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Synthesis of gold nanoparticles bearing the Thomsen–Friedenreich disaccharide: a new multivalent presentation of an important tumor antigen

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Abstract—Herein we describe the synthesis gold nanoshells encapsulated with up to 90 units of the Thomsen–Friedenreich (TF) tumor-associated carbohydrate antigen (TACA) disaccharide (Galβ1-3GalNAc-α-O-Ser/Thr) as well as the assembly of a suitably linked designer glycopeptide as a precursor to similar multivalent presentations on gold. The TF-coated nanoparticles are highly stable, water soluble, and easily handled. Improvements in the linker technology used to attach the disaccharide to the particles led to a robust multivalent platform for the presentation of this important carbohydrate. The antigen retains all recognition characteristics while displayed on this template as shown by several in vitro assays. This area of research could lead to the development of novel therapeutic agents that inhibit protein–carbohydrate interactions.
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1. Introduction

The Thomsen–Friedenreich disaccharide is a human tumor-associated carbohydrate antigen[1] present in about 90% of carcinomas but is rarely expressed in normal tissues.[2] Presentation of this antigen derives primarily from the truncation of O-linked glycans of the normal phenotype that are attached to the extracellular domain of cell surface (often mucin) proteins. The presence of smaller O-linked glycans on surface of carcinomas cell serves to reveal underlying peptide (and other) epitopes that would be masked in normal tissue. Not only is a powerful immune response mounted against these 'non-self' antigens on tumors, the TF antigen is also used as a diagnostic marker for preclinical tumor detection.[2] These properties have prompted several groups to develop immunotherapies and vaccine constructs based on the TF structure as well as its immediate precursor, TN antigen (GalNAcα-O-Ser/Thr).[3,4] In addition, the unmasking of peptide epitopes suggests that the synthesis of glycopeptide-based immunogens would be an important strategy to raise strong immune responses that more closely resemble the actual components presented in vivo.[5–7]

Recent studies by the Glinsky group have unequivocally proved the involvement of tumor cell TF structures in invasion and metastasis of breast and prostate tumors.[8] In particular, docking of tumors bearing TF structures to the microvascular endothelium is mediated by the β-galactosyl-binding protein galectin-3. These interactions are proposed to cause the critical heterotypic adhesion and homotypic aggregation of tumor cells that are important steps in the metastatic cascade as well as being required for cell survival and growth.[9–11] Both the adhesion events and cell growth are inhibited by TF function-blocking or anti-galectin-3 antibodies. Based on these results, the TF disaccharide and surrounding in vivo peptide sequences are prime candidates for the development of inhibitors of tumor cell growth and metastasis.[12]

The well known cluster glycoside effect[13] has spawned the construction of myriad multivalent glycopolymers for the study of carbohydrate–protein interactions and

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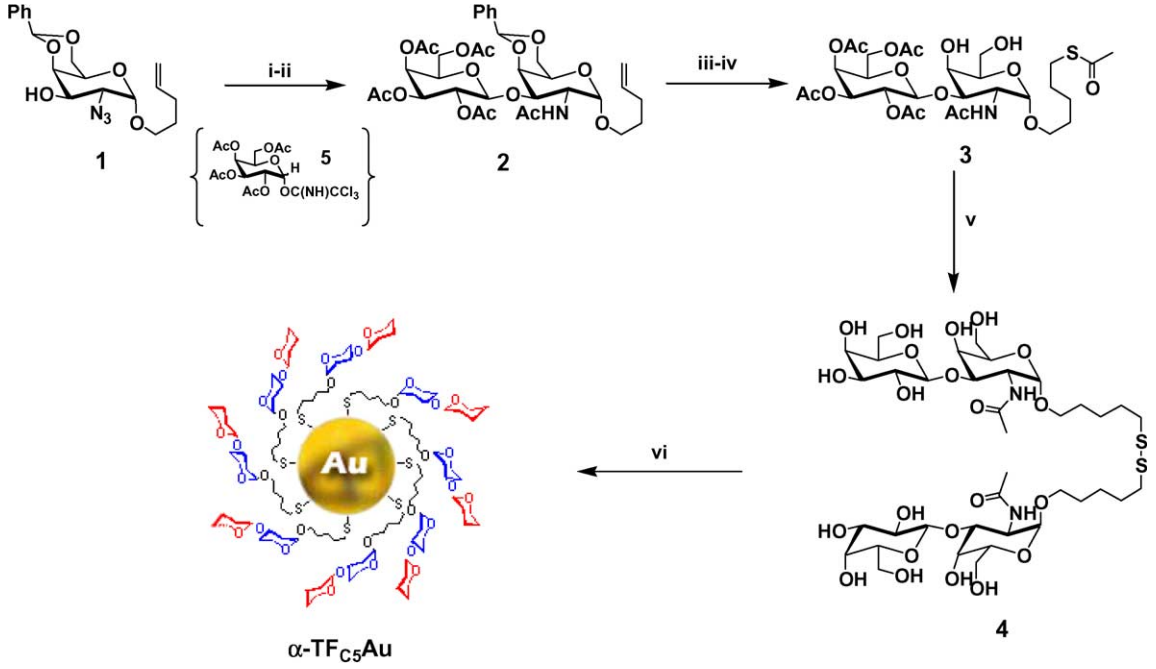
the development of infinitely more potent inhibitors of these processes as compared to monomeric sugars.[14,15] Since clustering of galectins while binding cellular sugar ligands seems to be critical for their function,[10] multivalent templates displaying galectin ligands could be extremely useful tools for unraveling separate biochemical functions. We have recently been interested in developing new multivalent systems to display TF antigen.[16,17] Applying the concept of 'glyconanotechnology' as originally put forth by the group of Penades and co-workers[18–21] and others,[22–25] we prepared 3-dimensional self assembled monolayers (3D-SAMs) of gold atoms stabilized by appropriately linked TF molecules. These assemblies present the antigen in a manner that is recognized by both lectins and antibodies, and they show marked antimetastatic activity in vivo. We have also improved on our synthesis of TF–glycoamino acid building blocks and applied these to the synthesis of a designer glycopeptide with a linker compatible with producing clusters of glycopeptides on 3D SAMs. This report describes the synthesis and some of the properties of these systems.

2. Results and discussion

2.1. Synthesis and evaluation of TF–AU particles

We have previously reported the synthesis of pentenyl glycosides from the 2-azido-1-nitrate of 3,4,6-triacetyl galactopyranose that were transformed to protected TF disaccharide analogues and TF glycoamino acids through simple and high-yielding protocols.[26] Scheme 1 briefly depicts the key intermediates involved and shows the transformation of the pentenyl group to a precursor for the synthesis of the gold nanoparticles. Intermediate **1** was treated as previously described (glycosylation with trichloroacetimidate **5**[27] and azide reduction) to give **2**.[26] Reaction of **2** with thiolacetic acid in the presence of AIBN smoothly and quantitatively gave thioacetate **3**, which upon removal of the protecting groups afforded disulfide dimer **4** as a precursor to nanoparticle synthesis.

Self-assembly to TF-coated gold particles (**α-TF$_{C5}$Au**; α- refers to the stereochemistry of the reducing end and C5 to the pentane linker) was carried out by slight modifications to now well documented procedures for the preparation of water soluble carbohydrate-encapsulated gold nanoparticles.[21–24] Briefly, a solution of disulfide (or corresponding monomeric thiol) and tetrachloroauric acid ($HAuCl_4$) in water is treated at 0 °C with an excess of $NaBH_4$ and stirred for a short time (1–2 h). Purification is performed by ultrafiltration or centrifugation with methanol (gold SAMs are insoluble in MeOH but freely soluble in water). Figure 1A shows a comparison of the ^{1}H NMR spectrum of **α-TF$_{C5}$Au** with the dimer TF-linked thiol (prepared by DTT treatment of disulfide **4**). All the features of the monomeric sugar are present in **α-TF$_{C5}$Au** but exhibit T_2 broadening due to the size of the particle and clustering of the sugars. The UV spectrum of **α-TF$_{C5}$Au** in Figure 1B shows the characteristic plasmon band at 520 nm and selected transmission electron micrographs (TEM) that reveal that the size of the particles is between 1 and 2 nm are shown in Figure 1C. Particles can be fine tuned to various sizes from 1 to 12 nm depending on reaction conditions (reductant, temperature, time). Similar transformations from **1** led to TN-coated gold nanoparticles (data not shown, to be published elsewhere).



Scheme 1. Reagents and conditions: (i) **7**, TMSOTf/CH_2Cl_2/molecular sieves, 90%; (ii) Zn, AcOH, Ac_2O, THF, 90%; (iii) 80% AcOH, 83%; (iv) AcSH/AIBN/1,4-dioxane, quant.; (v) cat NaOMe/MeOH, 97%; (vi) $NaBH_4$, $HAuCl_4$, H_2O, 0 °C.

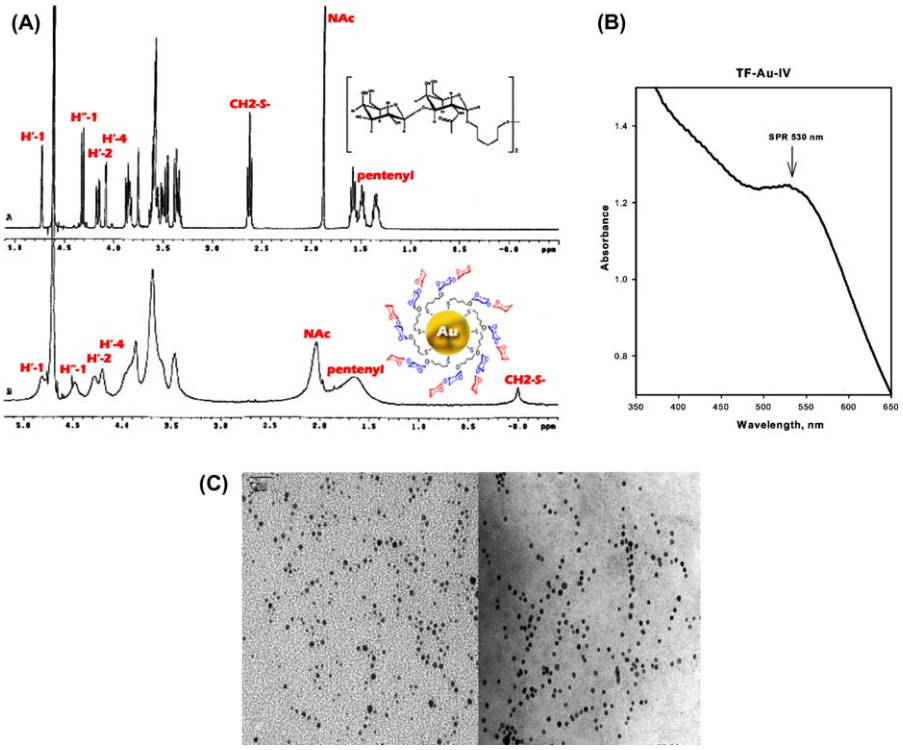


Figure 1. (A) Comparison of the ^{1}H NMR spectra of compound **4** and **α-TF$_{C5}$Au**. Details are observable for most protons in the spectrum of **α-TF$_{C5}$Au**. (B) UV spectrum of **α-TF$_{C5}$Au** showing the characteristic plasmon band at 520 nm. (C) Transmission electron micrographs of **α-TF$_{C5}$Au** showing the uniform size and dispersion of the particles.

2.2. Refinement of the linker

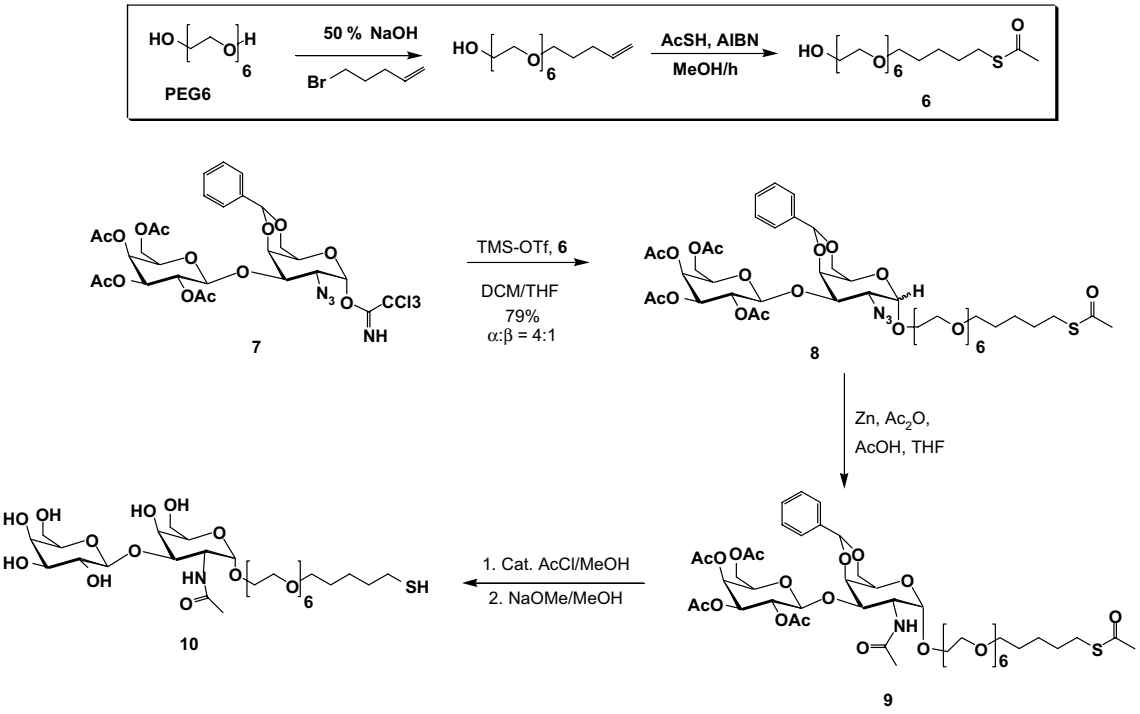
Although various tests (vide infra) confirmed the functionality and recognition of the antigen in the pentanethiol-linked particles, we reasoned that a simple pentyl chain may not be the optimum linker for future biological studies. In addition, there is evidence that the linker can play a role in the increased potency observed with specific multivalent carbohydrate templates.[28] Hence we prepared other particles based on a synthetic linker that retained the C5 chain and added a polyethylene glycol (PEG) spacer between this chain and the antigen. PEGylation of peptides, proteins, and orally available drugs can impart highly desirable effects such as longer circulating half lives, protection from immunogenicity, and increased water solubility.[29–31] Following the work of Palegrosdemang et al.,[32] we prepared a *hexa*-PEG (PEG6) precursor **6** armed with a masked thiol on one end and a free acceptor hydroxyl group at the other (Scheme 2). Glycosylation of **6** with the known trichloroimidate **7** afforded a 79% yield of a separable α/β (4:1) mixture of the glycoconjugate **8**, which was processed as before through **9** to the thiol **10** in high overall yield. Gold particles were assembled with both anomers of this ligand identically as for **α-TF$_{C5}$Au**. These particles (**α-TF$_{PEG6}$Au**, **βTF$_{PEG6}$Au**) were extremely stable for months at room temperature. All further studies were performed with PEG6-linked particles.

2.3. In vitro functional assays

Several assays were performed that validated the functional nature of the antigen on the particles. These included lectin affinity chromatography (LAC), agglutination of rhodamine-labeled peanut agglutinin (PNA), and aggregation of a TF-specific antibody[33] (see Experimental section for details). The TF-coated particles were retained on agarose-bound PNA affinity gels but did not interact with agarose-bound *Pisum sativum* agglutinin (PSA), an α-mannose-specific lectin. Either **α-TF$_{C5}$Au** or **α-TF$_{PEG6}$Au** could be eluted from the gel with competing amounts of galactose. As a testament to the recognition capacity and specificity of PNA to the antigen on the particles, the **β-TF$_{PEG6}$Au** (vide supra) did not bind to the column, confirming the requirement of the natural α-stereochemistry at the reducing end of the antigen for interaction with the protein (Fig. 2A). Strong agglutination of rhodamine-labeled PNA was observed by confocal microscopy after treatment with **α-TF$_{PEG6}$Au** whereas no change was observed when rhodamine-labeled PSA was used in the same assay (Fig. 2B). These results were confirmed by agglutination of the divalent monoclonal IgG(3) antibody F11 with the **α-TF$_{PEG6}$Au** (data not shown). We have performed both anti-tumor and anti-metastatic bioassays with **TF$_{PEG6}$Au** and have shown that these particles have both in vitro activity against tumor growth at specific doses and almost completely inhibit lung metastasis in vivo against an implanted metastatic breast cancer cell line (Heimburg, Barchi, Svarovsky, Rittenhouse-Olson, unpublished results).

2.4. TF-glycopeptide precursors for multivalent assembly on gold

As mentioned briefly above, TF and other O-linked TACA's are covalently attached to specific cell surface



Scheme 2.

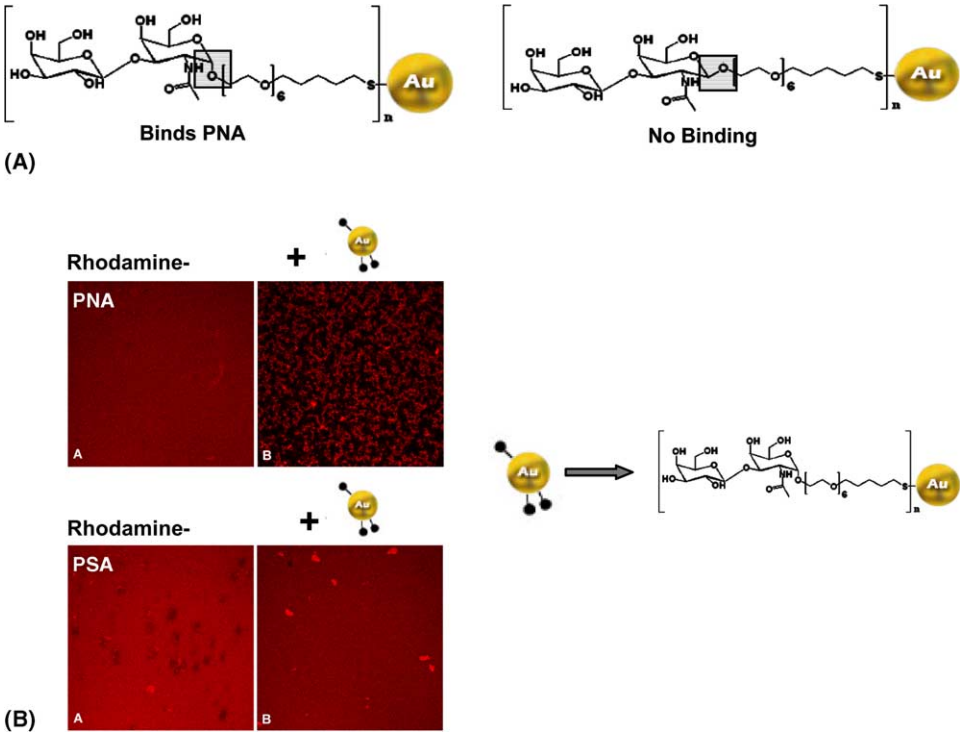


Figure 2. (A) Structures **α**- and **β-TF$_{PEG6}$Au** highlighting the anomeric stereochemistry and the binding propensity toward PNA. (B) TEM's of Rhodamine-PNA and Rhodamine-PSA with (right) and without (left) addition of **α-TF$_{PEG6}$Au**. Also shown is the definition of the schematic for the gold particles.

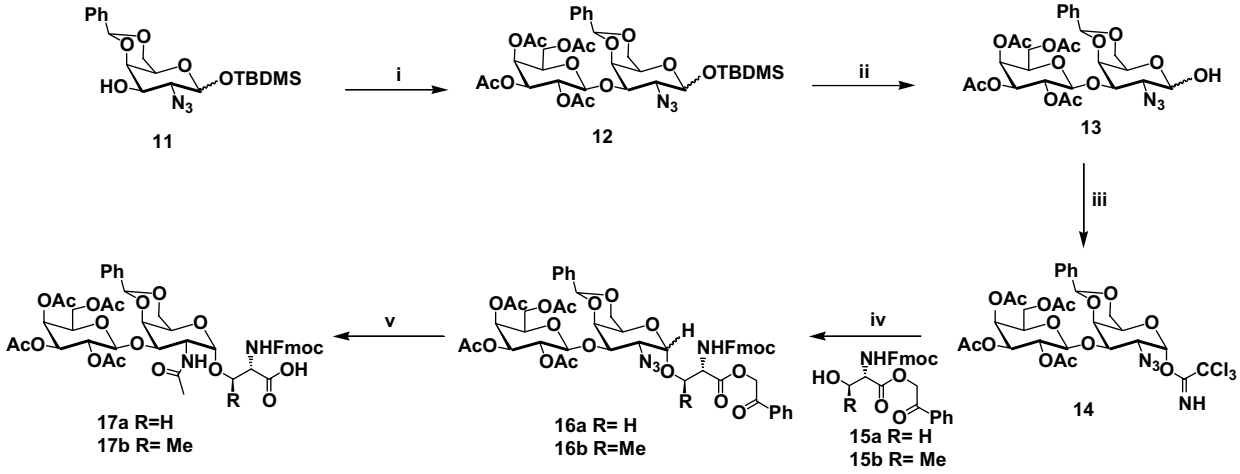
proteins such as mucins. Hence, when an immune response is mounted against a particular TACA, the glycans are presented in the context of the protein backbone and its surrounding sequence at the point of attachment. It seems reasonable then to assume that the ideal construction to develop as an immunogen in a putative vaccine preparation would be a combination of both the sugar and the peptide sequence, that is, a

glycopeptide. As shown above, thiol-stabilized gold nanoparticles are extremely useful templates for multivalent presentation. It was our thought that relevant tumor-associated *glycopeptides* could similarly be displayed on these particles and we sought to capitalize on this strategy by constructing suitably linked glycosylated peptides to be displayed on gold 3D-SAM's.

In our previous work that outlined the preparation of **2**, we also reported the synthesis of serine and threonine glycoamino acid building blocks for use in glycopeptide synthesis.[26] Although **2** is a versatile intermediate for both glycosylations and linker chemistry, the pentenyl group proved to be partly problematic as a glycosyl donor to amino acid acceptors with respect to yields (43–64% with threonine and serine donors, respectively). Over the past 20 years, there have been a host of published efficient syntheses of TF antigen neoglycoconjugates[14,16,34–39] and TF glycoamino acids.[5,7,36,40–44] In our continuing efforts toward high-yielding syntheses of these building blocks, we revisited some of the work of Schmidt[39,45] and found some marked improvements in specific steps. Most notably, we have succeeded in obtaining extremely high yields and stereoselectivities in the glycosylation of Fmoc-serine and threonine phenacyl esters prepared according to Luning et al.[44,46] (Scheme 3). Compound **11**[47] (derived from D-galactose through, (1) triacetyl galactal azidonitration, (2) removal of the anomeric nitro group with hydrazine hydrate, (3) silylation, (4) deacetylation, and (5) benzylidenation in 30% overall yield) was glycosylated with **5** at −45 °C to give the known[48] disaccharide **12** quantitatively and with exclusive β-stereochemistry. When the reaction was run at 0 °C, a 1:1 mixture of anomers was formed. The once troublesome anomeric desilylation step,[39,49] that was later optimized on a related analogue by Schmidt's group,[45] was refined by the use of triethylamine/trihydrofluoride complex in THF,[50] which removed the TBDMS group cleanly and quantitatively to give **13**, which was subsequently converted to the trichloroacetimidate **14**[7] in 95% yield by the procedure of Ren and Liu.[27] Glycosylations with serine and threonine derivatives **15a** and **15b**[44] proceeded with excellent yield and stereoselectivity, but under strictly optimized conditions. Reactions in CH_2Cl_2 at −40 °C gave a 1:1 mixture of anomers **17a** or **17b**, whereas reaction at the same temperature in THF gave exclusively α-product, but in low yield (40%). Raising the temperature to 25 °C afforded a 2:1 mixture of α/β products if the reaction was run in CH_2Cl_2; however, in a 3:1 mixture of CH_2Cl_2 to THF, the α/β ratio increased to 20:1 with yields consistently in the 90–95% range. Although more steps are involved in contrast to previous syntheses, the overall yield from simple D-galactose and the ease of most synthetic manipulations makes this scheme attractive for large syntheses of these critical building blocks. In addition, the use of the phenacyl ester for carboxyl protection on the amino acid allowed us to remove this group and reduce the azide in one simple high-yielding step to give the glycopeptide precursors **17a**,**b**.

2.5. Attempt at glycopeptide-coated nanoparticle synthesis

We proceeded to prepare a model glycopeptide precursor for presentation on gold nanoparticles (schematic in box, Fig. 3). This glycopeptide **18** was previously designed to bind to MHC-I isotypes where the carbohydrate was placed in an anchor position known to bind in a pocket of the T-cell receptor (TcR).[51] Glycopeptide **18** was shown to inhibit binding of a well-characterized K^b-binding peptide with a 4 nM IC_{50} and elicits a strong cytotoxic T-lymphocyte (CTL) response against the TF disaccharide.[51] We reasoned that a multivalent presentation of this glycopeptide may lead to an enhanced immune response and possibly more powerful glycopeptide-restricted CTL. For the synthesis of gold particles, we had the requirement that the peptide must be extended by a linker that could be processed to a terminal thiol group. We prepared the nona-glycopeptide **18** by standard peptide synthesis on 2-chlorotrityl resin with HATU/HOAt activation of individual amino acids. We succeeded in extending the N-terminus as shown in Figure 3B that led to a precursor to particle assembly



Scheme 3. Reagents and conditions: (i) **5**, TMSOTf/CH_2Cl_2/molecular sieves, −45 °C, 100%; (ii) $Et_3N{\cdot}3HF$, THF, 100%; (iii) CH_2Cl_2, CCl_3CN, DBU, 0 °C, 95%; (iv) 3:1 CH_2Cl_2:THF, **15a**,**b**, rt, 90–95%; (vi) 2:2:1 THF/AcOH/Ac_2O, Zn, rt, 79%.

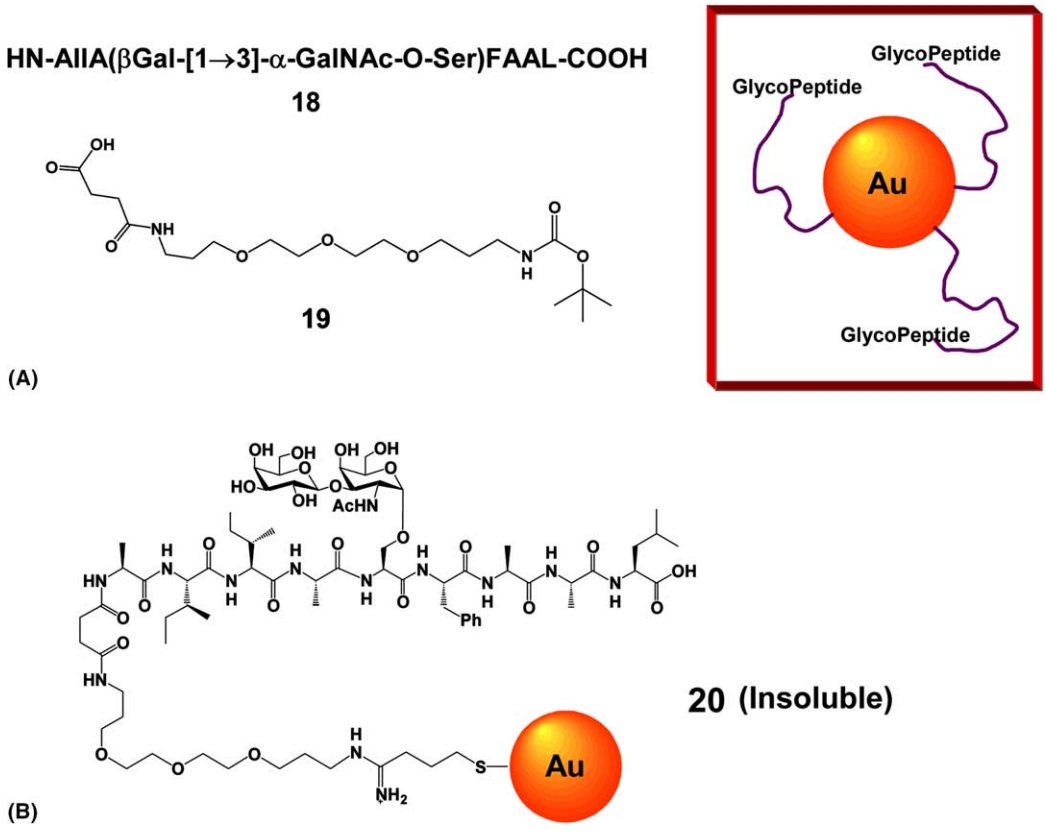


Figure 3. (Box) Schematic of a glycopeptide-coated gold particle. (A) Structure of the designer glycopeptide **18** and linker **19** used for the synthesis of the precursor to gold self assembly. (B) Structure of final product **20** used in the particle synthesis.

making use of the known spacer[52] **19** that was processed to a terminal thiolated glycopeptide **20** through iminothiolane[53] chemistry. Our initial attempts to synthesize glycopeptide-stabilized gold looked promising, but the insolubility of the resulting particle precluded characterization of the assembly. A previous synthesis of the homo serine analogue of **18** also reported solubility problems with the deprotected glycopeptide.[7] Our hope was to add enough hydrophilicity in the linker to overcome this drawback. Further research into solving this problem and the synthesis of other glycopeptide systems for multivalent presentations will be reported in due course.

3. Conclusion

We have successfully prepared gold nanoparticles coated with the important TF antigen in a robust and highly active form. These particles have interesting activities both in vitro and in vivo. The chemistry to prepare the TF precursors was high yielding and stereoselective and the convergence of our routes allows the synthesis of glycopeptide building blocks in an equally efficient manner. We are using similar chemistry to exploit these useful templates for the display of other biologically relevant glycans and glycopeptides. These systems should find extended use both as chemical and biological tools and have the potential to develop into anticancer therapeutics.[20,54,55]

4. Experimental

Melting points were determined on Fisher–Johns melting point apparatus and are uncorrected. R_f values refer to TLC performed on Analtech Uniplates GF precoated with silica gel 60 to a thickness of 0.25 mm. The spots were visualized by charring with a solution of ammonium molybdate (IV) tetrahydrate (12.5 g) and cerium (IV) sulfate tetrahydrate (5.0 g) in 10% aqueous H_2SO_4 (500 mL). Flash column chromatography (FCC) was performed under medium pressure using silica gel 60 (230–400 mesh, E. Merck) and usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Unless otherwise noted, chemicals were purchased from Aldrich–Sigma (Milwaukee, WI) and used without further purification.

NMR spectra were recorded on a Varian Inova-400 instrument with residual $CHCl_3$ (7.26 ppm) as the internal standard at frequencies of 399.74 MHz for ^1H and 100.51 MHz for ^{13}C. Assignments were based on gCOSY, TOCSY, ROESY, and ^{13}C/DEPT experiments. ^1H NMR data are tabulated in the order of multiplicity (s, singlet; d, doublet; dd, doublet of doublets; dt, double of triplets; t, triplet; q, quartet; m, multiplet; br s, broad signal), number of protons, and coupling constant(s) in hertz. IR spectra were recorded on a JASCO FT/IR-615 spectrophotometer. Specific optical rotations were determined using JASCO-P1010 polarimeter in 0.5 dm cuvette at 589 nm in chloroform. Five consecutive

measurements were taken and the average value is reported. Positive ion fast-atom bombardment mass spectra (FABMS) were obtained at an accelerating voltage of 6 kV and a resolution of 2000. Glycerol was used as the sample matrix, and ionization was effected by xenon atoms. Laser scanning confocal microscopy was performed on a Zeiss 510 confocal microscope (NCI-Frederick, Confocal Microscopy Facility, Image Analysis Lab). Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Analyses of some PEG-containing compounds (viz., **8**) did not meet the expected criteria but all other spectroscopic data were consistent with the desired structure. Transmission electron micrographs were performed on a Hitachi H-7000 microscope equipped with a Gatan digital camera operating at 75 kV.

4.1. 5-*O*-[2-Acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-1-α-thioacetyl pentane 3

A solution of 120 mg (0.17 mmol) of **2**[26] in 4 mL of anhydrous 1,4-dioxane was purged with argon for 20 min. To this deoxygenated solution 1.4 mL (2.55 mmol, 15 equiv) of triply-distilled thiolacetic acid was added followed by 30 mg (0.03 mmol) of AIBN. The reaction was left to stir under an argon atmosphere at 75 °C for 1 h. The reaction was quenched with cyclohexene (0.1 mL) and the solution was co-evaporated with xylenes (3×) under reduced pressure. FCC on silica gel with 3:1 EtOAc/hexanes provided 125 mg (99.8%) of white solid (7:3 mixture of amide rotamers). R_f 0.19 (3:1 EtOAc/hexanes); $[\alpha]_D = +157.8$ (*c* 0.22, $CHCl_3$); IR (neat) 3099.05, 1752.01, 1689.34, 1368.25, 1220.72; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.48–7.54 (m, 2H, Ph), 7.27–7.38 (m, 3H, Ph), 5.56 (d, 1H, J = 8.98Hz, NH), 5.52 (s, 1H, PhCH), 5.34 (d, 1H, J = 2.34 Hz, H″-4), 5.13–5.19 (m, 1H, H″-2), 4.92–4.98 (m, 2H, H″-3, H″-1), 4.76 (d, 1H, J = 7.81 Hz, β H″-1), 4.62 (m, 1H, H″-2), 3.90–4.30 (m, 6H, H′-6, H″-6, H′-4, H″-5), 3.88 (m, 1H, H′-3), 3.59 (br s, 1H, H″-5), 3.60–3.70 (m, 1H, –OCH_2CH$_2$CH$_2$CH$_2$CH$_2$SAc), 3.35–3.45 (m, 1H, –OCH_2CH$_2$CH$_2$CH$_2$CH$_2$SAc), 2.83 (t, 2H, J = 7.42 Hz, –OCH$_2$CH$_2$CH$_2$CH$_2$CH_2SAc), 2.30 (s, 3H, SAc), 1.93, 1.96, 2.00, 2.01, 2.11 (s, 15H, OAc, NHAc), 1.52–1.62 (m, 4H, –OCH$_2$CH$_2$CH_2CH_2CH$_2$SAc), 1.34–1.44 (m, 2H, –OCH$_2$CH_2CH$_2$CH$_2$CH$_2$SAc). FAB MS *m*/*z*: 784.0 (MH^+). Anal. Calcd for $C_{36}H_{49}NO_{16}S$: C 55.16; H 6.30; N 1.79. Found: C 54.89; H 6.32; N 1.87. A solution of 110 mg (0.14 mmol) of this product in 3 mL of 80% AcOH was stirred at 45 °C for 16 h. The reaction solution was concentrated at reduced pressure and co-evaporated twice with xylenes. The residue was purified by FCC on silica gel using 7% MeOH in CH_2Cl_2 to provide 69 mg (71% yield) of **3** as a white foam (7:3 mixture of amide rotamers). Mp 95–97 °C; R_f 0.46 (10% MeOH in CH_2Cl_2); $[\alpha]_D = +107.7$ (*c* 0.15, $CHCl_3$); IR (neat); 3552.24, 1750.08, 1688.37, 1656.55, 1545.67, 1370.18, 1220.72; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.33 (dd, 1H, J = 0.78, 3.51 Hz, H″-4), 5.11–5.18 (m, 1H, H″-2), 4.94 (dd, 1H, J = 3.51, 10.54 Hz, H′-3), 4.78 (d, 1H, J = 3.51 Hz, H′-1), 4.61 (d, 1H, J = 8.20 Hz, βH″-1), 4.51 (td, 1H, J = 3.90, 10.54 Hz, H′-2), 3.65–4.18 (m, 8H, H′-5, H′-3, H″-6, H″-6, H′-4, H″-5), 3.60–3.67 (m, 1H, –OCH_2CH$_2$CH$_2$CH$_2$CH$_2$SAc), 3.31–3.40 (m, 1H, –OCH_2CH$_2$CH$_2$CH$_2$CH$_2$SAc), 2.78–2.85 (m, 2H, –OCH$_2$CH$_2$CH$_2$CH$_2$CH_2SAc), 2.70 (br s, 2H, OH), 2.28 (s, 3H, SAc), 1.92, 1.94, 2.00, 2.02, 2.11 (s, 15H, OAc, NHAc), 1.50–1.62 (m, 4H, –OCH$_2$CH$_2$CH_2-CH_2CH$_2$SAc), 1.32–1.42 (m, 2H, –OCH$_2$CH_2CH$_2$-CH$_2$CH$_2$SAc), ^{13}C NMR (100 MHz, $CDCl_3$) δ = 196.46, 170.61, 170.39, 170.32, 169.72, 169.55, 101.86, 97.87, 78.38, 71.07, 70.82, 69.69, 69.37, 68.83, 67.89, 67.10, 62.88, 61.52, 48.06, 30.86, 29.57, 29.03, 28.88, 25.61, 23.56, 20.87, 20.81, 20.79, 20.72, 20.67. FAB MS *m*/*z*: 696.1 (MH^+). Anal. Calcd for $C_{29}H_{45}NO_{16}S$: C 50.06; H 6.52; N 2.01. Found: C 49.55; H 6.53; N 2.00.

4.2. TF-pentyl-disulfide 4

A solution of 300 mg (0.43 mmol) of **3** in 5 mL of MeOH was treated with 30 μL of 25% (w/v) NaOMe/MeOH. The reaction was stirred at rt and for 24 h with a slow flow of air bubbling through the solution. Initially, two spots were observed by TLC (R_f 0.13 and R_f 0.00 in 20% v/v MeOH/CH_2Cl_2). The higher R_f spot corresponded to the monomeric thiol while the lower spot corresponded to the desired dimer **4**. This mixture was completely converted to dimer **4** after 24 h. The reaction was carefully neutralized with strongly acidic Amberlite®-120, and evaporated under reduced pressure at 50 °C to give 200 mg (96%) of NMR pure **4**, which was further purified by reverse phase (C18) chromatography with 10% → 40% (v/v) MeOH/H_2O to give 187 mg (90%) of **4** as a white powder. The compound was soluble in both water and methanol. Mp 247–249 °C; $[\alpha]_D = +93.8$ (*c* 1.6, MeOH); IR (neat) 3379.64, 2944.77, 2827.13, 2112.64, 1746.23, 1218.79; ^{1}H NMR (400 MHz, CD_3OD) δ = 4.14 (d, 2H, J = 1.95Hz, NH), 3.87 (dd, 2H, J = 3.12, 10.93Hz, H′-2), 3.68 (m, 2H, [–OCH_2–]$_2$), 3.40 (m, 2H, –OCH_2–), 2.68 (t, 4H, J = 7.42 Hz, –CH_2S–SCH_2–), 1.94 (s, 6H, NHAc), 1.43–1.74 (m, 12H, [–OCH$_2$CH_2-CH_2CH_2CH$_2$S–]$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.77 (NHAc), 105.08 (C-1), 97.60, 77.76, 75.52, 73.52, 71.33, 70.85, 69.07, 68.83, 67.54, 61.64, 61.38, 49.19, 38.41, 28.89, 28.81, 24.93, 21.69. FAB MS *m*/*z*: 969.2 (MH^+). Anal. Calcd for $C_{38}H_{68}N_2O_{22}S_2$ (3 × H_2O) C, 44.61; H, 7.29; N, 2.74. Found: C 44.24; H 7.21; N 2.73.

4.3. General preparation of TF-linked gold nanoparticles

A solution of 1% $HAuCl_4$ (1.9 mL, 0.046 mmol in Au^{3+}) was added to 200 mL of degassed H_2O. To this solution was added 10 mg (0.010 mmol) of thiol **4** or disulfide **10** (0.020 mmol per thiol; ratio of reactants: TFSH/Au = 1:2.3). A cooled (0 °C) solution of 20 mg (0.53 mmol) of $NaBH_4$ in 25 mL H_2O was added dropwise to the reaction solution with rapid stirring. The resulting purple solution was stirred for an additional 1 h, purified by ultrafiltration on Millipore's Centriplus® YM-30 cartridges and freeze-dried.

4.4. Preparation of linker 6

A solution of 533 μL of 50% NaOH (6.7 mmol) was added to 10 g (35.5 mmol) of hexa(polyethyleneglycol) (Aldrich) at 100 °C. The reaction was stirred for 30 min at 100 °C and 5-bromo-1-pentene (1 g, 6.7 mmol) was quickly added and the reaction was stirred at 100 °C for 24 h. The reaction was diluted with water and extracted 6× with EtOAc. Combined organic extracts were evaporated and separated by FCC with 10:1 CH_2Cl_2/MeOH. This gave 1 g of monopentenylated hexa(polyethyleneglycol) as a clear liquid. R_f (10:1, CH_2Cl_2/MeOH) = 0.3; IR (neat); 3465 (br s), 2231, 2022, 1690; ^{1}H NMR (400 MHz, CD_3Cl) δ = 5.73–5.83 (m, 1H, C*H*=CH_2), 4.90–5.02 (m, 2H, C*H*=CH_2), 3.53–3.70 (m, 24H, PEG), 3.43 (t, 2H, J = 6.49 Hz, OC*H*$_2$-CH_2CH_2CH=CH_2), 2.08 (m, 2H, $OCH_2CH_2CH_2$-CH=CH_2), 1.65 (m, 2H, $OCH_2CH_2CH_2CH$=CH_2); ^{13}C NMR (100 MHz, CD_3Cl) δ = 72.49, 70.66, 70.59, 70.55, 70.52, 70.33, 70.07, 61.68, 30.19, 28.75. FAB MS m/z: 351.3 (MH^+), 373.3 (M + Na^+). Anal. Calcd for $C_{17}H_{34}O_7$: C, 58.26; H, 9.78. Found: C 58.30; H 9.78. A solution of 2.8 g (8 mmol) of this product in 30 mL MeOH was treated with 30 mmol (2.3 g, 2.3 mL) of freshly distilled HSAc. The solution was purged with argon for 20 min and 10 mg of AIBN was added. The reaction was irradiated with 360 nm light overnight, quenched with 1 mL of cyclohexene and evaporated. The residue was purified by FCC with 15:1 CH_2Cl_2/MeOH to afford 3 g (88%) of thioacetate **8** as a clear liquid. IR (neat); 3465 (br s), 1689; ^{1}H NMR (400 MHz, CD_3Cl) δ = 3.52–3.72 (m, 24H, PEG), 3.42 (t, 2H, J = 6.49 Hz, OC*H*$_2$$CH_2CH_2CH_2$-$CH_2$SAc), 2.84 (d, 2H, J = 6.95Hz, $OCH_2CH_2CH_2CH_2$-C*H*$_2$SAc), 2.60 (br s, 1H, OH), 2.29 (s, 3H, SAc), 1.56 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2$SAc), 1.39 (m, 2H, $OCH_2CH_2CH_2CH_2CH_2$SAc); ^{13}C NMR (100 MHz, CD_3Cl) δ = 195.91, 72.47, 71.09, 70.61, 70.57, 70.55, 70.36, 70.09, 61.72, 30.62, 29.34, 29.10, 29.01, 25.36; FAB MS m/z: 427.4 (MH^+). Anal. Calcd for $C_{19}H_{38}O_8S$: C, 53.50; H, 8.98. Found: C 52.17; H 8.91.

4.5. Thioacetic acid-[2-azido-2-deoxy-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-S-{5-[(penta-2-ethoxy)-2-hydroxy-ethoxy pentyl ester 8

A solution of 2.5 g (3.26 mmol) of **7** and 1 g (2.36 mmol) of **6** in CH_2Cl_2 was evaporated and co-evaporated with toluene and the residue was dried under vacuum overnight. The dried mixture was dissolved in 3:1 mixture of anhydrous CH_2Cl_2/THF (60:20 mL) and added via cannula to 2 g of flame-dried MS (4 Å). After stirring for 20 min 15 μL TMSOTf was quickly injected via syringe. After 30 min the reaction was quenched with Et_3N, filtered, and evaporated. The crude residue was purified by FCC with 20:1 CH_2Cl_2/MeOH to afford 1.9 g of **8** as a mixture of anomers (1.5 g of α-anomer and 400 mg of β-anomer, α/β = 3.75). Total yield 79%. Desired α-anomer (glassy solid) R_f (10:1 CH_2Cl_2/MeOH) = 0.8; 4:1 ratio of rotamers. NMR of the major rotamer is given. ^{1}H NMR (400 MHz, CD_3Cl) δ = 7.39–7.43 (m, 2H, Ph), 7.20–7.28 (m, 3H, Ph), 5.43 (s, 1H, PhCH), 5.28 (m, 1H, H-4″), 5.17 (dd, 1H, J = 7.88, 10.20 Hz, H-2″), 4.98 (d, 1H, J = 3.71 Hz, H-1′), 4.92 (m, 1H, H-3″), 4.67 (d, 1H, J = 7.88 Hz, H-1″), 4.26 (m, 1H, H-4′), 3.8–4.15 (m, 8H, H-2′, H-3′, H-6′, H-6″, H-5′, H-5″), 3.52 (m, 24H, PEG), 3.32 (m, 2H, OC*H*$_2$$CH_2CH_2$-$CH_2CH_2$SAc), 2.74 (m, 2H, $OCH_2CH_2CH_2CH_2$C*H*$_2$-SAc), 2.19 (s, 3H, SAc), 2.04, 1.94, 1.92, 1.86 (s, 12H, 4 × Ac), 1.46 (m, 4H, OCH_2C*H*$_2$$CH_2$C*H*$_2$$CH_2$SAc), 1.30 (m, 2H, OCH_2CH_2 C*H*$_2$$CH_2CH_2$SAc); ^{13}C NMR (100 MHz, CD_3Cl) δ = 195.86, 170.28, 170.23, 170.11, 169.38, 137.73, 128.81, 128.22, 128.08, 126.50, 126.13, 102.41, 100.58, 98.87, 75.81, 71.06, 70.77, 70.55, 70.50, 70.22, 70.07, 69.19, 68.67, 67.51, 66.97, 63.02, 62.39, 61.37, 58.84, 30.61, 29.64, 29.34, 29.09, 28.99, 25.35, 20.69, 20.67, 20.54; FAB MS m/z: 1031.9 (MH^+), 1069.9 (M+K^+).

4.6. Thioacetic acid-[2-acetamido-2-deoxy-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-S-{5-[(penta-2-ethoxy)-2-hydroxy-ethoxy]-pentyl ester 9

A solution of 1.5 g of **8** in 50 mL of 6:3:1 THF/AcOH/Ac_2O was treated with 15 g of Zn dust. After 4 h the reaction was filtered through a pad of Celite® and the zinc cake was washed with EtOAc. The filtrate was washed successively with water, satd $NaHCO_3$ and brine. The organic layer was dried over Mg_2SO_4, evaporated and purified by FCC with 20:1 CH_2Cl_2/MeOH to afford 1.2 g (79%) of **9** as a glassy solid. R_f (20:1 CH_2Cl_2/MeOH) = 0.2; $[\alpha]_D$ = +72.4 (c 1.37, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3Cl) δ = 7.52–7.57 (m, 2H, Ph), 7.30–7.40 (m, 3H, Ph), 5.96 (d, 1H, J = 9.27 Hz, NH), 5.56 (s, 1H, PhCH), 5.38 (m, 1H, H-4″), 5.20 (dd, 1H, J = 7.88, 10.66 Hz, H-2′), 4.99 (m, 2H, H-1′, H-3″), 4.77 (d, 1H, J = 7.88 Hz, H-1″), 4.67 (m, 1H, H-2′), 3.72–4.30 (m, 8H, H-2′, H-3′, H-6′, H-6″, H-5′, H-5″), 3.65 (m, 24H, PEG), 3.44 (t, 2H, J = 6.49 Hz, OC*H*$_2$$CH_2CH_2CH_2CH_2$SAc), 2.86 (t, 2H, J = 7.42 Hz, $OCH_2CH_2CH_2CH_2$C*H*$_2$SAc), 2.32 (s, 3H, NHAc), 2.15 (s, 3H, SAc), 2.05, 2.04, 1.98, 1.97 (s, 12H, 4 × Ac), 1.59 (m, 4H, OCH_2C*H*$_2$$CH_2$C*H*$_2$$CH_2$SAc), 1.42 (m, 2H, OCH_2CH_2C*H*$_2$$CH_2CH_2$SAc); ^{13}C NMR (100 MHz, CD_3Cl) δ = 170.27, 170.12, 169.53, 169.36, 137.74, 128.71, 128.10, 128.06, 126.62, 126.21, 126.06, 101.40, 100.59, 98.57, 75.70, 74.52, 71.05, 70.85, 70.74, 70.54, 70.52, 70.36, 70.06, 69.99, 69.27, 68.81, 67.23, 66.96, 63.13, 61.25, 18.12, 30.60, 29.32, 29.08, 28.98, 25.33, 23.39, 20.68, 20.53. FAB MS m/z: 1048.2 (MH^+). Anal. Calcd for $C_{48}H_{73}NO_{22}S$: C, 55.00; H, 7.02; N, 1.34; O, 33.58; S, 3.06. Found: C 54.57; H 7.06; N 1.43.

4.7. 5-Mercapto-pentyloxy-*O*-[2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranosyl]-(penta-2-ethoxy)-ethanol 10

A solution of 1.2 g (1.14 mmol) of **9** in 20 mL of dry MeOH was treated with a few drops of acetyl chloride

for 1.5 h, quenched with pyridine, evaporated, and purified by FCC with 20:1 → 10:1 CH_2Cl_2/MeOH to afford 700 mg (65%) of debenzylidenated **9** as a glassy solid. R_f (10:1 CH_2Cl_2/MeOH)= ; $[\alpha]_D = +47.6$ (*c* 0.60, MeOH); ^1H NMR (400 MHz, CD_3Cl) $\delta = 5.90$ (d, 1H, $J = 9.74$ Hz, NH), 5.37 (m, 1H, H-4″), 5.20 (dd, 1H, $J = 7.88$, 10.66 Hz, H-2″), 5.00 (m, 1H, H-3″), 4.86 (d, 1H, $J = 3.71$ Hz, H-1′), 4.66 (d, $J = 8.34$ Hz, H-1′), 4.57 (m, 1H, H-2′), 3.76–4.22 (m, 7H, H-3′, H-6′, H-6″, H-5″, H-5′), 3.54–3.72 (m, 24H, PEG), 3.45 (t, 2H, $J = 6.49$ Hz, $OCH_2CH_2CH_2CH_2CH_2SAc$), 2.86 (m, 2H, $OCH_2CH_2CH_2CH_2CH_2SAc$), 2.32 (s, 3H, NHAc), 2.16 (s, 3H, SAc), 2.08, 2.06, 1.99, 1.98 (s, 12H, 4 × Ac), 1.59 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2SAc$), 1.42 (m, 2H, $OCH_2CH_2CH_2CH_2CH_2SAc$); ^{13}C NMR (100 MHz, CD_3Cl) $\delta = 195.90$, 170.36, 170.17, 170.06, 169.59, 169.35, 101.63, 98.15, 78.10, 71.04, 70.77, 70.64, 70.56, 70.53, 70.48, 70.44, 70.20, 70.04, 69.97, 69.81, 69.10, 68.58, 67.05, 66.94, 62.51, 61.28, 47.81, 30.59, 29.31, 29.05, 28.96, 25.32, 23.30, 20.64, 20.60, 20.57, 20.50. FAB MS *m*/*z*: 961.4 (MH^+). Anal. Calcd for $C_{41}H_{69}NO_{22}S$: C, 51.29; H, 7.24; N, 1.46. Found: C 50.47; H 6.91; N 1.55. This compound (500 mg, 0.52 mmol) in 20 mL of dry MeOH was treated with 7 drops of 25% w/v NaOMe/MeOH and stirred at room temperature for an hour. The reaction was quenched with Amberlite®-120 ion-exchange resin, filtered, and evaporated. The residue was cleanly purified with 20% MeOH in CH_2Cl_2 to give 350 mg (90%) of **10** as a glassy solid. R_f (20% MeOH in CH_2Cl_2) = 0.20; $[\alpha]_D = +24.4$ (*c* 0.24, MeOH); ^1H NMR (400 MHz, D_2O) $\delta = 4.80$ (d, 1H, $J = 3.71$ Hz, H-1′), 4.35 (d, 1H, $J = 7.88$ Hz, H-1′), 4.24 (dd, 1H, $J = 3.71$, 10.66 Hz, H-2′), 4.12 (m, 1H, H-4″), 3.94 (dd, 1H, $J = 2.78$, 11.13 Hz, H-3′), 3.48–3.90 (m, 33H, 24H PEG, NH, H-3′, H-4′, H-5″, H-5″, H-6′, H-6″), 3.43 (m, 3H, $OCH_2CH_2CH_2CH_2CH_2SH$, H-2′); 2.44 (m, 2H, $OCH_2CH_2CH_2CH_2CH_2SH$), 1.91 (s, 3H, NHAc), 1.50 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2SH$), 1.33 (m, 2H, $OCH_2CH_2CH_2CH_2CH_2SH$); ^{13}C NMR (100 MHz, D_2O) $\delta = 174.44$, 101.75, 97.42, 97.30, 77.25, 74.92, 72.51, 70.87, 70.71, 70.57, 69.57, 69.11, 68.78, 68.69, 68.59, 68.48, 66.40, 61.16, 60.91, 48.45, 32.75, 27.98, 24.02, 23.66, 22.06, 22.04; FAB MS *m*/*z*: 750.3 (MH^+), 788.3 ($M+K^+$). Anal. Calcd for $C_{31}H_{59}NO_{17}S$: C, 49.65; H, 7.93; N, 1.87. Found: C 49.28; H 7.86; N 1.82.

4.8. 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-*tert*-butyldimethylsilyl-α-D-galactopyranoside 12

A dried mixture of 8.3 g (20 mmol) of **11**[47] and 12.7 g (25.8 mmol) of **5** was dissolved in 150 mL of anhydrous CH_2Cl_2 and the solution was transferred to a flask containing 5 g of flame-dried 4 Å MS via cannula under argon. After stirring for an hour at rt the reaction was cooled to −45 °C and 200 μL of TMSOTf was added in several portions via syringe. After 30 min at −45 °C the cold bath was removed and reaction was slowly allowed to warm to rt. The reaction was quenched with 0.3 mL Et_3N, diluted with CH_2Cl_2, filtered through Celite®, and concentrated. Purification by FCC with 2:1 EtOAc/hexanes gave 15 g (100%) of **12**[48] as a mixture of α,β isomers.

4.9. 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside 13

A solution of 15 g (20 mmol) of **12** in 250 mL of anhydrous THF was treated with 20 g (124 mmol) of $Et_3N{\cdot}3HF$. The reaction was stirred overnight at rt, diluted with EtOAc, washed with satd $NaHCO_3$, brine, and water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by FCC with 7:3 EtOAc/hexanes to give 12.5 g (100%) of hemiacetal **13**[56] as a mixture of α,β isomers.

4.10. Preparation of 14

A solution of 10.7 g (17.2 mmol) of **8** in 200 mL of anhydrous CH_2Cl_2 (Aldrich) was cooled to 0 °C and 20 mL (29 g, 200 mmol) of trichloroacetonitrile was added. After stirring for a few min, 0.5 mL of DBU was added. The reaction was stirred overnight, evaporated, and the residue was purified by FCC with 3:2 hexanes/EtOAc to give 12.5 g (95%) of pure **14**.[7]

4.11. N^{α}-Fluoren-9-ylmethoxycarbonyl-3-*O*-[2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-serine phenacyl ester 16a

A mixture of 1.05 g (2.36 mmol) of **15a** 2.05 g (2.67 mmol) of **14** was dissolved in dry CH_2Cl_2, evaporated, and dried under vacuum for 1 h. The dried mixture was dissolved 3:1 CH_2Cl_2/THF (60 mL) under argon and this solution was added under argon to 2 g of flame-dried molecular sieves (4′). After stirring for 30 min at rt 15 μL of TMSOTf was added at once. After 1 h, the reaction was quenched with few drops of Et_3N, filtered through Celite®, and evaporated. Purification by FCC with 1:1 → 2:1 EtOAc/hexanes gave 1.95 g (80%) of the α-isomer as a glassy solid. R_f (1:1, EtOAc/hexanes) = 0.33; $[\alpha]_D = +34.05$ (*c* 0.6 in $CHCl_3$); IR (neat) 2231.24, 2114.56, 2022.96, 1751.05 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) $\delta = 7.30$–7.90 (m, 18H, aromatic), 5.98 (d, 1H, $J = 8.25$ Hz, NH-Fmoc), 5.54 (s, 1H, PhCH), 5.30–5.50 (m, 3H), 5.28 (dd, 1H, $J_{1,2} = 7.90$ Hz, $J_{2,3} = 10.39$ Hz, H2″), 5.17 (d, 1H, $J = 2.95$ Hz, αH1′), 4.99 (dd, 1H, $J_{3,4} = 3.45$ Hz, $J_{2,3} = 10.39$ Hz, H3′), 4.77 (m, 1H, NHC*H*), 4.73 (d, 1H, $J_{1,2} = 7.94$ Hz, βH1″), 3.75–4.50 (m, 13H), 2.15, 2.03, 1.98 (s, 12H, Ac); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.34$, 170.15, 169.43, 143.66, 141.31, 137.65, 134.24, 133.89, 129.06, 128.89, 128.15, 127.85, 127.71, 127.18, 127.13, 126.13, 125.05, 120.12, 102.53, 100.62, 100.09, 75.79, 71.03, 70.82, 69.08, 68.65, 67.34, 66.98, 63.64, 61.41, 58.80, 54.55, 47.07, 20.72, 20.66, 20.57; FAB MS (*m*/*z*): 1051.1 (MH^+), 1023.1 (MH^+-N_2) Anal. Calcd for $C_{53}H_{54}N_4O_{19}$: C, 60.57; H, 5.18; N, 5.33. Found: C 60.26; H 5.21; N 5.16.

4.12. N^{α}-Fluoren-9-ylmethoxycarbonyl-3-*O*-[2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine phenacyl ester 16b

A mixture of 340 mg (0.74 mmol) of **15b** and 450 mg (3.22 mmol) of **14** was dissolved in minimum amount of dry CH_2Cl_2, evaporated and dried under vacuum for 1 h. The dried mixture was dissolved in 8 mL of 3:1 CH_2Cl_2/THF and added via cannula under argon to 550 mg of flame-dried molecular sieves (4′). After stirring for 20 min 20 μL of TMSOTf was added at once. After 30 min the reaction was quenched with few drops of Et_3N, filtered through Celite®, and evaporated. The residue was purified by FCC with 3:2 EtOAc/hexanes to give 600 mg (95%) of α-isomer, 30 mg (5%) of β-isomer (α/β = 19:1) as glassy solids. R_f (1:1 EtOAc/hexanes) = 0.33; $[\alpha]_D = +25.6$ (*c* 1.00, $CHCl_3$); IR (neat) 2232.20, 2115.53, 2022.96, 1750.08 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.30–7.94 (m, 18H, aromatic), 6.02 (d, 1H, J = 9.31 Hz, NH-Fmoc), 5.58 (s, 1H, PhCH), 5.48 (d, 1H, J = 3.73 Hz, α-H1′), 5.41 (m, 1H, H4″), 5.4–5.6 (m, 4H), 5.31 (dd, 1H, $J_{1,2}$ = 7.88 Hz, $J_{2,3}$ = 10.34 Hz, H2″), 5.04 (dd, 1H, $J_{2,3}$ = 3.39 Hz, $J_{3,4}$ = 10.37 Hz, H3″), 4.84 (d, 1H, J = 7.92 Hz, β-H1″), 4.55 (m, 1H, NHC*H*), 4.50–4.70 (m, 2H), 4.41 (m, 1H, H4′), 4.05–4.40 (m, 11H, H), 3.94 (m, 1H, H2′), 3.78 (m, 1H, H5′), 2.17, 2.03, 2.02, 1.99 (s, 12H, Ac), 1.42 (d, 3H, J = 6.39 Hz, Me-Thr); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.33, 170.14, 169.41, 141.33, 137.64, 134.11, 133.91, 128.97, 128.91, 128.17, 127.77, 127.12, 126.16, 125.19, 125.10, 120.02, 102.42, 100.69, 99.21, 75.95, 75.79, 71.08, 70.85, 69.16, 68.73, 67.32, 66.98, 66.80, 63.46, 61.34, 59.68, 58.60, 47.17, 20.72, 20.56, 19.27; FAB MS (*m*/*z*): 1065.1 (MH^+), 1037 (MH^+-N_2) Anal. Calcd for $C_{54}H_{56}N_4O_{19}$: C 60.90; H 5.30; N 5.26. Found: C 60.59; H 5.28; N 5.23.

4.13. N^{α}-Fluoren-9-ylmethoxycarbonyl-3-*O*-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-serine 17a[43]

A solution of 1.92 g (1.82 mmol) of **16a** in 50 mL of a 6:3:1 THF/AcOH/Ac_2O was treated with 15 g of Zn powder. In 4 h the reaction was filtered through a sintered glass funnel, concentrated, and purified by FCC with 92:7:1 $CHCl_3$/MeOH/AcOH to give 1.35 g (79%) of **17a** as a glassy solid.

4.14. N^{α}-Fluoren-9-ylmethoxycarbonyl-3-*O*-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine 17b[15]

A solution of 300 mg (0.28 mmol) **16b** in 10 mL of 2:2:1 THF/AcOH/Ac_2O was flushed with argon and treated with 800 mg of Zn powder at room temperature. The progress of the reaction was monitored by HPLC–MS. In 1 h ratio of the product to the starting material was 1:2. In 4 h reaction was complete and HPLC showed the desired product in 93% purity. The reaction solution was filtered through the sintered glass, concentrated, evaporated, and the residue was purified by FCC with 75:25:1 $CHCl_3$/MeOH/AcOH to give 210 mg (77%) of the final product as a mixture of three amide rotamers (NHAc, Fmoc-NH).

4.15. Solid-phase glycopeptide synthesis

The synthesis of peptide **18** was carried out manually using a disposable plastic syringe fitted with a Teflon filter and connected to a vacuum waste bottle via a 2-way Teflon valve. The synthesis was carried out starting from Leucine-derivatized 2-chlorotrityl resin (Novabiochem, 0.1 mmol/g). Subsequent amino acids were synthesized using N^{α}-Fmoc amino acids (5 equiv) in DMF with the addition of 4.9 equiv of HATU and HOAt as an acylation catalyst. Each coupling reaction was conducted for 1 h (monitoring with bromophenol blue). The Fmoc group was removed by treatment with 20% piperidine in DMF (3 and 10 min). Resin was washed with DMF between each coupling and deprotection step. The glycosyl amino acid building block **17a** (2 equiv) was coupled for 2 × 6 h. The N-terminal amino acid was coupled with the Boc-protected linker **19**. The completeness of all reactions was monitored using the Kaiser test.

The cleavage of the peptide from the resin with simultaneous Boc and benzylidene removal was achieved by the treatment with 50% TFA in dichloromethane for 2 h. The resin was washed (5 × 2 mL) with the cleavage cocktail, concentrated to ca. 1 mL and precipitated from cold ether. The precipitate was centrifuged, dissolved in 1:1 H_2O/MeCN and purified by preparative HPLC (eluant linear gradient 95:5 → 20:80 H_2O/MeCN over 60 min, t_R = 34 min) to give 28% of peptide the N-terminal-linked peptide as the free amine. MALDI-TOF-MS: 1712.17 for $C_{78}H_{1126}N_{12}O_{30}$ (MW = 1711.90). The tetra-acetylated glycopeptide was treated with 50 mM NaOMe in MeOH for 30 min. The reaction was quenched by addition of glacial acetic acid, the mixture evaporated, dissolved in a mixture of acetonitrile water, and purified by preparative HPLC (eluant linear gradient 95:5 → 20:80 H_2O/MeCN over 60 min, t_R = 30 min) to give 93% of the free N-terminal-linked glycopeptide. MALDI-TOF: 1543.13 for $C_{70}H_{118}N_{12}O_{26}$ (MW 1543.75). A solution of 5 mg (3.2 μmol) of this material 2.5 mL of 200 mM phosphate buffer at pH 8.0 was degassed by three pump-freeze-thaw cycles. To this solution was added 750 μL (1.9 mg) of a solution of Traut's reagent 2-iminothiolane in pH 8.0 buffer (10 mg/4 mL). After 1 h under argon the solution was concentrated and purified by preparative HPLC to afford monomeric thiol-modified peptide and the corresponding disulfide (eluant linear gradient 95:5 → 20:80 H_2O/MeCN over 60 min, t_R = 31 min for monomer, t_R = 32 min for dimer). MALDI-TOF-MS for monomer: 1646.05 for $C_{74}H_{126}N_{13}O_{26}S^+$ (MW = 1645.93). MALDI-TOF-MS for dimer: 3288.08 for $C_{148}H_{250}N_{26}O_{52}S_2^{2+}$ (MW = 3287.71). Preparation of glycopeptide-coated gold particles **20** (Fig. 3B) was carried out as per the general procedure. The solution turned deep purple as with other syntheses, but after

1 h, centrifugation and attempted re-dissolution with various solvents (H_2O, MeOH, DMSO) failed.

4.16. Lectin affinity chromatography (LAC)

Agarose-bound galactose-specific peanut agglutinin (PNA) and mannose/glucose-specific *Pisum sativum* agglutinin (PSA) were purchased from Vector Labs, Burlingame, CA (www.vectorlabs.com). Equal amounts (1 mL) of agarose-immobilized PSA and PNA were loaded onto two separate columns and washed with 10 bed volumes of 1 × PBS at pH 7.4 to wash out the lectin-stabilizing sugars. Solutions of the TF–gold particles in water (50 μL) were loaded onto each column and further washed with 150 μL of 1 × PBS. After 10 min each column was washed with 10 bed volumes of 1 × PBS in 1 mL aliquots. Each aliquot was compared by UV–vis spectroscopy to a solution of 50 μL of TF–gold particles in 1 mL 1 × PBS. No particles were eluted from the PNA column in any of the 10 × 1 mL aliquots while elution of particles from the PSA column was complete in the first 1 mL aliquot. Further, washing of the PNA column with several 1 mL aliquots of 200 mM galactose displaced the particles bound to the PNA thus indicating reversibility of the binding.

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