Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1925–1935
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Highly efficient preparation of tumor antigen-containing glycopeptide building blocks from novel pentenyl glycosides

Serge A. Svarovsky, Joseph J. Barchi, Jr.*

Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, Frederick, National Institutes of Health, 376 Boyles Street, Frederick, MD 21702, USA

Received 22 April 2003; accepted 13 June 2003

Abstract

O-Glycosylated amino acids containing the tumor-associated T(T_f)-antigen (β-D-Gal-(1→3)-α-D-GalNAc) disaccharide unit were conveniently synthesized in seven steps starting from D-galactose via an *n*-pentenyl glycoside (NPG) building block. Azidonitration of 3,4,6-tri-*O*-acetyl-D-galactal, followed by nitrate displacement with simultaneous acetate hydrolysis with sodium 4-penten-1-oxide, afforded *n*-pentenyl 2-deoxy-2-azidogalactoside (**3**) in near quantitative yield. Subsequent high-yielding transformations resulted in the synthesis of the key glycosyl donor *n*-pentenyl β-disaccharide **5** that was employed for the stereospecific preparation of glycosyl amino acids via NIS-promoted glycosylations with serine or threonine acceptors. The surprising utility of the reaction of sodium 4-penten-1-oxide with anomeric nitrates encouraged the detailed exploration of the action of a variety of nucleophiles on anomeric nitrates for the synthesis of useful 2-azido glycosyl donors directly from the 'classic' Lemieux azidonitration product of protected galactals. This expedient synthesis (28% overall yield from **1** to **7a**) that makes use of heretofore rarely exploited pentenyl 2′-azidoglycosides, should be a valuable entry in the armamentarium of routes to biologically relevant glycopeptides in both mono- and multivalent forms.
Published by Elsevier Ltd.

Keywords: T-Antigen; O-Glycopeptide; Stereoselective; Pentenylation

1. Introduction

Neoplastic transformation is generally accompanied by structural alterations in cell-surface oligosaccharides.[1] These changes are due to the altered expression of specific glycoprocessing enzymes that are characteristic of a particular tumorigenic phenotype. This results in the accumulation of unusual glycosidic structures that are recognized as non-self by the host immune system. These tumor-associated antigens (TAA) are often used as markers of the tumorigenic process. Several abnormal glycans are found attached to cell-surface mucin proteoglycans as truncated O-linked oligosaccharides. Two of the more prevalent TAAs that are often found on carcinoma-derived mucins, but much less frequently in normal tissues are the Thomsen–Friedenreich antigen (hereafter referred to as T antigen) (Gal-β(1→3)-α-D-GalNac-Ser/Thr) and its biological precursor T_n (α-D-GalNAc-Ser/Thr) antigen (as well as sialylated analogs).[2] Specific immuno-based therapies and vaccine constructs have been based on these structures.[3]

Due to the difficulty in isolating distinct, homogeneous and immunogenic glycopeptides from tumor tissue, the majority of research aimed at carbohydrate-based immunotherapy has relied on the use of semisynthetic or fully synthetic immunogens.[4] Since some recent results[5] in this field have shown clinical promise, it is highly desirable to develop quick, inexpensive, and, most importantly, highly stereoselective syntheses of tumor-associated carbohydrate structures as well as the glycosylated protein units (glycopeptides) from which they are derived. Numerous chemical approaches to these building blocks have been developed over the years.[6] Most of these procedures rely heavily on conventional glycosyl donors (e.g., anomeric bromides, trichloroacetimidates[6g] and thioglycosides[6a]) that do

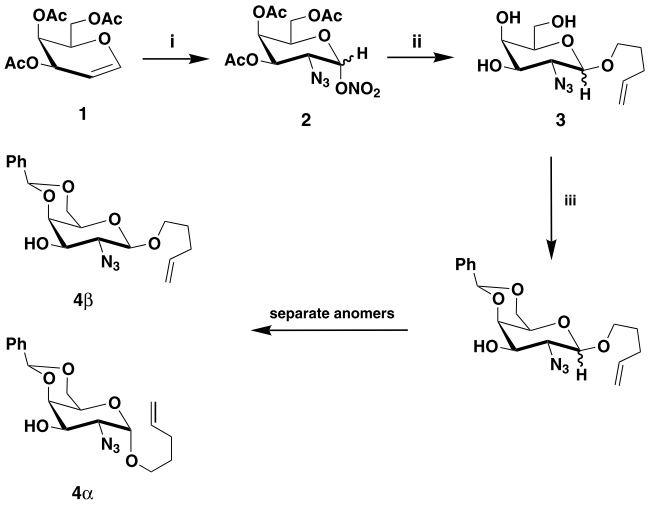
* Corresponding author. Fax: +1-301-8466033.
E-mail address: barchi@helix.nih.gov (J.J. Barchi, Jr.).

0008-6215/03/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/S0008-6215(03)00323-9

not offer orthogonality or access to conjugation of the saccharide to multivalent scaffolds, thus hindering the synthetic flexibility of these methods. In this paper we report a new route to T antigen glycoamino acid building blocks *via* hydrolytically stable *n*-pentenyl glycosides (NPGs).[7]

2. Results and discussion

Our approach involves a novel synthesis of precursors to 2-acetamido-NPGs and other glycosyl donors starting from the 'classic' azidonitration products of protected galactals developed by Lemieux.[8] Galactal **1** was synthesized most expediently in 75% overall yield in the one-pot procedure of Kozikowski and Lee,[9] starting from D-galactose. Azidonitration of **1** following Lemieux' procedure afforded a mixture of α- and β-nitrates **2** (Scheme 1) whose anomeric ratios (between 2:1 and 1:1 α/β) depended on subtle changes in reaction conditions. This mixture was then pentenylated and deacetylated in one convenient and nearly quantitative step using a solution of sodium metal (1.5 equiv) in 4-penten-1-ol as solvent/reactant to give the inseparable mixture of pentenyl glycosides **3**. Similar nitrate displacements using sodium methoxide[10] or other anions of heteroatoms have been previously described.[11] To our knowledge, this is a novel route for preparing pentenyl glycosides. The reaction evidently proceeded with complete inversion of configuration at the anomeric center: It was readily shown by NMR (see Section 3) that the ratio of anomeric configuration in the nitrate mixture was exactly reversed after reaction with the alkoxide (anomers separated after the next step, see below). However, the observed product composition does not rule out the possibility that the stereoselectivity associated with each individual substrate anomer is more variable and the reversal of stereochemistry observed was coincidental. Further proof for the stereospecificity of the reaction came when pure anomers were reacted similarly (see Section 3), and only pentenyl anomers with complete stereochemical inversion were observed. Benzylidenation of the mixture **3α,β** with benzaldehyde dimethylacetal afforded glycosyl acceptors **4α,β** in 70–75% yield using either *p*-toluenesulfonic acid or camphorsulfonic acid as a catalyst. At this point, the α/β anomeric mixture was easily separable ($\Delta R_f(\alpha/\beta) > 0.3$ in 1:1 hexanes–EtOAc), and the synthesis was carried forward independently with each anomer.



Scheme 1. (i) NaN_3, CAN, MeCN, 59–65%; (ii) Na, 4-penten-1-ol, 95%; (iii) $PhCH(OMe)_2$, PTSA, MeCN, 70–75%.

Encouraged by the efficiency of the reaction of sodium 4-penten-1-oxide to produce the NPG **3**, we undertook a more comprehensive exploration of the reaction of nitrate **2** with various nucleophiles (Fig. 1). Our primary objective was to examine the utility of preparing other glycosyl donors by nucleophilic nitrate displacement. Lemieux had used both bromide and chloride ion to prepare anomeric halides from the azidonitration product.[8] A survey of the literature revealed that anomeric xanthates[11d] and phosphates,[11b] two important classes of glycosyl donors, have been synthesized directly from glycosyl nitrates. A report of a C-glycosylation of the Lemieux' nitrate with allyltrimethylsilane and $BF_3 \cdot OEt_2$ has also appeared.[12]

We reasoned that the utility of the 2-azido-nitrate would be enhanced if other glycosyl donors could be prepared directly from the mixture of nitrate esters **2**. A series of nucleophiles were tested regarding their reactivity toward the compound **2**. We tested several nitrogen, sulfur, and selenium anions, as well as fluoride anions derived from different sources (TBAF, LiF, KF, spray-dried KF). With a wide variety of nucleophiles under a series of reaction conditions, the major product was simple nitrate removal to afford hemiacetal **8**. Sinaÿ and co-workers[11d] had previously reported that, although xanthic acid anions will displace an anomeric nitrate, simple thiols and base readily effected their reduction,[13] a transfomation that we also observed with all thiolate anions that we tested. The more potent reducing power of selenium was evident by the rapid (<1 min) and quantitative reduction of the nitrate mixture after treatment with benzene selenol in the presence of Hunig's base at room temperature. This is one of the more effective and rapid reductions of anomeric nitrates reported to date. The only positive result regarding nitrate displacement was with potassium thiocyanate (KTC), which gave a moderate yield (50%) of the anomeric TC **9**. In the interest of efficiency, we studied this reaction on the peracetylated material direct from the galactal azidonitration, although the presence of acetate esters was potentially a burden for many of the reaction conditions. Similar reaction of the

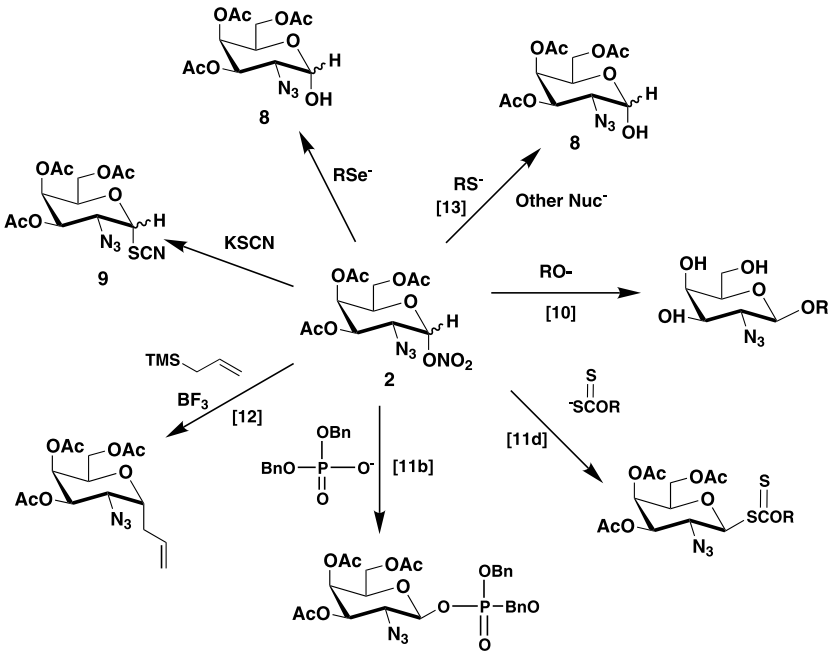
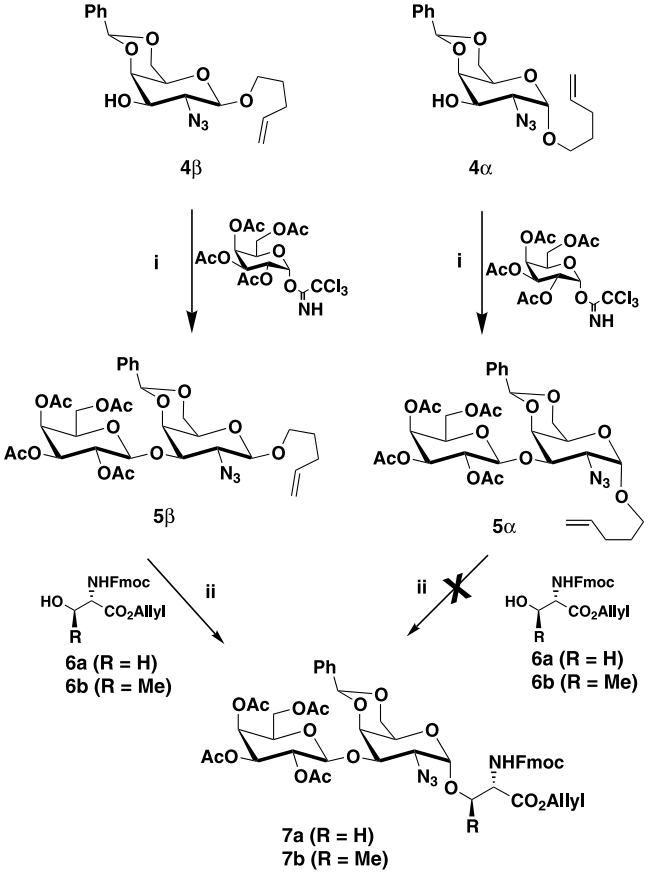


Fig. 1. Reactions of anomeric nitrates with various nucleophiles. Known procedures are marked with the appropriate reference in brackets over the reaction arrow.

nitrates when alternative protecting groups are employed is the subject of a future report.

Progression to a T-antigen-containing serine derivative proceeded smoothly from compound **4** (Scheme 2). Both acceptors **4α**,**β** coupled to 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose trichloroacetimidate (prepared in two steps from pentaacetyl galactose in 90% yield)[14] under standard glycosylation conditions[15] in nearly quantitative yield (95% recovered after chromatography) and with exclusive β stereoselectivity. The resulting pentenyl disaccharide donors **5α**,**β** were reacted with the protected amino acid acceptors **6a**,**b** using the standard NIS–stoichiometric TESOTf activation protocol pioneered by Fraser-Reid[16] to afford glycosyl amino acids **7a**,**b** in 64 and 43% yields, respectively. The reaction was only effective with the donor **5β** (see below). It is interesting to note that when catalytic amounts of TESOTf were employed, the coupling reaction proceeded with significantly lower efficiency.

Several aspects of glycosylations of the *n*-pentenyl 2′-azidodisaccharides with amino acid acceptors are noteworthy. A review of the literature reveals very few reports of glycosylations employing *n*-pentenyl 2′-azidoglycosyl donors of any sugar class.[17] Many of these reported poor yields or abject failure of reactions with various donors. Thus, we were pleased that good yields (consistent 60–65%) of glycosylation products were obtained with the β pentenyl glycoside **5β** with the serine derivative **6a**. Threonine **6b** also coupled but, as expected, in lower yield (43%). The reactions of **5b** with **6a**,**b** were essentially stereospecific: virtually no β-

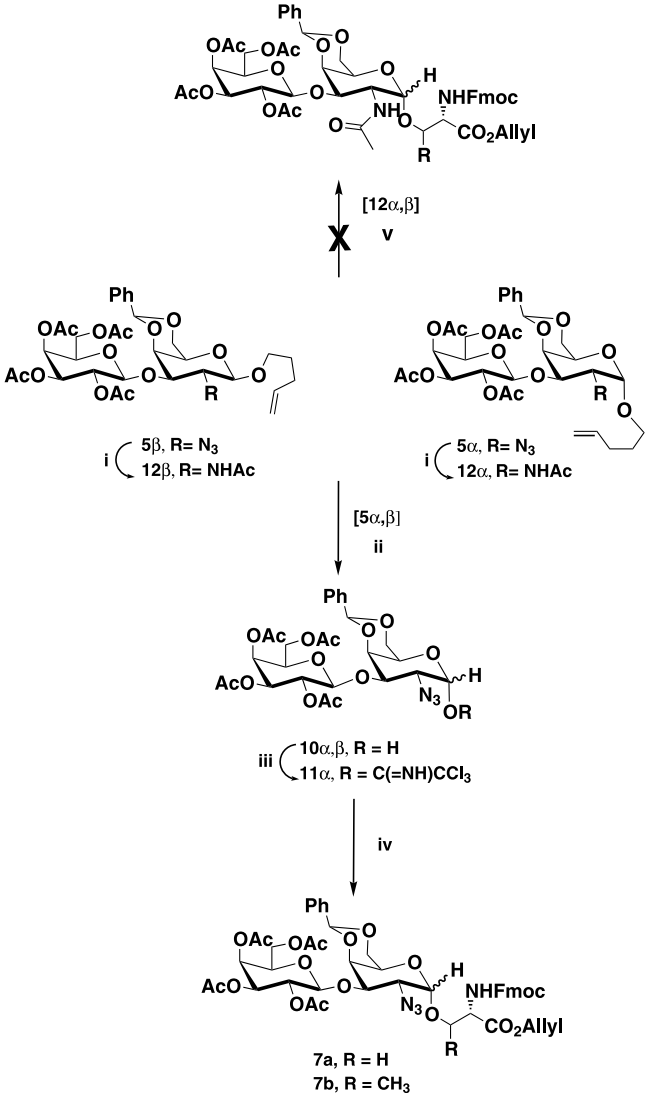


Scheme 2. (i) TMSOTf, CH_2Cl_2, MS, 90–95%; (ii) NIS, TESOTf, CH_2Cl_2, MS, 65% (R = H), 43% ($R = CH_3$).

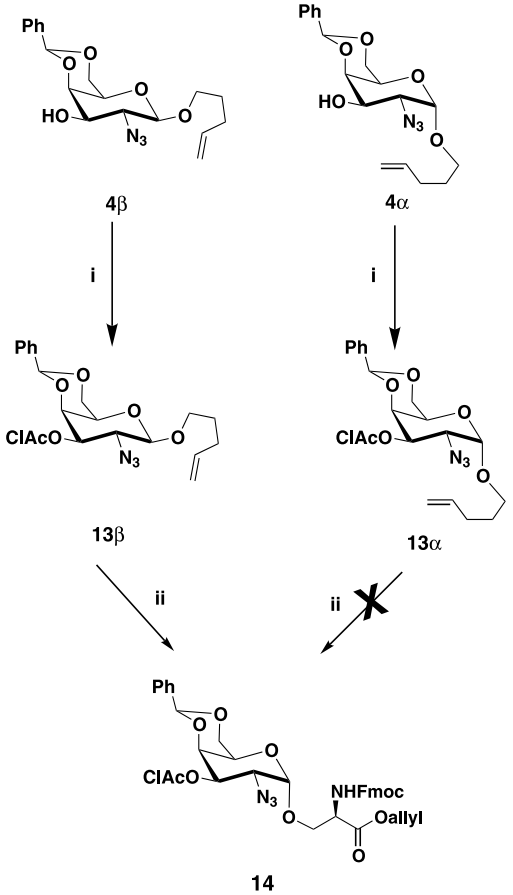
anomer of the glycosylated amino acids was detected during purification. To our surprise, the α-anomer **5α** gave very poor yields (< 10%) and much slower reaction rates with both **6a** and **6b**. The unexpected resistance to glycosylation of **5α** can be overcome by removal of the pentenyl group with CH_3CN–H_2O–NBS[18] to **10α**,**β**, followed by trichloroacetimidate formation to the donor **11α** (this was also accomplished with **5β**) and reaction with the aforementioned acceptors to give good yields of both serine and threonine T-antigen-containing amino acids (Scheme 3). It should be noted that although the yields of these glycosylations are superior to those with the pentenyl donors, the stereoselectivity was not as high (7% and 14% of the β-anomer for the serine and threonine products, respectively) These results also mirror those of Qui and co-workers[6h] who proceeded through similar trichloracetimidate intermediates to both serine and threonine-coupled T antigen building blocks. The ease and efficiency of these additional steps highlights the adaptability of this route when faced with specific 'unresponsive' NPGs.

We reasoned that the *cis*-1,2 disposition of the *n*-pentenyl and azido groups in the α-pentenyl glycosides may facilitate interaction of these two functionalities during the activation step of the glycosylation.[19] Accordingly, both anomers **5α**,**β** were reduced to the 2′-acetamido sugars **12α**,**β** with excess zinc in 10:5:1 THF–AcOH–Ac_2O[20] (80–88%) and subjected to glycosylation reactions with the protected serine **6a** (Scheme 3). Surprisingly, even with the donor **12β**, very poor yields, if any, were realized under identical glycosylation conditions. These results may highlight the 'disarming' effect of the 2′-acetamido or azido group for this system under activating conditions.

Preparation of T_n-containing amino acids was realized from common intermediate **4** *via* NPG **13** (Scheme 4). Reaction under identical NIS–stoichiometric TESOTf conditions with **6a** gave known chloroacetate **14** in a yield similar to that obtained during the preparation of **7a**. Similar to the reaction of NPG **5α** with **6a**, pentenyl



Scheme 3. (i) Zn–AcOH–Ac_2O–THF, 80–88%; (ii) CH_3CN, H_2O, NBS, 60%; (iii) CCl_3CN, CH_2Cl_2, DBU, 95%; (iv) **6a**,**b**, TMSOTf, CH_2Cl_2, MS, 90–95%; (v) **6a**, NIS, TESOTf, CH_2Cl_2, MS.



Scheme 4. (i) $(ClCH_2CO)_2O$, Pyr, CH_2Cl_2, 93–97%; (ii) **6a**, NIS, TESOTf, CH_2Cl_2, MS, 51% (**13β**), < 5% (**13α**).

glycoside **13α** gave very poor yields of **14**. This procedure could be thought of as an alternative assembly strategy for construction of the T antigen disaccharide and other higher order sugar derivatives. While this route offers little advantage in overall yield over other methods,[6] the presence of the pentenyl glycoside also facilitates further manipulations of this intermediate (*vide infra*).

Pentenyl glycosides have been used as orthogonal protecting groups that also may be used as 'spacers' to attach pendant oligosaccharides to relevant biological probes or proteins through modifications of the terminal olefin.[21] This is another advantage of the pentenyl group, whereas preparation of T_n and T antigens clusters may be elaborated *via* derivatives of compounds **4α** and **5α**, respectively (Scheme 5). Thus, using a wide range of chemistries, the pentenyl group may be transformed into various functional groups for further manipulations or to synthesize multivalent T_n, T-containing constructs. Progress toward the development of multivalent T-antigen displays will be outlined in a future report.

In conclusion, the highly useful preparation of pentenyl glycosides from anomeric nitrates allows efficient construction of glycoamino acid building blocks in high overall yield (28% from commercial material to **7a**). The stereoselectivity and adaptability of this route should facilitate the synthesis of biologically relevant glycopeptides on a large scale.

3. Experimental

3.1. Abbreviations

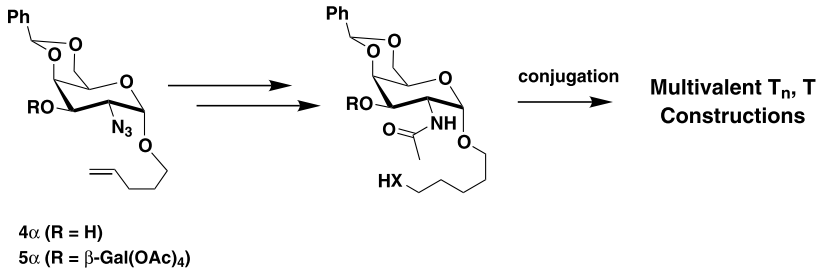
CAN (ceric ammonium nitrate); DBU (1,8-diazabicyclo[5.4.0]undec-7-ene); DIPEA (*N*,*N*-diisopropylethylamine); EtOAc (ethyl acetate); NBS (*N*-bromosuccinimide); TMSOTf (trimethylsilyl trifluoromethanesulfonate); TESOTf (triethylsilyl trifluoromethanesulfonate); α-Gal-TCA (1-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) 2,2,2-trichloroacetimidate; TLC (thin-layer chromatography); FC (flash chromatography); MS (molecular sieves).

3.2. General methods

Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. R_f values refer to TLC performed on Analtech Uniplates GF precoated with Silica Gel 60 to a thickness of 0.25 mm. The spots were visualized by charring with a solution of ammonium molybdate(IV) tetrahydrate (12.5 g) and cerium(IV) sulfate tetrahydrate (5.0 g) in 10% aq H_2SO_4 (500 mL). All purifications were performed by flash chromatography (FC) under medium pressure using Silica Gel 60 (230–400 mesh, E. Merck) unless stated otherwise. These purifications usually employed a stepwise solvent polarity gradient, correlated with TLC mobility.

NMR spectra were recorded on Varian Inova 400 instrument with residual $CHCl_3$ (7.26 ppm) as the internal standard at frequencies of 399.74 MHz for ^1H and 100.51 MHz for ^{13}C. Assignments were based on gCOSY, ROESY, and ^{13}C/DEPT experiments. ^1H NMR data are tabulated in the order of multiplicity (s, singlet; d, doublet; dd, doublet of doublets; dt, double of triplets; t, triplet; q, quartet; m, multiplet; brs, broad signal), number of protons, and coupling constant(s) in hertz. IR spectra were taken with a JASCO FT/IR-615 spectrometer. Specific optical rotations were determined using a JASCO-P1010 polarimeter in 0.5-dm cuvettes at 589 nm in chloroform. Five consecutive measurements were taken, and the average value is given. Positive-ion fast-atom bombardment mass spectra (FABMS) were obtained at an accelerating voltage of 6 kV and a resolution of 2000. Glycerol was used as the sample matrix, and ionization was effected by xenon atoms. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Unless otherwise noted, all chemicals were purchased from Aldrich–Sigma (Milwaukee, WI) and used without further purification. *N*-Iodosuccinimide (NIS) was recrystallized twice from dioxane–carbon tetrachloride prior to use. Activated, powdered 4Å molecular sieves (average diameter, 2–3 μm, CAS # 70955-01-0) were flame dried and used for sensitive glycosylation reactions.



Scheme 5.

3.3. 4-Pentenyl 2-azido-2-deoxy-β-D-galactopyranoside (3β)

Sodium metal (46 mg, 2 mmol) was dissolved in 5 mL of 4-penten-1-ol at room temperature (rt), and a solution of 506 mg (1.33 mmol) of **2α** in a minimum amount of 4-penten-1-ol was added. After 5 min, TLC (1:1 hexanes–ethyl acetate) indicated complete consumption of **2α**. The reaction was stirred for an additional 30 min and carefully neutralized with weakly acidic ion-exchange resin (Amberlite IRC-50). The mixture was filtered, and excess of 4-penten-1-ol was distilled at 50 °C (16–18 mbar) and recycled. The residual yellow syrup was purified by FC (5–10% MeOH in CH_2Cl_2) to afford 364 mg (99.2%) of the title compound as a amorphous solid. R_f 0.2 (10% MeOH in CH_2Cl_2, v/v); $[\alpha]_D$ +53° (*c* 1.13, $CHCl_3$); IR (neat): 3321.78, 2977.55, 2916.81, 2866.67, 2111.67, 1642.09; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73–5.85 (m, 1 H, –C*H*=CH_2), 4.93–5.05 (m, 2 H, –CH=C*H*$_2$), 4.27 (d, 1 H, *J* 7.95 Hz, βH-1), 3.37–4.02 (m, 8 H, H-2, H-3, H-4, H-5, H-6, –OC*H*$_2$-CH_2CH_2CH=CH_2), 2.11–2.19 (m, 2 H, –C*H*$_2$CH=CH_2), 1.67–1.79 (m, 2 H, –OCH$_2$C*H*$_2$$CH_2$CH=$CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.09 (–*C*H=CH_2), 115.29 (–CH=*C*H$_2$), 102.71 (C-1), 74.01, 72.34, 69.84, 69.27, 64.36 (–O*C*H$_2$$CH_2CH_2$CH=$CH_2$), 63.12 (C-6), 30.19, 28.93 (–OCH$_2$*C*H$_2$*C*H$_2$CH=CH_2). FABMS: *m*/*z* (relative intensity) 274.1 (MH^+), 547.2 (M_2H^+); Anal. Calcd for $C_{11}H_{19}N_3O_5$: C, 48.35; H, 7.01; N, 15.38. Found: C, 48.05; H, 7.00; N, 15.36.

When a mixture of nitrates **2** (**2α/2β** = 1.45) was used in the above procedure, *n*-pentenyl glycosides **3** were obtained with nearly exact reversal of anomeric configuration (**3β/3α** = 1.5). At this stage the anomers were inseparable and were used as a mixture in the next step.

3.4. 4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (4β)

Compound **3β** (610 mg, 2.25 mmol) was dissolved in 15 mL of anhyd acetonitrile, and the solution was cooled to 0 °C. Dimethoxytoluene (0.5 mL, 0.74 g, 4.9 mmol) was added, and the reaction was stirred for 5 min. *p*-Toluenesulfonic acid (PTSA) (9.4 mg, 0.057 mmol) was added to the reaction mixture at once, and the reaction was stirred at 0 °C for 3 h. The solution was diluted with EtOAc, quenched with a few drops of Et_3N and concentrated in vacuo. The resulting light-yellow syrup was purified by FC (2:1→1:1 hexanes–ethyl acetate) to afford 646 mg (80%) of the title compound as a white solid. R_f 0.28 (1:1 hexanes–ethyl acetate); $[\alpha]_D$ −11.6° (*c* 1.0, $CHCl_3$); IR (neat): 3447.13, 3070.12, 2872.45, 2109.74, 1640.16. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.53 (m, 5 H, Ph), 5.74–5.86 (m, 1 H, –C*H*=C*H*$_2$), 5.52 (s, 1 H, C*H*Ph), 4.93–5.06 (m, 2 H, –CH=C*H*$_2$), 4.29 (dd, 1 H, *J* 1.56, 12.5 Hz, H-6a), 4.27 (d, 1 H, *J* 7.81 Hz, H-1), 4.12 (dd, 1 H, *J* 1.2, 3.7 Hz, H-4), 4.07 (dd, 1 H, *J* 1.95, 12.5 Hz, H-6b), 3.90 (dt, 1 H, *J* 6.2, 9.5 Hz, OC*H*$_2$$CH_2CH_2$CH=$CH_2$), 3.47–3.61 (m, 2 H, H-2, H-3), 3.54 (dt, 1 H, *J* 6.8, 9.5 Hz, OC*H*$_2$$CH_2CH_2$CH=$CH_2$), 3.38 (m, 1 H, H-5), 2.59 (d, 1 H, *J* 8.5 Hz, OH), 2.11–2.20 (m, 2 H, –C*H*$_2$CH=CH_2), 1.66–1.82 (m, 2 H, –C*H*$_2$$CH_2$CH=$CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.23 (–*C*H=CH_2); 137.52, 129.56, 128.50, 126.59 (Ph), 115.22 (–CH=*C*H$_2$), 102.35 (Ph*C*H), 101.63 (C-1), 74.79, 71.59, 69.62, 69.24, 66.70 (–O*C*H$_2$$CH_2CH_2$CH=$CH_2$), 64.35 (C-6), 30.25, 28.88 (–*C*H$_2$*C*H$_2$CH=CH_2). FABMS: *m*/*z* (relative intensity) 362.2 (MH^+). Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.77; H, 6.39; N, 11.58.

3.5. 4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (4α)

The title compound was obtained from the mixture of **3α,β** (α/β = 3:2) using the procedure above in 83% (33% **4α** + 50% **4β**) yield. Upon benzylidenation, **4α** was easily separated from **4β** by FC and obtained as white crystals, mp 67–69 °C. R_f 0.64 (1:1 hexanes–ethyl acetate); $[\alpha]_D$ +153° (*c* 1.14, $CHCl_3$); IR (neat): 3495.35, 2908.13, 2106.85, 1640.16; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.51 (m, 5 H, Ph), 5.76–5.87 (m, 1 H, –C*H*=CH_2), 5.58 (s, 1 H, C*H*Ph), 4.97–5.08 (m, 2 H, –CH=C*H*$_2$), 5.00 (d, 1 H, *J* 3.32 Hz, αH-1), 4.22–4.28 (m, 2 H, H-4, H-6a), 4.16 (td, 1 H, *J* 3.9, 10.5 Hz, H-3), 4.06 (dd, 1 H, *J* 1.95, 12.88 Hz, H-6b), 3.67–3.74 (m, 2 H, –OC*H*$_2$$CH_2$-$CH_2CH$=$CH_2$, H-5), 3.45–3.54 (m, 2 H, –OC*H*$_2$$CH_2$-$CH_2CH$=$CH_2$, H-2), 2.43 (d, 1 H, *J* 10.54 Hz, OH), 2.12–2.21 (m, 2 H, –C*H*$_2$CH=CH_2), 1.69–1.79 (m, 2 H, –C*H*$_2$$CH_2$CH=$CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.06 (–*C*H=CH_2), 137.51, 129.56, 128.53, 126.41 (Ph), 115.32 (–CH=*C*H$_2$), 101.50, 98.92, 75.75, 69.48, 68.15, 67.56, 62.98, 60.90 (C-6), 30.40, 28.78 (–OCH$_2$*C*H$_2$*C*H$_2$CH=CH_2); FABMS: *m*/*z* (relative intensity) 362.2 (MH^+). Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 60.10; H, 6.44; N, 11.59.

3.6. 4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (5β)

Compound **4β** (225 mg, 0.62 mmol) was combined with 437 mg (0.89 mmol) of α-Gal-TCA,[14] evaporated twice with dry toluene, and dried overnight under vacuum. The dried reagents were dissolved in 5 mL of anhyd CH_2Cl_2 and transferred via canula to a two-neck flask containing 500 mg of activated 4Å molecular sieves (flame-dried and flushed with argon immediately prior to use). The reaction mixture was stirred at 0 °C for 30 min under argon. TMSOTf (10 μL, 0.06 mmol) was carefully added to the reaction mixture. After 20 min,

TLC (1:1 hexanes–ethyl acetate) showed only one spot corresponding to the product. The mixture was diluted with CH_2Cl_2 and quenched with a few drops of Et_3N. The solution was filtered through a short pad of Celite®, washed successively with satd $NaHCO_3$, brine, and water and dried over $MgSO_4$. Purification by FC (2:3 hexanes–EtOAc) afforded 422 mg (98.5%) of the product as a white solid: mp 83–85 °C; R_f 0.15, 0.43 (1:1, 2:3 hexanes–ethyl acetate); $[\alpha]_D$ +11.5° (*c* 1.53, $CHCl_3$); IR (neat): 2876.31, 2114.56, 1747.19, 1640.16, 1367.28, 1216.86; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48–7.53 (m, 2 H, Ph), 7.28–7.37 (m, 3 H, Ph), 5.79 (m, 1 H, –C*H*=CH_2), 5.36 (m, 1 H, H″-4), 5.23 (m, 1 H, H″-2), 4.92–5.04 (m, 3 H, –CH=C*H*$_2$, H″-3), 4.79 (d, 1 H, $J_{1,2}$ 8.2 Hz, βH″-1), 4.25 (d, 1 H, *J* 7.8 Hz, H′-1), 4.00–4.32 (m, 5 H, H′-4, H′-6, H″-6), 3.92–3.99 (m, 1 H, –OC*H*$_2$CH$_2$CH$_2$CH=CH_2), 3.86 (m, 1 H, H″-5), 3.77 (dd, 1 H, *J* 7.81, 10.54 Hz, H′-2), 3.45–3.53 (m, 1 H, –OC*H*$_2$CH$_2$CH$_2$CH=CH_2), 3.42 (dd, 1 H, *J* 3.51, 10.54 Hz, H′-3), 3.34 (bs, 1 H, H′-5), 1.95, 2.01, 2.04, 2.13 (4s, 12 H, CH_3), 2.08–2.17 (m, 2 H, –C*H*$_2$CH=CH_2), 1.65–1.78 (m, 2 H, –C*H*$_2$CH$_2$CH=CH_2), ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.53, 170.50, 170.38, 169.50, 138.23, 137.85, 129.05, 128.30, 126.41, 115.19 (–CH=*C*H$_2$); 102.55, 102.48, 102.18, 100.94, 78.65, 76.52, 75.24, 71.11, 71.02, 69.48, 69.18, 68.93, 67.16, 66.75, 62.59, 61.51, 30.23, 28.85, 20.91, 20.87, 20.77. FABMS: *m*/*z* (relative intensity): 692.2 (MH^+). Anal. Calcd for $C_{32}H_{41}N_3O_{14}$: C, 55.57; H, 5.97; N, 6.08. Found: C, 55.13; H, 6.12; N, 5.73.

3.7. 4-Pentenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-a-D-galactopyranoside (5α)

Compound **4α** (550 mg, 1.52 mmol) and 970 mg (1.97 mmol) of α-Gal-TCA[14] were dissolved in 12 mL of anhyd CH_2Cl_2 containing 600 mg of 4Å molecular sieves under argon. The mixture was cooled to 0 °C and stirred for 15 min prior to addition of 20 μL (0.12 mmol, 0.06 equiv) of TMSOTf. After 35 min, the reaction was quenched with a few drops of Et_3N, and the molecular sieves were filtered on a short pad of Celite®. The filtrate was successively washed with satd $NaHCO_3$, brine, and H_2O and dried over $MgSO_4$. After evaporation, the residue was purified by flash chromatography on silica gel with 3:2 hexanes–EtOAc to give 960 mg (91% yield) of white solid: mp 125–127 °C; R_f 0.21 (3:2 EtOAc–hexanes); $[\alpha]_D$ +54.7° (*c* 1.00, $CHCl_3$); IR (neat): 2108.78, 1747.19, 1367.28, 1216.86, 1044.26; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.38 (m, 3 H, Ph), 7.48–7.52 (m, 2 H, Ph), 5.72–5.84 (m, 1 H, –C*H*=CH_2), 5.38 (dd, 1 H, *J* 1.17, 3.51 Hz, H″-4), 5.52 (s, 1 H, PhCH), 4.94–5.05 (m, 4 H, –CH=C*H*$_2$, αH′-1 ($J_{1,2}$ 3.51 Hz), H″-3), 4.76 (d, 1 H, *J* 8.20 Hz, βH″-1), 4.35 (d, 1 H, *J* 2.73 Hz, H′-4), 4.22 (dd, 1 H, *J* 1.56, 12.49 Hz, H′-6a), 4.18 (dd, 1 H, *J* 6.24, 11.32 Hz, H″-6a), 4.05–4.13 (m, 2 H, H″-6b, H′-3), 4.02 (dd, 1 H, *J* 1.56, 12.49 Hz, H′-6b), 3.77 (dd, 1 H, *J* 3.51, 10.54 Hz, H′-2), 3.67–3.74 (m, 1 H, –OC*H*$_2$CH$_2$CH$_2$CH=CH_2), 3.65 (bs, 1 H, H′-5), 3.46–3.54 (m, 1 H, –OC*H*$_2$CH$_2$CH$_2$CH=CH_2), 2.10–2.17 (m, 2 H, –C*H*$_2$CH=CH_2), 1.95, 2.01, 2.03, 2.13 (s, 12 H, Ac), 1.68–1.76 (m, 2 H, –OCH$_2$C*H*$_2$CH$_2$CH=CH_2), ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.48, 170.34, 169.61, 137.98, 137.86, 129.04, 128.31, 126.33, 115.34, 102.65, 100.84, 98.90, 76.06, 71.26, 69.39, 68.88, 68.13, 67.18, 63.28, 61.61, 59.05, 30.40, 28.77, 20.91, 20.76. FABMS: *m*/*z* (relative intensity): 692.2 (MH^+). Anal. Calcd for $C_{32}H_{41}N_3O_{14}\cdot H_2O$: C, 54.16; H, 6.11; N, 5.92. Found: C, 53.96; H, 5.78; N, 5.71

3.8. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-galactopyranosyl]-L-serine allyl ester (7a)

3.8.1. Method A. Disaccharide **5β** (50 mg, 0.07 mmol) was combined with **6a** (24 mg, 0.065 mmol) and evaporated with dry toluene (2 × 5 mL) before drying under vacuum overnight. The mixture was dissolved in 3 mL of anhyd CH_2Cl_2 and transferred into 100 mg of flame-dried 4Å molecular sieves. The solution was treated with 18 mg (0.08 mmol) of freshly recrystallized *N*-iodosuccinimide, followed by TESOTf (17 μL, 0.07 mmol). After 30 min the reaction mixture was diluted with 10 mL of CH_2Cl_2, quenched with one drop of Et_3N, and filtered. The filtrate was washed consecutively with 10% $Na_2S_2O_3$, satd $NaHCO_3$, and water, and dried over $MgSO_4$. The residue was purified by flash chromatography (2:1 petroleum ether–acetone) to give 40 mg (64%) of **7a** as an amorphous solid.

3.8.2. Method B. A mixture of 300 mg (0.39 mmol) of **11α** with 240 mg (0.65 mmol, 1.67 equiv) of **6a** was dissolved in 5 mL of dry toluene and rotoevaporated to dryness. This procedure was repeated twice. The dried mixture was then dried under vacuum at 40 °C overnight, dissolved in 6 mL of anhyd CH_2Cl_2 and transferred via canula to 500 mg of flame-dried 4Å molecular sieves. The solution was cooled to −15 °C (thermostat), and 15 μL of TMSOTf was added. In 10 min TLC showed no disaccharide **11α**. After 20 min the reaction was diluted with CH_2Cl_2, quenched with two drops of Et_3N, filtered through a short pad of Celite® and concentrated. The residue was flash-chromatographed with 1:1 hexanes–EtOAc to afford 250 mg (66%) of **7a** as a white amorphous solid along with 27 mg (7%) of β-anomer (α/β = 9:1). Identification of the product was ascertained by correlation of the ^{1}H NMR spectrum to literature reports[22] and by elemental and mass spectral analysis.

3.9. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-galactopyranosyl]-L-threonine allyl ester (7b)

3.9.1. Method A. Compound **5β** (110 mg, 0.16 mmol) and 73 mg (0.19 mmol) of **6b** were combined, and the mixture was azeotropically dried twice with toluene and then dried at 40 °C under vacuum. The mixture was dissolved in 5 mL of anhyd CH_2Cl_2, and the solution was transferred into 200 mg of flame-dried 4Å molecular sieves. The resulting suspension was stirred under argon for a few minutes, and 36 mg (0.16 mmol) of freshly recrystallized *N*-iodosuccinimide was added, followed by 40 μL (0.17 mmol) of TESOTf was added by syringe. After 30 min, the reaction was diluted with CH_2Cl_2 and quenched with a few drops of Et_3N. The molecular sieves were filtered through a short pad of Celite®. The filtrate was washed with $Na_2S_2O_3$, water, dried over $MgSO_4$ and concentrated. The residue was purified on silica gel with 3:1 petroleum ether–acetone to afford 66 mg (43%) of the title compound as a white solid.

3.9.2. Method B. Compound **11α** (100 mg, 0.13 mmol) was combined with 60 mg (0.16 mmol, 1.2 equiv) of **6b**, azeotropically dried twice with dry toluene and dried under vacuum at 40 °C for 12 h. The mixture was dissolved in 6 mL of anhyd CH_2Cl_2 and transferred into 200 mg of flame-dried 4Å molecular sieves. The solution was cooled to −15 °C, and 6 μL of TMSOTf was added. After 20 min, the reaction was diluted with CH_2Cl_2, quenched with two drops of Et_3N, filtered through a short pad of Celite® and concentrated. The residue was purified by FC with 1.3:1 hexanes–EtOAc to afford 86 mg (67%) of the desired α-anomer and 18 mg (14%) of the β-anomer. Identification of the product was ascertained by correlation of the ^{1}H NMR spectrum to literature reports[23] and by elemental and mass spectral analysis.

3.10. Hydrolysis of 2 with phenyl selenide

To a solution of 100 mg (0.63 mmol) of PhSeH in 3 mL of anhyd CH_3CN solution of a mixture of **2α,β** 50 mg (0.13 mmol) in minimum amount of CH_3CN was added, followed by one drop of DIPEA. The solution became yellow in color. TLC taken immediately after addition showed only one product corresponding to hemiacetal **8**, and no remaining **2α,β**. The reaction was diluted with 10 mL of EtOAc, washed with satd NH_4Cl and water, then dried over $MgSO_4$ and concentrated. Purification by flash chromatography (1:1 hexanes–EtOAc) afforded 40 mg (93%) of hemiacetal **8** as a clear oil. Identification of the product was ascertained by correlation of the ^{1}H NMR spectrum to literature reports[24] and by elemental and mass spectral analysis.

3.11. 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α,β-D-galactopyranosyl thiocyanate (9)

A mixture of 50 mg (0.13 mmol) of nitrates **2** (α/β = 3:2) was dissolved in 2 mL of anhyd CH_3CN. Potassium thiocyanate (58 mg, 0.6 mmol) was added, followed by 150 mg of 18-crown-6. The reaction was stirred at rt overnight and diluted with ether. The organic layer was washed with brine and water, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography with 3:1 hexanes–EtOAc to give 31 mg (50%, incomplete reaction) of **9** as a clear oil (1:1 mixture of α- and β-anomers). R_f 0.50 (1:1 hexanes–EtOAc), IR (neat): 2161.81 (SCN), 2116.49 (N_3), 1748.16 (Ac); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.10 (d, 1 H, $J_{1,2}$ 5.08 Hz, αH-1), 5.47 (m, 1 H, αH-4), 5.39 (m, 1 H, βH-4), 5.04 (dd, 1 H, *J* 3.12, 10.93 Hz, αH-3), 4.94 (dd, 1 H, *J* 3.12, 10.15 Hz, βH-3), 4.51 (d, 1 H, *J* 9.76 Hz, βH-1), 4.43 (m, 1 H, H-5), 4.33 (dd, 1 H, *J* 5.08, 10.54 Hz, αH-2), 4.10–4.21 (m, 4 H, βH-6, αH-6), 3.94–4.04 (m, 2 H, βH-2, H-5), 2.03, 2.04, 2.05, 2.06, 2.15, 2.16 (s, 18 H, Ac), ^{13}C NMR (100 MHz, $CDCl_3$) (β-anomer **9** from α-nitrate **2**): δ 170.49, 170.14, 169.70, 107.57 (SCN), 83.86, 76.05, 72.99, 66.24, 61.35, 60.58, 20.82, 20.74, 20.71; FABMS: *m/z* (relative intensity): 373.1 (MH^+). Anal. Calcd for $C_{13}H_{16}N_4O_7S$: C, 41.93; H, 4.33; N, 15.05. Found: C, 42.32; H, 4.38; N, 14.68.

3.12. 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl) 2,2,2,-trichloroacetimidate (11α)

A mixture of free pyranoses **10α,β**[25] (770 mg, 1.23 mmol) was dissolved in 20 mL of anhyd CH_2Cl_2, the solution was cooled to 0 °C, and 2 mL (2.88 g, 20 mmol) of CCl_3CN was added, followed by eight drops of DBU. After 30 min, the solution was evaporated and purified by FC with 1:1 hexanes–EtOAc to give 811 mg (85%) of a white solid (no β-anomer was detected). R_f 0.60 (2:1 EtOAc–hexanes); IR (neat): 3305.39, 2113.60, 1746.23, 1709.59; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1 H, = NH), 7.47–7.52 (m, 2 H, Ph), 7.27–7.37 (m, 3 H, Ph), 6.52 (d, 1 H, *J* 3.12 Hz, αH′-1), 5.52 (s, 1 H, PhCH), 5.37 (m, 1 H, H″-4), 5.27 (dd, 1 H, *J* 8.20, 10.54 Hz, H″-2), 4.98–5.03 (m, 1 H, H″-3), 4.80 (d, 1 H, *J* 7.81 Hz, βH″-1), 4.42 (m, 1 H, H′-4), 3.88–4.25 (m, 6 H, H′-2, H′-3, H′-6, H″-6), 3.86 (m, 1 H, H″-5), 3.82 (bs, 1 H, H′-5), 1.94, 2.00, 2.01, 2.13 (s, 12 H, Ac). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.46, 170.43, 170.28, 169.57, 160.62, 137.66, 129.19, 128.38, 126.31, 102.37, 100.88, 95.99, 75.99, 75.40, 71.27, 71.19, 68.91, 67.23, 65.60, 61.73, 58.75, 20.92, 20.89, 20.74; FABMS: *m/z* (relative intensity): 606.2 (MH^+). Anal. Calcd for $C_{34}H_{45}NO_{15}$: C, 52.01; H, 5.33; N, 6.74. Found: C, 51.77; H, 5.43; N, 6.47

3.13. 4-Pentenyl [2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)]-α-D-galactopyranoside (12α)

Compound **5α** (625 mg, 0.90 mmol) in 15 mL of THF, 7 mL of AcOH, and 2.5 mL of Ac_2O was treated with 5 g of zinc dust at rt. The reaction mixture was stirred for 1 h, diluted with EtOAc and filtered through a short pad of Celite®. The filtrate was washed consecutively with water, satd $NaHCO_3$ and brine, then dried over $MgSO_4$ and concentrated. The residue was purified by FC using 5:1 EtOAc–hexanes to give 540 mg (85% yield) of **12α** as a white solid (7:3 mixture of rotamers). R_f 0.24 (4:1 EtOAc–hexanes); $[\alpha]_D$ +154° (*c* 0.52, $CHCl_3$); IR (neat): 2934.16, 1747.19, 1661.37, 1536.99, 1368.25; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.55 (m, 2 H, Ph), 7.26–7.39 (m, 3 H, Ph), 5.77 (m, 1 H, –C*H*=CH_2), 5.53 (d, 1 H, *J* 8.98 Hz, NH), 5.51 (s, 1 H, PhCH), 5.34 (d, 1 H, *J* 2.34 Hz, H″-4), 5.13–5.19 (m, 1 H, H″-2), 4.86–5.04 (m, 4 H, H″-3, H′-1, –CH=CH_2), 4.75 (d, 1 H, *J* 7.81 Hz, βH″-1), 4.57–4.66 (m, 1 H, H′-2), 3.91–4.27 (m, 6 H, H′-6, H″-6, H′-4, H″-5), 3.86–3.91 (m, 1 H, H′-3), 3.63–3.70 (m, 1 H, –OC$H_2$$CH_2CH_2CH$=$CH_2$), 3.60 (bs, 1 H, H′-5), 3.39–3.47 (m, 1 H, –OC$H_2$$CH_2$-$CH_2CH$=$CH_2$), 2.10 (m, 2 H, –$OCH_2CH_2CH_2$CH=$CH_2$), 1.94, 1.95, 2.00, 2.01, 2.11 (s, 15 H, Ac, NHAc), 1.62–1.72 (m, 2 H, –OCH_2CH_2CH_2CH=CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.53, 170.42, 170.35, 169.67, 169.61, 137.98, 137.86, 129.02, 128.33, 126.41, 126.26, 115.35, 101.26, 100.92, 98.35, 75.84, 74.44, 71.14, 71.03, 69.08, 67.74, 67.15, 63.24, 61.57, 48.43, 30.55, 30.47, 28.77, 23.66, 20.91, 20.89, 20.75. FABMS: *m*/*z* (relative intensity): 708.3 (MH^+). Anal. Calcd for $C_{34}H_{45}NO_{15}\cdot H_2O$: C, 56.27; H, 6.53; N, 1.93. Found: C, 56.32; H, 6.44; N, 1.91

3.14. 4-Pentenyl [2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)]-β-D-galactopyranoside (12β)

A solution of **5β** (500 mg, 0.72 mmol) was treated identically as described for 5α above. After purification (4:1 → 5:1 EtOAc–hexanes) 440 mg (86% yield) of **12β** was obtained as white solid. R_f 0.18 (4:1 EtOAc–hexanes); $[\alpha]_D$ +32.2° (*c* 1.17, $CHCl_3$); IR (neat): 1743.33, 1660.41, 1552.42, 1367.28, 1216.86; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.50 (m, 2 H, Ph), 7.24–7.32 (m, 3 H, Ph), 5.66–5.77 (m, 1 H, –C*H*=CH_2), 6.02 (d, 1 H, *J* 7.03 Hz, NH), 5.47 (s, 1 H, PhCH), 5.29 (d, 1 H, *J* 3.51 Hz, H″-4), 5.11–5.17 (m, 1 H, H″-2), 5.03 (d, 1 H, *J* 8.20 Hz, βH′-1), 4.86–4.97 (m, 3 H, H″-3, –CH=CH_2), 4.71 (d, 1 H, *J* 8.20 Hz, βH″-1), 4.61–4.67 (m, 1 H, H′-3), 4.19–4.26 (m, 2 H, H′-6a, H′-4), 3.94–4.10 (m, 3 H, H′-6b, H″-6), 3.82 (bs, 1 H, H″-5), 3.80–3.86 (m, 1 H, –OC$H_2CH_2CH_2CH$=CH_2), 3.42 (m, 1 H, –OC$H_2$$CH_2$-$CH_2CH$=$CH_2$), 3.39 (bs, 1 H, H′-5), 3.28–3.35 (m, 1 H, H′-2), 2.00 (m, 2 H, –OCH_2CH_2CH_2CH=CH_2), 1.87, 1.90, 1.95, 1.96, 2.07 (s, 15 H, Ac, NHAc), 1.45–1.62 (m, 2 H, –OCH_2CH_2CH_2CH=CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.19, 170.49, 170.45, 170.23, 169.39, 138.20, 138.07, 128.97, 128.23, 126.36, 115.07 (CH=CH_2), 101.42, 100.77, 99.11, 76.15, 75.80, 71.19, 71.01, 69.47, 69.20, 69.07, 67.32, 66.57, 61.87, 54.61, 30.16, 28.83, 23.87 (CH_3C(O)NH), 20.97, 20.85, 20.71 (CH_3C(O)O); FABMS: *m*/*z* (relative intensity): 708.3 (MH^+). Anal. Calcd for $C_{34}H_{45}NO_{15}$: C, 57.70; H, 6.41; N, 1.98. Found: C, 57.53; H, 6.46; N, 2.17.

Glycosylations of **6a**,**b** with **12α**,**β** were carried out under exactly the same conditions as outlined in Section 3.9.1 (vide supra).

3.15. 4-Pentenyl 2-azido-3-*O*-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-β-D-galactopyranoside (13β)

To a solution of **4β** (300 mg, 0.83 mmol) in 10 mL of anhyd CH_2Cl_2 was added 0.1 mL of dry pyridine. The reaction mixture was cooled to 0 °C, and 170 mg (1 mmol) of chloroacetic anhydride was slowly added. After stirring overnight, the reaction was diluted with EtOAc, washed with 1 M HCl, twice with brine and water, and then dried over $MgSO_4$ and evaporated. Separation on silica gel with 4:1 hexanes–EtOAc yielded 340 mg (93%) of **12β** as a clear oil. R_f 0.10 (4:1 hexanes–EtOAc); $[\alpha]_D$ +23.9° (*c* 0.92, $CHCl_3$); IR (neat): 2114.56 (N_3), 1763.58 (Ac); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.46 (m, 2 H, Ph), 7.38–7.32 (m, 3 H, Ph), 5.85–5.74 (m, 1 H, –CH_2C*H*=CH_2), 5.49 (s, 1 H, PhCH), 5.06–4.94 (m, 2 H, –CH_2CH=CH_2), 4.73 (dd, 1 H, *J* 3.51, 10.93 Hz, H-3), 4.35 (d, 1 H, *J* 7.81 Hz, βH-1), 4.33–4.27 (m, 2 H, H-6a, H-4), 4.13 (d, 2 H, ClCH_2CO), 4.03 (m, 1 H, H-6b), 3.98 (m, 1 H, –OC$H_2CH_2CH_2CH$=CH_2), 3.89 (dd, 1 H, *J* 7.81, 10.93 Hz, H-2), 3.50–3.49 (m, 1 H, –OC$H_2CH_2CH_2CH$=CH_2), 3.44 (m, 1 H, H-5), 2.19–2.12 (m, 2 H, –CH_2CH=CH_2), 1.82–1.66 (m, 2 H, –CH_2CH_2CH=CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.20, 138.14, 137.53, 129.39, 128.44, 126.45, 115.29, 102.38, 101.14, 73.91, 72.60, 69.73, 69.11, 66.32, 60.48, 40.95, 40.67, 30.22, 28.84; FABMS: *m*/*z* (relative intensity): 438.1 (MH^+). Anal. Calcd for $C_{20}H_{24}ClN_3O_6$: C, 54.86; H, 5.52; Cl, 8.10; N, 9.60; Found: C, 55.64; H, 5.44; N, 9.30.

3.16. 4-Pentenyl 2-azido-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-α-D-galactopyranoside (13α)

To a solution of **4α** (300 mg, 0.83 mmol) in 10 mL of anhyd CH_2Cl_2 was added 0.1 mL of dry pyridine. The reaction mixture was cooled to 0 °C, and 170 mg (1 mmol) of chloroacetic anhydride was slowly added. After stirring overnight, the reaction was diluted with EtOAc, washed with 1 M HCl, twice with brine and

water, and dried over $MgSO_4$ and evaporated. Separation on silica gel with 4:1 hexanes–EtOAc yielded 351 mg (97%) of **13α** as a clear oil. R_f 0.30 (4:1 hexanes–EtOAc); $[\alpha]_D$ +105.5° (*c* 0.37, $CHCl_3$); IR (neat): 2108.78 (N_3), 1761.65 (Ac); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.44 (m, 2 H, Ph), 7.38–7.31 (m, 3 H, Ph), 5.85–5.73 (m, 1 H, $-CH_2CH{=}CH_2$), 5.50 (s, 1 H, PhCH), 5.35 (dd, 1 H, *J* 3.51, 10.93 Hz, H-3), 5.04 (d, 1 H, *J* 3.51 Hz, αH-1), 5.06–4.94 (m, 2 H, $-CH_2CH{=}CH_2$), 4.48 (m, 1 H, H-4), 4.23 (dd, 1 H, *J* 1.17, 12.88 Hz, H-6a), 4.12 (s, 2 H, $ClCH_2CO$), 4.04 (dd, 1 H, *J* 1.56, 12.49 Hz, H-6b), 3.92–3.87 (m, 1 H, H-2), 3.74 (m, 1 H, H-5), 3.76–3.69 (m, 1 H, $-OCH_2CH_2CH_2CH{=}CH_2$), 3.54–3.47 (m, 1 H, $-OCH_2CH_2CH_2CH{=}CH_2$), 2.18–2.11 (m, 2 H, $-CH_2CH{=}CH_2$), 1.76–1.68 (m, 2 H, $-CH_2CH_2CH{=}CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.17, 137.99, 137.59, 129.35, 128.43, 126.31, 115.42, 100.93, 98.66, 73.36, 71.57, 69.29, 68.28, 62.62, 57.33, 40.93, 30.39, 28.73; FABMS: *m*/*z* (relative intensity): 438.1 (MH^+). Anal. Calcd for $C_{20}H_{24}ClN_3O_6$: C, 54.86; H, 5.52; N, 9.60; Found: C, 55.20; H, 5.51; N, 9.36.

3.17. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2-azido-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-α-D-galactopyranosyl]-*O*-L-serine allyl ester (14)

A mixture of 88 mg (0.20 mmol) of compound **13** and 115 mg (0.31 mmol) of Fmoc-Ser-OAll was azeotropically dried with toluene three times. The mixture was then dried under vacuum at 40 °C overnight, and then dissolved in 4 mL of anhyd CH_2Cl_2. To this solution 35 mg (0.20 mmol) of freshly recrystallized NIS was added under argon, and the reaction was stirred at rt for several minutes before 67 μL (0.20 mmol) of TESOTf was added via syringe. After 1 h the reaction mixture was diluted with 10 mL of CH_2Cl_2, quenched with one drop of Et_3N, and filtered. The filtrate was washed consecutively with 10% $Na_2S_2O_3$, satd $NaHCO_3$, and water, and then dried over $MgSO_4$. The residue was purified by FCC to give 73 mg (51%) of compound **14** as an amorphous solid. Identification of the product was ascertained by correlation of the ^{1}H NMR spectrum to that reported in the literature.[26]

Acknowledgements

We thanks Drs James Kelley and Cong Lai for mass spectra and the Center for Cancer Research, National Cancer Institute for an FTE loan for S.A.S.

References

1. (a) Hakomori, S. *Cancer Res.* **1985**, *45*, 2405–2414;
(b) Kim, Y. J.; Varki, A. *Glycoconjugate J.* **1997**, *14*, 569–576;
(c) Barchi, J. J. *Curr. Pharm. Des.* **2000**, *6*, 485–501.
2. (a) Dahlenborg, K.; Hultman, L.; Carlsson, R.; Jansson, B. *Int. J. Cancer* **1997**, *70*, 63–71;
(b) Yamashita, Y.; Chung, Y. S.; Horie, R.; Kannagi, R.; Sowa, M. *J. Natl. Cancer Inst.* **1995**, *87*, 441–446;
(c) Jansson, B.; Borrebaeck, C. A. K. *Cancer Immunol. Immunother.* **1992**, *34*, 294–298.
3. (a) Adluri, S.; Helling, F.; Ogata, S.; Zhang, S. L.; Itzkowitz, S. H.; Lloyd, K. O.; Livingston, P. O. *Cancer Immunol. Immunother.* **1995**, *41*, 185–192;
(b) Koganty, R. R.; Reddish, M. A.; Longenecker, B. M. *Drug Discovery Today* **1996**, *1*, 190–198.
4. (a) Allen, J. R.; Harris, C. R.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 1890–1897;
(b) Deshpande, P. P.; Danishefsky, S. J. *Nature* **1997**, *387*, 164–166;
(c) Maclean, G. D.; Bowen-Yacyshyn, M. B.; Samuel, J.; Meikle, A.; Stuart, G.; Nation, J.; Poppema, S.; Jerry, M.; Koganty, R.; Wong, T.; Longenecker, B. M. *J. Immunother.* **1992**, *11*, 292–305;
(d) Ragupathi, G.; Howard, L.; Cappello, S.; Koganty, R. R.; Qiu, D. X.; Longenecker, B. M.; Reddish, M. A.; Lloyd, K. O.; Livingston, P. O. *Cancer Immunol. Immunother.* **1999**, *48*, 1–8.
5. Lloyd, K. O. *Drug News Perspect.* **2000**, *13*, 463–470.
6. (a) Luning, B.; Norberg, T.; Tejbrant, J. *Glycoconjugate J.* **1989**, *6*, 5–19;
(b) Komba, S.; Meldal, M.; Werdelin, O.; Jensen, T.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1999**, 415–419;
(c) St. Hilaire, P. M.; Cipolla, L.; Franco, A.; Tedebark, U.; Tilly, D. A.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3559–3564;
(d) Paulsen, H.; Schleyer, A.; Mathieux, N.; Meldal, M.; Bock, K. *J. Chem. Soc. Perkin Trans. 1* **1997**, 281–293;
(e) Glunz, P. W.; Hintermann, S.; Williams, L. J.; Schwarz, J. B.; Kuduk, S. D.; Kudryashov, V.; Lloyd, K. O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 7273–7279;
(f) George, S. K.; Holm, B.; Reis, C. A.; Schwientek, T.; Clausen, H.; Kihlberg, J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 880–885;
(g) Rademann, J.; Schmidt, R. R. *Carbohydr. Res.* **1995**, *269*, 217–225;
(h) Qiu, D.; Gandhi, S. S.; Koganty, R. R. *Tetrahedron Lett.* **1996**, *37*, 595–598;
(i) Jiaang, W. T.; Chang, M. Y.; Tseng, P. H.; Chen, S. T. *Tetrahedron Lett.* **2000**, *41*, 3127–3130.
7. Fraser-Reid, B.; Udodong, U. E.; Wu, Z. F.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927–942.
8. Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244–1249.
9. Kozikowski, A. P.; Lee, J. *J. Org. Chem.* **1990**, *55*, 863–870.
10. (a) Paulsen, H.; Paal, M.; Schultz, M. *Tetrahedron Lett.* **1983**, *24*, 1759–1762;
(b) Paulsen, H.; Paal, M. *Carbohydr. Res.* **1984**, *135*, 53–69.

11. (a) Paulsen, H.; Deessen, U. V.; Tietz, H. *Carbohydr. Res.* **1985**, *137*, 63–78;
(b) Illarionov, P. A.; Torgov, V. I.; Shibaev, V. N. *Izv. Akad. Nauk. Ser. Khim.* **2000**, *49*, 1924–1928;
(c) Paulsen, H.; Paal, M. *Carbohydr. Res.* **1984**, *135*, 53–70;
(d) Marra, A.; Gauffeny, F.; Sinaÿ, P. *Tetrahedron* **1991**, *47*, 5149–5160.
12. Bertozzi, C.; Bednarski, M. D. *Tetrahedron Lett.* **1992**, *33*, 3109–3112.
13. Gauffeny, F.; Marra, A.; Shun, L. K. S.; Sinaÿ, P.; Tabeur, C. *Carbohydr. Res.* **1991**, *219*, 237–240.
14. Ren, T.; Liu, D. *Tetrahedron Lett.* **1999**, *40*, 7621–7625.
15. Paulsen, H.; Ranwald, W.; Weichert, U. *Liebigs Ann. Chem.* **1988**, 75–86.
16. Madsen, R.; Udodong, U. E.; Roberts, C.; Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1554–1565.
17. (a) Anilkumar, G.; Nair, L. G.; Olsson, L.; Daniels, J. K.; Fraser-Reid, B. *Tetrahedron Lett.* **2000**, *41*, 7605–7608;
(b) Olsson, L.; Jia, J. J.; Fraser-Reid, B. *J. Org. Chem.* **1998**, *63*, 3790–3792.
18. Mach, M.; Schlueter, U.; Mathew, F.; Fraser-Reid, B.; Hazen, K. C. *Tetrahedron* **2002**, *68*, 7345–7354.
19. (a) Benati, L.; Montevecchi, P. C. *J. Org. Chem.* **1981**, *46*, 4570–4573;
(b) Bach, T.; Schlummer, B.; Harms, K. *Chem. Commun. (Cambridge)* **2000**, 287–288.
20. Nakamura, K.; Ishii, A.; Ito, Y.; Nakahara, Y. *Tetrahedron* **1999**, *55*, 11253–11266.
21. Buskas, T.; Soderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958–963.
22. Wang, Z.-G.; Zhang, X.-U.; Ho, Y.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1996**, *295*, 25–39.
23. George, S. K.; Schwientek, T.; Holm, B.; Reis, C.; Clausen, E.; Kihlberg, J. *J. Am. Chem. Soc.* **2001**, *123*, 11117–11125.
24. Frische, K.; Meldal, M.; Werdelin, O.; Mouritsen, S.; Jensen, T.; Galli-Stompino, L.; Bock, K. *J. Pept. Sci.* **1996**, *2*, 212–222.
25. Grundler, G.; Schmidt, R. *Liebigs Ann. Chem.* **1984**, *11*, 1826–1847.
26. Wang, Z.-G.; Zhang, X.-F.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1996**, *4*, 1901–1908.