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Synthesis and Cell-Selective Antitumor Properties of Amino Acid Conjugated Tumor-Associated Carbohydrate Antigen-Coated Gold Nanoparticles

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Abstract

The Thomsen Friedenreich antigen (TF_{ag}) disaccharide is a tumor-associated carbohydrate antigen (TACA) found primarily on carcinoma cells and rarely expressed in normal tissue. The TF_{ag} has been shown to interact with Galectin-3 (Gal-3), one in a family of β -galactoside binding proteins. Galectins have a variety of cellular functions, and Gal-3 has been shown to be the sole galectin with anti-apoptotic activity. We have previously prepared gold nanoparticles (AuNP) coated with the TF_{ag} in various presentations as potential anti-adhesive therapeutic tools or antitumor vaccine platforms. Here we describe the synthesis of TF_{ag}-glycoamino acid conjugates attached to gold nanoparticles through a combined alkane/PEG linker, where the TF_{ag} was attached to either a serine or threonine amino acid. Particles were fully characterized by a host of biophysical techniques, and along with a control particle carrying hydroxyl-terminated linker units, were evaluated in both Gal-3 positive and negative cell lines. We show that the particles bearing the saccharides selectively inhibited tumor cell growth of the Gal-3 positive cells significantly more than the Gal-3 negative cells. In addition, the threonine-attached TF particles were more potent than the serine-attached constructs. These results support the use of AuNP as antitumor therapeutic platforms, targeted against cell lines that express specific lectins that interact with TF_{ag}.

1. Introduction

Tumor-associated carbohydrate antigens (TACAs) are glycan structures presented primarily on tumor cells and nearly absent on their normal counterparts.^{1,2} These unusual structures arise from the aberrant expression of different glycosyltransferases in the transformed phenotype, leading to either extension (N-linked) or truncation (O-linked) of cell-surface

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Supplementary Data

Additional DLS, TEM and analysis data for gold nanoparticles and NMR data for all intermediates, including the gold nanoparticle conjugates. Supplementary Data can be found in the online version of this manuscript.

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glycans.^{2,3} As the name implies, these structures are targets of the human immune system (antigens), since they differ from “self” oligosaccharides. As a result, both active and passive immunotherapeutic approaches against many of these glycan structures have been explored by several groups.^{3–13} To date however, no vaccine or antibody therapies targeting TACAs has been translated to the clinic.

TACA expression can be a result of changes in several different steps in the glycoprocessing machinery, including increased/decreased sialylation^{14–24} or fucosylation^{25–29}, increased N-linked glycan branching, altered O-linked glycolipid (ganglioside) compositions^{30–34} and truncated mucin-type O-glycans.^{16,35–50} These structures, in part, may modify the physical and chemical properties of the tumor cell, leading to altered cell adhesion and signal transduction, often resulting in enhanced aggressiveness and metastatic potential. Consequently, altered tumor glycosylation is a target of many anticancer therapeutic strategies, including inhibition of glycosyltransferases^{51,52} to, in effect, remodel the aberrant glycans toward more “normal” compositions. Altered tumor glycans may also adversely affect cell adhesion, which is another target of therapeutic intervention.⁵³

The Thomsen Freidenreich TACA (herein referred to as TF_{ag}, for “TF antigen”) is a simple, truncated disaccharide, viz., Galβ1-3GalNAc-α-Serine/Threonine, that is prominently displayed on tumor cells but rarely found on normal tissue.⁵⁴ TF_{ag} is an excellent target of anticancer therapeutic intervention, as it acts as a tumor antigen as well as a mediator of metastasis (via lectin-mediated adhesive events) in several solid tumor types.^{55–58} Hence, a plethora of approaches have been explored to exploit TF_{ag} as a target for both active^{4,5,8} and passive^{59–61} immunotherapy; in addition to strategies that inhibit cell adhesion.^{57,58,62,63} It is now well established that TF_{ag} engages a specific galectin, Galectin-3 (Gal-3), during the metastatic spread of certain TF_{ag}-bearing tumors, and that this interaction can dictate the aggressiveness of the tumor.^{55,63–66} Since the majority of biologically relevant carbohydrate-protein interactions require multivalent binding for enhanced avidity⁶⁷, many of these studies have utilized platforms where the TF_{ag} or a TF_{ag} mimic is displayed in multiple copies for a more potent inhibitory effect.

Our laboratory has been interested in developing new multivalent platforms to display the TF_{ag} in various contexts^{68–71}, as potential vaccine constructs or inhibitors of cell adhesion. We have utilized gold nanoparticles (AuNPs) as our “standard” platform for their ease of synthesis coupled with the ability to attach a variety of molecular families to their surface. In the past several years, the AuNP field has exploded with a variety of constructions that have extremely useful biological/therapeutic utility,^{72–74} even one that has found its way to clinical trials.^{75,76} Decoration of AuNPs with glycan-based molecules took hold in ~2001 and has advanced our understanding of multivalent carbohydrate-protein interactions.^{77,78} TF_{ag}-coated AuNPs from our lab have been prepared both with simple linkers and with the TF_{ag} in the context of mucin-derived glycopeptides; a design that elicits an immune response in mice toward the glycosylated units.⁷¹ Herein we describe the design and synthesis of AuNPs with the TF_{ag} O-linked to the amino acids (serine (Ser) or threonine (Thr)) to which they are commonly presented on cell surface proteins. We hypothesized that cytotoxicity of these particles against tumor cells could be dependent both on the expression of Gal-3 in the cells as well as the amino acid used for conjugation.

2. Results and Discussion

2.1 Synthesis of TF_{ag}-Amino Acid Conjugates

In our original design, we synthesized TF_{ag}-coated AuNPs where the anomeric center was α -linked to a simple short polyethylene glycol segment with a terminal thiol for attachment to the nanoparticle surface.⁶⁸ In a biological setting, the glycan is attached to a protein through the hydroxyl group of either serine or threonine, and hence this saccharide-amino acid conjugate is often thought of as the actual “antigenic” structure, not solely the carbohydrate (TACA). We redesigned the synthesis of our TF_{ag}-conjugates to accommodate a single amino acid. This design makes use of the TF_{ag}-glycoamino acid and an appropriate linker for nanoparticle attachment. We chose to synthesize both Ser and Thr conjugated TF_{ag} ligands for coating the nanoparticle’s surface, since both our group^{79–81} and others^{82,83} have shown differential activity and conformational preferences in glycopeptides that are dependent on the glycan attachment to either of these amino acids (Figure 1A).

In our design, the NH₂ group of both amino acids was N-acetylated to mimic a peptide bond. As we have done previously, we also prepared “control” AuNPs that bear only the linker unit terminated by a hydroxyl group in place of the glycan (Figure 1B). The synthesis of the molecules used for AuNP coating is shown in Scheme 1.

Commercially available heptaethylene glycol **1** was mono-protected with one equivalent of 5-bromo-1-pentene affording **2** in 52% yield. Functionalization of the remaining hydroxyl group as the mesylate followed by replacement with azide gave **4** in high yield. Reduction to the amine with lithium aluminum hydride followed by Boc protection and addition of the elements of thioacetic acid across the double bond resulted in compound **6**. Removal of the Boc group with TFA followed by a peptide coupling reaction with peracetylated Fmoc-TF-Serine or Threonine (each either prepared by us⁸⁴ or purchased from Sussex Research, Ottawa, Canada) afforded the appropriately-linked TF_{ag} for preparation of gold nanoparticles. We employed coupling conditions that were determined previously by our laboratory to minimize racemization of the α -carbon of the amino acid.^{85,86} Final stages for the preparation of the desired AuNP ligand included Fmoc deprotection with piperidine, acetylation of the resulting amino group with acetic anhydride in methanol and Zemplén deprotection of all O- and S-protected acetates resulting in thiols **10a** and **10b**. For the preparation of the control ligand, compound **2** was processed directly to the thioacetate **11** and hydrolyzed to **12**; this compound was used directly for AuNP synthesis.

2.2 Preparation and Characterization AuNPs

Preparations of AuNPs were accomplished by sodium borohydride reduction of gold salts in a methanol solution (Figure 2). Initial attempts to prepare these materials in water failed to produce uniform particles which were further limited due to aggregation and instability. Using degassed methanol and specific concentrations/ratios of gold salt to carbohydrate thiol conjugate yielded much improved uniformity and stability (see experimental details). Hence, compounds **10a**, **10b** or **12** were employed to prepare small size AuNPs by reduction of HAuCl₄ with sodium borohydride in degassed methanol. Ultrafiltration to remove residual small molecules yielded dark red powders. Transmission Electron Microscopy (TEM)

analysis of all AuNPs showed good size distribution. Using two separate methods, we determined that the size of the particles fell in the 1.5–3.5 nm range (Supporting information), consistent with the preparation method, with TF_{ag}-Ser-AuNPs < PEG-AuNPs (control) TF_{ag}-Thr-AuNPs. In addition, dynamic light scattering (DLS) experiments showed the hydrodynamic diameter to be, on average, slightly over 5 nm for the TF_{ag}-Ser-AuNPs and TF_{ag}-Thr-AuNPs particles but larger (13 nm) for PEG-AuNPs; zeta potential measurements were all slightly negative (–9 to –16 mV). Figure 2 shows the TEM micrographs and DLS curves, and Table 1 list various physical data for each particle (core size, zeta potential, hydrodynamic diameter).

All TF_{ag}-coated particles are evaluated for the presence of TF_{ag} using lectin affinity chromatography with lectin-coated agarose gel (Vector labs, Burlingame, CA) according to the procedure outlined in Materials and Methods. Agarose-bound peanut agglutinin (PNA; TF_{ag}-specific) and pisum sativum (PSA; mannose/glucosamine-specific) were employed. The TF_{ag}-containing particles were found to only bind to the PNA-agarose in a simple visual test (data not shown). These TF_{ag}-containing particles could be eluted from the column with 200 mM galactose. Control PEG-linked particles did not bind either column. We next estimated ligand coverage on each TF_{ag}-containing particle by carbohydrate analysis using acid hydrolysis and High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD). All analyses showed the expected 1:1 ratio of galactose to N-acetylgalactosamine molarity, and comparison to standard curves allowed calculation of the nanomoles of sugar present on the particles. Using the diameter derived from the TEM data, the average number of gold atoms contained in each nanoparticle (assuming particles are essentially “spherical”) can be calculated. This can be combined with the number of nanomoles of ligand present (derived from the HPAE-PAD data) to calculate a weight percentage attributed to the ligands in the entire AuNP complex (see Supporting Information). Based on this data, the TF_{ag}-Ser- and TF_{ag}-Thr-AuNPs have 20 and 70 copies of ligands, respectively. Although the TF-threonine-coated particles were twice the diameter of the serine-containing constructs, the relative nanomoles of ligand contained on the TF_{ag}-Thr-AuNPs was approximately 65% of the relative coating on the TF_{ag}-Ser-AuNPs (i.e., 0.24 nmol/ug vs. 0.35 nmol/ug for Thr vs. Ser, respectively); this is due to the difference in the number of surface atoms on particles whose average diameters differ by a factor of 2. Interestingly, although the PEG-AuNPs showed approximately similar core diameters as the TF_{ag}-Thr-AuNPs, DLS data showed the hydrodynamic diameter was more than double those of the sugar-containing particles. We attribute this to possible aggregation of these AuNPs to small oligomers in solution through non-specific hydrogen bonding of the many hydroxyl groups covering the nanoparticle surface.

3. Cytotoxicity and Apoptosis Assays

Tumors bearing the TF_{ag} have been shown to be more aggressive, where the presence of this disaccharide helps to mediate metastatic spread of various solid tumor types. Increased metastasis is mediated by TF_{ag} interaction with Gal-3, a member of the β -galactoside-binding proteins that maintain a variety of functions in normal cells and whose aberrant expression is associated with several pathologies, including cancer.⁶⁵ A report by Glinsky recently showed that mimics of the TF_{ag} inhibit clonogenic survival of the highly metastatic

and Gal-3 positive MDA-MB-435 cell line, in addition to synergistic activity with paclitaxel to inhibit survival and induce apoptosis.⁸⁷ Induction of apoptosis was thought to proceed through inhibition of Gal-3 signaling by the TF_{ag} mimic. We thus evaluated the AuNPs as cytotoxins against two separate lymphoma cell lines, one Gal-3 positive (SU-DHL-6) and the other Gal-3 negative (RAJI) using the MTS viability assay according to the experimental procedure in Materials and Methods. Figure 3A outlines the results and shows that not only are the particles more cytotoxic to the Gal-3 positive cell line, but the TF_{ag}-Thr-AuNPs were found to be 3–4 times more active than the TF_{ag}-Ser-AuNPs in these cells. Furthermore, the TF_{ag}-Thr-AuNPs maintain moderate activity against the Gal-3 negative RAJI cells, while the TF_{ag}-Ser-AuNPs were inactive at the concentrations tested. The “control” PEG-AuNPs (PNP) displaying simple free hydroxyl groups were inactive in both cell lines.

The TF_{ag}-Thr-coated particles were next evaluated to determine if their mode of cytotoxicity was due to simple necrosis or through an apoptotic pathway. This was done by staining treated cells with Annexin V, which binds to phosphatidylserine displayed at the surface of cells, and is a marker for apoptotic action. Co-staining cells with propidium iodide, and analysis by flow cytometry, allowed for the differentiation of cells which were viable, necrotic (damaged), or in early and late stage apoptosis (Figure 3B). Results show that treatment of RAJI (Gal-3 negative) cells with TF_{ag}-Thr-AuNPs at low (10 μ M) and high (100 μ M) concentrations for 6 hours resulted in limited induction of both necrotic and apoptotic cytotoxic activity. Conversely, the Gal-3 positive SU-DHL-6 cells showed a significant increase in the percentage of analyzed cells in early apoptosis upon exposure to 10 μ M of the particles compared to the control, and more than twice the number of apoptotic cells when treated at high concentrations. These results suggest the selective interaction of TF_{ag}-Thr-AuNPs with Gal-3 expressing cancer cells, leading to the induction of apoptosis and cell death.

4. Discussion

Carbohydrate protein interactions are notoriously weak, and nature circumvents this weak affinity through the use of “multivalent” interactions that increase the avidity of these binding events by as much as several orders of magnitude.^{62,67} Multivalent constructs presenting carbohydrates have been used for decades as valuable biochemical tools; they have revealed mechanistic details of the so-called “cluster glycoside effect” and have been useful as potential therapeutic agents that inhibit deleterious carbohydrate-protein binding events. In this work, we have used gold nanoparticles as platforms for the multivalent displays of a specific TACA, the TF_{ag}, that is directly involved in metastasis of various tumors of the breast, prostate and pancreas.

Specifically, we synthesized the TF antigen on AuNPs in a manner that included the amino acids that the sugars are naturally attached to on cell surface proteins. Although both the TF_{ag}-Thr-AuNPs and the control PEG-AuNPs were very similar in core size, the TF_{ag}-Ser-AuNPs were much smaller, indicative of a faster self-assembly process with the strong NaBH₄ reducing agent. Despite this, uniformity was quite reasonable as shown by both TEM and DLS analysis. The TF_{ag}-Thr-AuNPs, however, contained a lower copy number of

ligands coated on their surface, relative to the number of surface atoms in the respective AuNPs. This is perhaps not surprising in light of the different conformational preferences for serine- and threonine-linked glycopeptides (vide supra): Rotation about the glycosidic torsion angles in both the Tn antigen (simple α -GalNAc) and the TF_{ag}-linked glycoaminoacids/glycopeptides are much more restricted relative to the same serine-derived conjugates.⁸⁰ This may lead to more facile attachment of the more accommodating serine conjugates, while steric considerations may restrict the number of ligands that can be attached with the threonine conjugates. We have also prepared similar particles with doping of the hydroxyl-terminated linker moiety and see more consistent coating on these particles (Barchi and Biswas, unpublished data).

Quite intriguing is the fact that the TF_{ag}-Thr-AuNPs showed increased *in vitro* cytotoxic activity towards lymphoma cells relative to those of the TF_{ag}-Ser-AuNPs. This may be a consequence of the higher number of ligands attached to the larger TF_{ag}-Thr AuNPs compared to the TF_{ag}-Ser AuNPs. The TF_{ag}-Thr AuNPs were active in the low micromolar range, which is reasonable potency for a carbohydrate-based molecular interaction. Recent structural work on Gal-3/TF_{ag} complexes has shown that *in vitro* dissociation constants for this carbohydrate-protein interaction are ~47 μ M for monomeric TF-Threonine.⁸⁸ If a “direct” relationship exists between binding Gal-3 and cytotoxicity in SU-DHL-6 cells, coating AuNPs with TF_{ag}-Thr would have increased potency by about one order of magnitude compared to the dissociation constant concentration. While not conclusive and requiring further testing, the cellular toxicity data described here suggests a selective interaction of the particles with Gal-3. Moreover, the fact that hydroxyl-coated particles are completely inactive, and that the TF_{ag}-Thr-AuNPs are more active than those with serine, strongly suggests that the presence and conformation of both the sugar and Gal-3 is critical to biological activity. In addition, although not performed with the cells lines in this study, previous unpublished work from our laboratory showed that monomeric TF-threonine or serine is approximately 100-fold less active as a cytotoxic agent in another Gal-3 positive breast cancer cell line. Both *in vitro* and *in vivo* work with other particles that present TF_{ag} in different ways is in progress and is the subject of future manuscripts.

5. Conclusion

The use of gold nanoparticles for display of various biologically relevant carbohydrates is a very active area of scientific interest. The work described here outlines the importance of both a specific threonine-linked TACA (TF_{ag}) for cytotoxicity of different tumor cell lines, as well as positive Galectin-3 expression. Displaying TACA's on AuNPs is a valid and important concept for delivery, where presentation of different carbohydrates may mimic the arrangement of molecules on the cell surface. In addition, the use of AuNP dramatically alters the pharmacokinetic properties of the antigen relative to administration of simple saccharides, potentially promoting improved biological activity. Thus the use of these constructs should shed light on various cellular mechanisms regarding carbohydrate-protein interactions and may lead to valuable therapeutic agents in the future. Follow up *in vivo* work with these and other particles will be reported in due course.

6. Materials and Methods

6.1 General Methods

Heptaethylene Glycol (**1**) was purchased from Biomatrik Inc. (Jiaxing, Zhejiang, China) and used as it is. Amicon ultra tubes were purchased from Millipore Ltd. Most all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Tetrachloroauric acid (HAuCl_4) was purchased from Strem Chemicals (Newburyport, MA). Agarose-bound galactose-specific peanut agglutinin (PNA) and mannose/glucose-specific Pisum sativum agglutinin (PSA) were purchased from Vector Labs (Burlingame, CA). Cell lines were purchased from ATCC, Manassas, VA, USA. NMR spectra were recorded on a Varian Inova instrument at 400 MHz with an inverse H-X probe. Liquid chromatography/Mass spectral (LC/MS) data were acquired on an Agilent 1100 in electrospray mode. Samples were analyzed for their sizes and zeta potentials in 10 mM NaCl at 25°C with a Malvern ZetaSizer NanoZS (Malvern Instruments Ltd, Westborough, MA) equipped with a 633 nm laser and a back-scattering detector in an automated mode. Hydrodynamic size of the samples was measured by using the Stokes-Einstein equation. The zeta potential of the samples was calculated from the electrophoretic mobility using the Smoluchowski approximation

6.2. 3,6,9,12,15,18,21-heptaohexacos-25-en-1-ol (**2**)

To an anhydrous THF solution of NaH (0.39 g of 45% in mineral oil, 10.1 mmol) was added heptaethylene glycol (**1**, 3 g, 9.2 mmol) drop wise at 4 °C. After 30 min, 1-bromopentene (1.18 mL, 10.1 mmol) was added slowly to the above solution. The reaction mixture allowed to warm to room temperature and then stirred under N_2 for 14 h. The reaction was quenched by addition of methanol at 0 °C, the solvent was evaporated and 200 mL of water was added to the residue which was then extracted with ethyl acetate (100 mL, 3X). The organic layers were washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. Purification by flash column chromatography over silica gel with 1% methanol in dichloromethane gave pure colorless oil **2**: Rf: 0.41 (4% MeOH in DCM); yield: 1.86 g, 52%, ^1H NMR (400 MHz; CDCl_3) δ 5.82 (ddtd, $J = 16.9, 10.2, 6.6, 1.9$ Hz, 1H), 5.07–4.90 (m, 2H), 3.79–3.53 (m, 28H), 3.47 (td, $J = 6.7, 1.8$ Hz, 2H), 2.61 (t, $J = 6.2$ Hz, 1H), 2.11 (dtt, $J = 8.0, 6.7, 1.4$ Hz, 2H), 1.74–1.62 (m, 2H); ^{13}C NMR (CDCl_3 ; 100 MHz) δ 132.2, 114.6, 77.4, 76.9, 76.6, 70.6, 70.5, 70.0, 69.9, 50.6, 30.2, 28.7; MS m/z [$\text{M} + \text{H}$] $^+$ calcd for $\text{C}_{19}\text{H}_{39}\text{O}_8$ 394.2, found 394.2; MS m/z [$\text{M} + \text{Na}$] $^+$ calcd for $\text{C}_{19}\text{H}_{38}\text{O}_8\text{Na}$ 417.2, found 417.2.

6.3. 3,6,9,12,15,18,21-heptaohexacos-25-en-1-yl methanesulfonate (**3**)

Compound **2** (1.5 g, 3.8 mmol) and triethylamine (2.6 mL, 19 mmol) were stirred together in 50 mL dry dichloromethane at 0 °C for 15 min followed by slow addition of methanesulfonylchloride (1.5 mL, 19 mmol). The reaction mixture was then stirred for 4 h at room temperature and filtered. The filtrate was evaporated under reduced pressure and flash column chromatography over silica gel with 2.5% methanol in dichloromethane yielded pure colorless oil **3**: Rf: 0.61 (5% MeOH in DCM); yield: 1.78 g, 99%, ^1H NMR (400 MHz; CDCl_3) δ 5.77 (ddt, $J = 16.9, 10.2, 6.6$ Hz, 1H), 5.04–4.85 (m, 2H), 4.39–4.30 (m, 2H), 3.77–3.69 (m, 2H), 3.68–3.58 (m, 23H), 3.57–3.51 (m, 2H), 3.42 (t, $J = 6.7$ Hz, 2H), 3.04 (d, $J = 0.6$ Hz, 3H), 2.07 (dtt, $J = 8.0, 6.6, 1.4$ Hz, 2H), 1.66–1.55 (m, 2H); ^{13}C

NMR (CDCl₃; 100 MHz) δ 138.2, 114.6, 70.6, 70.5, 70.4, 70.0, 69.2, 68.9, 37.7, 30.1, 28.7; MS m/z MS m/z [M + Na]⁺ calcd for C₂₀H₄₀O₁₀SNa 495.2, found 495.2.

6.4. 1-azido-3,6,9,12,15,18,21-heptaoxaheptacos-25-ene (4)

Compound **3** (1.7 g, 3.6 mmol) and NaN₃ (1.6 g, 25.2 mmol) were heated in dry DMF at 75 °C for 6h. The reaction mixture was filtered through celite and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel chromatography using 2% MeOH in dichloromethane as eluent to yield pure **4** as a colorless oil: Rf: 0.31 (5% MeOH in DCM); yield: 1.5 g, 100%, ¹H NMR (400 MHz; CDCl₃) δ 5.77 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H), 5.03 – 4.85(m, 2H), 3.65 – 3.51 (m, 26H), 3.42 (t, J = 6.7 Hz, 2H), 3.34 (dd, J = 5.6, 4.6 Hz, 2H), 2.07 (dt, J = 8.0, 6.6, 1.5 Hz, 2H), 1.69 – 1.57 (m, 2H); ¹³C NMR (CDCl₃; 100 MHz) δ 138.2, 114.6, 70.6, 70.5, 70.0, 69.9, 50.6, 30.2, 28.7; MS m/z MS m/z [M + Na]⁺ calcd for C₁₉H₃₇N₃O₇Na 442.2, found 442.2.

6.5. *tert*-butyl (3,6,9,12,15,18,21-heptaoxaheptacos-25-en-1-yl)carbamate (5)

An anhydrous THF solution (50 mL) of compound **4** (1.5 g, 3.57 mmol) was added dropwise to an ice cold LAH solution (10.5 mL, 1M THF) and stirred for 2 h at 4°C. Methanol (11 mL) was added to quench the reaction and the solvent was evaporated under reduced pressure. The crude material was used as-is to synthesize **5**. Pyridine (0.63 mL) and crude **4** were dissolved in dry DCM and stirred for 15 min followed by addition of Boc₂O (3 g, 13.74 mmol). After 3 h, the solvent was evaporated and the residue was partitioned between ethyl acetate and water. The layers were separated and the aqueous layer was extracted with ethyl acetate (75 mL, 3X). The organic layers were combined and dried over Na₂SO₄. Evaporation of the solvent under reduced pressure yielded pure **5**: Rf: 0.59 (5% MeOH in DCM); yield: 1.33 g, 76%, ¹H NMR (400 MHz; CDCl₃) δ 5.77 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H), 5.05 – 4.87 (m, 2H), 3.66 – 3.48 (m, 26H), 3.42 (t, J = 6.7 Hz, 2H), 3.27 (q, J = 5.6 Hz, 2H), 2.07 (dt, J = 8.0, 6.6, 1.5 Hz, 2H), 1.64 (dq, J = 8.5, 6.7 Hz, 2H), 1.40 (s, 9H); ¹³C NMR (CDCl₃; 100 MHz) δ 138.2, 114.6, 70.6, 70.5, 70.4, 70.2, 70.1, 70.0, 40.5, 30.1, 28.7, 28.3; MS m/z [M + Na]⁺ calcd for C₂₄H₄₇NO₉Na 516.3, found 516.3.

6.6. S-(2,2-dimethyl-4-oxo-3,8,11,14,17,20,23,26-octaoxa-5-azahentriacontan-31-yl)ethanethioate (6)

Compound **5** (1.25 g, 2.53 mmol), thioacetic acid (1.3 mL, 17.7 mmol) and a catalytic amount of AIBN were dissolved in dry DMF and reacted in a photo reactor for 6h. The solvent was coevaporated with toluene (3X) followed by silica gel chromatography of the residue (3% methanol in DCM) to yield pure **6**: Rf: 0.47 (7% MeOH in DCM); yield: 1.26 g, 87%, ¹H NMR (400 MHz; CDCl₃) δ 3.64 – 3.55 (m, 24H), 3.54 – 3.47 (m, 4H), 3.40 (t, J = 6.6 Hz, 2H), 3.26 (q, J = 5.4 Hz, 2H), 2.85 – 2.79 (m, 2H), 2.27 (s, 3H), 1.59 – 1.48 (m, 4H), 1.40 (s, 9H); ¹³C NMR (CDCl₃; 100 MHz) δ 195.8, 142.9, 71.0, 70.5, 70.4, 70.2, 70.1, 70.0, 40.3, 29.3, 29.0, 28.3, 28.9; MS m/z [M + Na]⁺ calcd for C₂₆H₅₁NO₁₀SNa 592.3, found 592.3.

6.7 N- α -Fmoc-O- α -(2,3,4,6-tetra-O-acetyl-D-galactopyranosyl- β -(1-3)-2-acetamido-2-deoxy-3,4,6-tetra-O-acetyl-D-galactopyranosyl)-L-threonine-(9,12,15,18,21,24,27-hepta-oxa-3-thioacetyl-30-azatetracontan-33-yl) (8A)

The Boc group of **6** (1.25 g, 2.19 mmol) was cleaved with 1.5 mL of 50% TFA in DCM at room temperature. After 6 h, the solvent was evaporated under high vacuum and free amine (**7**) was used without further purification to synthesize **8**. To an anhydrous ice-cold DMF solution of **7** (0.35 g, 0.59 mmol), TF_{ag}-Thr (0.61 g, 0.64 mmol), HATU (0.24 g, 0.65 mmol) and HOAT (0.88 g, 0.65 mmol) was added 2,4,6, trimethylpyridine drop-wise until the pH reached 8.5. The reaction mixture was stirred for 5 h under argon and the solvent was evaporated under reduced pressure. The crude residue was purified on a Waters preparative HPLC system using Phenomenex 30 X 75 mm 10 μ Luna C-18 column (0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) at a flow rate of 30 mL/min. A linear gradient from 5% – 100% solvent B was employed for 6 min and then held for 2 min before reequilibration. Absorbance was monitored at 254 nm to give a white glassy solid **8A** (t_r = 7.69 min): yield: 0.63 g, 73%, ¹H NMR (400 MHz; CD₃OD) δ 8.09 (s, 1H), 7.84 – 7.75 (m, 2H), 7.66 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.6 Hz, 2H), 7.33 – 7.27 (m, 2H), 7.11 (d, J = 9.0 Hz, 1H, NH), 5.39 – 5.29 (m, 2H), 5.00 – 4.92 (m, 2H), 4.65 (d, J = 7.0 Hz, 1H, H-1''), 4.56 (dd, J = 10.7, 6.2 Hz, 1H), 4.46 (dd, J = 10.8, 6.3 Hz, 1H), 4.36 – 4.14 (m, 8H), 4.14 – 4.07 (m, 2H), 4.01 – 3.89 (m, 2H), 3.63 – 3.44 (m, 28H), 3.41 (td, J = 6.5, 1.7 Hz, 2H), 2.86 – 2.78 (m, 2H), 2.26 (d, J = 1.8 Hz, 3H), 2.08 (dd, J = 11.2, 2.6 Hz, 6H), 2.03 – 1.95 (m, 12H), 1.90 (s, 3H), 1.53 (dq, J = 8.6, 7.0 Hz, 4H), 1.42 – 1.34 (m, 2H), 1.20 (d, J = 6.3 Hz, 3H); ¹³C NMR (CD₃OD; 100 MHz) δ 170.7, 170.6, 169.6, 141.2, 127.4, 126.8, 124.7, ¹³C NMR (100 MHz, CD₃OD) δ 170.77, 170.58, 169.64, 141.23, 127.43, 126.80, 124.71, 124.6, 119.6, 100.9, 70.7, 70.5, 70.2, 70, 69.7, 67.1, 62.8, 60.8, 29.1, 28.7, 28.3, 24.9, 19.4, 19.3, 19.2, 19.0, 17.8. MS m/z [M + Na]⁺ calcd for C₆₆H₉₅N₃O₂₈SNa 1432.5, found 1432.5.

6.8. N- α -acetyl-O- α -(2,3,4,6-tetra-O-acetyl-D-galactopyranosyl- β -(1-3)-2-acetamido-2-deoxy-3,4,6-tetra-O-acetyl-D-galactopyranosyl)-L-threonine-(9,12,15,18,21,24,27-hepta-oxa-3-thioacetyl-30-azatetracontan-33-yl)(9A)

Compound **8A** was treated with 5% piperidine in DMF for 1 min to deprotect the Fmoc group. The solvent was evaporated under reduced pressure and the crude material used as-is for acetylation to prepare **9A** by addition of acetic anhydride (0.23 mL, 2.23 mmol) (0.53 g, 0.446 mmol) in anhydrous methanol. The reaction was stirred for 12 h and the solvent was evaporated under reduced pressure. Silica gel chromatography (4.5% methanol/DCM) purification yielded oily **9A**: R_f: 0.55 (10% MeOH in DCM); yield: 0.52 g, 95%, ¹H NMR (400 MHz; CDCl₃) δ 6.91 (s, 1H), 6.41 (s, 1H), 5.31 (d, J = 3.1 Hz, 2H), 5.04 (dd, J = 10.6, 7.7 Hz, 1H), 4.90 (dd, J = 10.5, 3.4 Hz, 1H), 4.79 (d, J = 3.7 Hz, 1H, H-1'), 4.56 (d, J = 7.9 Hz, 1H, 1-H''), 4.53 – 4.42 (m, 2H), 4.19 – 4.10 (m, 4H), 4.05 (dd, J = 11.2, 7.8 Hz, 2H), 3.94 – 3.82 (m, 2H), 3.67 – 3.54 (m, 28H), 3.39 (t, J = 6.6 Hz, 2H), 2.81 (t, J = 7.3 Hz, 2H), 2.27 (d, J = 0.6 Hz, 3H), 2.12 – 2.10 (m, 3H), 2.09 – 1.96 (m, 18H), 1.91 (s, 3H), 1.59 – 1.49 (m, 4H), 1.41 – 1.33 (m, 2H), 1.23 (d, J = 6.3 Hz, 3H); ¹³C NMR (CDCl₃; 100 MHz) δ 195.8, 170.4, 170.1, 169.4, 101.0, 100.7, 71.0, 70.4, 70.0, 69.2, 68.6, 67.9, 66.7, 63.1, 60.8, 48.6, 39.5, 30.5, 29.3, 29.0, 28.9, 25.3, 23.2, 20.7, 20.5, 18.2; MS m/z [M + H]⁺ calcd for

$C_{53}H_{88}N_3O_{27}S$ 1230.5 found 1230.5; MS m/z $[M + Na]^+$ calcd for $C_{53}H_{87}N_3O_{27}SNa$ 1252.5, found 1252.5.

6.9. N- α -acetyl-O- α -(D-galactopyranosyl- β -(1-3)-2-acetamido-2-deoxy-D-galactopyranosyl)-L-threonine-(9,12,15,18,21,24,27-hepta-3-thia-30-azatetratricontan-33-yl) (10A)

Compound **9A** (0.5 g, 0.5 mmol) was dissolved in degassed methanol and 0.5 M NaOMe was added drop wise until the solution pH reached 8.5. The reaction was stirred for 4 h under argon and neutralized by Amberlite IR-120 resin. The solution was filtered through celite carefully under argon and the solvent was evaporated under argon flow to give pure **10A**: yield: 0.31 g, 79%, ^1H NMR (400 MHz; CD_3OD) δ 4.49 (d, J = 2.6 Hz, 1H, H-1'), 4.37 (dd, J = 10.9, 3.9 Hz, 1H), 4.32 (d, J = 7.6 Hz, 1H, H-1''), 4.19 (dd, J = 6.4, 2.7 Hz, 1H), 4.12 – 4.09 (m, 1H), 3.93 – 3.87 (m, 1H), 3.84 (dd, J = 10.9, 3.0 Hz, 1H), 3.78 (dd, J = 3.2, 1.0 Hz, 1H), 3.73 – 3.65 (m, 4H), 3.63 – 3.52 (m, 26H), 3.51 – 3.38 (m, 8H), 2.67 (t, J = 7.2 Hz, 1H), 2.47 (t, J = 7.1 Hz, 2H), 2.06 (s, 3H), 2.01 (s, 3H), 1.62 – 1.50 (m, 4H), 1.49 – 1.38 (m, 2H), 1.25 (d, J = 6.4 Hz, 3H); ^{13}C NMR (D_2O); 100 MHz) δ 174.5, 174.0, 171.4, 104.6, 98.8, 76.8, 75.1, 74.9, 72.4, 70.9, 70.7, 70.4, 69.6, 69.0, 68.5, 61.1, 60.9, 57.9, 48.4, 38.9, 38.0, 28.0, 24.0, 22.2, 21.7, 18.0; MS m/z $[M + H]^+$ calcd for $C_{39}H_{74}N_3O_{20}S$ 936.4, found 936.4; MS m/z $[M + Na]^+$ calcd for $C_{39}H_{73}N_3O_{20}SNa$ 958.4, found 958.4.

N- α -Fmoc-O- α -(2,3,4,6-tetra-O-acetyl-D-galactopyranosyl- β -(1-3)-2-acetamido-2-deoxy-3,4,6-tetra-O-acetyl-D-galactopyranosyl)-L-serine-(9,12,15,18,21,24,27-hepta-3-thioacetyl-30-azatetratricontan-33-yl) (8B)

Same procedure used as that for **8A** except for the use of TF_{ag} -Ser (t_r = 7.5 min), yield 77%, ^1H NMR (400 MHz; $CDCl_3$) δ 7.73 (d, J = 7.6 Hz, 2H), 7.55 (d, J = 7.5 Hz, 2H), 7.37 (t, J = 7.4 Hz, 2H), 7.28 (td, J = 7.4, 1.1 Hz, 2H), 5.35 – 5.27 (m, 2H), 5.08 – 5.02 (m, 1H), 4.89 (dt, J = 11.4, 5.7 Hz, 1H), 4.77 (d, J = 3.6 Hz, 1H), 4.55 – 4.34 (m, 4H), 4.18 (t, J = 6.7 Hz, 1H), 4.06 (dq, J = 12.2, 6.3, 5.6 Hz, 4H), 3.96 – 3.71 (m, 6H), 3.63 – 3.44 (m, 26H), 3.39 (q, J = 8.1, 6.6 Hz, 4H), 2.81 (t, J = 7.3 Hz, 2H), 2.27 (s, 3H), 2.10 (d, J = 8.6 Hz, 6H), 2.05 – 1.87 (m, 15H), 1.59 – 1.48 (m, 4H), 1.41 – 1.29 (m, 2H); ^{13}C NMR (CD_3OD ; 100 MHz) δ 195.9, 170.4, 170.0, 169.5, 155.9, 143.6, 141.2, 127.8, 127.0, 124.8, 120.0, 100.8, 99.1, 71.0, 70.2, 70, 68.6, 67.7, 66.7, 62.6, 60.9, 47.0, 39.4, 30.5, 29.3, 29.0, 28.9, 25.3, 20.7, 20.6, 20.5; MS $[M + H]^+$ calcd for $C_{65}H_{94}N_3O_{28}S$ 1396.6, found 1396.6; MS m/z $[M + Na]^+$ calcd for $C_{65}H_{93}N_3O_{28}SNa$ 1418.5, found 1418.5.

6.10. N- α -acetyl-O- α -(2,3,4,6-tetra-O-acetyl-D-galactopyranosyl- β -(1-3)-2-acetamido-2-deoxy-3,4,6-tetra-O-acetyl-D-galactopyranosyl)-L-serine-(9,12,15,18,21,24,27-hepta-3-thioacetyl-30-azatetratricontan-33-yl) (9B)

Same procedure used as that for **9A**: Rf: 0.55 (10% MeOH in DCM); yield 95%, ^1H NMR (400 MHz; CD_3OD) δ 5.39 (d, J = 3.4 Hz, 1H), 5.33 (dd, J = 3.3, 1.2 Hz, 1H), 5.01 (d, J = 3.3 Hz, 1H), 5.00 – 4.96 (m, 1H), 4.79 – 4.77 (m, 1H), 4.74 (d, J = 7.5 Hz, 1H, H-1''), 4.59 (dd, J = 5.5, 4.6 Hz, 1H), 4.39 – 4.34 (m, 1H), 4.17 – 4.10 (m, 4H), 4.04 – 3.98 (m, 2H), 3.95 – 3.85 (m, 2H), 3.66 (dd, J = 10.6, 5.7 Hz, 1H), 3.63 – 3.55 (m, 24H), 3.56 – 3.49 (m, 4H), 3.44 (td, J = 6.4, 3.4 Hz, 2H), 3.37 (td, J = 5.2, 2.3 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.27 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.03 – 1.99 (m, 9H), 1.97 (s, 3H), 1.90 (d, J = 0.9 Hz, 4H),

1.55 (p, $J = 7.2$ Hz, 4H), 1.44 – 1.36 (m, 2H); ^{13}C NMR; MS m/z [M + Na] $^+$ calcd for $\text{C}_{52}\text{H}_{85}\text{N}_3\text{O}_{27}\text{SNa}$ 1238.5, found 1238.5.

6.11. N- α -acetyl-O- α -(D-galactopyranosyl- β -(1-3)-2-acetamido-2-deoxy-D-galactopyranosyl)-L-threonine-(9,12,15,18,21,24,27-heptaosa-3-thia-30-azatetatriacontan-33-yl) (10B)

Same procedure was followed as that for **10A**: yield 77.7%, ^1H NMR (400 MHz; D_2O) δ 4.73 (d, $J = 3.7$ Hz, 1H, H-1'), 4.42 (t, $J = 4.8$ Hz, 1H), 4.31 (d, $J = 7.7$ Hz, 1H, H-1''), 4.16 (dd, $J = 11.0, 3.7$ Hz, 1H), 4.08 (d, $J = 3.1$ Hz, 1H), 3.88 – 3.72 (m, 4H), 3.68 – 3.61 (m, 1H), 3.61 – 3.56 (m, 4H), 3.55 – 3.41 (m, 28H), 3.41 – 3.32 (m, 4H), 3.26 (t, $J = 5.4$ Hz, 2H), 2.60 (t, $J = 7.2$ Hz, 1H), 2.39 (t, $J = 7.1$ Hz, 1H), 1.92 (s, 3H), 1.87 (s, 3H), 1.43 (dq, $J = 13.7, 6.9$ Hz, 4H), 1.27 (q, $J = 8.0$ Hz, 2H); ^{13}C NMR (D_2O ; 100 MHz) δ 174.26, 171.31, 129.40, 104.57, 98.00, 76.90, 74.90, 72.43, 70.81, 70.49, 69.49, 69.02, 68.68, 68.50, 67.31, 60.87, 48.39, 39.00, 32.64, 27.87, 23.91, 23.55, 22.00, 21.68; MS m/z [M + H] $^+$ calcd for $\text{C}_{38}\text{H}_{72}\text{N}_3\text{O}_{20}\text{S}$ 921.4, found 922.4; MS m/z [M + Na] $^+$ calcd for $\text{C}_{38}\text{H}_{71}\text{N}_3\text{O}_{20}\text{S Na}$ 944.4, found 944.4.

6.12. S-(1-hydroxy-3,6,9,12,15,18,21-heptaohexacosan-26-yl) ethanethioate (11)

Compound **11** was synthesized following the same photo catalytic method as that for preparation of **6** from **5**: yield 87%, ^1H NMR (400 MHz; CDCl_3) δ 3.73 – 3.50 (m, 28H), 3.41 (t, $J = 6.6$ Hz, 2H), 2.87 – 2.79 (m, 2H), 2.28 (s, 3H), 1.62 – 1.49 (m, 4H), 1.44 – 1.31 (m, 2H); ^{13}C NMR (CDCl_3 ; 100 MHz) δ 194.9, 72.4, 71.0, 70.6, 70.5, 70.3, 70, 69.1, 30.6, 29.3, 29.0, 25.3; MS m/z [M + H] $^+$ calcd for $\text{C}_{21}\text{H}_{43}\text{O}_9\text{S}$ 471.2, found 472.2; MS m/z [M + Na] $^+$ calcd for $\text{C}_{21}\text{H}_{42}\text{O}_9\text{SNa}$ 493.2 found 493.2.

6.13. 26-mercapto-3,6,9,12,15,18,21-heptaohexacosan-1-ol (12)

Same procedure was followed as that for **10**: yield: 79%, ^{13}C NMR (101 MHz, CD_3OD) δ 72.19, 70.64, 70.11, 70.10, 70.08, 70.07, 70.04, 69.91, 69.74, 60.75, 38.18, 28.86, 28.57, 24.63; MS m/z [M + H] $^+$ calcd for $\text{C}_{19}\text{H}_{41}\text{O}_8\text{S}$ 429.2, found 429.2; MS m/z [M + Na] $^+$ calcd for $\text{C}_{19}\text{H}_{40}\text{O}_8\text{SNa}$ 493.2 found 451.2.

6.14. Synthesis of Gold Nanoparticles

$\text{TF}_{\text{ag}}\text{-Thr/Ser}$ (**10A**, **10B** or **12**, 2 eq.) in degassed MeOH (7X MeOH/ μmol of $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$) was added to a solution of $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ (1 mg/mL in water). This mixture was stirred for 10 min at 4 °C and then 20 equivalent of ice cold NaBH_4 (1 mg/mL in water) was added drop-wise over 20 min. The reaction was stirred at 0°C for 30 min under argon and then slowly warmed to room temperature. The reaction was further stirred for 3 h at RT under argon and the solvent was evaporated under reduced pressure. The concentrated residue was purified using Amicon 10 kDa Centricon ultrafiltration system. The nanoparticle pellet was redispersed in water and freeze-dried to yield pure AuNPs as a red gummy solid.

6.15. Lectin affinity chromatography

Equal amounts (1 mL) of agarose-immobilized PSA and PNA were loaded onto two separate columns and washed with 10 bed volumes of HEPES buffer at pH 7.4 to wash out

the lectin-stabilizing sugars. Solutions of the TF-AuNPs particles in water (50 μ L) were loaded onto each column and further washed with 150 μ L of 1 X HEPES. After 10min each column was washed with 10 bed volumes of HEPES in 1 mL aliquots. A solution of 200 mM galactose in buffer was used to elute AuNPs that were retained on the column.

6.16. Carbohydrate Analysis

AuNP samples were dissolved in water to a concentration of 1 mg/ml. An aliquot of 100 μ L was mixed with an equal volume of 4M TFA and the mixture heated at 100° C for 3 hrs. The sample was then centrifuged to remove solid particles and the supernatant lyophilized. The residue from lyophilization was reconstituted in 100 μ L of water (1 μ g/ μ L concentration). Analysis was performed on a Dionex ICS 5000 HPAEC instrument by injecting an aliquot of the hydrolysate corresponding to 1 μ g of the original sample. Calculated peak areas were an average of duplicate runs, and these were compared to those of a standards based on known concentrations of monosaccharides. A CarboPac™ PA10 (250 x4 mm) analytical column was used and elution was by an isocratic 18 mM NaOH solution.

6.17. Cytotoxicity of TF-Au Nanoparticles and Cellular Apoptosis

Toxicity of TF_{ag}-AuNPs towards SU-DHL-6 (galectin-3 positive) and RAJI (galectin-3 negative) lymphoma cells was assessed using the MTS viability assay. Briefly, cells were plated at 20 x 10³ cells/well in a 96 well plate and incubated overnight. To each well was added 10 μ L of a concentrated solution of TF_{ag}-Thr-AuNPs, TF_{ag}-Ser-AuNPs or PEG-AuNPs prepared in DI water to achieve final concentrations of 0.001 – 100 μ M. Blank or 20% DMSO containing media was used as negative and positive controls, respectively. Samples were incubated for 24 hours, followed by centrifugation of treated cells at 2,500 rpm for 10 minutes at 4°C to pellet. The supernatant was removed and 100 μ L of fresh media containing 20 μ L MTS reagent was added, followed by incubation of the cells for 4 hours. Absorbance was then read at 490 nm, following manufacturer's instructions (Promega, Madison, WI), using a UV plate reader (Biotek, Winooski, VT). The absorbance of the negative controls was subtracted from each sample as a blank, and percent viability calculated using the equation: (Absorbance_{treated cells}/Absorbance_{untreated cells}) × 100. IC₅₀ values were computed using the Graphpad 5.0 software package and represented as the average of three independent experiments ± standard deviation.

Induction of apoptosis in these cell lines upon treatment with TF_{ag}-Thr-AuNPs was assessed using the Annexin V/Dead Cell Apoptosis Kit following the manufacturer's instructions (Life Technologies, Grand Island, NY). Specifically, 5 x 10⁵ RAJI or SU-DHL-6 cells in 1mL media were placed in flow cytometry tubes and allowed to incubate overnight. Cells were then centrifuged at 2,000 rpm for 5 minutes to pellet, and fresh media containing 10 μ M or 100 μ M of TF_{ag}-Thr-AuNPs was added to each tube and then incubated for 6 hours. After incubation cells were pelleted and washed with 200 μ L PBS, followed by washing with a 1X annexin binding buffer solution. Cells were then suspended in 100 μ L of fresh binding buffer and 5 μ L of an Alexa Fluor 488-Annexin V solution was added. Cells were incubated for 15 minutes before washing, followed by addition of 100 μ L binding buffer containing 1 μ L of a propidium iodide solution. Before analysis by flow cytometry, samples were diluted with 400 μ L binding buffer and gently vortexed. To establish gating regions for

flow cytometry analysis, untreated cells were left unlabeled, treated with each individual stain, or double stained, and signal plotted as Alexa Fluor 488 versus propidium iodide (AF/PI) fluorescence intensity. Results are presented as the percentage of total analyzed cells detected in the ++ (late apoptosis), +/- (early apoptosis), -/+ (damaged) or -- (viable) gating regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Gold nanoparticles coated with Thomsen Friedenreich antigen glycoaminoacids were prepared
- Selective cytotoxicity of tumor cells expressing Galectin-3 was observed
- Selectivity was also seen for threonine constructs over those attached to serine

Figure 1. (A) Serine and threonine-conjugated TF_{ag} AuNPs use in this study. (B) Structure of the control linker-conjugated particles.

Figure 2. (A) Preparation of AuNPs. (B) TEM, (C) ^1H NMR and (D) DLS analysis of $\text{TF}_{\text{ag}}\text{-Thr-AuNPs}$. (E) Carbohydrate analysis of the two TF_{ag} -bearing AuNPs.

Figure 3.
 (A) MTS cell viability assay for both RAJI and SU-DHL-6 lymphoma cells, with corresponding IC₅₀ values for each formulation. (B) Stages of apoptosis resulting from treatment of cells with TF_{ag}-Thr-AuNPs. Significant increase in apoptotic cells due to treatment was only observed in Gal-3 positive cells.

Scheme 1.

a) Pentenyl bromide, sodium hydride, THF, 4 °C (0.5 h) to RT, 14 h, 52%; b) Thioacetic acid, AIBN, DMF, hv, 6h, 87%; c) NaOMe/MeOH, pH 8.5, 4h 79%; d) Methanesulfonyl Chloride, triethylamine, dichloromethane, 4h, 99%; e) sodium azide, DMF, 75°C, 6 h, 99%; f) i. lithium aluminum hydride, THF, 4 °C, 2 h; ii. Boc₂O, pyridine, dichloromethane, 3 h, 76% over 2 steps; g) 50% TFA, dichloromethane, 2 min; then HOAT/HATU, 2,4,6-trimethylpyridine in DMF, 5 h, **8A**-73%; **8B**-77%. h) i. Piperidine, DMF, 1 min; ii. Ac₂O, MeOH, 12 h 95% over 2 steps.

Table 1

DLS, zeta potential and core size data for AuNPs.

Formulations	Average hydrodynamic diameter (nm)	Zeta Potential (mV)	Core Size (nm) ^a
TF _{ag} -Ser-AuNP	5.5	– 16.2	1.44/1.91
TF _{ag} -Thr-AuNP	5.3	– 12.5	3.24/3.82
HO-PEG-AuNP	13	– 9.0	2.76/3.36

^aCore size was calculated by two methods: Back calculated from area (first number) and using the maximum diameter measurement (both calculated in ImageJ, NIH)