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Glycoamino Acid Analogues of the Thomsen−Friedenreich Tumor-Associated Carbohydrate Antigen: Synthesis and Evaluation of Novel Antiproliferative Factor Glycopeptides

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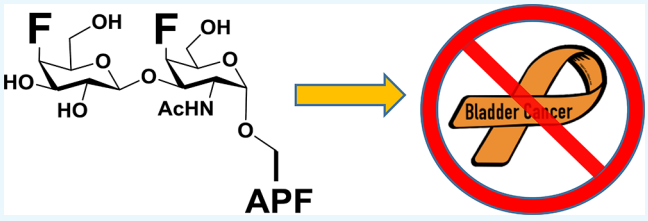
Supporting Information

ABSTRACT: Glycoamino acid analogues of the Thomsen−Friedenreich antigen disaccharide, where the 4′ and 4″ hydroxyl groups were substituted with fluorine or hydrogen, were synthesized and incorporated into the asialylated antiproliferative factor (*as*-APF), a biologically active form of APF, a glycopeptide found in the urine of patients with interstitial cystitis. Various strategies were employed to incorporate the fluorine atom at the 4-positions of either the galactose or *N*-acetylgalactosamine unit of the disaccharide antigen, based on stereochemistry and reactivity. These glycopeptides were evaluated in antiproliferative assays on both primary normal bladder epithelial cells and T24 bladder carcinoma cells. Unlike many previously published substitutions to APF, mono-4′-fluorination of the GalNAc residue did not affect the activity, whereas fluoro-derivatives of the galactose 4″-position or both 4′ and 4″ hydroxyls showed a reduced potency relative to the monosubstituted GalNAc derivative. A fourth compound where the 4″ position of galactose was deoxygenated showed a lower potency than the parent and monosubstituted compounds. These results suggest that specific substitutions in the sugar moieties in the APF can be tolerated, and the glycomimetic design of APF analogues can include fluorine in the GalNAc sugar of the disaccharide.



INTRODUCTION

All mammalian cell surfaces are coated with oligosaccharide chains (termed "glycans") that are presented in various guises through covalent attachment to proteins and lipids.[1] Proteins primarily display two types of glycosylation: N-linked (glycan with a reducing end anomeric "*β*" N-linkage to the carboxamide of an asparagine residue) and O-linked (glycan with a reducing end anomeric "*α*" O-linkage to the hydroxyl group of a serine or threonine residue); and these sugars contribute to a variety of protein functions (e.g., folding, infection, cell−cell communication, and immune responses).[2] Many of these glycan chains are highly modified during various disease states, such as cancer,[3] with many of these aberrant glycans becoming targets of the immune system and are hence known as tumor-associated carbohydrate antigens (TACAs). Two prevalent O-linked TACAs expressed on many solid tumors are Tn[4] (Thomsen nouvelle, GalNAc*α*-*O*-Ser/Thr, Tn_{ag}) and its extended core-1 structure, TF (Thomsen−Friedenreich, Gal*β*1-3GalNAc*α*-*O*-Ser/Thr, TF_{ag}) antigens.[5] Truncation of larger normal cell oligosaccharide chains to these smaller glycan structures serves to expose underlying peptide sequences within the extracellular domains of cell-surface proteins (e.g., mucins). As a result, these novel epitopes can be recognized by the immune system, and thus various strategies to design immunotherapies against TACAs have been evaluated in recent years.[6−14] In addition, TF_{ag} has been directly tied to the metastasis of breast,[15] prostate,[16−18] and pancreatic[19] cancer through its interaction with tumor-derived galectin-3.

Although modified TACAs such as TF_{ag} have been used in immunological/antitumor studies outlined above, TF_{ag} is also found on selected natural products, which have biological activity that is dependent on the presence of the disaccharide. The conotoxins,[20] cod glycopeptide,[16] and the antiproliferative factor (APF) from interstitial cystitis (IC) patients[21] all contain TF_{ag}, which contributes directly to their biological function. Hence, modified versions of TF_{ag} and their use in synthetic

Received: July 18, 2017
Accepted: August 24, 2017
Published: September 8, 2017

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

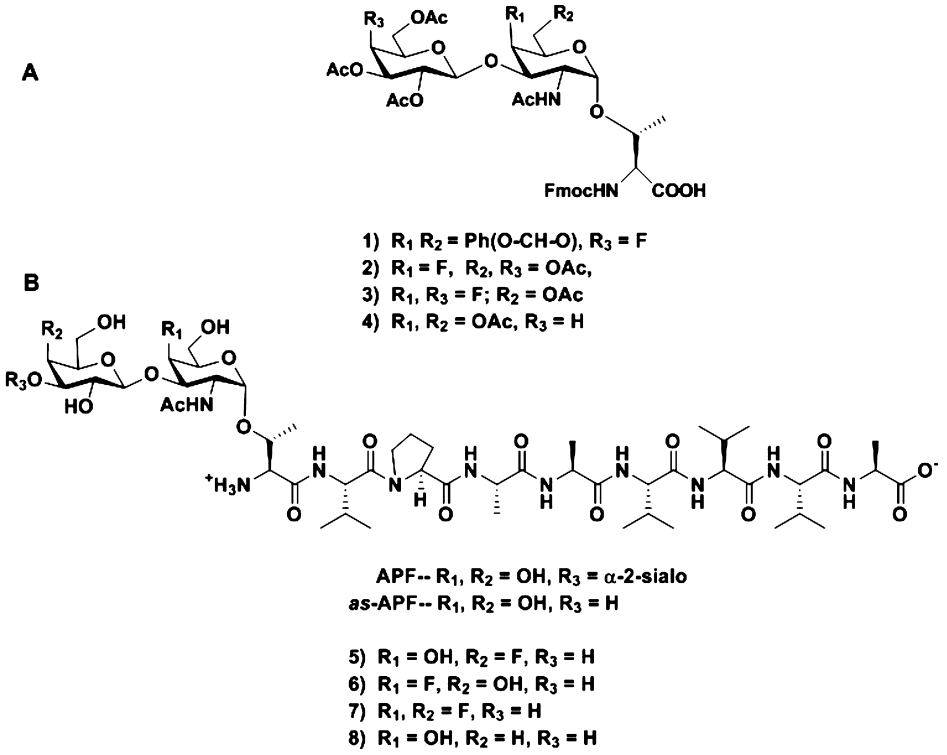
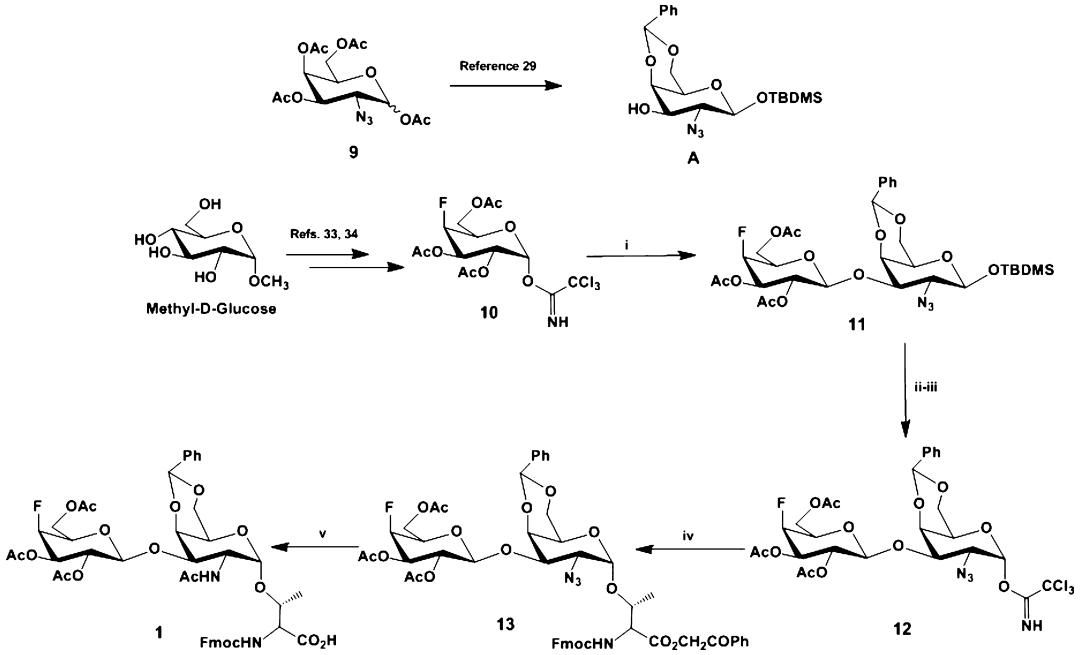


Figure 1. (A) Target glycoamino acids and (B) glycopeptides used in this study. APF and asialylated APF (*as*-APF) are labeled accordingly.

Scheme 1[a]



[a]Reagents and conditions: (i) **A**, TMS-OTf, dichloromethane (DCM), 4 Å molecular sieves (MS), 67% (ii) $Et_3N{\cdot}3HF$, tetra-*n*-butylammonium fluoride (TBAF), tetrahydrofuran (THF), 76%; (iii) CCl_3CN, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), DCM, 76%; (iv) **B**, TMS-OTf, DCM/THF 3:1, 4 Å MS, 74.4%; and (v) Zn, THF/AcOH/Ac_2O (6:3:1) 71.4%.

congeners of these active glycopeptides may serve to influence either the mode of action or the potency of these agents. Our group has extensively studied APF, a highly potent sialylated TF_{ag}-containing glycopeptide, discovered in the urine of patients with IC/painful bladder syndrome (PBS), a debilitating disease of the bladder. The APF treatment of normal bladder (NB) cells causes cellular changes that are reminiscent of the disease.[22,23] In addition, APF is not only highly potent against NB epithelial cells but also a powerful antiproliferative agent against a variety of tumor cell lines in vitro.[24] Our lab has prepared several analogues of this glycopeptide and has developed a solid structure−activity relation profile for the

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

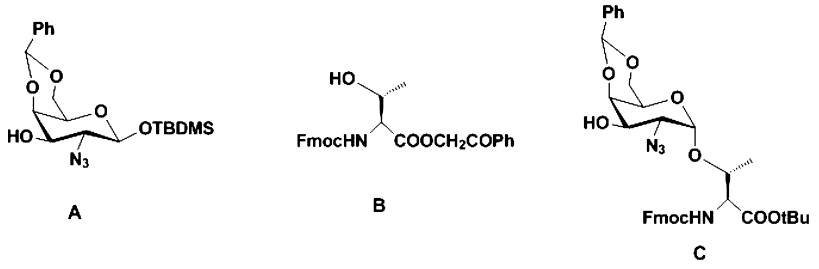
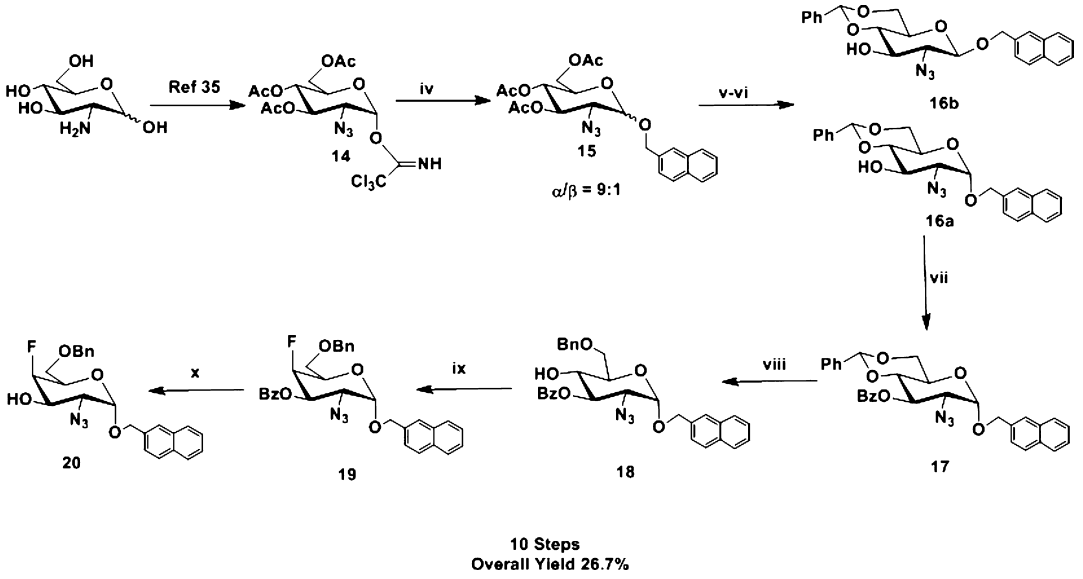


Figure 2. Various acceptors used for the synthesis of (A) compound **11**, (B) compounds **13**, **29**, and **30**, and (C) compound **32**.

Scheme 2[a]



[a]Reagents and conditions: (i)−(iii) ref 35; (iv) 2-napthylmethanol, TMS-OTf, DCM/ether (2:1) 87.3%; (v) 25% NaOMe/MeOH, 82%; (vi) benzaldehyde dimethylacetal, camphor-10-sulfonic acid (CSA), dimethylformamide (DMF), **16a**, 73.6%, **16b**, 8.4%; (vii) benzoyl chloride/pyridine, 91.6%; (viii) $NaBH_3CN$, 4 Å MS, THF; then 2 M HCl in ether, 85.7%; (ix) DAST, DCM, 84%; and (x) 25% NaOMe/MeOH, 94.3%.

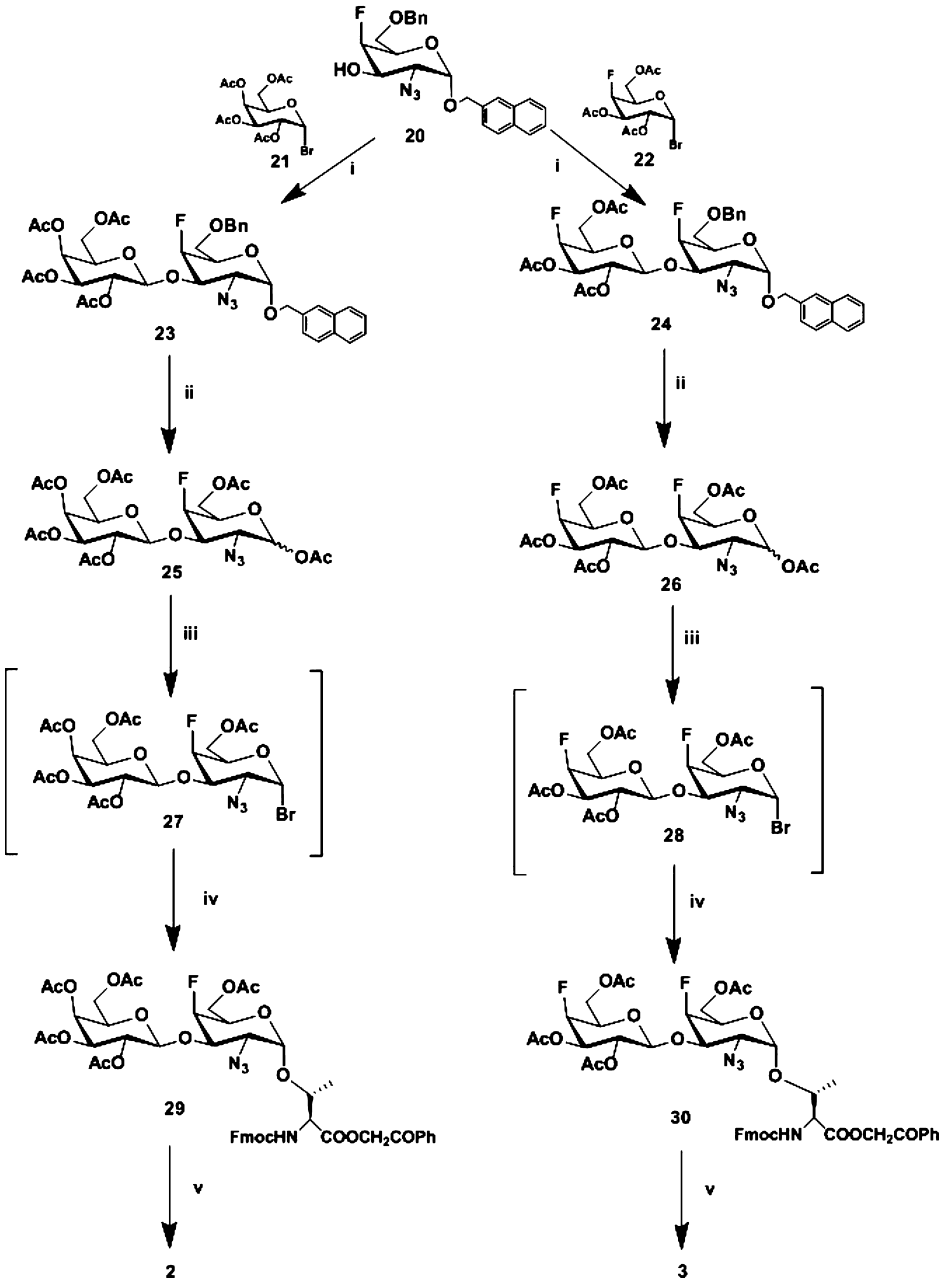
antiproliferative activity of APF.[25,26] Although we previously documented that a TF_{ag} disaccharide moiety and proper stereochemistry are critical for its activity, we had not prepared analogues where selected functional groups of the sugars were substituted. In past reports on immunological studies of TF_{ag}-containing mucin glycopeptides, specific functional groups of TF_{ag} were substituted with a fluorine atom, which is often used as a surrogate for both hydroxyl groups and hydrogen atoms.[9,27,28] However, the axial 4′-position is more difficult to replace with fluorine, and hence only one study of a TF antigen with a 4″-fluoro modification on the galactose residue was recently reported.[6] Herein, we describe our own synthesis of a modified version of the previously reported 4″-fluoro-galactose glycoamino acids (GAAs) along with three other analogues (two fluorinated and one 4″-deoxy) of the TF_{ag} and their incorporation into GAAs (Figure 1A). We used these new GAAs as building blocks in Fmoc-based solid-phase peptide synthesis (SPPS) to prepare novel analogues of APF (Figure 1B) and studied the effect that these substitutions had on the antiproliferative activity.

RESULTS AND DISCUSSION

4″-F-Galβ1-3GalNAc TF GAA (1). The synthesis of both monomeric 4′-fluoro-galactose and 4′-fluoro-galactosamine derivatives has been described in the literature (vide infra); however, reports for the synthesis of GAAs, with these congeners as precursors for the glycopeptide synthesis, are sparse. A very recent report by Hoffmann-Röder and co-workers outlined the first synthesis of MUC1-based glycopeptides using a TF-antigen derivative with a 4″-fluoro-galactose moiety.[6] The 4″-fluoro group imparted resistance to hydrolysis by β-galactosidase, and the authors were able to prepare MUC1 glycopeptide conjugates of this analogue with the tetanus toxoid protein1 and generate an immune response in mice.[6] We have prepared a similar 4″-F-Gal-GalNAc-Thr GAA building block via an alternative process outlined in Scheme 1. The known galactosamine precursor **A** (Figure 2) used for our synthesis was initially derived from our previous work on an improved TF_{ag} synthesis,[29,30] but arrived via a slightly shorter procedure, which we found as more efficient and avoids an azidonitration step. Starting from galactosamine hydrochloride, access to acetylated 2-azido galactose peracetate derivative **9** proceeded smoothly via the diazo transfer reaction of Wong,[31] using the modified imidazole-based transfer reagent of Goddard-Borger and Stick.[32] Adapting from our previous work, this was processed in six high-yielding steps to the known[30] acceptor **A**. The known 4′-fluoro-galactosyl donor **10** was simply prepared in five steps by the method of Koch and Chambers[33] through the selective acetylation of the 2′, 3′, and 6′ positions of methyl-D-glucose via a stannyl intermediate and subsequent diethylaminosulfur trifluoride (DAST) fluorination of the sole free 4′ hydroxyl group to afford the 2,3,6-triacetyl-4-

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

Scheme 3[a]



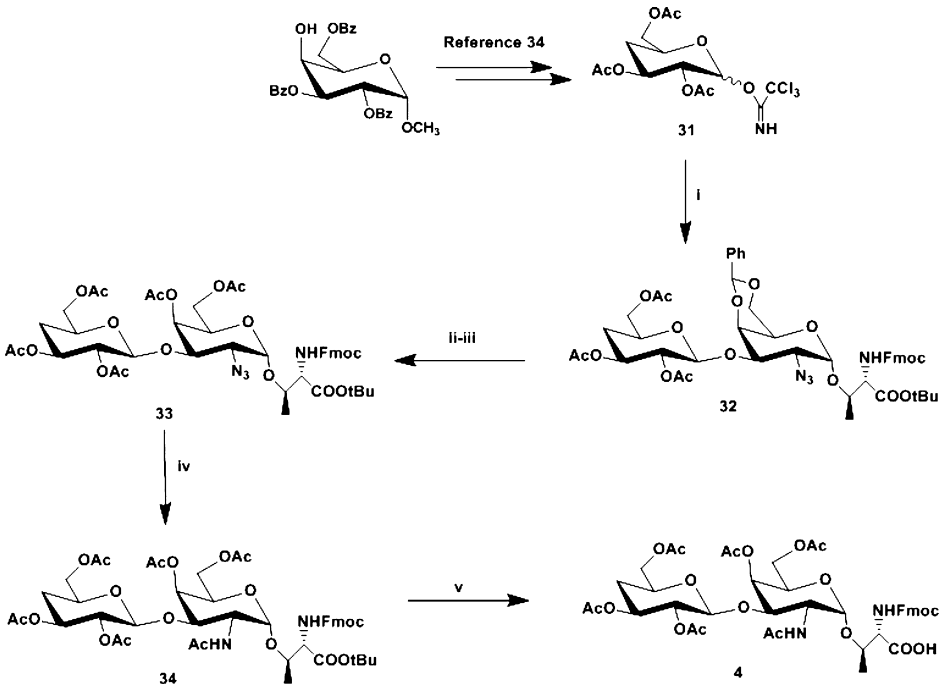
[a]Reagents and conditions: (i) AgOTf, DCM, 4 Å MS, 55% (**23**) 60% (**24**); (ii) 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), DCM/H_2O (20:1), then Ac_2O, pyridine, 53% (**25**) 47% (**26**); (iii) TMSBr, $BiBr_3$, DCM, 98% (**27**) 87% (**28**); (iv) **B**, $AgClO_4$, DCM, 4 Å MS; 97% (**29**, α/β; 56:41) 95% (**30**, α/β; 51:44); and (v) Zn, THF/AcOH/AC_2O (6:3:1) 84% (**2**) 93% (**3**).

F-methyl galactoside. Acetolysis to the anomeric acetate, followed by its selective removal with hydrazine acetate and trichloroimidate formation afforded **10**[34] in 66% overall yield. Glycosylation with acceptor **A** gave β1-3 TF_{ag}-disaccharide derivative **11** (67%). Transformation of **11** into a glycoamino acid building block involved tert-butyldimethylsilyl removal and trichloroimidate formation to **12**, glycosylation to **13** with the protected threonine derivative **B** (Figure 2), and concomitant azide reduction/carboxylate deprotection, which afforded α-glycoamino acid reagent **1** in 32% overall yield.

4″-F- and 4′,4″-Difluoro Galβ1-3GalNAc TF GAAs (2 and 3). *N*-Acetyl galactosamine derivatives bearing an axial 4′ fluorine are known in the literature; but to prepare compound **2**, the TF_{ag}-glycoamino acid with 4′-F GalNAc (as well as **3** with both axial hydroxyls substituted with fluorine), our strategy necessitated some modified anomeric chemistry to obtain an efficient fluorination at the 4′ and 4″ positions (Scheme 2). Similar to galactosamine, glucosamine hydrochloride was transformed into the peracetylated 2-azido derivative. Selective removal of anomeric acetate, donor formation to the known trichloroimidate **14**,[35] and glycosylation with 2-naphthalenemethanol afforded a 9:1 inseparable α/β mixture of naphthyl glycosides **15** in 76% overall yield (three steps). The naphthyl system was installed because previous reports suggested that fluorination by the displacement of a hydroxy derivative at C4′ in a gluco-configuration

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

Scheme 4[a]



[a]Reagents and conditions: (i) **C**, TMS-OTf, 1,2-dichloroethane, 4 Å MS, 70%; (ii) iodine, MeOH/H_2O, reflux; (iii) acetic anhydride, pyridine, 73.4%, two steps; (iv) chloroform/pyridine/thioacetic acid (1:1:1), 88.7%; and (v) 95% aq trifluoroacetic acid (TFA), 94.9%.

would be facilitated by bulky anomeric appendages that are in the α position.[36] Hence, acetate hydrolysis followed by 4′,6′ benzylidene formation resulted in a now separable mixture of alcohols **16a** and **16b**. Pure α anomer **16a** was 3′-benzoylated to yield **17**, and the 4′-position was unlocked by the reductive opening of the 4′,6′-benzylidene ring, allowing smooth conversion of compound **18** with DAST in DCM to 4′-fluorinated galactosamine analogue **19** in 84% yield. This is one of the high-yielding 4′-axial fluorinations of a 2-azido sugar reported to date.[37−40] Removal of the 3′-benzoate ester afforded acceptor **20**.

The synthesis of glycoamino acids **2** and **3** (Scheme 3) converged at this point using **20** as a common acceptor. Disaccharide formation between **20** and a galactose donor however proved problematic. We attributed this to the possible "disarming" of the C3′ hydroxyl group reactivity through an inductive or conformational effect imparted by the axial fluorine atom. Several methods were attempted to enhance the reactivity of the acceptor hydroxyl group, but none proved expedient or high-yielding. Optimum yields were obtained by the "classical" method of reacting peracetylated galactosyl bromide **21** or fluorinated derivative **22**[33] with the acceptor under the auspices of silver triflate catalysis, with yields of pure β-disaccharides **23** or **24** of 60 and 55%, respectively. We were pleased at this moderate but an acceptable yield and the stereoselectivity in these novel reactants; although this seemingly simple 1,3 glycosylation using an assisting acetate at O2′ of the galacto-donor should result in near-exclusive beta products, several have reported mixtures and hydrolysis products that hamper the efficiency of this coupling.[27,41,42] Another bright spot in this method was that approximately 15% of the precious fluorinated acceptor could be recovered during purification. Elaboration to the appropriate GAAs **2** and **3** involved the following straightforward sequence. Reductive removal of both naphthyl and benzyl groups followed by acetylation afforded compounds **25** and **26**. Formation of anomeric bromides **27** and **28** and glycosylation with amino acid **B** and silver perchlorate afforded α-linked GAAs **29** and **30**. Simultaneous azide reduction, N-acetylation, and phenacyl ester removal, as above, afforded GAAs **2** and **3** in a high yield. The only downside to this highly efficient sequence is the amino acid glycosylation step: although this proceeds in a nearly quantitative yield for both fluorinated derivatives, the anomeric ratio only slightly favored the α product (reagents and conditions, Scheme 3), even if the anomers were easily separable. The stereoselectivity of the glycosylation undoubtedly suffered because of the lack of any steric or electronic bias in the 4-fluoro donor. The timing of this step, however, was difficult to avoid because it was necessary to fluorinate the sugar building blocks before the introduction of the amino acid portion of the molecule. Hence, any structural features that may bias the glycosylation toward specific anomers are removed before this step. Glycoamino acid formation at the monomer stage of the 4′-fluoro galactosamine derivatives did not enhance the anomeric ratio in a favorable way.

4″-Deoxy-Galβ1-3GalNAc TF GAA (4). We also prepared the TF antigen GAA (**4**) deoxygenated at the galactose 4′ position. Commercially available methyl(2,3,6-tri-*O*-benzoyl)-galactopyranoside (Scheme 4) was processed through the 4′-thiocarbonylimidazole derivative with tri-*N*-butyl tin hydride and azobisisobutyronitrile in refluxing toluene to afford the known methyl-(2,3,6-tri-*O*-benzoyl)-4′-deoxyglucoside.[34] The benzoyl groups were replaced with acetates, and the anomeric center was processed similar to the preparation of **10** above to afford 4′-deoxy gluco-donor **31**[34] in nearly 50% yield over seven steps. Glycosylation with the known Fmoc/*t*-butyl protected acceptor **C**[43] (Figure 2) proceeded to give β-disaccharide **32** in 70% yield. Simple, high-yield processing of **32** through **33** (benzylidene removal and acetylation) and **34**

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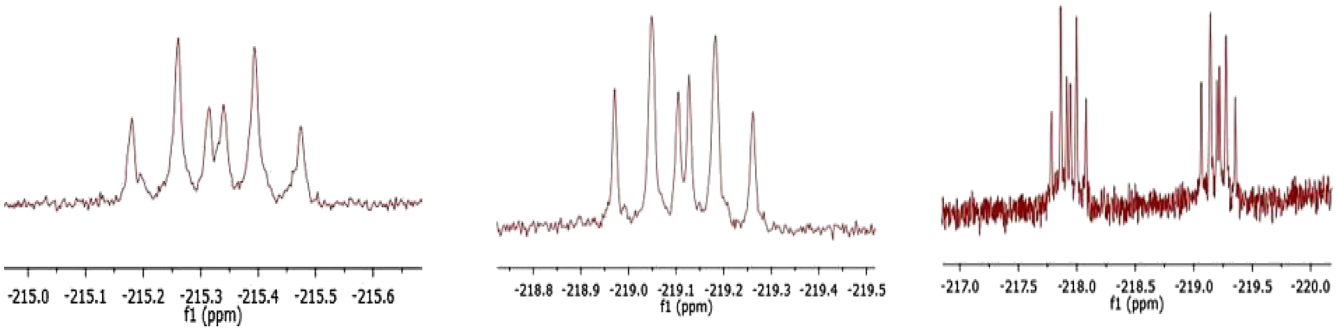

Figure 3. Proton-coupled fluorine 19 spectra of compounds **5**, **6**, and **7** (left to right). Chemical shifts are referenced to external TFA at −76.55 ppm.

(azide reduction/acetylation) afforded, after the *t*-butyl group hydrolysis, final GAA **4** (65% over four steps).

Synthesis and Characterization of APF Peptides. With the GAAs in hand, four new APF peptide analogues were synthesized by standard Fmoc-based SPPS methods. Procedures for amino acid coupling were similar to those in our previous reports,[26,44] with minor variations. The peptides were synthesized under microwave radiation on a CEM Liberty peptide synthesizer using 2-chlorotrityl resin. Amino acids were double-coupled to the resin to ensure the complete reaction of each amino acid and to avoid the production of deletion sequences. Coupling of the glycoamino acid was performed manually under the conditions we had developed previously.[45] The final peptides were deprotected on resin with hydrazine hydrate, cleaved from the resin, and high-performance liquid chromatography (HPLC) purified. All glycopeptides were >95% pure by the HPLC analysis (see the Supporting Information). All purified glycopeptides were characterized by high-resolution mass spectrometry and multiple nuclear magnetic resonance (NMR) experiments. Both ^{1}H and ^{13}C spectra are shown for compounds **5**−**8** in the Supporting Information along with the assignment tables for fluorinated derivatives **5**−**7**. The ^{19}F spectra for the fluorinated final APF derivatives are shown in Figure 3. Inspection shows that the coupling constants for each monofluoro derivative are very similar, indicating that substitution does not affect the ring pucker to any substantial degree. We have performed comprehensive modeling and NMR studies on fluorinated as-APF analogues **5**−**7**, and the results from these studies will be reported in a future manuscript.

Antiproliferative Activity of APF Analogues. The four glycopeptide analogues were tested for antiproliferative activity against NB epithelial cells and the T24 bladder carcinoma cell line (Figures 4 and 5), as performed in the previous studies of APF analogues.[21,22] Determination of cell proliferation was performed with the WST-1 assay.[46,47] This is similar to other chromogenic assays but produces a formazan product that is water-soluble (MTT product is insoluble) and more stable than other (XTT) products (see e.g., refs 48 and 49). Replacement of the 4-OH group of galactosamine with fluorine (compound **6**) resulted in a compound whose potency was very similar to that of the parent *as*-APF, whereas remaining analogues **5**, **7**, and **8** were more than 3-fold less potent than **6** (Figure 1B). We also tested our new analogues against T24 bladder cancer cells as our previous studies have shown that these tumor cells are nearly as sensitive to the activity of APF as NB cells.[21,24] The results were similar to the data for NB cells: compound **6** was the most potent with **5**, **7**, and **8** being ∼3 to 5-fold less potent (Figure 5).

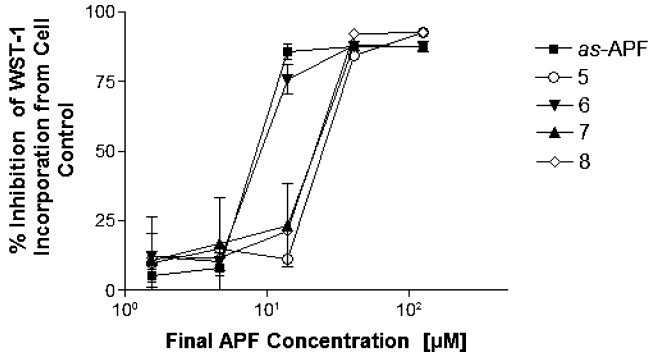

Figure 4. Antiproliferative WST-1 assay on derivatives **5**−**8** relative to *as*-APF against NB epithelial cells. The error bars are standard deviations based on a minimum of three separate experiments.

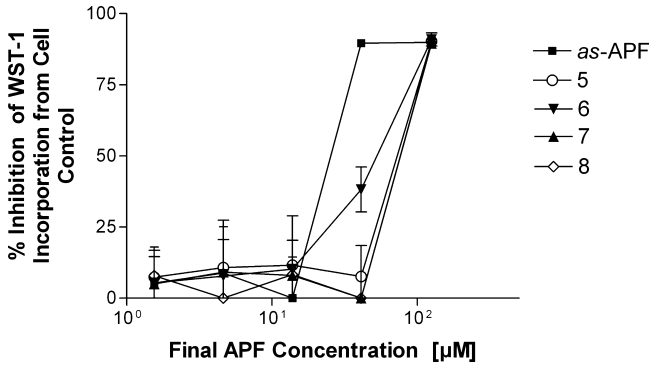

Figure 5. Antiproliferative WST-1 assay on derivatives **5**−**8** relative to *as*-APF against T24 bladder carcinoma cells. The error bars are standard deviations based on two separate experiments.

These results shed additional light on the requirements for the antiproliferative activity of various functional aspects of the sugar portion of the molecule. We had previously shown that both disaccharide and α linkage to the peptide were absolutely necessary for the maintenance of cellular activity.[44] In addition, one of our original analogues that retained full activity contained a lactosamine unit (Galβ1-4GlcNAc, unpublished results) instead of TF_{ag}, strongly suggesting that the reducing sugar can tolerate changes and still remain active, whereas the galactose moiety at the nonreducing end remained important for activity. The results presented here also showed that modification of the 4″-position of the galactose ring is detrimental to activity. Coupled with our previous results, indicating that the removal of the galactose moiety rendered the *as*-APF inactive,[44] our current data therefore confirm the

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

importance of a galacto-type stereochemistry/functionality in the nonreducing sugar for optimal *as*-APF activity. Replacement of either hydrogens or hydroxyl groups with fluorine is a common substitution in drug discovery. The fluorine atom is highly compact and electronegative, making it a suitable isostere for either a small H atom or a hydrogen bonding/accepting OH group. Fluorine atoms can only accept hydrogen bonds, and this property can be used as an advantage in various medicinal chemistry campaigns. The strong electronegativity of fluorine can also have dramatic effects on the conformation and/or binding properties of small molecules.

CONCLUSIONS

We prepared several threonine glycoamino acid analogues of the TF_{ag} disaccharide and used them to prepare analogues of a unique antiproliferative agent, APF. The synthesis of the GAAs entailed exploring a variety of glycosylation conditions that were amenable to the modified reactivity of the intermediates along the synthetic routes. All molecules were efficiently prepared, and all GAAs were incorporated into the APF peptide sequence by microwave-assisted SPSS. The additional structure−activity relationships that were gleaned from this study will help us design novel glycomimetics of APF for the future design of inhibitors for IC/PBS as well as therapeutic leads against various malignancies.

MATERIALS AND METHODS

General. All solvents were purchased from either VWR (Radnor, PA) or Thermo Fisher Scientific (Waltham, MA) and dried using a glass contour room temperature drying system; the reagents/chemicals were purchased from Sigma-Aldrich (St Louis, MO), and amino acids and peptide synthesis reagents were purchased from Bachem (Torrance, CA). Thin-layer chromatography analyses were performed on Analtech (Newark, DE) glass-backed plates for ultraviolet detection at 245 nm. Purification of intermediates was performed either with manually packed silica gel columns or on an ISCO CombiFlash+ system. Final glycoaminoacid and glycopeptide products were purified on a Waters 2545 Prep HPLC system interfaced to a 2767 sample manager. Liquid chromatography/mass spectrometric analysis was performed with either a Shimadzu 2020 LCMS system or an Agilent 1100 LCMS system.

Routine ^{1}H and ^{13}C NMR data were collected on a Bruker NanoBay 400 MHz spectrometer, and ^{19}F data were collected at 376 MHz on the same instrument with a multinuclear SMART Probe at 25 °C and processed with MestreNova software (Santiago de Compostela, Spain). The spectra were referenced to the residual protonated solvent peak. Final glycopeptides were analyzed at 500 MHz on a Bruker AVANCE III spectrometer with a triple resonance cryoprobe (TCI). Experiments for resonance assignments included 1-D proton and carbon, along with 2-dimensional COSY, TOCSY, HSQC, and HMBC data. Data were collected in both 9:1 H_2O/D_2O and 100% D_2O for assignment purposes.

(*tert*-Butyldimethylsilyl) 2-Azido-2-deoxy-3*β*-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4,6-*O*-benzylidene-*β*-D-galactopyranoside (11). To a solution of **10**[34] (0.406 g; 0.89 mmol) and **A**[29] in dry DCM (60 mL) was added dried 4 Å MS. The mixture was cooled to −78 °C, and TMSOTf (50 *μ*L; 0.28 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 20 h. The reaction was cooled to −20 °C and quenched with Et_3N (0.10 mL). The mixture was filtered through a celite pad and concentrated under vacuum. The crude product was purified over silica gel chromatography using a gradient of EtOAc in hexanes (25−50%), which yielded pure disaccharide **11** (0.419 g; 67.0%). **^{1}H NMR** (400 MHz, chloroform-*d*): *δ* 7.55−7.50 (m, 2H, Ar(C***H***)), 7.41−7.32 (m, 3H, Ar(C***H***)), 5.53 (s, 1H, Ph−C***H***), 5.31 (dd, *J* = 10.4, 7.9 Hz, 1H, ***H2′***), 4.97 (ddd, *J* = 27.6, 10.5, 2.7 Hz, 1H, ***H3′***), 4.90 (dd, *J* = 50.2, 2.7 Hz, 1H, ***H4′(F)***), 4.82 (d, *J* = 7.9 Hz, 1H, ***H1′***), 4.54 (d, *J* = 7.6 Hz, 1H, ***H1***), 4.39 (dd, *J* = 11.2, 6.1 Hz, 1H, ***H6a′***), 4.26 (dd, *J* = 12.4, 1.5 Hz, 1H, ***H6a***), 4.24−4.17 (m, 2H, ***H6b′, H4***), 4.04 (dd, *J* = 12.4, 1.7 Hz, 1H, ***H6b***), 3.80 (dt, *J* = 26.0, 6.6 Hz, 1H, ***H5′***), 3.71 (dd, *J* = 10.6, 7.6 Hz, 1H, ***H2***), 3.45 (dd, *J* = 10.6, 3.5 Hz, 1H, ***H3***), 3.35 (s, 1H, ***H5***), 2.11 (s, 3H, OAc(CH_3)), 2.09 (s, 3H, OAc(CH_3)), 2.07 (s, 3H, OAc(CH_3)), 0.94 (s, 9H, Si−*t*Bu(3× CH_3)), 0.17 (s, 3H, Si−CH_3), 0.16 (s, 3H, Si−CH_3). **^{13}C NMR** (101 MHz, chloroform-*d*): *δ* 170.58, 170.46, 169.31, 137.90, 128.95, 128.26, 126.38, 102.03, 100.87, 97.59, 85.80 (d, $J_{C4,F}$ = 187.0 Hz), 78.05, 75.22, 71.57 (d, $J_{C3,F}$ = 17.7 Hz), 71.06 (d, $J_{C5,F}$ = 18.3 Hz), 69.23, 68.80, 66.79, 64.85, 61.37 (d, $J_{C6,F}$ = 5.8 Hz), 25.82, 20.91, 20.86, 20.78, −3.90, −4.77. **HRMS**: $C_{31}H_{44}FN_3O_{12}Si$ ESI M + H calcd: 698.2751; observed, 698.2744.

(Trichloroacetimido)-2-azido-2-deoxy-3*β*-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4,6-*O*-benzylidene-*α*-D-galactopyranoside (12). To a solution of **11** (0.30 g; 0.42 mmol) in anhydrous THF (15 mL) was added $Et_3N{\cdot}3HF$ (1.5 mL; 9.10 mmol). The reaction was stirred at room temperature for 18 h and then treated with a solution of TBAF (1 M, 1.0 mL, 1.0 mmol). After 2 h of stirring, the mixture was diluted with ethyl acetate (60 mL), washed with water (1 × 25 mL), and dried. After filtration and concentration, the residual intermediate was used directly in the next step. This intermediate was dissolved in dry DCM (15 mL) and cooled in an ice bath. Trichloroacetonitrile (0.46 mL; 4.58 mmol) and DBU (0.05 mL; 0.344 mmol) were added. The ice bath was removed, and the mixture was stirred for 20 h. The solvent was removed in vacuo and purified by flash chromatography using ethyl acetate/hexanes (1:4 to 2:1) to give 179 mg of product **12** (75.8%). **^{1}H NMR** (400 MHz, chloroform-*d*): *δ* 8.68 (s, 1H, $Cl_3CC(NH)$), 7.27−7.48 (m, 5H, ***Ph***), 6.49 (d, *J* = 2.7 Hz, 1H, ***H1***), 5.50 (s, 1H, PhC***H***), 5.31 (dd, *J* = 8.1, 10.3 Hz, 1H, ***H2′***), 4.92 (ddd, *J* = 2.7, 10.3, 27.7 Hz, 1H, ***H3′***), 4.79 (dd, *J* = 50.3, 2.7 Hz, 1H, ***H4′(F)***), 4.41 (dist d, 1H, ***H4***), 4.33 (ddd, *J* = 11.2, 6.2, 0.8 Hz, 1H, ***H6′a***), 4.23 (dd, *J* = 12.5, 1.5 Hz, 1H, ***H3***), (ddd, *J* = 11.2, 6.7 Hz, 1H, ***H6′b***), 4.14−4.06 (m, apparent AB of ABX, 2H, ***H6a, H6b***), 3.97 (dd, *J* = 12.8, 2.7 Hz, 1H, ***H2***), 3.82 (br s, 1H, ***H5***), 3.80 (br dt, *J* = 26.0, 6.7 Hz, ***H5′***), 2.043 (s, 3H, ***OAc***), 2.015 (s, 3H, ***OAc***), 1.989 (s, 3H, ***OAc***). **^{13}C NMR** (101 MHz, chloroform-*d*): *δ* 169.4, 169.3, 168.3, 136.4, 128.0, 127.2, 125.1, 100.8, 99.7, 94.8, 84.6 ($J_{C4,F}$ = 187 Hz), 74.4 ($J_{C3,F}$ = 20 Hz), 70.5, 70.4, 70.3, 70.1, 67.7 ($J_{C5,F}$ = 15.4 Hz), 64.4, 60.44 ($J_{C6,F}$ = 5.3 Hz), 19.8, 19.7 (2× C). **HRMS**: compound is unstable in the mass spectrometer and used as is.

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-*α*-[2-azido-2-deoxy-3-*O*-*β*-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4,6-*O*-benzylidene-*α*-D-galactopyranosyl]-L-threonine Phenacyl Ester (13).** To a solution of **12** (0.120 g; 0.164 mmol) and **A** (0.10 g; 0.217 mmol) in dry DCM/THF (3:1, 12 mL) was added activated 4A MS. The mixture was stirred at room temperature for 30 min. After

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

cooling to −78 °C, TMSOTf (0.020 mL; 0.113 mmol) was added. The mixture was stirred at −40 °C for 1 h and then quenched with triethylamine (0.020 mL; 0.121 mmol). The mixture was diluted with DCM (20 mL) and then filtered through a celite pad. The filtrate was concentrated and then purified by flash chromatography using ethyl acetate/hexanes (1:2 → 2:1) to give 0.126 g of product **13** (74.4%). **^{1}H NMR** (400 MHz, chloroform-*d*): δ 7.83 (d, *J* = 7.7 Hz, 2H, Ar(C***H***)), 7.71 (d, *J* = 7.6 Hz, 2H, Ar(C***H***)), 7.55 (m, 3H, Ar(C***H***)), 7.4−7.50 (overlapping d and t, 4H, Ar(C***H***)), 7.21−7.36 (m, 7H, Ar(C***H***)), 5.94 (d, *J* = 9.4 Hz, 1H, N***H***-Fmoc), 5.50 (s, 1H, PhC***H***), 5.39 (d, *J* = 3.5 Hz, 1H, ***H1***), 5.38 (center of AB q, 2H, PhCO***CH***$_2$), 5.29 (dd, *J* = 8.2, 10.2 Hz, 1H, ***H2′***), 4.90 (dist ddd, *J* = 2.3, 10.0, 27.3 Hz, 1H, ***H3′***), 4.79 (d, *J* = 7.8 Hz, 1H, ***H1′***), 4.78 (dd, *J* = 50.3, 2.3 Hz, 1H, ***H4′***), 4.55 (m, 1H, Thr(***CHβ***)), 4.48 (dist dd, 1H, ***H6′a***), 4.46 (dd, *J* = 10.1, 6.6 Hz, 1H, ***H6′b***), 4.36 (dd, *J* = 11.3, 6.4 Hz, 1H, Fmoc(C***H***H)), 4.34 (br s, 1H, Fmoc(C***H***)), 4.26 (AB q, 2H, ***H6a,b***), 4.22 (m, 1H, Thr(C***Hα***)), 4.15 (dd, *J* = 11.3, 6.7 Hz, 1H, Fmoc(CH***H***)), 3.96−4.04 (m, 2H, ***H2***, ***H5***), 3.88 (dd, *J* = 10.9, 3.5 Hz, 1H, ***H3***), 3.77 (dt, *J* = 25.8, 6.7 Hz, 1H, ***H5′***), 3.70 (br s, 1H, ***H4***), 2.04 (s, 3H, OAc(C***H***$_3$)), 1.99 (s, 3H, OAc(C***H***$_3$)), 1.95 (s, 3H, 2× OAc(C***H***$_3$)), 1.34 (d, *J* = 6.5 Hz, 3H, Thr(C***H***$_3$)). **^{13}C NMR** (400 MHz, chloroform-*d*): δ 190.1, 169.4, 169.2, 169.0, 168.3, 155.8, 142.9, 142.7, 140.3, 136.6, 133.1, 132.9, 128.0, 127.9, 127.8, 127.2, 127.1, 126.8, 126.7, 126.1, 126.0, 125.1, 124.3, 124.2, 124.1, 119.0, 112.5, 100.9, 99.6, 98.2, 84.6 ($J_{C4,F}$ = 187.2 Hz), 74.8, 74.7, 74.5, 70.5 ($J_{C3,F}$ = 17.6 Hz), 70.1, 69.9, 68.1, 67.6, 66.3, 65.8, 62.5, 60.2 ($J_{C6,F}$ = 5.4 Hz), 58.7, 57.6, 46.1, 19.7, 19.6, 18.3. **HRMS**: $C_{52}H_{53}FN_4O_{17}$ ESI M + H calcd: 1025.3463; observed, 1025.3463.

N*-(9*H*-Fluren-9-yl)-methoxycarbonyl-*O*-α-[2-acetamido-2-deoxy-3-*O*-β-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4,6-*O*-benzylidine-D-galactopyranosyl]-L-threonine (1).** To a solution of **13** (0.104 g, 0.101 mmol) in THF/acetic acid/acetic anhydride (6:3:1, 30 mL) was added activated zinc (1.0 g). The mixture was stirred at room temperature for 20 h. After filtration, the filtrate was concentrated and purified by flash chromatography using ethyl acetate/acetic acid (50:1 to 15:1) to give 0 0668 g of product **1** (71.4%). **^{1}H NMR** (500 MHz, methanol-d_4): δ 7.82 (d, *J* = 7.6 Hz, 2H, Ar(CH***)), 7.70 (dd, *J* = 7.7, 3.6 Hz, 2H, Ar(C***H***)), 7.51 (d, *J* = 2.3 Hz, 1H, Ar(C***H***)), 7.50 (s, 1H, Ar(C***H***)), 7.41 (td, *J* = 7.5, 3.0 Hz, 2H, (2× Ar(C***H***))), 7.37−7.28 (m, *J* = 3.2 Hz, 5H, (5× Ar(C***H***))), 5.59 (s, 1H, Ph−C***H***), 5.11 (dd, *J* = 10.2, 7.8 Hz, 1H, ***H2′***), 5.02 (ddd, *J* = 27.4, 10.4, 2.6 Hz, 1H, ***H3′***), 4.94 (d, *J* = 3.7 Hz, 1H, ***H1***), 4.87 (dd, *J* = 50.4, 2.7 Hz, 1H, ***H4′(F)***), 4.56 (qd, *J* = 10.9, 6.2 Hz, 2H, Fmoc(C***H***$_2$)), 4.46 (dd, *J* = 11.2, 3.4 Hz, 1H, ***H2***), 4.41 (d, *J* = 6.4 Hz, 1H, Thr(C***Hβ***)), 4.40−4.34 (m, 2H, ***H6a′***, Thr(C***Hα***)), 4.29 (t, *J* = 6.3 Hz, 1H, Fmoc(C***H***)), 4.26−4.19 (m, 2H, ***H6b′***, ***H4***), 4.13 (q, *J* = 12.5 Hz, 2H, ***H6***(C***H***$_2$)), 3.97−3.86 (m, 2H, ***H5′***, ***H3***), 3.76 (s, 1H, ***H5***), 2.06 (s, 3H, OAc(C***H***$_3$)), 2.05 (s, 3H, OAc(C***H***$_3$)), 2.00 (s, 3H, OAc(C***H***$_3$)), 1.97 (s, 3H, OAc-(C***H***$_3$)), 1.23 (d, *J* = 6.3 Hz, 3H, Thr(C***H***$_3$)). **^{13}C NMR** (126 MHz, methanol-d_4): δ 173.13, 172.06, 171.56, 171.12, 159.08, 145.36, 145.19, 142.70, 139.63, 129.75, 128.96, 128.85, 128.84, 128.21, 127.46, 126.13, 126.02, 121.02, 120.99, 102.84, 102.04, 101.30, 87.84 (d, $J_{C4,F}$ = 184.6 Hz), 77.03, 76.89, 76.22, 72.67 (d, $J_{C3,F}$ = 17.5 Hz), 72.35 (d, $J_{C5,F}$ = 17.6 Hz), 70.38, 70.23, 67.69, 64.94, 62.94 (d, $J_{C6,F}$ = 5.6 Hz), 23.36, 20.83, 20.74, 20.46, 19.35. **HRMS**: $C_{46}H_{51}FN_2O_{17}$ ESI M + H calcd: 923.3239; observed, 923.3239.

(2-Napthylmethyl) 2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranoside (15). To a solution of 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranosyl trichloroacetimidate (**14**;[35] 9.13 g; 19.19 mmol) in DCM/diethylether (2:1, 150 mL) were added 2-naphthylmethanol (2.50 g; 15.80 mmol) and activated 4 Å MS (5 g). The mixture was cooled to −40 °C, and TMSOTf (0.67 mL; 3.79 mmol) was added dropwise and stirred for 30 min at the same temperature. The reaction was allowed to warm to room temperature for over 30 min and then cooled to 0 °C before being quenched with trimethylamine (0.70 mL). The mixture was filtered through a celite pad and concentrated under vacuum. The crude product was purified by flash chromatography (toluene/acetone, 10:1) to give **15** as a mixture of isomers (7.90 g; 87.30%) (*note: the α-isomer is assigned; the β-isomer H1 is assigned*). **^{1}H NMR** (400 MHz, chloroform-*d*): δ 7.91−7.80 (m, 4H, Ar(C***H***)), 7.55−7.47 (m, 3H, Ar(C***H***)), 5.55 (dd, *J* = 10.6, 9.2 Hz, 1H, ***H3(α)***), 5.10 (d, *J* = 3.8 Hz, 1H, ***H1(α)***), 5.07 (d, *J* = 10.6 Hz, 1H, ***H4(α)***), 4.92 (d, *J* = 12.0 Hz, 1H, Nap-C***H***H), 4.80 (d, *J* = 12.0 Hz, 1H, Nap-C***H***H), 4.49 (d, *J* = 8.1 Hz, 0.1H, ***H1(β)***), 4.27 (dd, *J* = 12.2, 4.2 Hz, 1H ***H6a(α)***), 4.08 (ddd, *J* = 10.1, 4.2, 2.4 Hz, 1H, ***H5(α)***), 4.03 (dd, *J* = 12.2, 2.3 Hz, 1H, ***H6b(α)***), 3.35 (dd, *J* = 10.6, 3.6 Hz, 1H, ***H2(α)***), 2.09 (s, 3H, OAc(CH$_3$)), 2.09 (s, 3H, OAc(C***H***$_3$)), 2.03 (s, 3H, OAc(C***H***$_3$)). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 170.71, 170.12, 169.81, 133.49, 133.32, 133.26, 128.69, 128.11, 127.88, 127.53, 126.51, 126.46, 126.03, 100.47, 96.77, 70.61, 70.36, 68.68, 67.98, 61.94, 61.06, 20.86. **HRMS**: $C_{23}H_{25}N_3O_8$ ESI M + NH_4 calcd: 489.1980; observed, 489.1967.

(2-Napthylmethyl) 2-Azido-2-deoxy-4,6-*O*-benzylidine-α-D-glucopyranoside (16a) and 1-(2-Napthylmethyl) 2-Azido-2-deoxy-4,6-*O*-benzylidine-β-D-glucopyranoside (16b). To a solution of (2-naphthylmethyl) 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranoside (**15**; 7.90 g; 16.77 mmol) in methanol (70 mL) was added a solution of 25% sodium methoxide in methanol (4 mL). The reaction mixture was stirred at room temperature for 3 h and then cooled in an ice bath before being neutralized with 2 M HCl/diethylether solution. The reaction mixture was concentrated under vacuum and purified by flash chromatography (DCM/MeOH 15:1) to give (2-naphthylmethyl)-2-azido-2-deoxy-glucopyranoside (4.75 g; 82.0%). This material (13.75 mmol) was dissolved in dry DMF (60 mL) and cooled in an ice bath to 0 °C. To this solution were added benzaldehyde dimethylacetal (6.5 mL; 43.10 mmol) and (+) CSA (0.65 g; 2.79 mmol). The mixture was stirred at room temperature for 4 h. The mixture was diluted with EtOAc (150 mL) and washed with saturated aqueous ammonium chloride (2 × 70 mL). The organic layer was dried, concentrated in vacuo, and purified by flash chromatography (EtOAc/hexanes 1:4) to give desired α-isomer **16a** (4.39 g; 73.6%) and β-isomer **16b** (0.50 g; 8.4%). **16a—^{1}H NMR** (400 MHz, chloroform-*d*): δ 7.90−7.81 (m, 4H, Ar-C***H***), 7.55−7.45 (m, 5H, Ar(C***H***)), 7.43−7.32 (m, 3H, Ar(C***H***)), 5.55 (s, 1H, PhC***H***), 5.05 (d, *J* = 3.7 Hz, 1H, ***H1(α)***), 4.95 (d, *J* = 12.2 Hz, 1H, Nap-C***H***H), 4.78 (d, *J* = 12.1 Hz, 1H, Nap-C***H***H), 4.33 (t, *J* = 10.1, 9.6 Hz, 1H, ***H3***), 4.28 (dd, *J* = 10.2, 5.2 Hz, 1H, ***H6a***), 3.98 (td, *J* = 9.9, 4.9 Hz, 1H, ***H5***), 3.76 (t, *J* = 10.3 Hz, 1H, ***H6b***), 3.56 (t, *J* = 9.3 Hz, 1H, ***H4***), 3.32 (dd, *J* = 10.0, 3.7 Hz, 1H, ***H2***). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 136.98, 133.94, 133.33, 133.29, 129.55, 128.62, 128.55, 128.10, 127.90, 127.22, 126.45, 126.41, 126.35, 125.90, 102.29, 97.53, 82.02, 70.14, 69.05, 68.98, 63.28, 62.80. **HRMS**: $C_{24}H_{23}N_3O_5$ ESI M + H calcd: 434.1710; observed, 434.1702.

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

16b—^{1}**H NMR** (400 MHz, chloroform-*d*): δ 7.93−7.81 (m, 4H, Ar-C*H*), 7.59−7.43 (m, 5H, Ar-C*H*), 7.43−7.32 (m, 3H, Ar-C*H*), 5.56 (s, 1H, PhC*H*), 5.11 (d, *J* = 11.8 Hz, 1H, Nap-C*H*H), 4.87 (d, *J* = 11.8 Hz, 1H, Nap-C*H*H), 4.56 (d, *J* = 7.9 Hz, 1H, ***H1(β)***), 4.39 (dd, *J* = 10.5, 5.0 Hz, 1H, ***H6a***), 3.83 (t, *J* = 10.3 Hz, 1H, ***H6b***), 3.66 (t, *J* = 9.2 Hz, 1H, ***H3***), 3.59 (t, *J* = 9.1 Hz, 1H, ***H4***), 3.51 (t, *J* = 9.3, 8.0 Hz, 1H, ***H2***), 3.43 (td, *J* = 9.6, 5.0 Hz, 1H, ***H5***). ^{13}**C NMR** (101 MHz, $CDCl_3$): δ 136.91, 133.89, 133.35, 133.32, 129.54, 128.59, 128.54, 128.13, 127.89, 127.27, 126.42, 126.39, 126.36, 125.94, 102.18, 101.19, 80.77, 72.21, 71.70, 68.70, 66.69, 66.39. **HRMS**: $C_{24}H_{23}N_3O_5$ ESI M + H calcd: 434.1710; observed, 434.1702.

(2-Napthylmethyl) 2-Azido-2-deoxy-3-*O*-benzoyl-4,6-*O*-benzylidine-α-D-glucopyranoside (17). To a solution of (2-naphthylmethyl) 2-azido-2-deoxy-4,6-*O*-benzylidene-2-α-D-glucopyranoside (**16a**; 4.39 g, 10.10 mmol) in dry pyridine (100 mL) was added benzoyl chloride (4.80 mL, 41.30 mmol). The reaction was stirred at room temperature for 4 h; the solvent was concentrated to 30 mL and diluted with EtOAc (150 mL). This organic solution was washed with water (2 × 75 mL), dried, and concentrated in vacuo. Purification by flash chromatography (EtOAc/hexanes, 1:4 followed by EtOAc/hexanes/DCM 1:4:4) gave product **17** (4.95 g; 91.6%). ^{1}**H NMR** (400 MHz, chloroform-*d*): δ 8.08 (d, *J* = 7.5 Hz, 2H, Ar-C*H*), 7.91−7.83 (m, 4H, Ar(C*H*)), 7.60−7.54 (m, 2H, Ar(C*H*)), 7.53−7.48 (m, 2H, Ar(C*H*)), 7.47−7.38 (m, 4H, Ar-C*H*), 7.33−7.27 (m, 3H, Ar-C*H*), 5.94 (t, *J* = 9.9 Hz, 1H, ***H3***), 5.53 (s, 1H, −PhC*H*), 5.16 (d, *J* = 3.6 Hz, 1H, ***H1***), 5.01 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.84 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.31 (dd, *J* = 10.3, 4.9 Hz, 1H, ***H6a***), 4.20−4.09 (m, 1H, ***H5***), 3.88−3.76 (m, 2H, ***H4, H6b***), 3.40 (dd, *J* = 10.4, 3.6 Hz, 1H, ***H2***). ^{13}**C NMR** (101 MHz, $CDCl_3$): δ 165.56, 136.93, 133.65, 133.36, 133.33, 130.07, 129.72, 129.18, 128.69, 128.51, 128.32, 128.15, 127.90, 127.47, 126.45, 126.38, 126.27, 126.05, 101.82, 97.78, 79.84, 70.18, 69.72, 68.97, 63.24, 62.04. **HRMS**: $C_{31}H_{27}N_3O_6$ ESI M + Na calcd: 580.1792; observed, 580.1786.

(2-Napthylmethyl) 2-Azido-2-deoxy-3-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (18). To a solution of (2-naphthylmethyl) 2-azido-2-deoxy-3-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**17**; 3.59 g; 6.67 mmol) in dry THF (70 mL) and activated 4 Å MS (1.0 g) was added sodium cyanoborohydride (96.71 g; 106.77 mmol), and the mixture was stirred at room temperature for 25 min. After cooling to 0 °C, 2.0 M HCl/diethylether solution was added dropwise, until the mixture attained pH 3; the ice bath was removed, and the reaction was stirred at room temperature for 1 h. After filtration through a celite pad, the filtrate was diluted with EtOAc (150 mL), washed with water (2 × 75 mL) and saturated $NaHCO_3$ solution (2 × 75 mL), dried, filtered, and concentrated in vacuo. Purification by flash chromatography (EtOAc/hexanes 1:4 then EtOAc/hexanes/DCM 1:4:4) gave product **18** (3.01 g; 85.7%). ^{1}**H NMR** (400 MHz, chloroform-*d*): δ 8.10 (d, *J* = 7.6 Hz, 2H, Ar(C*H*)), 7.90−7.80 (m, 4H, Ar(C*H*)), 7.60 (tt, *J* = 6.9, 1.3 Hz, 1H, Ar(C*H*)), 7.54 (dd, *J* = 8.4, 1.6 Hz, 1H, Ar-C*H*), 7.52−7.43 (m, 4H, Ar-C*H*), 7.39−7.27 (m, 5H, Ar(C*H*)), 5.64 (dd, *J* = 10.6, 8.7 Hz, 1H, ***H3***), 5.15 (d, *J* = 3.6 Hz, 1H, ***H1***), 4.96 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.81 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.67−4.53 (AB-doublet, 2H, Bn(CH_2), 3.98 (dt, *J* = 9.8, 3.7 Hz, 1H, ***H5***), 3.91 (td, *J* = 9.3, 3.0 Hz, 1H, ***H4***), 3.77 (qd, *J* = 10.5, 3.7 Hz, 2H, ***H6(CH$_2$)***), 3.46 (dd, *J* = 10.6, 3.6 Hz, 1H, ***H2***), 2.96 (d, *J* = 4.1 Hz, 1H, O*H*). ^{13}**C NMR** (101 MHz, $CDCl_3$): δ 167.29, 137.90, 133.94, 133.72, 133.31, 133.27, 130.17, 129.33, 128.63, 128.59, 128.56, 128.13, 127.96, 127.84, 127.34, 126.39, 126.30, 126.07, 96.96, 74.45, 73.85, 71.03, 70.74, 69.97, 69.31, 61.27. **HRMS**: $C_{31}H_{29}N_3O_6$ ESI M + H calcd: 540.2129; observed, 540.2118.

(2-Napthylmethyl) 2-Azido-2-deoxy-3-*O*-benzoyl-4-fluoro-4-deoxy-6-*O*-benzyl-α-D-glucopyranoside (19). To a solution of (2-naphthylmethyl) 2-azido-2-deoxy-3-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (**18**; 1.65 g; 3.05 mmol) in dry DCM (70 mL) was added 4-dimethylaminopyridine (0.375 g, 3.06 mmol). The solution was cooled to −40 °C, and DAST (1.01 mL; 7.64 mmol) was added dropwise. The mixture was stirred at room temperature for 20 h and diluted with DCM (100 mL); the organic layer was washed with saturated $NaHCO_3$ solution (75 mL) and dried. After filtration and concentration in vacuo, the material was purified by silica gel chromatography (EtOAc/hexanes 1:10 to 1:6) to give fluorinated product **19** (1.33 g; 84%). ^{1}**H NMR** (400 MHz, chloroform-*d*): δ 8.14−8.07 (m, 2H, Ar(C*H*)), 7.90−7.80 (m, 4H, Ar(C*H*)), 7.60 (tt, *J* = 7.0, 1.3 Hz, 1H, Ar(C*H*)), 7.56−7.44 (m, 5H, Ar(C*H*)), 7.40−7.27 (m, 5H, (Ar(C*H*)), 5.61 (ddd, *J* = 26.3, 11.1, 2.5 Hz, 1H, ***H3***), 5.18 (d, *J* = 3.5 Hz, 1H, ***H1***), 5.11 (dd, *J* = 50.7, 2.6 Hz, 1H, ***H4(F)***), 4.95 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.83 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.60−4.48 (AB-doublet, 2H, Bn(CH_2)), 4.21 (dt, *J* = 29.5, 6.7 Hz, 1H, ***H5***), 3.91 (dd, *J* = 11.1, 3.5 Hz, 1H, ***H2***), 3.70 (dd, *J* = 9.7, 6.9 Hz, 1H, ***H6a***), 3.61 (ddd, *J* = 9.7, 6.3, 1.2 Hz, 1H, ***H6b***). ^{13}**C NMR** (101 MHz, chloroform-*d*): δ 165.61, 137.71, 133.78, 133.60, 133.19, 133.16, 130.01, 129.12, 128.52, 128.47, 128.00, 127.82, 127.74, 127.62, 127.22, 126.28, 126.20, 125.92, 96.97, 86.87 (d, $J_{C4,F}$ = 184.7 Hz), 73.56, 70.15, 69.67 (d, $J_{C3,F}$ = 17.6 Hz), 68.42 (d, $J_{C6,F}$ = 18.2 Hz), 67.63 (d, *J* = 5.5 Hz), 57.89 (d, *J* = 2.3 Hz). **HRMS**: $C_{31}H_{28}FN_3O_5$ ESI M + NH_4 calcd: 559.2351; observed, 559.2344.

(2-Napthylmethyl) 2-Azido-2-deoxy-4-fluoro-4-deoxy-6-*O*-benzyl-α-D-glucopyranoside (20). To a solution of (2-naphthylmethyl) 2-azido-2-deoxy-3-*O*-benzoyl-4-fluoro-6-*O*-benzyl-α-D-galactopyranoside (**19**; 1.32 g; 2.43 mmol) in MeOH (50 mL) was added a solution of 25% sodium methoxide in MeOH (3.0 mL). The mixture was stirred at room temperature for 3 h, cooled in an ice bath, and neutralized with 2 M HCl/ether solution. The mixture was concentrated in vacuo and purified by flash chromatography (EtOAc/hexanes 1:4 to 1:3) to give compound **20** as a white foam (1.00 g; 94.3%). ^{1}**H NMR** (400 MHz, chloroform-*d*): δ 7.83 (dd, *J* = 10.3, 7.0 Hz, 4H, Ar(C*H*)), 7.57−7.42 (m, 3H, Ar(C*H*)), 7.33 (h, *J* = 7.9 Hz, 5H, Ar(C*H*)), 5.09 (d, *J* = 3.6 Hz, 1H, ***H1***), 4.89 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.89 (dd, *J* = 50.1, 2.5 Hz, 1H, ***H4(F)***), 4.77 (d, *J* = 12.1 Hz, 1H, Nap-C*H*H), 4.60−4.48 (m, 2H, Bn(CH_2)), 4.26−3.98 (m, 2H, ***H3***, ***H5***), 3.71 (dd, *J* = 9.5, 7.1 Hz, 1H, ***H6a***), 3.65−3.56 (m, 1H, ***H6b***), 3.51 (dd, *J* = 10.7, 3.5 Hz, 1H, ***H2***), 2.20 (dd, *J* = 9.0, 1.3 Hz, 1H, O*H*). ^{13}**C NMR** (101 MHz, chloroform-*d*): δ 137.87, 134.05, 133.33, 133.26, 128.62, 128.57, 128.10, 128.00, 127.87, 127.79, 127.23, 126.44, 126.34, 125.97, 97.15, 89.39 (d, $J_{C4,F}$ = 180.7 Hz), 73.75, 70.27, 68.69 (d, $J_{C3,F}$ = 18.1 Hz), 67.89, 67.83, 67.64, 60.81. **HRMS**: $C_{24}H_{24}FN_3O_4$ ESI M + H − N_2 calcd: 410.1762; observed, 410.1759.

(2-Napthylmethyl) 2-Azido-2-deoxy-3β-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-4-fluoro-4-deoxy-6-*O*-benzyl-α-D-glucopyranoside (23). To a solution of **21** (0.18 g; 0.43 mmol) and **20** (0.119 g; 0.27 mmol) in dry DCM (10 mL) was added dried 4 Å MS (0.30 g). The mixture was cooled to −30 °C, and AgOTf (0.20 g; 0.78 mmol) was added and stirred for 3 h. Additional bromide donor **21** (0.26 g; 0.60 mmol) and

DOI: 10.1021/acsomega.7b01018
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AgOTf (0.40 g; 1.55 mmol) were added and stirred for another 2 h. The mixture was filtered through a celite pad, concentrated in vacuo, and purified by flash chromatography (EtOAc/hexanes (1:4) to (1:2)) to give desired product **23** (0.114 g; 54% yield). **^{1}H NMR** (400 MHz, chloroform-*d*): δ 7.88−7.80 (m, 4H), 7.51 (dt, J = 6.0, 3.4 Hz, 3H), 7.38−7.29 (m, 5H, Ar(C***H***)), 5.38 (d, J = 3.4 Hz, 1H, ***H4′***)), 5.31 (dd, J = 10.4, 7.9 Hz, 1H, ***H2′***), 5.10 (d, J = 3.5 Hz, 1H, ***H1***)), 5.01 (dd, J = 10.4, 3.4 Hz, 1H, ***H3′***), 4.98 (dd, J = 49.9, 2.7 Hz, 1H, ***H4(F)***), 4.90 (d, J = 13.0 Hz, 1H, Nap-C***H***H), 4.78 (d, J = 11.9 Hz, 1H, Nap-C***H***H), 4.74 (d, J = 7.9 Hz, 1H, ***H1′***), 4.59−4.49 (m, 2H, Bn(C***H***$_2$)), 4.11 (d, J = 6.6 Hz, 2H, ***H6′(CH$_2$)***), 4.09−3.97 (m, 2H, ***H3, H5***), 3.91 (t, J = 6.6 Hz, 1H, ***H5′***), 3.72−3.64 (m, 2H, ***H2, H6a***), 3.61 (dd, J = 9.7, 6.2 Hz, 1H, ***H6b***), 2.16 (s, 3H, OAc(C***H***$_3$)), 2.08 (s, 3H, OAc(C***H***$_3$)), 1.98 (s, 3H, OAc-(C***H***$_3$)), 1.91 (s, 3H, OAc(C***H***$_3$)). **^{13}C NMR** (101 MHz, chloroform-*d*): δ 170.43, 170.41, 170.25, 169.57, 137.87, 133.92, 133.30, 133.29, 128.62, 128.61, 128.07, 128.00, 127.87, 127.78, 127.56, 126.49, 126.43, 126.17, 102.92, 97.14, 88.71 (d, $J_{C4,F}$ = 185.1 Hz), 76.45 (d, $J_{C3,F}$ = 18.0 Hz), 73.75, 71.06, 71.01, 70.33, 68.86 (d, $J_{C5,F}$ = 18.4 Hz), 68.69, 68.02 (d, $J_{C6,F}$ = 5.4 Hz), 67.00, 61.42, 59.28 (d, $J_{C2,F}$ = 2.5 Hz), 20.82, 20.80, 20.70, 20.61. **HRMS**: $C_{38}H_{42}FN_3O_{13}$ ESI M + NH_4 calcd: 785.3040; observed, 785.3060.

(2-Napthylmethyl)2-azido-2-deoxy-3β-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4-fluoro-4-deoxy-6-*O*-benzyl-α-D-glucopyranoside (24). To a mixture of **22**[33] (0.50 g; 1.1 4 mmol) and 4 Å MS (0.30 g) in dry DCM (12 mL) at −30 °C was added AgOTf (0.650 g; 2.5 2 mmol). The reaction was stirred for 30 min before adding a solution of **20** (0.612 g, 1.64 mmol) in dried DCM (12 mL). The reaction was stirred at −30 °C for 30 min and then at room temperature for 60 min. After filtration through a celite pad, the filtrate was concentrated in vacuo and purified by silica gel flash chromatography to give product **24** (0.50 g; 60.1%). **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.77 (dd, J = 11.9, 7.4 Hz, 4H, Ar(C***H***)), 7.46−7.40 (m, 3H, Ar(C***H***)), 7.33−7.20 (m, 5H, Ar(C***H***)), 5.30 (dd, J = 10.4, 8.0 Hz, 1H, ***H2′***), 5.02 (d, J = 3.5 Hz, 1H, ***H1***), 4.89 (dd, J = 49.4, 2.4 Hz, 1H, ***H4(F)***), 4.87 (ddd, J = 27.6, 10.3, 2.5 Hz, 1H, ***H3′***), 4.82 (d, J = 11.9 Hz, 1H, Nap-C***H***H), 4.76 (d, J = 50.6 Hz, 1H, ***H4′(F)***), 4.70 (d, J = 11.0 Hz, 1H, Nap-C***H***H), 4.68 (d, J = 7.2 Hz, 1H, ***H1′***), 4.52−4.44 (m, 2H, Bn(C***H***$_2$)), 4.23 (dd, J = 10.83, 6.75 Hz, 1H, ***H6′a***), 4.14 (dd, J = 11.3, 6.5 Hz, 1H ***H6′b***), 4.04−3.91 (m, 2H, ***H3, H5***), 3.73 (dt, J = 26.0, 6.6 Hz, 1H, ***H5′***), 3.66−3.58 (m, 2H, ***H6a, H2***), 3.54 (ddd, J = 9.6, 6.4, 1.4 Hz, 1H, ***H6b***), 2.04 (s, 3H, OAc(C***H***$_3$)), 2.01 (s, 3H, OAc(C***H***$_3$)), 1.87 (s, 3H, OAc(C***H***$_3$)). **^{13}C NMR** (126 MHz, chloroform-*d*): δ 170.56, 170.47, 169.44, 137.87, 133.92, 133.30, 133.29, 128.63, 128.61, 128.09, 128.00, 127.87, 127.81, 127.59, 126.49, 126.43, 126.19, 102.56, 97.08, 88.69 (d, J_{C4-F} = 185.3 Hz), 85.68 (d, $J_{C4'-F}$ = 187.1 Hz), 76.30 (d, J_{C3-F} = 18.1 Hz), 73.77, 71.53 (d, $J_{C3'-F}$ = 17.8 Hz), 71.15 (d, $J_{C5'-F}$ = 18.2 Hz), 70.30, 68.91 (d, $J_{C5'-F}$ = 18.5 Hz), 68.59, 68.03 (d, J_{C6-F} = 5.3 Hz), 61.43 (d, $J_{C6'-F}$ = 5.6 Hz), 59.32 (d, J_{C2-F} = 3.0 Hz), 20.86, 20.79, 20.68. **HRMS**: $C_{36}H_{39}F_2N_3O_{11}$ ESI M + NH_4 calcd: 745.2891; observed, 745.2879.

2-Azido-2-deoxy-3β-(2,3,4,6-tetra-*O*-acetyl-D-galacto-pyranosyl)-4-fluoro-4-deoxy-1,6-di-*O*-acetyl-α-D-gluco-pyranoside (25). To a solution of **23** (0.45 g; 0.59 mmol) in DCM/water (20:1, 15.75 mL) was added DDQ (0.95 g; 4.10 mmol). The mixture was stirred at room temperature for 18 h; more DDQ (0.60 g; 2.64 mmol) was added and stirring was continued for 2 h. The solvent was removed in vacuo, and the crude material was coevaporated with methanol (2 × 10 mL). Purification over silica gel using a gradient of EtOAc/hexanes (50:50 to 100:0) gave the 1-hydroxy intermediate. This compound was taken in anhydrous pyridine (10 mL), and acetic anhydride (1 mL) was added. The reaction was stirred for 3 h, diluted with EtOAc (100 mL), washed with water (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by flash chromatography (EtOAc/hexanes 1:2 to 1:1) gave product **25** (0.193 g; 53.0%) (*assignments to the α-isomer are labeled (A) and to the β-isomer are labeled (B)*). **^{1}H NMR** (400 MHz, chloroform-*d*): δ 6.31 (d, J = 3.1 Hz, 1H, ***H1(A)***), 5.46 (dd, J = 8.5, 0.8 Hz, 1H, ***H1(B)***), 5.42−5.37 (m, 2H, ***H4′(A), H4′(B)***), 5.30 (t, J = 8.1 Hz, 1H, ***H2′(A/B)***), 5.27 (t, J = 8.4 Hz, 1H, ***H2′(A/B)***), 5.04 (d, J = 3.4 Hz, 1H, ***H3′(A/B)***), 5.01 (d, J = 3.4 Hz, 1H, ***H3′(A/B)***), 4.95 (dd, J = 49.75, 1.9 Hz, 1H, ***H4F(A)***), 4.81 (dd, J = 49.31, 2.5 Hz, 1H, ***H4F(B)***), 4.78 (d, J = 7.9 Hz, 1H, ***H1′(A/B)***), 4.75 (d, J = 7.9 Hz, 1H, ***H1′(A/B)***), 4.29−4.15 (m, 6H, ***H6′(A), H6′(B), H6a(A), H6a(B)***), 4.15−4.00 (m, 3H, ***H6b(A), H6b(B, H5(A))***), 3.98−3.88 (m, 4H, ***H5′(A), H5′(B), H3(A), H2(A)***), 3.87−3.74 (m, 2H, ***H5(B), H2(B)***), 3.51 (ddd, J = 26.5, 10.5, 2.6 Hz, 1H, ***H3(B)***), 2.19 (s, 3H, OAc(C***H***$_3$)), 2.18 (s, 3H, OAc(C***H***$_3$)), 2.17 (s, 6H, 2× OAc(C***H***$_3$)), 2.07 (s, 9H, 3× OAc(C***H***$_3$)), 2.06 (s, 3H, OAc(C***H***$_3$)), 2.05 (s, 3H, OAc(C***H***$_3$)), 2.04 (s, 3H, OAc(C***H***$_3$)), 1.98 (s, 6H, 2× OAc(C***H***$_3$)). **^{13}C NMR** (101 MHz, chloroform-*d*): δ 170.65, 170.45, 170.42, 170.36, 170.22, 169.48, 169.44, 168.90, 168.65, 102.68 (d, J = 7.4 Hz), 92.78, 90.67, 88.02 (d, $J_{C4A,F}$ = 186.2 Hz), 86.19 (d, $J_{C4B,F}$ = 186.4 Hz), 78.57 (d, $J_{C3B,F}$ = 18.1 Hz), 76.03 (d, $J_{C3A,F}$ = 18.0 Hz), 72.18 (d, $J_{C5B,F}$ = 18.2 Hz), 71.24, 71.11, 70.98, 69.43 (d, $J_{C5A,F}$ = 18.4 Hz), 68.73, 68.69, 67.02, 66.92, 61.94 (d, $J_{C6,F}$ = 6.9 Hz), 61.83 (d, $J_{C6,F}$ = 6.2 Hz), 61.50, 61.40, 61.20, 58.56 (d, $J_{C2A,F}$ = 2.9 Hz), 21.07, 21.04, 20.85, 20.83, 20.82, 20.77, 20.67. **HRMS:** $C_{24}H_{32}FN_3O_{15}$ ESI M + NH_4 calcd: 639.2156; observed, 639.2156.

2-Azido-2-deoxy-3β-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4-fluoro-4-deoxy-1,6-di-*O*-acetyl-α-D-glucopyranoside (26). Compound **24** was processed identically as 23 (above) to provide **26** (0.225 g; 46.9%) as a mixture of isomers. (*Assignments to the α-isomer are labeled (A) and to the β-isomer are labeled (B)*). **^{1}H NMR** (400 MHz, chloroform-*d*): δ 6.31 (d, J = 3.0 Hz, 1H, ***H1(A)***), 5.45 (dd, J = 8.5, 0.7 Hz, 1H, ***H1(B)***), 5.34 (dt, J = 10.1, 8.2 Hz, 2H, ***H3′(A), H3′(B)***), 5.03−4.74 (m, 8H, ***H2′(A), H2′(B), H4(A), H4(B), H4′(A), H4′(B), H1′(A), H1′(B)***), 4.48−4.32 (m, 2H, ***H6a(A), H6a(B)***), 4.28−4.16 (m, 6H, ***H6b(A), H6b(B), H6′(A), H6′(B)***), 4.07 (dt, J = 28.2, 6.3 Hz, 1H, ***H5(A)***), 3.99−3.74 (m, 6H, ***H3(A), H2(A), H5(B), H5′(A), H5′(B)***), 3.53 (ddd, J = 26.4, 10.5, 2.5 Hz, 1H, ***H3(B)***), 2.18 (s, 3H, OAc(C***H***$_3$)), 2.16 (s, 3H, OAc(C***H***$_3$)), 2.11 (s, 6H, 2× OAc(C***H***$_3$)), 2.09 (s, 3H, OAc(C***H***$_3$)), 2.08 (s, 6H, 2× OAc(C***H***$_3$)), 2.07 (s, 6H, 2× OAc(C***H***$_3$)), 2.07 (s, 3H, OAc(C***H***$_3$)). **^{13}C NMR** (101 MHz, chloroform-*d*): δ 170.63, 170.50, 170.47, 170.44, 169.34, 169.29, 168.91, 168.67, 102.26, 92.75, 90.65, 87.95 (d, $J_{C4,F}$ = 186.37 Hz), 87.06 (d, $J_{C4,F}$ = 186.89 Hz), 85.73 (d, $J_{C'4,F}$ = 187.22 Hz), 85.60 (d, $J_{C'4,F}$ = 187.29 Hz), 78.39 (d, $J_{C3(B),F}$ = 18.0 Hz), 75.74 (d, $J_{C3(A),F}$ = 18.0 Hz), 72.14 (d, $J_{C5(B),F}$ = 18.3 Hz), 69.41 (d, $J_{C5(A),F}$ = 18.4 Hz), 68.64, 68.60, 61.87 (d, $J_{C6(A),F}$ = 6.0 Hz), 61.78 (d, $J_{C6(A),F}$ = 5.8 Hz), 61.48, 61.38 (d, $J_{C6(A),F}$ = 5.7 Hz), 61.12 (d, $J_{C6(A),F}$ = 5.8 Hz), 58.57 (d, $J_{C6(A),F}$ = 2.9 Hz), 21.06, 21.03, 20.84, 20.80,

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

20.75. **HRMS**: $C_{22}H_{29}F_2N_3O_{13}$ ESI M + NH_4 calcd: 599.2007; observed, 599.2002.

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-α-[2-azido-2-deoxy-3-*O*-β-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-4-fluoro-4-deoxy-6-*O*-acetyl-D-galactopyranosyl]-L-threonine Phenacyl Ester (29).** To a solution of **25** (0.143 g; 0.23 mmol) in dry DCM (10 mL) was added TMSBr (0.125 mL; 0.94 mmol) dropwise along with $BiBr_3$ (0.012 g; 0.026 mmol). The mixture was stirred at room temperature for 3 h and poured onto ice cold $NaHCO_{3(aq)}$ (50 mL). After extraction with DCM (2 × 50 mL), the combined organic layers were washed with aqueous $NaCl_{(sat)}$ (1 × 40 mL) and water (1 × 40 mL), dried, and concentrated. Quick purification through a short column of silica (EtOAc/hexanes 1:2) gave intermediate bromide **27** (0.146 g, 98%), which was used immediately. To a cold (−30 °C) solution of bromide **27** (0.045 g; 0.062 mmol) in dry DCM (8 mL) were added dried MS and Fmoc-threonine phenacyl ester **B** (0.045 g; 0.097 mmol). The mixture was warmed to room temperature, and silver perchlorate (0.055 g; 0.265 mmol) was added. The reaction was stirred in the dark for 4 h and then filtered through a celite pad; the filtrate was concentrated in vacuo. Purification by flash chromatography (EtOAC/hexanes, 1:1 to 2:1) gave 0.0354 g (56%) of product **29**. **^{1}H NMR** (500 MHz, chloroform-*d*): δ 7.91 (d, *J* = 7.7 Hz, 2H, Ar(CH***)), 7.77 (d, *J* = 7.6 Hz, 2H, Ar(C***H***)), 7.63 (q, *J* = 7.0 Hz, 3H, Ar(C***H***)), 7.50 (t, *J* = 7.7 Hz, 2H, Ar(C***H***)), 7.40 (t, *J* = 7.5 Hz, 2H, Ar(C***H***)), 7.32 (t, *J* = 7.5 Hz, 2H, Ar(C***H***)), 5.90 (d, *J* = 9.4 Hz, 1H, N***H***-Fmoc), 5.57 (d, *J* = 16.4 Hz, 1H, PhCO−C***H***H), 5.52 (d, *J* = 3.8 Hz, 1H, ***H1***), 5.40 (d, *J* = 3.4 Hz, 1H, ***H4′***), 5.36 (d, *J* = 16.4 Hz, 1H, PhCO−C***H***H), 5.31 (dd, *J* = 10.5, 7.9 Hz, 1H, ***H2′***), 5.03 (dd, *J* = 10.4, 3.4 Hz, 1H, ***H3′***), 4.94 (dd, *J* = 49.6, 2.5 Hz, 1H, ***H4(F)***), 4.80 (d, *J* = 7.9 Hz, 1H, ***H1′***), 4.64 (qd, *J* = 8.25, 6.70, 1.75 Hz, 1H, Thr(C***Hβ***)), 4.57 (dd, *J* = 9.6, 1.9 Hz, 1H, Thr(C***Hα***)), 4.53 (dd, *J* = 10.6, 7.0 Hz, 1H, Fmoc(C***H***H), 4.34 (dd, *J* = 10.4, 7.5 Hz, 1H, Fmoc(C***H***H)), 4.30−4.24 (m, 3HFmoc(C***H***), ***H6***(CH_2)), 4.22 (dd, *J* = 11.3, 6.4 Hz, 1H, ***H6a′***), 4.15−4.06 (m, 2H, ***H6b′, H5***), 4.01−3.89 (m, 2H, ***H3, H5′***), 3.77 (dd, *J* = 10.8, 3.8 Hz, 1H, ***H2***), 2.17 (s, 3H, OAc(CH_3)), 2.08 (s, 3H, OAc(CH_3)), 2.03 (d, *J* = 2.2 Hz, 6H, 2× OAc(CH_3)), 1.98 (s, 3H, 2× OAc(CH_3)), 1.41 (d, *J* = 6.5 Hz, 3H, Thr(CH_3)). **^{13}C NMR** (126 MHz, chloroform-*d*): δ 191.42, 170.66, 170.41, 170.21, 169.98, 169.51, 156.86, 143.95, 143.76, 141.43, 134.31, 133.93, 129.10, 127.89, 125.28, 125.19, 120.16, 102.73, 98.90, 88.62 (d, $J_{C4,F}$ = 185.5 Hz), 76.26, 75.89 (d, $J_{C3,F}$ = 18.1 Hz), 68.66, 67.75 (d, $J_{C5,F}$ = 18.2 Hz), 67.51, 66.97, 66.92, 62.65 (d, $J_{C6,F}$ = 5.5 Hz), 61.26, 60.50, 59.87, 58.63, 47.25, 20.84, 20.80, 20.75, 20.68, 19.22. **HRMS**: $C_{49}H_{53}FN_4O_{19}$ ESI M + H calcd: 1021.3361; observed, 1021.3349.

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-α-[2-acetamido-2-deoxy-3-*O*-β-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-4-fluoro-4-deoxy-6-*O*-acetyl-D-galactopyranosyl]-L-threonine (2).** To a solution of **29** (0.140 g; 0.146 mmol) in THF/AcOH/Ac_2O (6:3:1, 30 mL) was added activated Zn (2 g). The mixture was stirred at room temperature for 20 h. The mixture was filtered through a celite pad and washed with THF (10 mL). The filtrate was concentrated in vacuo and purified by flash chromatography using 2−4% acetic acid in ethyl acetate to give 0.106 g (84.2%) of compound **2**. **^{1}H NMR** (500 MHz, methanol-d_4): δ 7.82 (d, *J* = 7.7 Hz, 2H, 2× Ar(CH***)), 7.70 (t, *J* = 6.1 Hz, 2H, 2× Ar(C***H***)), 7.40 (d, *J* = 8.2 Hz, 2H, 2× Ar(C***H***)), 7.33 (q, *J* = 6.8 Hz, 2H, 2× Ar(C***H***)), 5.37 (d, *J* = 3.4 Hz, 1H, ***H4′***), 5.08 (dd, *J* = 10.2, 7.9 Hz, 1H, ***H2′***), 5.01 (dd, *J* = 10.5, 3.2 Hz, 1H, ***H3′***), 4.87 (4.86, 1H, ***H1***) (d, *J* = 49.5 Hz, 1H, ***H4(F)***), 4.67 (d, *J* = 7.8 Hz, 1H, ***H1′***), 4.61−4.50 (m, 2H, Fmoc(CH_2)), 4.37 (q, *J* = 6.8 Hz, 1H, Thr(C***Hβ***)), 4.35−4.25 (m, 2H, ***H2***, Fmoc(C***H***)), 4.23 (d, *J* = 6.6 Hz, 4H, Thr(C***Hα***), ***H6***(CH_2), ***H6a′***), 4.15−4.02 (m, 2H, ***H5, H6b′***), 3.97 (t, *J* = 6.6 Hz, 1H, ***H5′***), 3.80 (dd, *J* = 27.0, 11.5 Hz, 1H, ***H3***), 2.16 (s, 3H, OAc(CH_3)), 2.07 (s, 3H, OAc(CH_3)), 2.02 (s, 6H, 2× OAc(CH_3)), 1.99 (s, 3H, OAc(CH_3)), 1.94 (s, 3H, NHAc(CH_3)), 1.23 (d, *J* = 6.4 Hz, 3H, Thr(CH_3)). **^{13}C NMR** (101 MHz, methanol-d_4): δ 175.20, 173.48, 173.12, 172.34, 171.99, 171.91, 171.47, 171.08, 159.06, 145.35, 145.12, 142.70, 128.86, 128.22, 126.11, 126.01, 121.05, 121.02, 103.24, 100.89, 89.89 (d, $J_{C4,F}$ = 183.1 Hz), 77.54, 75.99 (d, $J_{C3,F}$ = 17.8 Hz), 72.33, 72.04, 70.13, 68.87 (d, $J_{C5,F}$ = 17.7 Hz), 68.68, 67.67, 63.85 (d, $J_{C6,F}$ = 5.5 Hz), 62.47, 59.93, 23.29, 20.79, 20.74, 20.66, 20.49, 20.46, 19.15. **HRMS**: $C_{43}H_{51}FN_2O_{19}$ ESI M + H calcd: 919.3143; observed, 919.3152.

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-α-[2-azido-2-deoxy-3-*O*-β-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4-fluoro-4-deoxy-6-*O*-acetyl-D-galactopyranosyl]-L-threonine Phenacyl Ester (30).** Similar to the procedure for the synthesis of **29**, compound **26** (0.084 g; 0.144 mmol) was processed through bromide **28** in 87% yield. This donor was reacted as is with amino acid **B** (0.110 g; 0.23 mmol) and $AgClO_4$ (0.140 g; 0.67 mmol) to give **30** (0.074 g; 51.0%). **^{1}H NMR** (500 MHz, chloroform-*d*): δ 7.91 (d, *J* = 7.8 Hz, 2H, Ar(CH***)), 7.77 (d, *J* = 7.5 Hz, 2H, Ar(C***H***)), 7.63 (q, *J* = 7.6, 7.0 Hz, 3H, Ar(C***H***)), 7.50 (t, *J* = 7.7 Hz, 2H, Ar(C***H***)), 7.40 (t, *J* = 7.5 Hz, 2H, Ar(C***H***)), 7.32 (t, *J* = 7.5 Hz, 2H, Ar(C***H***)), 5.93 (d, *J* = 9.4 Hz, 1H, Fmoc-N***H***), 5.57 (d, *J* = 16.4 Hz, 1H, PhCO−C***H***H), 5.52 (d, *J* = 3.7 Hz, 1H, ***H1***), 5.38 (m, 2H, H2′, PhCO−C***H***H), 4.97 (ddd, *J* = 25.9, 11.7, 2.36 Hz, 1H, ***H3′***), 4.93 (d, *J* = 50.88 Hz, 1H, ***H4(F)***), 4.85 (dd, *J* = 50.09, 2.36 Hz, 1H, ***H4′(F)***), 4.62 (q, *J* = 6.8 Hz, 1H, Thr(C***Hβ***)), 4.56 (d, *J* = 9.5 Hz, 1H, Thr(C***Hα***)), 4.53 (dd, *J* = 10.4, 7.0 Hz, 1H, Fmoc(C***H***H)), 4.41 (dd, *J* = 11.3, 6.3 Hz, 1H, ***H6a′***), 4.36−4.20 (m, 5H, Fmoc(C***H***H), ***H6***(CH_2), Fmoc(C***H***), ***H6b′***), 4.11 (dt, *J* = 28.9, 6.3 Hz, 1H, ***H5***), 3.96 (dd, *J* = 25.7, 10.6 Hz, 1H, ***H3***), 3.85 (dt, *J* = 26.0, 6.7 Hz, 1H, ***H5′***), 3.79 (dd, *J* = 10.7, 3.8 Hz, 1H, ***H2***), 2.11 (s, 3H, OAc(CH_3)), 2.08 (s, 3H, OAc(CH_3)), 2.07 (s, 3H, OAc(CH_3)), 2.04 (s, 3H, OAc(CH_3)), 1.42 (d, *J* = 6.4 Hz, 3H, Thr(CH_3)). **^{13}C NMR** (126 MHz, chloroform-*d*): δ 191.44, 170.67, 170.49, 170.43, 170.01, 169.37, 156.88, 143.98, 143.80, 141.43, 134.32, 133.94, 129.11, 127.90, 120.16, 102.35, 98.91, 88.56 (d, $J_{C4,F}$ = 185.6 Hz), 85.65 (d, $J_{C4',F}$ = 187.2 Hz), 76.26, 75.69 (d, $J_{C3,F}$ = 18.1 Hz), 71.47 (d, $J_{C3',F}$ = 18.2 Hz), 71.19 (d, $J_{C5',F}$ = 18.1 Hz), 68.58, 67.75 (d, $J_{C5,F}$ = 18.2 Hz), 67.54, 66.93, 62.62 (d, $J_{C6,F}$ = 5.8 Hz), 61.22 (d, $J_{C6',F}$ = 5.6 Hz), 59.93, 58.67, 47.26, 20.86, 20.83, 20.80, 20.78, 19.27. **HRMS**: $C_{41}H_{48}F_2N_2O_{17}$ ESI M + H calcd: 879.2994; observed, 879.3006.

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-α-[2-acetamido-2-deoxy-3-*O*-β-(2,3,6-tri-*O*-acetyl-4-fluoro-4-deoxy-D-galactopyranosyl)-4-fluoro-4-deoxy-6-*O*-acetyl-D-galactopyranosyl]-L-threonine (3).** To a solution of **30** (0.148 g; 0.15 mmol) in THF/AcOH/Ac_2O (6:3:1, 30 mL) was added activated zinc (1 g). The mixture was stirred at room temperature for 20 h. The mixture was filtered through a celite pad and washed with THF (10 mL). The filtrate was concentrated in vacuo and purified by flash chromatography using 2−4% acetic acid in ethyl acetate to give 0.123 g (93.1%) of compound **3**. **^{1}H NMR** (500 MHz, methanol-d_4): δ 7.82 (d,

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

J = 7.6 Hz, 2H, 2× Ar(C***H***)), 7.72−7.67 (m, 2H, 2× Ar(C***H***)), 7.41 (td, J = 7.5, 3.3 Hz, 2H, 2× Ar(C***H***)), 7.36−7.30 (m, 2H, 2× Ar(C***H***)), 5.09 (t, J = 9.0 Hz, 1H, ***H2′***), 5.02 (ddd, J = 27.3, 10.4, 2.4 Hz, 2H, ***H3′***), 4.87 (dd, J = 50.2, 2.1 Hz, 1H, ***H4′(F)***), 4.83 (dd, J = 50.0, 1.8 Hz, 1H, ***H4(F)***), 4.68 (d, J = 7.7 Hz, 1H, ***H1′***), 4.60−4.51 (m, 2H, Fmoc(CH_2)), 4.44−4.36 (m, 2H, ***H6a′***, Thr(C***Hβ***)), 4.34−4.27 (m, 2H, ***H2***, Fmoc(C***H***)), 4.26−4.21 (m, 3H, Thr(C***Hα***), ***H6***(CH_2)), 4.16 (dd, J = 11.4, 5.7 Hz, 1H, ***H6b′***), 4.09 (dt, J = 30.1, 7.0 Hz, 1H, ***H5***), 3.88 (dt, J = 26.9, 6.5 Hz, 1H, ***H5′***), 3.79 (dd, J = 27.0, 12.0 Hz, 1H, ***H3***), 2.07 (s, 3H, OAc(CH_3)), 2.06 (s, 6H, 2× OAc(CH_3)), 2.01 (s, 3H, OAc(CH_3)), 1.98 (s, 3H, NHAc(CH_3)), 1.23 (d, J = 6.4 Hz, 3H, Thr(CH_3)). **^{13}C NMR** (126 MHz, methanol-d_4): δ 173.11, 172.34, 172.00, 171.50, 171.01, 159.11, 145.35, 145.12, 142.71, 128.86, 128.22, 126.12, 126.02, 121.05, 121.01, 102.97, 100.87, 89.87 (d, $J_{C4,F}$ = 184.2 Hz), 87.74 (d, $J_{C4',F}$ = 183.8 Hz), 77.44, 75.99 (d, $J_{C3,F}$ = 18.0 Hz), 72.46 (d, J = 17.5 Hz), 72.30 (d, J = 17.7 Hz), 70.22, 68.88 (d, J = 18.0 Hz), 67.67, 63.89 (d, J = 5.0 Hz), 62.67 (d, J = 5.8 Hz), 59.86, 23.28, 20.77, 20.70, 20.66, 20.47, 19.17. **HRMS**: $C_{41}H_{48}F_2N_2O_{17}$ ESI M + H calcd: 879.2994; observed, 879.3006.

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-*α*-[2-azido-2-deoxy-3-*O*-*β*-(2,3,6-tri-*O*-acetyl-4-deoxy-D-galactopyranosyl)-4,6-*O*-benzylidene-*α*-D-galactopyranosyl]-L-threonine *tert*-Butyl Ester (32).** To a solution of **31**[34] (0.162 g, 0.372 mmol) in dry 1,2-dichloroethane (5 mL) containing activated 4A MS was added a solution of **C**[43] (0.198 g, 0.294 mmol) in 1,2-dichloroethane (8 mL). The mixture was cooled to −78 °C, and TMSOTf (0.020 mL, 0.11 mmol) was added. After stirring for 2 h, the reaction was quenched with triethyl amine (0.020 mL, 0.143 mmol). The mixture was diluted with DCM (25 mL) and filtered through a celite pad. The filtrate was concentrated and purified by flash chromatography using ethyl acetate/hexanes (1:2 to 1:1) to give 0.194 g (70.0%) of compound **32**. **^{1}H NMR** (500 MHz, methanol-d_4): δ 7.82 (d, J = 7.6 Hz, 2H, 2× Ar(CH***)), 7.70 (t, J = 6.9 Hz, 2H, 2× Ar(C***H***)), 7.50 (d, J = 7.0 Hz, 2H, 2× Ar(C***H***)), 7.41 (t, J = 7.5 Hz, 2H, 2× Ar(C***H***)), 7.38−7.30 (m, 5H, phenyl***H***), 5.58 (s, 1H, Ph−C***H***−), 5.14 (d, J = 3.6 Hz, 1H, ***H1***), 5.06 (td, J = 10.6, 5.4 Hz, 1H, ***H3′***), 4.81 (d, J = 7.9 Hz, 1H, ***H1′***), 4.53 (d, J = 3.3 Hz, 1H, ***H4***), 4.46 (dd, J = 10.6, 6.8 Hz, 1H, Fmoc-***CH***)), 4.38 (dd, J = 10.7, 7.0 Hz, 1H, Fmoc-***CH***), 4.34 (dd, J = 6.5, 3.5 Hz, 1H, Thr-***CHβ***), 4.27 (m, 2H, Fmoc-***CH***, ***H6′A***), 4.18 (td, J = 9.0, 7.7, 4.1 Hz, 2H, ***H3***, and Thr-***CHα***), 4.15−4.07 (m, 3H, H6(***C***H_2), ***H6′B***), 3.81 (s, 1H, ***H5***), 3.77 (dd, J = 10.9, 3.7 Hz, 1H, ***H2***), 2.09 (dd, J = 12.6, 5.4 Hz, 1H, ***H4A***), 2.03 (s, 3H, O***Ac***), 1.99 (s, 3H, O***Ac***), 1.64 (q, J = 12.1 Hz, 1H, ***H4B***), 1.51 (s, 9H, ***t-Bu***), 1.29 (d, J = 6.3 Hz, 3H, Thr-***CH***$_3$). **^{13}C NMR** (126 MHz, methanol-d_4): δ 172.25, 171.80, 171.64, 170.86, 158.92, 145.21, 142.62, 139.56, 129.89, 129.04, 128.87, 128.23, 128.20, 127.46, 120.98, 103.50, 102.08, 101.10, 83.60, 77.60, 77.20, 77.10, 73.80, 72.22, 70.99, 70.25, 68.16, 66.33, 64.88, 61.20, 60.85, 33.15, 28.27, 20.95, 20.89, 20.78, 19.39. **HRMS**: $C_{48}H_{56}N_4O_{16}$ ESI M + H calcd: 945.3764; observed, 945.3728.

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-*α*-[2-azido-2-deoxy-3-*O*-*β*-(2,3,6-tri-*O*-acetyl-4-deoxy-D-galactopyranosyl)-4,6-di-*O*-acetyl-D-galactopyranosyl]-L-threonine *tert*-Butyl Ester (33).** To a solution of **32** (0.269 g, 0.284 mmol) in methanol (20 mL) containing few drops of water was added iodine (0.240 g). The mixture was heated at reflux for 5 h and then cooled to room temperature. The mixture was diluted with ethyl acetate (100 mL), washed with saturated sodium bicarbonate solution (2 × 50 mL) and brine (2 × 50 mL), dried, and filtered. The filtrate was concentrated in vacuo to give the crude intermediate diol that was used as recovered. The diol was dissolved in pyridine (15 mL), and acetic anhydride (2 mL) was added. The mixture was stirred at room temperature for 20 h and then poured onto ice water (40 mL). After extraction with ethyl acetate (2 × 50 mL), the organic layer was washed with saturated sodium bicarbonate solution (1 × 50 mL), dried, and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography using ethyl acetate/hexanes (1:1 to 2:1) to give 0.196 g (73.4%) of product **33**. **^{1}H NMR** (500 MHz, methanol-d_4): δ 7.82 (d, J = 7.5 Hz, 2H, Ar(CH***), 7.70 (t, J = 6.4 Hz, 2H, Ar(C***H***), 7.41 (t, J = 7.6 Hz, 2H Ar(C***H***), 7.32 (td, J = 7.6, 2.9 Hz, 2H, Ar(C***H***), 5.50 (d, J = 3.2 Hz, 1H, H4), 5.09 (d, J = 3.8 Hz, 1H, ***H1***), 5.04 (p, J = 6.6 Hz, 1H, ***H3′***), 4.74 (m, 2H, ***H1′***, ***H2′***), 4.46−4.36 (m, 2H, Fmoc(***CH***$_2$)), 4.30 (dd, J = 6.4, 3.5 Hz, 1H, Thr(***CHβ***)), 4.28−4.24 (m, 2H, ***H5***, Fmoc(***CH***)), 4.23−4.07 (m, 3H, ***H6a***, ***H6′(a,b)***), 3.95 (dd, J = 11.6, 7.9 Hz, 1H, ***H6b***), 3.81 (dd, J = 11.2, 5.3 Hz, 1H, ***H5′***), 3.69 (dd, J = 10.8, 3.7 Hz, 1H, ***H2***), 2.10 (s, 3H, ***OAc***), 2.08 (s, 4H, ***OAc***, ***H4(a)***), 2.01 (s, 3H, ***OAc***), 2.00 (s, 3H, ***OAc***), 1.59 (q, J = 12.1 Hz, 1H, ***H4(b)***), 1.50 (s, 9H, ***t-Bu***), 1.30 (d, J = 6.5 Hz, 3H, Thr(***CH***$_3$)). **^{13}C NMR** (126 MHz, methanol-d_4): δ 172.55, 172.26, 171.76, 171.67, 171.54, 170.76, 158.94, 145.22 (d, J = 8.4 Hz), 142.61, 128.87, 128.86, 128.24, 128.21, 126.26, 126.23, 121.02, 121.00, 102.58, 100.68, 83.58, 77.73, 76.15, 73.94, 71.98, 71.58, 70.79, 69.09, 68.21, 66.28, 64.16, 61.49, 61.17, 33.29, 28.28, 20.95, 20.85, 20.77, 20.72, 20.70, 19.20. **HRMS**: $C_{45}H_{56}N_4O_{18}$ ESI M + H calcd: 945.3662; observed, 945.3622.

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-*α*-[2-acetamido-2-deoxy-3-*O*-*β*-(2,3,6-tri-*O*-acetyl-4-deoxy-D-galactopyranosyl)-4,6-di-*O*-acetyl-D-galactopyranosyl]-L-threonine *tert*-Butyl Ester (34).** To a solution of **33** (0.262 g, 0.278 mmol) in chloroform (1.2 mL) were added pyridine (1.2 mL) and thioacetic acid (1.2 mL). The mixture was stirred at room temperature for 20 h and then concentrated in vacuo. The residue was taken in ethyl acetate (70 mL), washed with 1 N HCl (1 × 30 mL), saturated sodium bicarbonate solution (1 × 30 mL), and brine (1 × 30 mL), and dried. After filtration, the solvent was concentrated, and the residue was purified by flash chromatography using ethyl acetate/hexanes (1:1 → 1:0) to give 0.235 g (88.7%) of product **34**. **^{1}H NMR** (400 MHz, methanol-d_4): δ 7.82 (d, J = 7.6 Hz, 2H, Ar(CH***), 7.69 (dd, J = 7.7, 2.8 Hz, 2H, Ar(C***H***), 7.41 (td, J = 7.4, 2.8 Hz, 2H, Ar(C***H***), 7.33 (td, J = 7.6, 5.1 Hz, 2H, Ar(C***H***), 5.37 (d, J = 3.2 Hz, 1H, ***H4***), 4.94 (ddd, J = 11.5, 9.6, 5.4 Hz, 1H, ***H3′***), 4.79 (d, J = 4.0 Hz, 1H, ***H1***), 4.65 (dd, J = 9.7, 7.8 Hz, 1H, ***H2***), 4.62−4.54 (m, 2H, Fmoc(***CH***$_2$)), 4.52 (d, J = 7.7 Hz, 1H, ***H1′***), 4.35−4.25 (m, 3H, ***H2***, Fmoc(***CH***), Thr(***CHβ***)), 4.20 (td, J = 11.0, 10.2, 5.8 Hz, 2H, ***H6a′***, Thr(***CHα***)), 4.16−4.06 (m, 3H, ***H6a***, ***H6b′***, ***H5***), 3.99 (dd, J = 11.3, 7.5 Hz, 1H, ***H6b***), 3.84 (dd, J = 11.1, 3.3 Hz, 1H, ***H3***), 3.74 (dd, J = 11.7, 5.7 Hz, 1H, ***H5′***), 2.09 (s, 3H, ***OAc***), 2.07 (m, 4H, ***H4***(C***H***H), ***OAc***), 2.05 (s, 3H, ***OAc***), 2.00 (s, 3H, ***OAc***), 1.99 (s, 3H, ***OAc***), 1.96 (s, 3H, NH***Ac***), 1.59 (q, J = 12.0 Hz, 1H, ***H4***(CH***H***)), 1.44 (s, 9H, ***t-Bu***), 1.24 (d, J = 6.3 Hz, 3H, Thr(***CH***$_3$)). **^{13}C NMR** (101 MHz, methanol-d_4): δ 172.89, 172.45, 172.28, 171.92, 171.77, 171.29, 170.88, 159.10, 145.21 (d, J = 16.1 Hz), 142.70, 128.86, 128.22, 126.10, 125.97, 121.03, 102.36, 100.67, 83.61, 76.57, 74.22, 73.66, 71.77, 71.30, 70.73, 68.87, 67.57, 66.06, 64.03, 60.73, 33.31, 28.38, 23.41, 20.90, 20.80, 20.74, 20.72, 20.67, 19.58. **HRMS**: $C_{47}H_{60}N_2O_{19}$ ESI M + H calcd: 957.3863; observed, 957.3817.

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-α-[2-acetamido-2-deoxy-3-*O*-β-(2,3,6-tri-*O*-acetyl-4-deoxy-D-galactopyranosyl)-4,6-di-*O*-acetyl-D-galactopyranosyl]-L-threonine (4).** A solution of **34** (0.235 g; 0.24 mmol) and 95:5 TFA/H_2O (5 mL) was stirred at room temperature for 60 min. The mixture was concentrated under vacuum at 15 °C and then coevaporated with toluene (3 × 5 mL) at 20 °C. The crude product was purified by silica gel chromatography using a gradient of ethyl acetate/acetic acid (50:1 to 50:3) to give compound **4** (0.208 g; 94.9%). **^{1}H NMR** (400 MHz, methanol-d_4): δ 7.82 (d, *J* = 7.6 Hz, 2H, Ar(CH***)), 7.69 (dd, *J* = 7.6, 3.2 Hz, 2H, Ar(C***H***)), 7.41 (t, *J* = 7.4 Hz, 2H, Ar(C***H***)), 7.33 (td, *J* = 7.3, 2.4 Hz, 2H, Ar(C***H***)), 5.38 (d, *J* = 3.3 Hz, 1H, ***H4***), 4.95 (ddd, *J* = 11.4, 9.5, 5.4 Hz, 1H, ***H3′***), 4.88 (d, *J* = 4.1 Hz, 1H, ***H1***), 4.66 (dd, *J* = 9.6, 7.8 Hz, 1H, ***H2′***), 4.61−4.46 (m, 3H, ***H1′***, Fmoc(CH_2)), 4.36 (q, *J* = 6.7 Hz, 1H, Thr(C***Hβ***), 4.32−4.24 (m, 2H, ***H2***, Fmoc(C***H***)), 4.24−4.16 (m, 3H, ***H5***, Thr(C***Hα***), ***H6a′***), 4.13 (dd, *J* = 11.4, 5.0 Hz, 2H, ***H6b′***, ***H6a***), 3.97 (dd, *J* = 11.4, 7.6 Hz, 1H, ***H6b***), 3.89 (dd, *J* = 11.1, 3.3 Hz, 1H, ***H3***), 3.77 (dd, *J* = 11.0, 5.2 Hz, 1H, ***H5′***), 2.14−2.05 (m, 7H, ***H4′***(C***H***), 2× OAc(CH_3)), 2.04 (s, 3H, OAc(CH_3)), 2.00 (s, 3H, OAc(CH_3)), 1.99 (s, 3H, OAc(CH_3)), 1.98 (s, 3H, NHAc(CH_3)), 1.59 (q, *J* = 12.0 Hz, 1H, ***H4′***(C***H***)), 1.23 (d, *J* = 6.4 Hz, 2H, Thr(CH_3)). **^{13}C NMR** (101 MHz, methanol-d_4): δ 172.49, 172.32, 171.96, 171.79, 171.34, 159.05, 145.37, 145.17, 142.86−142.48 (m), 128.84, 128.21, 126.13, 126.01, 121.01 (d, *J* = 3.0 Hz), 102.42, 100.76, 74.46, 73.70, 71.83, 71.41, 70.72, 68.81, 67.62, 66.14, 64.09, 33.34, 23.29, 20.74, 19.20. **HRMS**: $C_{43}H_{52}N_2O_{19}$ ESI M + H calcd: 901.3237; observed, 901.3193.

Loading of the C-Terminal Amino Acid on Chlorotrityl Chloride Resin. 2-Chlorotrityl chloride resin (1 g; loading capacity 1.5 mmol/g) was placed in a 10 mL polypropylene fritted column, suspended in dry DCM for 15 min, and further washed with dry DCM (2 × 7 mL) and then with dry DMF (3 × 7 mL). Fmoc-Ala-OH (0.467 g, 1.5 mmol) was dissolved in dry DMF (5 mL) and added to the resin, followed by diisopropylethylamine (DIPEA, 520 μL, 3.0 mmol). The resin was shaken with this mixture at room temperature for 45 min, after which the reagents were drained and the resin was washed with dry DMF (3 × 7 mL) followed by dry DCM (5 × 7 mL). The coupling and washing steps were repeated, and the resin was dried under vacuum overnight. The alanine loading was calculated, using a method reported previously by Gude et al., to be 0.4 mmol/g.[50]

Synthesis of Octapeptide (H-VPAAVVVA-*CTC-Resin*). SPPS was performed on a CEM Liberty microwave peptide synthesizer with a CEM Discover microwave generator. Fmoc-Ala-CTC resin (250 mg, 0.1 mmol) was swelled at room temperature for 30 min and then loaded into the peptide synthesizer, and the following seven amino acids in the sequence (10 equiv each) were double-coupled under microwave catalysis using 0.5 M (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (HATU) and 2 M DIPEA (20 W; 50 °C for 10 min, 2×). Fmoc-deprotection was affected by microwaves in the presence of 20% piperidine in *N*-methyl-2-pyrrolidone with 0.1% hydroxybenzotriazole (35 W; 50 °C for 2 min; 2×). The resin-attached octapeptide (Fmoc-VPAAVVVA) was retrieved from the synthesizer, washed with dry DCM (3 × 5 mL), and dried overnight. The Fmoc-number following the synthesis of the octapeptide was determined to be 0.3 mmol/g. The terminal Fmoc-group was removed manually by shaking the resin with 20% piperidine in DMF (7 mL, 30 min; 2×). The deprotected resin was washed with dry DMF (3 × 7 mL) followed by dry DCM (3 × 7 mL).

General Method for Coupling GAAs 1-4 to H-VPAAVVVA-*CTC-Resin*. Fmoc-GlycoAA-OH (0.9 equiv) and HATU (0.9 equiv) were dissolved in DMF (2 mL) and added to the resin (0.5 μmol) at room temperature. Finally, 2,4,6-trimethyl pyridine (1.5 equiv) was added to the mixture, and the resin was shaken at room temperature for 3 h. The reagents were drained, and the resin was washed with DMF (3 × 5 mL). The Fmoc-group was removed using 20% piperidine in DMF (2 × 2 mL). The resin was washed with DMF (3 × 5 mL) and then treated with 10% hydrazine hydrate in DMF for 4 h. The resin was filtered and washed again with DMF (3 × 5 mL) followed by DCM (5 × 5 mL) and then dried. The glycopeptide was cleaved off the resin by treatment with TFA/H_2O (95:5; 2 mL) for 2 h. The TFA solution was collected, and the resin was washed with TFA (2 × 1 mL). The TFA solutions were combined, and the acid was removed under vacuum at 20 °C. The residue was redissolved in Milli-Q water, lyophilized to a white foam, and purified by preparative HPLC (see the Supporting Information).

WST-1 Cell Proliferation Assay. Primary NB epithelial cells were grown from bladder tissue biopsies, as previously described.[21] For this study, the cells were obtained from either the bladder biopsies of patients who were at least 18 years old or from the cadaveric tissue, in accordance with the guidelines of the Institutional Review Board of the University of Maryland School of Medicine. None of the cell donors had any history of a functional bladder disorder. T24 (HTB-4) cells were obtained from ATCC and cultured, as previously described.[24]

Bladder epithelial and carcinoma cell proliferations were measured using a WST-1 assay (Roche) according to the manufacturer's instructions. For this assay, NB cells were plated onto 96-well tissue culture plates (VWR) at a density of 1.5 × 10^4 cells/well, cultured in minimum essential medium (MEM) containing 10% heat-inactivated fetal bovine serum (FBS), 1% antibiotic/antimycotic solution, and 1% L-glutamine (all from Sigma) at 37 °C in a 5% CO_2 atmosphere overnight; T24 cells were plated at a density of 1.5 × 10^3 cells/well, cultured in McCoy's 5A medium (Invitrogen) containing 10% heat inactivated FBS, 1% antibiotic/antimycotic solution, 1% L-glutamine, and 2.2 g/L sodium bicarbonate (all from Sigma). The next day, the medium was changed to serum-free MEM medium (NB cells) or serum-free McCoy's medium (T24 cells), and the cells were cultured for an additional 24 h. On the third day, APF derivatives were diluted in acetonitrile/H_2O (double-distilled) (1:1) and added to the serum-free cell medium; cell controls received acetonitrile/H_2O (double distilled) (1:1) alone. The cells were then incubated at 37 °C in a 5% CO_2 atmosphere for an additional 48 h, after which the WST-1 reagent was applied to the cells and incorporation was measured at 450 nm with the reference wavelength at 690 nm, using a Molecular Devices microplate reader.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01018.

HPLC conditions; yields for glycoamino acid coupling reactions; ^{1}H and ^{13}C NMR spectra for all new compounds; HPLC purity traces for glycopeptides **5**−

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

8; and full NMR assignments for glycopeptides **5**, **6**, and **7** (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported in part by funding from the Intramural Research Program of the NIH, the National Cancer Institute, the Center for Cancer Research (M.A.S., S.A., and J.J.B.), as well as in part by Merit Review funding from the United States (U.S.) Department of Veterans Affairs Biomedical Laboratory Research and Development Service, Office of Research and Development (S.K.K.). The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

REFERENCES

(1) Bertozzi, C. R.; Rabuka, D. Structural Basis of Glycan Diversity. In *Essentials of Glycobiology*, 2nd ed.; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor: NY, 2009.

(2) Varki, A.; Lowe, J. B. Biological Roles of Glycans. In *Essentials of Glycobiology*, 2nd ed.; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor: NY, 2009.

(3) Varki, A.; Kannagi, R.; Toole, B. P. Glycosylation Changes in Cancer. In *Essentials of Glycobiology*, 2nd ed.; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. E., Eds.; Cold Spring Harbor: NY, 2009.

(4) Springer, G. F.; Desai, P. R. Tn Epitopes, Immunoreactive with Ordinary Anti-Tn Antibodies, on Normal, Desialylated Human Erythrocytes and on Thomsen-Friedenreich Antigen Isolated Therefrom. *Mol. Immunol.* **1985**, *22*, 1303−1310.

(5) Springer, G. F.; Tegtmeyer, H. On the Origin of Anti-Thomsen-Friedenreich (T) Antibodies. *Naturwissenschaften* **1980**, *67*, 317−318.

(6) Johannes, M.; Reindl, M.; Gerlitzki, B.; Schmitt, E.; Hoffmann-Röder, A. Synthesis and biological evaluation of a novel MUC1 glycopeptide conjugate vaccine candidate comprising a 4′-deoxy-4′-fluoro-Thomsen-Friedenreich epitope. *Beilstein J. Org. Chem.* **2015**, *11*, 155−161.

(7) Bourgault, J. P.; Trabbic, K. R.; Shi, M. C.; Andreana, P. R. Synthesis of the tumor associative α-aminooxy disaccharide of the TF antigen and its conjugation to a polysaccharide immune stimulant. *Org. Biomol. Chem.* **2014**, *12*, 1699−1702.

(8) Hevey, R.; Ling, C.-C. Recent advances in developing synthetic carbohydrate-based vaccines for cancer immunotherapies. *Future Med. Chem.* **2012**, *4*, 545−584.

(9) Hoffmann-Röder, A.; Johannes, M. Synthesis of a MUC1-glycopeptide−BSA conjugate vaccine bearing the 3′-deoxy-3′-fluoro-Thomsen−Friedenreich antigen. *Chem. Commun.* **2011**, *47*, 9903−9905.

(10) Heimburg-Molinaro, J.; Almogren, A.; Morey, S.; Glinskii, O. V.; Roy, R.; Wilding, G. E.; Cheng, R. P.; Glinsky, V. V.; Rittenhouse-Olson, K. Development, Characterization, and Immunotherapeutic Use of Peptide Mimics of the Thomsen-Friedenreich Carbohydrate Antigen. *Neoplasia* **2009**, *11*, 780−792.

(11) Qiu, L.; Li, J.; Yu, S.; Wang, Q.; Li, Y.; Hu, Z.; Wu, Q.; Guo, Z.; Zhang, J. A novel cancer immunotherapy based on the combination of a synthetic carbohydrate-pulsed dendritic cell vaccine and glycoengineered cancer cells. *Oncotarget* **2015**, *6*, 5195−5203.

(12) Sørensen, A. L.; Reis, C. A.; Tarp, M. A.; Mandel, U.; Ramachandran, K.; Sankaranarayanan, V.; Schwientek, T.; Graham, R.; Taylor-Papadimitriou, J.; Hollingsworth, M. A.; Burchell, J.; Clausen, H. Chemoenzymatically synthesized multimeric Tn/STn MUC1 glycopeptides elicit cancer-specific anti-MUC1 antibody responses and override tolerance. *Glycobiology* **2006**, *16*, 96−107.

(13) Liakatos, A.; Kunz, H. Synthetic glycopeptides for the development of cancer vaccines. *Curr. Opin. Mol. Ther.* **2007**, *9*, 35−44.

(14) Hanisch, F.-G.; Ninkovic, T. Immunology of O-glycosylated proteins: Approaches to the design of a MUC1 glycopeptide-based tumor vaccine. *Curr. Protein Pept. Sci.* **2006**, *7*, 307−315.

(15) Yu, L. G.; Andrews, N.; Zhao, Q.; McKean, D.; Williams, J. F.; Connor, L. J.; Gerasimenko, O. V.; Hilkens, J.; Hirabayashi, J.; Kasai, K.; Rhodes, J. M. Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion. *J. Biol. Chem.* **2007**, *282*, 773−781.

(16) Guha, P.; Kaptan, E.; Bandyopadhyaya, G.; Kaczanowska, S.; Davila, E.; Thompson, K.; Martin, S. S.; Kalvakolanu, D. V.; Vasta, G. R.; Ahmed, H. Cod glycopeptide with picomolar affinity to galectin-3 suppresses T-cell apoptosis and prostate cancer metastasis. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 5052−5057.

(17) Glinskii, O. V.; Sud, S.; Mossine, V. V.; Mawhinney, T. P.; Anthony, D. C.; Glinsky, G. V.; Pienta, K. J.; Glinsky, V. V. Inhibition of Prostate Cancer Bone Metastasis by Synthetic TF Antigen Mimic/Galectin-3 Inhibitor Lactulose-L-Leucine. *Neoplasia* **2012**, *14*, 65−73.

(18) Glinsky, V. V.; Glinsky, G. V.; Rittenhouse-Olson, K.; Huflejt, M. E.; Glinskii, O. V.; Deutscher, S. L.; Quinn, T. P. The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium. *Cancer Res.* **2001**, *61*, 4851−4857.

(19) Senapati, S.; Chaturvedi, P.; Chaney, W. G.; Chakraborty, S.; Gnanapragassam, V. S.; Sasson, A. R.; Batra, S. K. Novel Interaction of MUC4 and Galectin: Potential Pathobiological Implications for Metastasis in Lethal Pancreatic Cancer. *Clin. Cancer Res.* **2011**, *17*, 267−274.

(20) Gerwig, G.; Hocking, H.; Stöcklin, R.; Kamerling, J.; Boelens, R. Glycosylation of Conotoxins. *Mar. Drugs* **2013**, *11*, 623.

(21) Keay, S. K.; Szekely, Z.; Conrads, T. P.; Veenstra, T. D.; Barchi, J. J.; Zhang, C.-O.; Koch, K. R.; Michejda, C. J. An antiproliferative factor from interstitial cystitis patients is a frizzled 8 protein-related sialoglycopeptide. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11803−11808.

(22) Keay, S.; Kaczmarek, P.; Zhang, C.-O.; Koch, K.; Szekely, Z.; Barchi, J. J.; Michejda, C. Normalization of Proliferation and Tight Junction Formation in Bladder Epithelial Cells from Patients with Interstitial Cystitis/Painful Bladder Syndrome by d-Proline and d-Pipecolic Acid Derivatives of Antiproliferative Factor. *Chem. Biol. Drug Des.* **2011**, *77*, 421−430.

(23) Zhang, C.-O.; Wang, J.-Y.; Koch, K. R.; Keay, S. Regulation of tight junction proteins and bladder epithelial paracellular permeability by an antiproliferative factor from patients with interstitial cystitis. *J. Urol.* **2005**, *174*, 2382−2387.

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632

(24) Koch, K. R.; Zhang, C.-O.; Kaczmarek, P.; Barchi, J.; Guo, L.; Shahjee, H. M.; Keay, S. The effect of a novel frizzled 8-related antiproliferative factor on in vitro carcinoma and melanoma cell proliferation and invasion. *Invest. New Drugs* **2012**, *30*, 1849−1864.
(25) Mallajosyula, S. S.; Adams, K. M.; Barchi, J. J.; MacKerell, A. D. Conformational Determinants of the Activity of Antiproliferative Factor Glycopeptide. *J. Chem. Inf. Model.* **2013**, *53*, 1127−1137.
(26) Kaczmarek, P.; Tocci, G. M.; Keay, S. K.; Adams, K. M.; Zhang, C.-O.; Koch, K. R.; Grkovic, D.; Guo, L.; Michejda, C. J.; Barchi, J. J. Structure−Activity Studies on Antiproliferative Factor (APF) Glycooctapeptide Derivatives. *ACS Med. Chem. Lett.* **2010**, *1*, 390−394.
(27) Wagner, S.; Mersch, C.; Hoffmann-Röder, A. Fluorinated Glycosyl Amino Acids for Mucin-Like Glycopeptide Antigen Analogues. *Chem.—Eur. J.* **2010**, *16*, 7319−7330.
(28) Mersch, C.; Wagner, S.; Hoffmann-Röder, A. Synthesis of Fluorinated Analogues of Tumor-Associated Carbohydrate and Glycopeptide Antigens. *Synlett* **2009**, 2167−2171.
(29) Svarovsky, S. A.; Barchi, J. J. Highly efficient preparation of tumor antigen-containing glycopeptide building blocks from novel pentenyl glycosides. *Carbohydr. Res.* **2003**, *338*, 1925−1935.
(30) Svarovsky, S. A.; Szekely, Z.; Barchi, J. J. Synthesis of gold nanoparticles bearing the Thomsen−Friedenreich disaccharide: a new multivalent presentation of an important tumor antigen. *Tetrahedron: Asymmetry* **2005**, *16*, 587−598.
(31) Alper, P. B.; Hung, S.-C.; Wong, C.-H. Metal catalyzed diazo transfer for the synthesis of azides from amines. *Tetrahedron Lett.* **1996**, *37*, 6029−6032.
(32) Goddard-Borger, E. D.; Stick, R. V. An efficient, inexpensive, and shelf-stable diazotransfer reagent: Imidazole-1-sulfonyl azide hydrochloride. *Org. Lett.* **2007**, *9*, 3797−3800.
(33) Koch, K.; Chambers, R. J. An improved synthesis of 4-deoxy-4-fluoro-d-galactopyranosyl derivatives. *Carbohydr. Res.* **1993**, *241*, 295−299.
(34) van Dorst, J. A. L. M.; van Heusden, C. J.; Voskamp, A. F.; Kamerling, J. P.; Vliegenthart, J. F. G. Synthesis of Hex p-(1 → 4)-*β*-d-Glc pNAc-(1 → 2)-*α*-d-Man p-(1 → O)(CH2)7CH3 probes for exploration of the substrate specificity of glycosyltransferases: Part I, Hex = *β*-d-Gal, 4-deoxy-*β*-d-Gal, 4-O-methyl-*β*-d-Gal, 4-deoxy-4-fluoro-*β*-d-Gal, or *β*-d-Glc. *Carbohydr. Res.* **1996**, *291*, 63−83.
(35) Zapata, A.; Martín-Lomas, M. Building blocks for the synthesis of glycosyl-myo-inositols involved in the insulin intracellular signalling process. *Carbohydr. Res.* **1992**, *234*, 93−106.
(36) Sarkar, A. K.; Brown, J. R.; Esko, J. D. Synthesis and glycan priming activity of acetylated disaccharides. *Carbohydr. Res.* **2000**, *329*, 287−300.
(37) Barthel, S. R.; Antonopoulos, A.; Cedeno-Laurent, F.; Schaffer, L.; Hernandez, G.; Patil, S. A.; North, S. J.; Dell, A.; Matta, K. L.; Neelamegham, S.; Haslam, S. M.; Dimitroff, C. J. Peracetylated 4-Fluoro-glucosamine Reduces the Content and Repertoire of N- and O-Glycans without Direct Incorporation. *J. Biol. Chem.* **2011**, *286*, 21717−21731.
(38) Xia, J.; Xue, J.; Locke, R. D.; Chandrasekaran, E. V.; Srikrishnan, T.; Matta, K. L. Synthesis of fluorinated mucin core 2 branched oligosaccharides with the potential of novel substrates and enzyme inhibitors for glycosyltransferases and sulfotransferases. *J. Org. Chem.* **2006**, *71*, 3696−3706.
(39) Thomas, R. L.; Abbas, S. A.; Matta, K. L. Synthesis of Uridine 5′-(2-Acetamido-2,4-Dideoxy-4-Fluoro-Alpha-D-Galactopyranosyl) Diphosphate and Uridine 5′-(2-Acetamido-2,6-Dideoxy-6-Fluoro-Alpha-D-Glucopyranosyl) Diphosphate. *Carbohydr. Res.* **1988**, *184*, 77−85.
(40) Thomas, R. L.; Abbas, S. A.; Piskorz, C. F.; Matta, K. L. Synthesis of 2-Acetamido-2,4-Dideoxy-4-Fluoro-3-O-*β*-D-Galactopyranosyl-D-Glucopyranose—a Potential Specific Substrate for (1→2)-*α*-L-Fucosyltransferase. *Carbohydr. Res.* **1988**, *175*, 158−162.
(41) Cato, D.; Buskas, T.; Boons, G.-J. Highly efficient stereospecific preparation of Tn and TF building blocks using thioglycosyl donors and the Ph2SO/Tf2O promotor system. *J. Carbohydr. Chem.* **2005**, *24*, 503−516.
(42) Dziadek, S.; Brocke, C.; Kunz, H. Biomimetic synthesis of the tumor-associated (2,3)-sialyl-T antigen and its incorporation into glycopeptide antigens from the mucins MUC1 and MUC4. *Chem.—Eur. J.* **2004**, *10*, 4150−4162.
(43) Mathieux, N.; Paulsen, H.; Meldal, M.; Bock, K. Synthesis of glycopeptide sequences of repeating units of the mucins MUC 2 and MUC 3 containing oligosaccharide side-chains with core 1, core 2, core 3, core 4 and core 6 structure. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2359−2368.
(44) Kaczmarek, P.; Keay, S. K.; Tocci, G. M.; Koch, K. R.; Zhang, C. O.; Barchi, J. J.; Grkovic, D.; Guo, L.; Michejda, C. J. Structure−activity relationship studies for the peptide portion of the bladder epithelial cell antiproliferative factor from interstitial cystitis patients. *J. Med. Chem.* **2008**, *51*, 5974−5983.
(45) Zhang, Y.; Muthana, S. M.; Farnsworth, D.; Ludek, O.; Adams, K.; Barchi, J. J.; Gildersleeve, J. C. Enhanced Epimerization of Glycosylated Amino Acids During Solid-Phase Peptide Synthesis. *J. Am. Chem. Soc.* **2012**, *134*, 6316−6325.
(46) Ishiyama, M.; Tominaga, H.; Shiga, M.; Sasamoto, K.; Ohkura, Y.; Ueno, K. A combines assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. *Biol. Pharm. Bull.* **1996**, *19*, 1518−1520.
(47) Mizoguchi, M.; Ishiyama, M.; Shiga, M.; Sasamoto, K. The development of new type oxidative and reductive chromogenic reagents in clinical analysis. *Bunseki Kagaku* **1996**, *45*, 111−124.
(48) Ngamwongsatit, P.; Banada, P. P.; Panbangred, W.; Bhunia, A. K. WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic Bacillus species using CHO cell line. *J. Microbiol. Methods* **2008**, *73*, 211−215.
(49) Tan, A. S.; Berridge, M. V. Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. *J. Immunol. Methods* **2000**, *238*, 59−68.
(50) Gude, M.; Ryf, J.; White, P. D. An accurate method for the quantitation of Fmoc-derivatized solid phase supports. *Lett. Pept. Sci.* **2002**, *9*, 203−206.

DOI: 10.1021/acsomega.7b01018
ACS Omega 2017, 2, 5618−5632