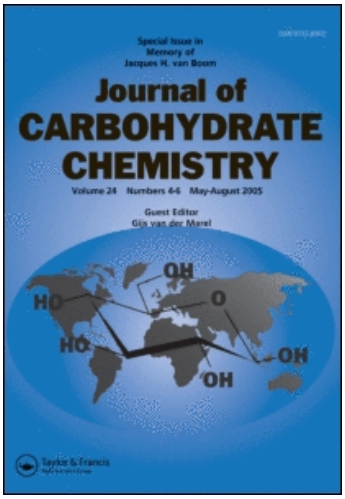

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Highly Efficient Stereospecific Preparation of Tn and TF Building Blocks Using Thioglycosyl Donors and the Ph_2SO/Tf_2O Promotor System

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Highly Efficient Stereospecific Preparation of Tn and TF Building Blocks Using Thioglycosyl Donors and the Ph_2SO/Tf_2O Promotor System

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The activation of 2-azido-2-deoxy Tn and TF thioglycosyl donors by the powerful thiophilic promoter system Ph_2SO/Tf_2O has been investigated. Glycosylation of an Fmoc-protected threonine derivative gave 1,2-cis glycosides in high yields and excellent stereoselectivities. The galactosylation of phenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside was achieved in high yield and without orthoester formation using a trichloroacetimidate donor carrying a 2-*O*-(2,5-difluorobenzoyl) group. The anomeric thiophenyl group of the constructed TF disaccharide could directly be activated by the van Boom promotor for the glycosylation of a threonine derivative.

Keywords Glycosylation, Thioglycoside, Tn antigen, TF antigen, Glycopeptide

INTRODUCTION

Mucins are a family of highly glycosylated proteins that are expressed on most epithelial cells.[1] The polypeptide backbone of mucins consists of highly conserved tandem repeats that are heavily *O*-glycosylated through clustered serine and threonine residues. The large and complex mucin-type carbohydrate structures serve as important recognition motifs for interactions with proteins.

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Dedicated to the memory of Professor Jacques H. van Boom.
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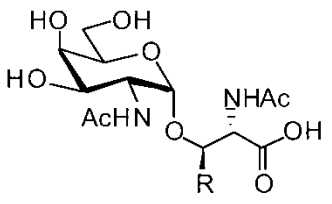
Although the glycoforms of mucins display extreme heterogeneity in regards to saccharide composition, length, and linkages, the biosynthesis of mucins is initiated by the addition of N-acetylgalactosamine to serine and threonine residues. Further elongation of this structure leads to the large family of O-glycans, classified into eight groups depending on their core stucture. This elongation takes place at the 3-O and/or 6-O-position by addition of galactose and/or polylactosamine residues followed by chain terminations with sialic acids, fucoses, or sulfation.

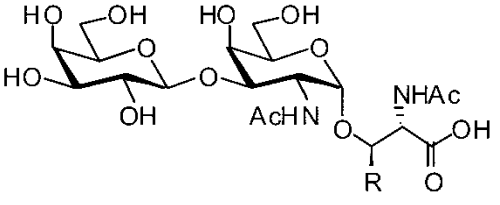
In malignant cells, altered expression levels of glycosyl transferases such as the downregulation of glucosaminyltransferases and concomitant up-regulation of sialyltransferases lead to simpler truncated forms of the glycans. This aberrant glycosylation has been correlated with specific disease states. For example, it is known that the presence of the Tn antigen and the related structure Gal*p*β(1-3)Gal*p*Nacα-Ser/Thr, also known as the TF antigen (Fig. 1), is common on human epithelial tumor cells,[2] such as colon and prostate cancers.[3] The presence of these antigens has spurred intense studies aimed at the development of immunotherapy for cancer.[4,5] However, the enormous structural diversity that is introduced by glycosylation renders the isolation of well-defined glycopeptides from natural sources an almost impossible task, thus presenting a major obstacle to the study of the structure-activity relationship of these compounds. It is thus not surprising that homogeneous synthetic glycopeptides would be most valuable tools for unraveling the specific roles of glycopeptides derived from mucins in biological processes.[6]

Our goal is to develop synthetic carbohydrate-based anticancer vaccines and to construct complex glycosulfopeptides derived from mucins. With this goal in mind, we needed a facile route to substantial quantities of Tn and TF building blocks that could be used for Fmoc solid phase synthesis. To this end, the formation of the α-glycosidic linkage between N-acetylgalactosamine and serine or threonine is a key step. This particular glycosylation has garnered much attention and has been extensively reviewed in the literature.[7–9] Here we report the use of the van Boom/van der Marel promotor



R= H or CH_3

1 Tn



R= H or CH_3

2 TF

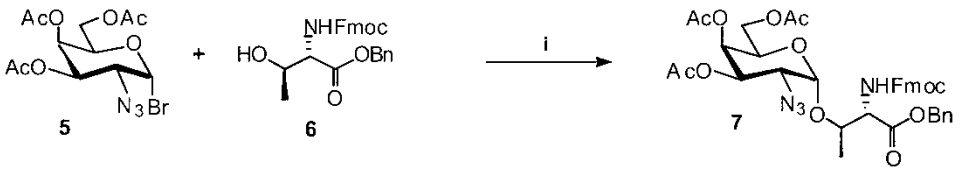
Figure 1: Tn and TF.

system for the activation of thioglycosides in the synthesis of Tn and TF derivatives useful for solid phase glycopeptide synthesis.

RESULTS AND DISCUSSION

Despite recent advances, the chemical synthesis of glycopeptides remains a difficult task.[10,11] A crucial step in any glycopeptide synthesis is the incorporation of the saccharide part to the peptide backbone. Currently, the most general synthetic methodology employs preformed glycosylated amino acids for the stepwise solid-phase synthesis of peptides. The protecting groups for these glycosylated amino acids must be carefully chosen and are rather limited, as the *O*-glycosidic bond is acid labile and the *O*-linked glycopeptide can undergo β-elimination upon treatment with strong bases. Presently, the use of acetyl esters as hydroxyl protection for the oligosaccharide part and N^{α}-Fmoc-protected amino acids is a standard technique in solid-phase glycopeptide synthesis. The formation of the α-glycosidic linkage between N-acetylgalactosamine and serine or threonine is a key step in the preparation of suitable glycosylated amino acid derivatives. For the installment of the 1,2-*cis* linkage, the nonparticipating 2-azido-2-deoxy group is commonly employed to mask the amino function. Recent reports have shown that 2-acetamido-2-deoxy galactose derivatives carrying a 4,6-benzylidene give α-selectivity in the preparation of Tn,[12] TF,[13] and sialyl-TF[14] building blocks. Anomeric halides[4,15–18] and trichloroacetimidates[19,20] are the most commonly used glycosyl donors to prepare the Gal*p*NAcα-Ser/Thr linkage.[8,9] A typical example of this glycosylation is the activation of fully acetylated 2-azido-2-deoxy bromides by $AgClO_4/Ag_2CO_3$ in the presence of serine or threonine derivatives (Sch. 1).[21] The reported yields and stereoselectivities are highly dependent on the protecting group patterns of both the saccharide donor and amino acid acceptor. Anomeric fluorides have also been utilized for the preparation of Tn derivatives with $Cp_2ZrCl_2/AgClO_4$ as promoters.[4,20,22] Although high yielding in glycosylations of both serine and threonine derivatives, the observed stereoselectivity was markedly decreased for the N^{α}-Fmoc-Ser-OBn derivative.[20]

The resulting poor stereoselectivities from the use of various TF disaccharide donors[4,23–25] for the glycosylation of serine and threonine derivatives led



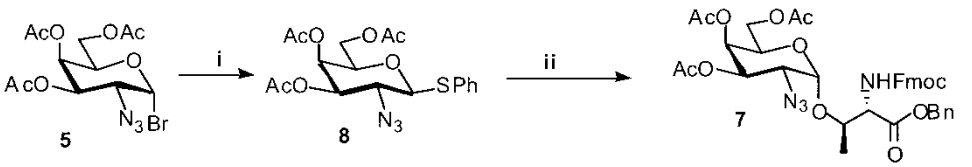
Scheme 1: (**i**): $AgClO_4/Ag_2CO_3$, DCM/toluene, 48 hr, 64%.

Danishefsky and coworkers to implement the "cassette method."[4] This method involves the use of a properly protected Tn derivative as a general acceptor in the synthesis of practically any *O*-linked glycopeptide.

Most reported methods depend on conventional labile donors that must be prepared just prior to glycosylation, thus clearly diminishing synthetic flexibility. More stable donors such as the *n*-pentenyl-[26] and seleno-glycosides[27] are capable of withstanding protecting group manipulations and may be directly activated for glycosidations, but have not found widespread use for the synthesis of Tn and TF building blocks.[28–32]

A new generation of thioglycosyl promoters has recently been introduced by Crich and coworkers.[33,34] In the most successful promoter system, 1-benzenesulfinylpiperidine (BSP) was reacted with triflic anhydride (Tf_2O) to form a sulfonium species, which could convert disarmed thioglycosides into reactive triflates at very low temperatures.[34] However, it was found that the BSP/Tf_2O promoter pair was unable to successfully activate the disarmed phenylthioglycosides of 2-azido-2-deoxy-mannose and 2-azido-2-deoxy-glucose. Thus, building upon the foundation of the Crich discovery, this promoter system was further refined by van Boom and van der Marel.[35,36] Reacting diphenylsulfoxide (Ph_2SO) with Tf_2O led to a highly electrophilic species capable of activating highly unreactive donors. This potent promoter resulted in high yields and excellent stereoselectivities. By exploiting differences in reactivity, it was shown to be useful in chemoselective glycosylation sequences with the BSP/Tf_2O promoter system. In light of this discovery, we wished to explore the use of thioglycosides as donors in the formation of the α-glycosidic linkage between 2-azido-2-deoxy derivatives of the Tn and TF saccharides.

To streamline the synthesis of Tn and TF derivatives, we wanted to explore the use of the Ph_2SO/Tf_2O promoter system for the direct activation of 2-azido-2-deoxy thiogalactoside donors in glycosylations with threonine derivatives. In an attempt to accomplish this, bromide **5**[37] was converted into the corresponding thiophenyl derivative by reaction with sodium thiophenolate in a mixture of dichloromethane and ethanol (Sch. 2).[38] Activation of thiophenyl glycoside **8**[39] with the Ph_2SO/Tf_2O promoter system for the glycosylation of N^{α}-Fmoc-Thr(OH)-OBn (**6**) proceeded with high efficiency and produced the glycosyl amino acid **7** in an excellent yield of 85%. The reaction proceeded



Scheme 2: (**i**): NaS Ph, EtOH/DCM, 3 hr, %; (**ii**): **6**, Ph_2SO/Tf_2O, DCM, − 60°C, 1 hr, 85%.

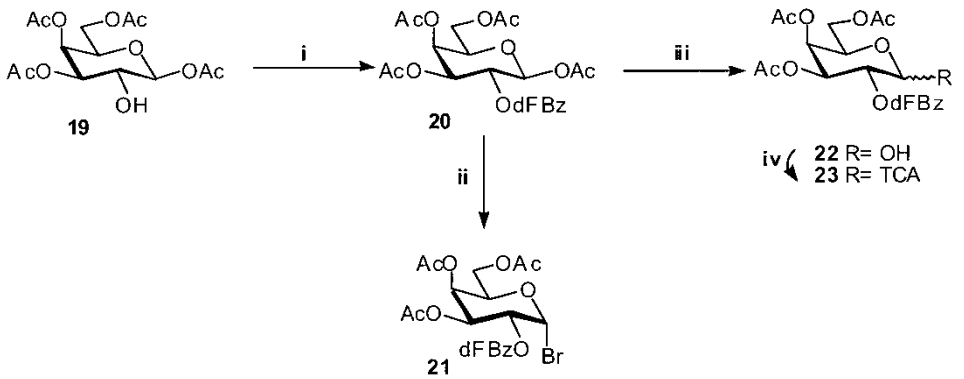
with complete stereochemical control as the α-anomer was formed exclusively. Compared to similar glycosylations using MeOTf,[28] DMTST,[28,29] or NBS/TBAOTf,[30–32] the Ph_2SO/Tf_2O promoter provides far superior yield and stereoselectivity. Encouraged by this result, we directed our attention to the preparation of the TF antigen. By deacetylation and the introduction of a 4,6-benzylidene, thioglycoside **8** was easily converted into the known acceptor **10**.[39] For the galactosylation, several glycosyl donors were investigated. The results are summarized in Table 1. The commonly used donor per-*O*-acetylated galactosyl bromide **11** activated by AgOTf[40] (entry 1) gave in our experiments unreliable results. Disaccharide **12** was often accompanied by a formation of substantial amounts of the corresponding orthoester. Changing the promoter system or anomeric leaving group did not improve the outcome (entries 2–4).

The fact that the Ph_2SO/Tf_2O promoter system uses nearly stoichiometric quantities of activator and the thioglycoside is converted into the corresponding triflate before the glycosyl acceptor is added to the reaction mixture,[35] prompted us to explore the possibility of using the readily available thiogalactoside **14**. Activation of **14** by Ph_2SO/Tf_2O in the presence of 2,5-di-^{t}butyl-4-methylpyridine (DTBMP) and subsequent reaction with acceptor **10** (entry 5) gave mainly the corresponding orthoester. We were encouraged to find only trace amounts of the disaccharide corresponding to the activation and self-coupling of acceptor **10**. In an attempt to avoid the orthoester formation, a similar reaction was performed with the omission of the base. Unfortunately, this reaction gave the same disappointing result (entry 6).

Replacing a 2-O-acetyl by a 2-O-benzoyl is a common way to avoid orthoester formation. Indeed, using fully benzoylated bromide **15** in an AgOTf-activated glycosylation of acceptor **10** gave disaccharide **16** in 76% yield (entry 7). However, due to the more severe basic conditions required for the removal of *O*-benzoates and in particular a 2-*O*-benzoate of galactose, this derivative would not be suitable for use in glycopeptide synthesis. Instead, we turned our attention to the 2,5-di-fluorobenzoyl group (dFBz) that was recently introduced for glycopeptide synthesis by Kihlberg and coworkers.[41] An advantage of this protecting group is that the difluorobenzoyl possesses a combination of the positive qualities associated with 2-O-benzoyl groups in glycosylations and the ease of removal of acetyl esters. Using the easily accessible fully difluorobenzoylated galactosyl bromide **17**[41] in an AgOTf-mediated reaction, the desired disaccharide **18** was obtained, but only in 51% yield (entry 8). The donor was found to be very unreactive and the reaction sluggish. An attempt to improve the reaction by gentle heating was unsuccessful. A positive feature of this donor was that no orthoester was isolated. The mediocre yield prompted us to prepare glycosyl donors **21** and **23** (Sch. 3). Carrying a C-2 dFBz and acetyl esters at the C-3, C-4, and C-6 position, it was believed that these donors would exhibit a higher reactivity. To accomplish

Table 1.

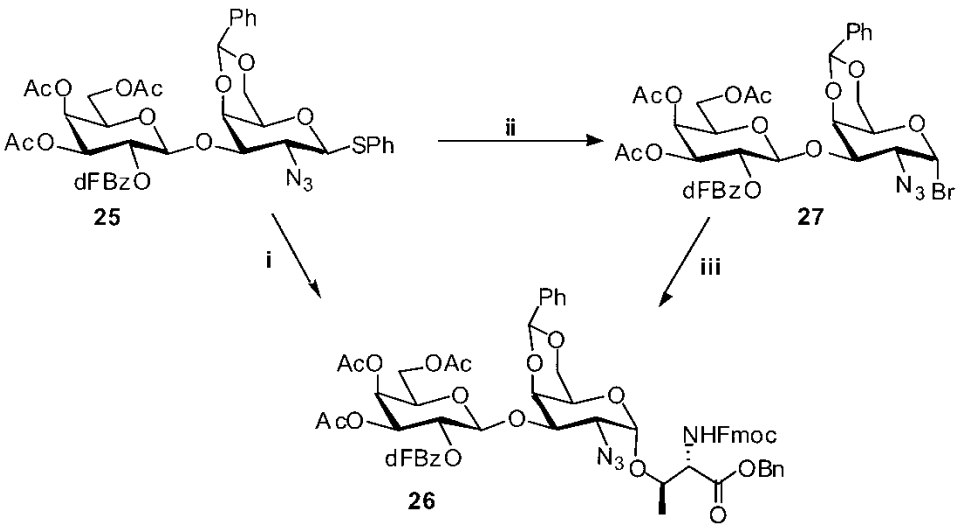
Entry	Donor	R_1	R_2	R_3	Promotor/solvent/temp.	Product
1	**11**	α-Br	Ac	Ac	AgOTf/DCM/−40°C-RT	Orthoester/**12**
2	**11**	α-Br	Ac	Ac	HgO/$HgCl_2$/DCM/50°C	Orthoester/**12**
3	**13**	α/β-OC(NH)CCl_3	Ac	Ac	TMSOT f/DCM/−20°C-RT	Orthoester/**12**
4	**14**	β-SEt	Ac	Ac	NIS/TMSOTf/DCM/−20°C-RT	Orthoester/**12**
5	**14**	β-SEt	Ac	Ac	Ph_2SO/Tf_2O/DTBMP/DCM/−60°C-RT	Orthoester
6	**14**	β-SEt	Ac	Ac	Ph_2SO/Tf_2O/DCM/−60°C-RT	Orthoester
7	**15**	α-Br	Bz	Bz	AgOTf/DCM/−40°C-RT	**16** (76%)
8	**17**	α-Br	dFBz	dFBz	AgOTf/DCM/−40°C-RT	**18** (51%)
9	**21**	α-Br	dFBz	Ac	AgOTf/DCM/−40°C-RT	**25** (63%)
10	**23**	α/β-OC(NH)CCl_3	dFBz	Ac	TMSOTf/DCM/−20°C-RT	**25** (74%)



Scheme 3: (**i**): dF BzCl, DMAP, pyridine, 18 hr, 96%; (**ii**): 30% HBr/HOAc, Ac_2O, 50°C, 89%; (**iii**): NH_2NH_2-HOAc, DMF, 60°C, 3 hr, 92%; (**iv**): Trichloroacetonitrile, DBU, DCM, 0oC, 96%.

this goal, alcohol **19**[42] was acylated with difluorobenzoyl chloride in the presence of 4-dimethylaminopyridine (DMAP), which afforded C-2 dFBz derivative **20** in 96% yield. Conversion of **20** into glycosyl donor **21** was achieved by treatment with 30% hydrogen bromide in acetic acid at 50°C. Selective cleavage of the anomeric acetate of **20** gave hemiacetal **22**, which was transformed into trichloroacetimidate **23** using standard conditions.[43] AgOTf activation of bromide **21** at −40°C in the presence of glycosyl acceptor **10** provided disaccharide **25** in 63% yield (entry 9). The superior reactivity of bromide **21** as compared to that of fully difluorobenzoylated bromide **17** was reflected by a slightly increased yield. The condensation of trichloroacetimidate **23** activated by TMSOTf at −20°C and alcohol **10** furnished disaccharide **25** in a further improved yield of 74%.

Having established a reliable and efficient route to the thiophenyl TF disaccharide, we chose to evaluate this glycosyl donor with construction of the α-O-linkage to threonine in mind. As depicted in Scheme 4, thioglycoside **25** was activated by Ph_2SO/Tf_2O in the presence of DTBMP at −60°C for the glycosylation of threonine derivative **6**. Remarkably, the reaction yielded exclusively the α-anomer product **26** in an excellent yield of 82%. It should be noted that these results are not only the best results reported for the TF disaccharide thioglycosides, but also the selectivity of this reaction is more superior than what is observed in most procedures using halides and trichloroacetimidates as glycosyl donors. To illustrate this feature, thiophenyl **25** was converted into the corresponding bromide **27** by treatment with molecular bromine. Subsequently, activation of the bromide with $AgClO_4$ in a glycosidation with threonine acceptor **6** gave **26** in an acceptable overall yield, but as expected, the α/β-selectivity was lowered and isolation of **26** required careful chromatography.



Scheme 4: (**i**): **6**, Ph_2SO/Tf_2O, DCM, −60°C, 1 hr, 82%; (**ii**): Br_2, DCM, 0°C; (**iii**): **6**, $AgClO_4$, DCM, rt, 48 hr, 68% yield over two steps.

In conclusion, we have described an efficient route for Tn and TF antigen building blocks that are useful in the solid-phase synthesis of glycopeptides derived from mucins. Using the promotor system introduced by van Boom and van der Marel for the activation of disarmed thioglycosides, we found that the activation of Tn and TF thioglycoside donors proceeded smoothly and provided the α-*O*-glycosidic bond to N^{α}-Fmoc-Thr benzyl ester in high yields and with exclusive formation of the α-product. Additionally, the TF derivative may serve as an intermediate for further extension in the synthesis of other mucin-derived glycopeptides.

EXPERIMENTAL

General

NIS was purchased from Fluka and recrystallized from dioxane/CCl_4. All other chemicals were purchased from Aldrich, Acros, and Fluka and used without further purification. Molecular sieves were activated at 145°C for 10 hr. All solvents employed were of reagent grade and dried by refluxing over appropriate drying agents. TLC was performed using Kieselgel 60 F_{254} (Merck) plates, with detection by UV light (254 nm) and/or by charring with 8% sulfuric acid in ethanol. Column chromatography was performed on silica gel (Merck, mesh 70–230). Extracts were concentrated under reduced pressure at ≤ 40°C (water bath). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova300 spectrometer and a Varian Inova500

spectrometer equipped with Sun workstations. ^{1}H spectra recorded in $CDCl_3$ were referenced to residue $CHCl_3$ at 7.26 ppm or TMS, and ^{13}C spectra to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made using standard 1D and gCOSY, gHSQC, and TOCSY 2D experiments. Positive ion matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid as a matrix.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine benzylester (7)**. To a solution of compound **8** (43 mg, 101 μmol) and Ph_2SO (58 mg, 284 μmol) in dry CH_2Cl_2 (5 mL) was added, at −60°C, trifluoromethanesulfonic anhydride (24 μL, 141 μmol). The reaction mixture was stirred for 10 min, after which a solution of acceptor **6** (87 mg, 202 μmol) in CH_2Cl_2 (1 mL) was added. The mixture was stirred at −60°C for 1 hr after which it was slowly warmed to rt and quenched by the addition of saturated aqueous $NaHCO_3$ (3 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel chromatography (hexane/EtOAc 3 : 1) yielded **7** (64 mg, 86.0 μmol, 85%); TLC (hexane/EtOAc 2 : 1) Rf = 0.39; NMR data was in agreement with reported data. HR MALDI-TOF MS: *m/z*: Calc for $C_{38}H_{40}N_4O_{12}$: 744.2643; found 767.2541 $[M + Na]^+$.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranose (20). To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (700 mg, 2.01 mmol) in dry pyridine (8 mL) was added 4-(dimethylamino)pyridine (49 mg, 0.402 mmol) and the solution was stirred at rt for 30 min. 2,5-Difluorobenzoyl chloride (0.5 mL, 4.02 mmol) was added dropwise over a 10-min period and the stirring was continued for 18 hr. The reaction was quenched by addition of methanol (4 mL) and after stirring for 1 hr, the solution was diluted with CH_2Cl_2 (120 mL) and washed with water (150 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried ($MgSO_4$) and evaporated to dryness. After purification by silica gel chromatography (hexane/EtOAc 8 : 1), **20** (942 mg, 1.93 mmol, 96%) was afforded as a white solid; TLC (hexane/EtOAc 1 : 1), $R_f = 0.68$; $[\alpha]_D + 20.0$ (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.55–7.05 (m, 3H, dFBz), 6.53 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 5.60–5.41 (m, 3H, H-2, H-3, H-4), 4.39 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.16–4.07 (m, 2H, H-6), 2.18, 2.14, 2.04, 2.03 (s, 12H, 4 × CH_3CO); ^{13}C, δ 20.7, 20.8, 20.9, 21.0 (4 × CH_3CO), 61.4 (C-6), 67.6 (C-5), 67.7 (C-3), 68.0 (C-4), 69.1 (C-2), 89.7 (C-1), 118.3–122.2 (aromatic C), 170.6, 170.3, 170.2, 170.1 (4 × CH3*C*O); HR MALDI-TOF MS: *m/z*: Calc for $C_{21}H_{22}F_2O_{11}$: 488.1130; found 511.1029 $[M + Na]^+$.

3,4,6-Tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranosyl bromide (21). Compound **20** (300 mg, 614 μmol) was dissolved in a mixture

of acetic acid (3 mL) and acetic anhydride (2 mL). Thirty-three percent hydrogen bromide in acetic acid (4 mL) was added and the mixture was stirred at 50°C for 6 hr. The solution was allowed to cool to rt, diluted with CH_2Cl_2 (100 mL), and washed with water (125 mL) and saturated aqueous $NaHCO_3$ (125 mL). The organic phase was dried ($MgSO_4$) and concentrated. Purification of the residue by silica gel chromatography (hexane/EtOAc 3 : 1) furnished **21** (278 mg, 545 μmol, 89%); TLC (hexane/EtOAc 2 : 1), $R_f = 0.51$; $[\alpha]_D + 31.4$ (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.61–7.08 (m, 3H, dFBz), 6.80 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.60–5.55 (m, 2H, H-3, H-4), 5.30 (dd, 1H, $J_{1,2}$ 2.2 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.54 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.25–4.09 (m, 2H, H-6), 2.17, 2.06, 1.98 (s, 9H, 3 × C*H*$_3$CO); ^{13}C, δ 20.7, 20.8, 20.9, (3 × *C*H$_3$CO), 61.0 (C-6), 67.3 (C-5), 68.2 (C-2), 69.0 (C-4), 71.5 (C-3), 87.8 (C-1), 118.4–122.7 (aromatic C), 170.6, 170.1, 169.9 (3 × CH$_3$*C*O); HR MALDI-TOF MS: m/z: Calc for $C_{19}H_{19}BrF_2O_9$: 508.0181; found 531.0079 $[M + Na]^+$.

3,4,6-Tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-D-galactopyranose (22). Hydrazine acetate (192 mg, 2.08 mmol) was added to a solution of **20** (925 mg, 1.89 mmol) in DMF (9 mL) heated at 60°C. The mixture was kept at 60°C for 3 hr, allowed to return to rt, diluted with EtOAc (75 mL), and washed with 20% aqueous NaCl (75 mL). The aqueous phase was extracted with EtOAc (40 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Silica gel chromatography purification (hexane/EtOAc 2 : 1) gave **22** (776 mg, 1.74 mmol, 92%); TLC (hexane/EtOAc 2 : 1), $R_f = 0.22$; $[\alpha]_D + 7.4$ (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.60–7.10 (m, 3H, dFBz), 5.68 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.59 (dd, ^1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.52 (d, 1H, $J_{4,5}$ 2.5 Hz, H-4), 5.36 (dd, 1H, $J_{1,2}$ 10.7 Hz, $J_{2,3}$ 3.6 Hz, H-2), 4.52 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.20–4.10 (m, 2H, H-6), 3.10 (bs, 1H, OH), 2.18, 2.17, 2.06 (s, 9H, 3 × C*H*$_3$CO); ^{13}C, δ 20.8, 20.9, 21.0 (3 × *C*H$_3$CO), 62.0 (C-6), 66.7 (C-5), 68.5 (C-3), 69.9 (C-4), 71.5 (C-2), 90.8 (C-1), 118.2–122.2 (aromatic C), 170.2, 170.4, 170.7 (3 × CH$_3$*C*O); HR MALDI-TOF MS: m/z: Calc for $C_{19}H_{20}F_2O_{10}$: 446.1025; found 469.0920 $[M + Na]^+$.

3,4,6-Tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranosyl trichloroacetimidate (23). Compound **22** (250 mg, 560 μmol) was dissolved in dry CH_2Cl_2 (5 mL) at 0°C, and trichloroacetonitrile (1.2 mL, 8.40 mmol) was added followed by 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU, 8 μL, 53.5 μmol). The mixture was stirred for 2 hr at 0°C, concentrated to dryness, and purified by silica gel chromatography (hexane/EtOAc/TEA 5 : 1 : 0.01) to yield **23** (317 mg, 538 μmol, 96%); TLC (hexane/EtOAc 2 : 1), $R_f = 0.39$; $[\alpha]_D + 26.25$ (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.57–7.06 (m, 3H, dFBz), 6.74 (d, 1H, $J_{1,2}$ 2.2 Hz, H-1), 5.55–5.70 (m, 3H, H-2, H-3,

H-4) 4.51 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.24–4.08 (m, 2H, H-6), 2.20, 2.03, 1.99 (s, 9H, 3 × C*H*$_3$CO); ^{13}C, δ 20.8, 20.9, 21.0 (3 × *C*H$_3$CO), 61.5 (C-6), 67.7 (C-3), 67.8 (C-4), 68.2 (C-2), 69.4 (C-5), 93.6 (C-1), 118.2–122.5 (aromatic C), 161.0 (C = NH) 170.1, 170.3, 170.5 (3 × CH3*C*O); HR MALDI-TOF MS: *m*/*z*: Calc for $C_{21}H_{20}Cl_3F_2NO_{10}$: 589.0121; found 612.0019 $[M + Na]^+$.

Phenyl 2-Azido-4,6-O-benzylidene-2-deoxy-3-O-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (25). Trimethylsilyl trifluoromethane sulfonate (7 μL, 38.7 μmol) was added, at −20°C and under argon, to a stirred mixture of **10** (35 mg, 91.1 μmol), **23** (75 mg, 128 μmol), and 4Å molecular sieves in CH_2Cl_2 (5 mL). The reaction was allowed to slowly return to rt and was quenched with triethylamine. The reaction was diluted with CH_2Cl_2 (50 mL), filtered through Celite, and evaporated to dryness. The residue was purified by silica gel chromatography (hexane/EtOAc 2 : 1) to furnish **25** (55 mg, 67.4 μmol, 74%); TLC (hexane/EtOAc 1 : 1) $R_f = 0.65$; $[\alpha]_D + 10.6$ (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.70–7.05 (m, 13H, dFBz, 2Ph), 5.51 (s, 1H, PhC*H*), 5.48 (t, 1H, $J_{1,2}$ 10.1 Hz, H-2′), 5.42 (d, 1H, $J_{3,4,5}$ 3.0 Hz, H-4′), 5.15 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz, H-3′), 4.91 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1′), 4.42–4.34 (m, 2H, H-5′, H-1), 4.26 (d, 1H, $J_{3,4,5}$ 2.5 Hz, H-4), 4.17–3.94 (m, 4H, H-5, H-6, 2H-6′), 3.73 (t, 1H, $J_{1,2,3}$ 9.9 Hz, H-2), 3.54 (dd, 1H, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.0 Hz, H-3), 3.46 (m, 1H, H-6), 2.14, 2.05, 1.93 (s, 9H, 3 × C*H*$_3$CO); ^{13}C, δ 20.7, 20.9, 21.0 (3 × *C*H$_3$CO), 60.0 (C-2), 61.7 (C-6), 67.2 (C-4′), 70.0 (C-2′), 70.1 (C-5′) 71.2 (C-3′), 74.9 (C-4), 81.1 (C-3), 86.0 (C-1), 101.0 (Ph*C*H), 102.3 (C-1′), 126.6–137.9 (aromatic C), 170.3, 170.4, 170.5 (3 × CH$_3$*C*O); HR MALDI-TOF MS: *m*/*z*: Calc for $C_{38}H_{37}F_2N_3O_{13}S$: 813.2015; found 836.1913 $[M + Na]^+$.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-Azido-4,6-O-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzylester (26).** To a solution of compound **25** (64 mg, 79 μmol), Ph_2SO (45 mg, 221 μmol), and 2,5-di-*tert*-butyl-3-methylpyridine (49 mg, 235 μmol) in dry CH_2Cl_2 (4 mL) was added trifluoromethanesulfonic anhydride (19 μL, 112 μmol) at −60°C. The mixture was stirred for 10 min, after which a solution of acceptor **6** (68 mg, 158 μmol) in CH_2Cl_2 (1.5 mL) was added. The reaction was stirred at −60°C for 1 hr and then it was slowly warmed to rt and quenched by the addition of saturated aqueous $NaHCO_3$ (2 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Purification by silica gel chromatography (hexane/EtOAc 3 : 1) gave **26** (74 mg, 65 μmol, 82%); TLC (hexane/EtOAc 1 : 1) $R_f = 0.67$; $[\alpha]_D + 21.0$ (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.60–6.97 (m, 21H, dFBz, Ph, Bn, Fmoc), 5.71 (d, 1H, J 9.3 Hz, NH), 5.54 (t, 1H, $J_{1,2,3}$ 9.8 Hz, H-2′), 5.45 (d, 1H, $J_{4,5}$ 2.5 Hz H-4′), 5.18 (dd, 1H, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 2.9 Hz, H-3′), 5.13 (s, 1H, PhC*H*), 4.93 (d, 1H, $J_{1,2}$ 7.8 Hz H-1′),

4.89 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 4.50–4.13 (m, 10 H, Fmoc 3H, H_α, H_β, H-4, H-5, H-6_{ax}, H-6′), 4.02–3.97 (m, 2H, H-3, H-5′), 3.73 (dd, 1H, $J_{1,2}$ 2.9 Hz, $J_{2,3}$ 10.3 Hz, H-2), 3.63 (m, 1H, H-6_{eq}), 2.18, 2.03, 1.93 (s, 9H, 3 × CH_3CO), 1.27 (d, 3H, CH_3); ^{13}C, δ 19.2 (CH_3), 20.8, 20.9, 21.0 (3 × CH$_3$CO), 59.3 (Cα), 59.8 (C-2), 63.6 (C-6), 69.2-66.2 (C-4′, CH_2-Fmoc), 70.1 (C-2′), 71.3 (C-5′, C-3′), 75.8 (C-3), 75.6 (C-4), 76.3 (Cβ), 99.2 (C-1), 101.0 (CHPh), 102.4 (C-1′), 117.9–130.2 (aromatic C), 169.2–171.1 (5 × C = O); HR MALDI-TOF MS: m/z: Calc for $C_{58}H_{56}F_2N_4O_{18}$: 1134.3558; found 1157.3450 $[M + Na]^+$.

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