (m, 5H, Ph); 5.57–5.46 (m, 3H, H-1, H-2, H-4); 5.17 (dd, 1H, J = 3.4, 10.6 Hz, H-3); 4.61 (m, 1H, H-5); 4.43 (dd, 1H, J = 6.5, 11.1 Hz, H-6a); 4.23 (dd, 1H, J = 7.2 Hz, H-6b); 3.94 (d, 1H, J = 3.3 Hz, OH); 2.77–2.54 (m, 4H, OCO(CH_2)$_2$); 2.16–2.01 (3s, 9H, OCOCH_3 and $COCH_3$). FAB MS: *m*/*z* 489 $[M+Na]^+$.

3.12. *O*-(3,4-Di-*O*-acetyl-6-*O*-benzoyl-2-*O*-levulinyl-α,β-D-galactopyranosyl)trichloroacetimidate 15

To a solution of **14** (2.69 g, 5.78 mmol) in dry CH_2Cl_2 (27 mL), Cl_3CCN (11 mL, 0.11 mol), and catalytic DBU were added. After stirring at room temperature

for 1 h, the mixture was concentrated in vacuo and the residue was purified by chromatography over a short silica gel column (3:2 hexane/AcOEt) to yield **15** (2.92 g, 83%) as a mixture of anomers. Data for the α anomer: TLC 0.26 (3:2 hexane/AcOEt). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (s, 1H, N*H*); 8.00–7.39 (m, 5H, Ph); 6.60 (d, 1H, *J* = 3.3 Hz, H-1); 5.66 (br d, 1H, H-4); 5.49 (dd, 1H, *J* = 3.0, 10.8 Hz, H-3); 5.42 (dd, 1H, H-2); 4.59 (m, 1H, H-5); 4.44 (dd, 1H, *J* = 6.9, 11.4 Hz, H-6a); 4.31 (dd, 1H, *J* = 5.7 Hz, H-6b); 2.73–2.46 (m, 4H, OCO(CH_2)$_2$); 2.18–2.05 (3s, 9H, OCOCH_3 and COCH_3). FAB MS: *m*/*z* 632 [M+Na]$^+$.

3.13. 4-Pentenyl 2-*N*-(3,4-di-*O*-acetyl-6-*O*-benzoyl-2-*O*-levulinyl-α-D-galactopyranosyl)acetimido-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside 16

To a mixture of **9** (140 mg, 0.21 mmol) and **15** (190 mg, 0.31 mmol) in dry CH_2Cl_2 (4 mL), TMSOTf (16 μL, 0.093 mmol) was added. After stirring for 6 h, the reaction was neutralized with Et_3N and concentrated to dryness. The residue was purified by flash chromatography (hexane/AcOEt 1:1) to yield **16** (112 mg, 48%). TLC 0.39 (1:1 hexane/AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06–7.38 (m, 10H, Ph); 6.41 (d, 1H, *J* = 3.0 Hz, H-1c); 5.65 (m, 1H, –*CH*=CH$_2$); 5.57 (d, 1H, *J* = 1.5 Hz, H-4c); 5.45–5.38 (m, 2H, H-2c, H-3c); 5.32–5.25 (m, 2H, H-3b, H-4b); 5.16 (d, 1H, *J* = 3.5 Hz, H-1b); 5.10 (dd, 1H, *J* = 10.5 Hz, H-2b); 4.90–4.80 (m, 2H, –CH=C*H*$_2$); 4.67–4.58 (m, 2H, H-6a, H-6′a); 4.47 (m, 1H, H-5b); 4.35 (m, 1H, H-5c); 4.29–4.19 (m, 3H, H-1a, H-6c, H-6′c); 3.86 (d, 1H, *J* = 2.5 Hz, OH); 3.77–3.68 (m, 2H, –CH$_2$–O–, H-3a); 3.58 (m, 1H, H-5a); 3.50 (m, 1H, H-4a); 3.39–3.30 (m, 2H, –CH$_2$–O–, H-2a); 2.77–2.51 (m, 4H, OCO(CH_2)$_2$); 2.15–1.94 (7s, 21H, –COCH_3, –N=C–CH_3); 2.01–1.52 (m, 4H, CH$_2$); 1.09 (d, 3H, *J* = 6.5 Hz, H-6b). FAB MS: *m*/*z* 1136 [M+Na]$^+$.

3.14. Phenyl 4-*O*-(3,4-di-*O*-acetyl-6-*O*-benzoyl-2-*O*-levulinoyl-β-D-galactopyranosyl)-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 18

To a mixture of **17**[17] (1.47 g, 1.89 mmol) and **15** (2.75 g, 4.5 mmol) in dry Et_2O (20 mL), TMSOTf (20 μL, 0.11 mmol) was added. After stirring for 3 h, the reaction was neutralized with Et_3N and concentrated to dryness. The residue was purified by flash chromatography (hexane/AcOEt 3:2), to yield **18** (850 mg, 37%). TLC 0.27 (hexane/AcOEt 1:1). $[\alpha]_D^{20} = -49.6$ (*c* 1, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–6.96 (m, 19H, Ph); 5.46 (d, 1H, *J* = 10.6 Hz, H-1a); 5.43 (d, 1H, *J* = 3.3 Hz, H-4c); 5.39 (d, 1H, *J* = 2.9 Hz, H-4b); 5.18 (dd, 1H, *J* = 3.3, 10.9 Hz, H-3b); 5.14 (dd, 1H, *J* = 8.3, 10.1 Hz, H-2c); 5.08 (m, 1H, *J* = 6.4 Hz, H-5b); 4.98–4.92 (m, 3H, H-1b, H-6a, H-3c); 4.85–4.74 (m, 3H, H-2b, H-3a, H-6c); 4.63–4.60 (m, 2H, H-1c, H-6′c); 4.37–4.33 (m, 2H, H-2a, H-6′a); 4.02 (t, 1H, *J* = 9.3 Hz, H-4a); 3.93 (m, 1H, H-5a); 3.78 (t, 1H, *J* = 6.9 Hz, H-5c); 2.78–2.54 (m, 4H, CO–CH$_2$–CH$_2$–CO); 2.16, 2.15, 2.03, 1.99, 1.92, 1.84 (6s, 18H, –COCH_3); 1.20 (d, 3H, *J* = 6.6 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.8, 171.0, 170.7, 170.4, 170.1, 169.8, 166.0, 165.7 (C=O); 134.4–123.8 (Ph); 100.8 (C-1c); 95.4 (C-1b); 83.8 (C-1a); 75.6, 72.4, 71.7, 71.4, 70.9, 69.1, 68.4, 67.6, 67.0, 64.3 (C-5b); 62.5 (C-6a); 61.0 (C-6c); 55.6, 37.7, 29.7, 27.8, 20.8, 20.7, 20.6, 20.5 (CO–CH$_2$–CH$_2$–CO, COCH_3); 15.9 (C-6b). FAB MS: *m*/*z* calcd for $C_{61}H_{63}NO_{24}S$: 1248.3358; found: 1248.3283 [M+Na]$^+$.

3.15. Phenyl 4-*O*-(3,4-di-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranosyl)-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 19

To a solution of **18** (730 mg, 0.59 mmol) in dry CH_2Cl_2 (30 mL), a 0.4 N solution of hydrazine acetate in MeOH (3 mL, 1.2 mmol) was added. After stirring for 2 h, acetyl acetone (0.2 mL) was added and the mixture concentrated to dryness. The residue was purified by flash chromatography (hexane/AcOEt 3:2), to yield **19** (600 mg, 89%). TLC 0.22 (hexane/AcOEt 1:1). $[\alpha]_D^{20} = -54.5$ (*c* 1, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–6.97 (m, 19H, Ph); 5.46–5.44 (m, 2H, H-1a, H-4c); 5.37 (d, 1H, *J* = 2.9 Hz, H-4b); 5.18 (dd, 1H, *J* = 3.3, 10.9 Hz, H-3b); 5.10 (m, 1H, *J* = 6.6 Hz, H-5b); 5.04 (d, 1H, *J* = 11.8 Hz, H-6a); 4.95 (d, 1H, *J* = 4.0 Hz, H-1b); 4.90–4.81 (m, 3H, H-2b, H-3a, H-3c); 4.72 (dd, 1H, *J* = 8.1, 11.5 Hz, H-6′a); 4.59 (dd, 1H, *J* = 6.2, 11.5 Hz, H-6c); 4.55 (d, 1H, *J* = 7.9 Hz, H-1c); 4.43 (dd, 1H, *J* = 4.9, 11.8 Hz, H-6′a); 4.37 (t, 1H, *J* = 10.7 Hz, H-2a); 4.04 (t, 1H, *J* = 9.5 Hz, H-4a); 3.95–3.92 (m, 1H, H-5a); 3.84 (t, 1H, *J* = 6.9 Hz, H-5c); 3.71 (t, 1H, *J* = 7.9 Hz, H-2c); 2.98 (br s, 1H, OH); 2.15, 2.03, 1.99, 1.92, 1.84 (5s, 15H, –COCH_3); 1.13 (d, 3H, *J* = 6.6 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.7, 170.4, 170.3, 169.7, 166.1, 166.0 (C=O); 134.6–123.8 (Ph); 103.0 (C-1c); 95.5 (C-1b); 83.7 (C-1a); 77.9 (C-5a); 75.8, 73.1, 72.7, 71.6, 71.2, 69.7, 68.3, 67.6, 67.1, 64.1 (C-5b); 62.8 (C-6a); 61.2 (C-6c); 55.5 (C-2a); 20.8, 20.7, 20.6, 20.5 (COCH_3); 16.2 (C-6b). MALDI-TOF MS: *m*/*z* calcd for $C_{56}H_{57}NO_{22}SNa$: 1150.2985; found: 1150.3020 [M+Na]$^+$.

3.16. Phenyl 4-*O*-[3,4-di-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-β-D-galactopyranosyl]-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 20

To a solution of **6**[19] (950 mg, 2.1 mmol) in dry CH_2Cl_2 (5 mL), TMS–I (300 μL, 2.1 mmol) was added at room temperature and stirred for 30 min. This solution was added to a solution of **19** (460 mg, 0.408 mmol) and 2,6-di-*tert*-butylpyridine (495 μL, 2.1 mmol) in dry CH_2Cl_2 (5 mL), and the resulting mixture overnight at 40 °C. MeOH (30 mL) was added and stirred for one more hour. The mixture was neutralized with Et_3N, diluted with CH_2Cl_2 (20 mL), and washed with H_2O (15 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers concentrated to dryness. The resulting mixture was passed through column chromatography (hexane/AcOEt 1:1) to recover unreacted acceptor **19** (220 mg, 48%). Elution

with acetone yielded the deprotected tetrasaccharide which, after solvent evaporation, was treated with Ac_2O (2 mL), pyridine (4 mL), and a catalytic amount of DMAP. After stirring for 3 h, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with HCl 1 N solution (2×15 mL) and H_2O (2×15 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and the mixture was purified by flash chromatography (1:1 hexane/AcOEt), to yield **20** (280 mg, 49%). TLC 0.30 (hexane/AcOEt 1:1). $[\alpha]_D^{20} = -63.6$ (*c* 0.33, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06–6.91 (m, 19H, Ph); 5.43–5.33 (m, 5H, H-1a, H-1d, H-4b, H-4c, H-4d); 5.29 (dd, 1H, $J = 3.1$, 11.0 Hz, H-3d); 5.17 (dd, 1H, $J = 3.1$, 10.9 Hz, H-3b); 5.07–4.99 (m, 4H, H-2d, H-3c, H-5b, H-6a); 4.92–4.80 (m, 3H, H-1b, H-2b, H-3a); 4.72 (dd, 1H, $J = 8.0$, 11.5 Hz, H-6c); 4.60–4.55 (m, 2H, H-5d, H-6′c); 4.37 (t, 1H, $J = 10.2$ Hz, H-2a); 4.17 (t, 1H, $J = 9.5$ Hz, H-4a); 3.86–3.79 (m, 3H, H-2c, H-5a, H-5c); 2.15, 2.14, 2.03, 1.97, 1.96, 1.95, 1.91, 1.84 (8s, 24H, –$COCH_3$); 1.31 (d, 3H, $J = 6.5$ Hz, H-6d); 1.20 (d, 3H, $J = 6.5$ Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.7, 170.5, 170.4, 170.0, 169.8, 169.5, 165.9, 165.6 (C=O); 134.6–123.8 (Ph); 100.6 (C-1c); 96.6 (C-1d); 95.9 (C-1b); 84.0 (C-1a); 77.2, 74.6 (C-4a); 73.3, 73.2, 71.4, 71.3, 68.3, 68.0, 67.4, 67.3, 67.0, 65.4, 64.3, 62.5, 60.9, 55.8 (C-2a); 20.8, 20.6, 20.5 ($COCH_3$); 16.0 (C-6b); 15.7 (C-6d). FAB MS: *m/z* calcd for $C_{68}H_{73}NO_{29}S$: 1422.3887; found: 1422.3776 $[M+Na]^+$.

3.17. 5-Bromo-pentyl 4-*O*-[3,4-di-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-β-D-galactopyranosyl]-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 21

To solution of **20** (50 mg, 36 μmol), 5-bromopentan-1-ol (100 mg, 0.6 mmol), NIS (35 mg, 0.159 mmol), and 4 Å molecular sieves in dry CH_2Cl_2 (1 mL) at −30 °C, TfOH (4 μL, 40 μmol) was added. The mixture was allowed to warm to room temperature in 2 h. Then, more NIS (35 mg, 0.159 mmol) and TfOH (4 μL, 40 μmol) were added and stirred for 2 h. The solution neutralized with Et_3N, diluted with CH_2Cl_2 (20 mL), and washed with saturated aqueous $Na_2S_2O_3$ (15 mL) and H_2O (15 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo, and the mixture was purified by flash chromatography (3:2 hexane/AcOEt), to yield **21** (45 mg, 86%). TLC 0.32 (hexane/AcOEt 1:1). $[\alpha]_D^{20} = -104.2$ (*c* 0.83, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08–7.24 (m, 14H, Ph); 5.43–5.35 (m, 4H, H-1d, H-4b, H-4c, H-4d); 5.24 (dd, 1H, $J = 3.2$, 11.0 Hz, H-3d); 5.20 (dd, 1H, $J = 3.2$, 11.0 Hz, H-3b); 5.08–4.96 (m, 4H, H-2d, H-3c, H-5b, H-6a); 5.00 (d, 1H, $J = 8.6$ Hz, H-1a); 4.97 (d, 1H, $J = 3.8$ Hz, H-1b); 4.84 (dd, 1H, $J = 3.8$, 11.0 Hz, H-2b); 4.80 (t, 1H, $J = 9.6$ Hz, H-3a); 4.72–4.67 (m, 2H, H-1c, H-6c); 4.61–4.56 (m, 2H, H-5d, H-6′c); 4.45 (dd, 1H, $J = 3.3$, 12.4 Hz, H-6′a); 4.31–4.25 (m, 2H, H-2a, H-4a); 3.84–3.75 (m, 4H, H-2c, H-5a, H-5c, 1CH_2–O); 3.35 (m, 1H, 1CH_2–O); 3.00 (m, 2H, CH_2–Br); 2.16, 2.14, 2.04, 1.98, 1.96, 1.95, 1.95, 1.85 (8s, 24H, –$COCH_3$); 1.57–1.05 (m, 6H, –CH_2–); 1.33 (d, 3H, $J = 6.5$ Hz, H-6d); 1.21 (d, 3H, $J = 6.5$ Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.7, 170.6, 170.4, 170.0, 169.8, 165.9, 165.6 (C=O); 134.5–123.6 (Ph); 100.4 (C-1c); 98.3 (C-1a); 96.5 (C-1d); 95.7 (C-1b); 74.4 (C-4a); 73.3, 73.1, 72.9, 72.2, 71.4, 71.3, 71.2, 69.5, 68.2, 67.9, 67.6, 67.5, 67.1, 65.3, 64.2, 62.1, 60.9, 56.7 (C-2a); 33.2 (CH_2Br); 32.2, 29.6, 28.3, 24.5, 20.8, 20.6, 20.5 (–CH_2–, $COCH_3$); 16.0 (C-6b); 15.7 (C-6d). MALDI-TOF MS: *m/z* calcd for $C_{67}H_{78}NO_{30}BrNa$: 1478.3684; found: 1478.3712 $[M+Na]^+$.

3.18. 5-Thioacetyl-pentyl 4-*O*-[3,4-di-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-β-D-galactopyranosyl]-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-6-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 22

A solution of **21** (225 mg, 0.168 mmol), KSAc (90 mg, 0.79 mmol), and a catalytic amount of NBu_4I in butanone (20 mL) was stirred for 3 h at 60 °C. The mixture was diluted with AcOEt (40 mL) and washed with H_2O (2×25 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo, and the mixture was purified by flash chromatography (1:1 hexane/AcOEt), to yield **22** (228 mg, 93%). TLC 0.30 (hexane/AcOEt 1:1). $[\alpha]_D^{20} = -87.1$ (*c* 0.75, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07–7.24 (m, 14H, Ph); 5.42–5.35 (m, 4H, H-1d, H-4b, H-4c, H-4d); 5.24 (dd, 1H, $J = 3.0$, 11.0 Hz, H-3d); 5.20 (dd, 1H, $J = 3.2$, 11.2 Hz, H-3b); 5.08–4.95 (m, 6H, H-1a, H-1b, H-2d, H-3c, H-5b, H-6a); 4.85–4.78 (m, 2H, H-2b, H-3a); 4.71–4.65 (m, 2H, H-1c, H-6c); 4.59–4.55 (m, 2H, H-5d, H-6′c); 4.44 (dd, 1H, $J = 3.2$, 12.2 Hz, H-6′a); 4.30–4.24 (m, 2H, H-2a, H-4a); 3.83–3.70 (m, 4H, H-2c, H-5a, H-5c, 1CH_2–O); 3.33 (m, 1H, 1CH_2–O); 2.49–2.37 (m, 2H, CH_2–SAc); 2.23, 2.16, 2.13, 2.03, 1.98, 1.96, 1.95, 1.95, 1.84 (9s, 27H, –$COCH_3$); 1.37–1.00 (m, 6H, –CH_2–); 1.33 (d, 3H, $J = 6.5$ Hz, H-6d); 1.21 (d, 3H, $J = 6.5$ Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.7, 170.8, 170.7, 170.6, 170.3, 170.0, 169.8, 165.8, 165.6 (C=O); 134.5–123.6 (Ph); 100.3 (C-1c); 98.4 (C-1a); 96.5 (C-1d); 95.7 (C-1b); 73.4 (C-4a); 73.3, 73.2, 72.9, 72.2, 71.4, 71.3, 71.2, 69.6, 68.2, 67.9, 67.6, 67.5, 67.1, 65.3, 64.2, 62.1, 60.9, 56.7 (C-2a); 30.6, 28.9, 28.7, 28.6, 25.0, 20.8, 20.6, 20.5 (–CH_2–, $COCH_3$); 16.0 (C-6b); 15.7 (C-6d). MALDI-TOF MS: *m/z* calcd for $C_{69}H_{81}NO_{31}SNa$: 1474.4406; found: 1474.4386 $[M+Na]^+$.

3.19. 5,5′-Dithio bis{pentyl 4-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranoside} 23

To a solution of **22** (40 mg, 27 μmol) in 2-butanol (4 mL), ethylenediamine (0.8 mL) was added and stirred overnight at 90 °C. The mixture was concentrated and coevaporated with MeOH three times and lyophilized. The yellow syrup was treated with Ac_2O (2 mL), pyridine (4 mL), and a catalytic amount of DMAP. After stirring for 5 h, the reaction was concentrated in vacuo and the mixture was purified by flash chromatography (AcOEt), to yield **23** (28 mg, 82%). TLC 0.30 (AcOEt). $[\alpha]_D^{20} = -123.8$ (*c* 1, CH_2Cl_2). ^{1}H NMR (500 MHz,

$CDCl_3$): δ 5.52 (d, 1H, J = 8.2 Hz, NH); 5.38–5.29 (m, 5H, H-1b, H-1d, H-4b, H-4c, H-4d); 5.16–5.10 (2dd, 1H, J = 3.1, 11.0 Hz, H-3b, H-3d); 5.00–4.89 (m, 4H, H-2b, H-2d, H-3c, H-5b); 4.67 (d, 1H, J = 8.2 Hz, H-1a); 4.58 (dd, 1H, J = 1.8, 12.1 Hz, H-6a); 4.48–4.39 (m, 3H, H-1c, H-5d, H-6c); 4.25–4.14 (m, 3H, H-3a, H-6′a, H-6′c); 3.86–3.70 (m, 4H, H-4a, H-2c, H-5c, $1CH_2$–O); 3.53–3.40 (m, 3H, H-2a, H-5a, $1CH_2$–O); 2.63 (t, 2H, J = 7.3 Hz, CH_2–SAc); 2.12, 2.11, 2.09, 2.04, 1.98, 1.95, 1.94, 1.93, (11s, 33H, –$COCH_3$); 1.69–1.31 (m, 6H, –CH_2–); 1.18–1.16 (2d, 6H, J = 6.6 Hz, H-6b, H-6d). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.6, 170.5, 170.4, 170.1, 169.9, 169.7 (C=O); 100.5, 99.9 (C-1b, C-1d); 96.3 (C-1a); 95.6 (C-1c); 73.4 (C-4a); 74.6, 74.0, 73.4, 73.0, 72.8, 71.4, 71.1, 70.9, 69.6, 68.3, 68.0, 67.8, 67.5, 66.9, 65.1, 64.0, 62.3, 60.8, 50.0, 38.7, 29.0, 28.7, 24.8, 23.5, 21.1, 20.9, 20.8, 20.7, 20.6, 20.6 (–CH_2–, $COCH_3$); 15.9, 15.7 (C-6b, C-6d). MALDI-TOF MS: m/z calcd for $C_{102}H_{148}N_2O_{58}S_2Na$: 2415.8027; found: 2415.8059 $[M+Na]^+$.

3.20. 5,5′-Dithio bis{pentyl 4-*O*-[2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-3-*O*-(α-L-fucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside} 1

To a solution of **23** (25 mg, 10 μmol) in CH_2Cl_2 (1 mL), a 0.1 N solution of MeONa in MeOH (2 mL, 0.2 mmol) was added and was stirred overnight. The mixture was neutralized with Amberlite IR-120H$^+$, filtrate, and concentrated in vacuo to yield **1** (28 mg, 82%). TLC 0.30 (MeOH). ^{1}H NMR (500 MHz, MeOD): δ 5.52 (d, 1H, J = 8.2 Hz, NH); 5.38–5.29 (m, 5H, H-1b, H-1d, H-4b, H-4c, H-4d); 5.16–5.10 (2dd, 1H, J = 3.1, 11.0 Hz, H-3b, H-3d); 5.00–4.89 (m, 4H, H-2b, H-2d, H-3c, H-5b); 4.67 (d, 1H, J = 8.2 Hz, H-1a); 4.58 (dd, 1H, J = 1.8 Hz, 12.1 Hz, H-6a); 4.48–4.39 (m, 3H, H-1c, H-5d, H-6c); 4.25–4.14 (m, 3H, H-3a, H-6′a, H-6′c); 3.86–3.70 (m, 4H, H-4a, H-2c, H-5c, $1CH_2$–O); 3.53–3.40 (m, 3H, H-2a, H-5a, $1CH_2$–O); 2.63 (t, 2H, J = 7.3 Hz, CH_2-SAc); 2.12, 2.11, 2.09, 2.04, 1.98, 1.95, 1.94, 1.93, (11s, 33H, –$COCH_3$); 1.69–1.31 (m, 6H, –CH_2–); 1.18–1.16 (2d, 6H, J = 6.6 Hz, H-6b, H-6d). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.6, 170.5, 170.4, 170.1, 169.9, 169.7 (C=O); 100.5, 99.9 (C-1b, C-1d); 96.3 (C-1a); 95.6 (C-1c); 73.4 (C-4a); 74.6, 74.0, 73.4, 73.0, 72.8, 71.4, 71.1, 70.9, 69.6, 68.3, 68.0, 67.8, 67.5, 66.9, 65.1, 64.0, 62.3, 60.8, 50.0, 38.7, 29.0, 28.7, 24.8, 23.5, 21.1, 20.9, 20.8, 20.7, 20.6, 20.6 (–CH_2–, $COCH_3$); 15.9, 15.7 (C-6b, C-6d). MALDI-TOF MS: m/z calcd for $C_{62}H_{108}N_2O_{38}S_2Na$: 1575.5914; found: 1575.5933 $[M+Na]^+$.

3.21. Le^y-functionalized gold glyconanoparticle 2

To a solution of **1** (13 mg, 17 μmol) in MeOH (1.4 mL), an aqueous 0.025 M solution of $HAuCl_4$ (120 μL) was added. Then, aqueous 1 N solution of $NaBH_4$ (90 μL) was added in several portions with rapid shaking. The black suspension formed was shaken for an additional 2 h and the methanolic layer was separated by decantation. The black solid was dissolved in water (300 μL) and purified by centrifugal filtering (AMICON MW 30,000, 15 min, 14000 rpm). The process was repeated twice, until the nanoparticles were free of salts and starting materials. The residue in the AMICON filter was dissolved in 500 μL of water and lyophilized to afford 1.8 mg of Le^y-functionalized gold glyconanoparticle **2**. ^{1}H NMR (500 MHz, D_2O): 5.24–5.09 (2 br s, 2H, H-1b, H-1d); 4.87 (br s, 1H, H-5 b or d); 4.50 (br s, 2H, H-1a, H-1c); 4.24 (br s, 1H, H-5 b or d); 2.02 (m, 6H, –$COCH_3$). TEM: average diameter 1.52 nm, 116–140 gold atoms.[27]

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