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Synthesis and glycan priming activity of acetylated disaccharides

Arun K. Sarkar [1], Jillian R. Brown, Jeffrey D. Esko *

Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, La Jolla, CA 92093, USA

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Abstract

Five disaccharides related in structure to the glycans of vertebrate mucins have been chemically synthesized using orthogonal blocking, coupling and deblocking techniques. These include 2-naphthylmethyl 3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**6**), 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**14**), 2-naphthylmethyl2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**20**), 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**23**) and 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**27**). These per-O-acetylated compounds were fed to U-937 cells to test their ability to prime oligosaccharide synthesis, inhibit glycoprotein biosynthesis and alter adhesion to E-selectin expressed on endothelial cells. The results show that **6**, **14**, and **20** served as substrates for oligosaccharide synthesis. The generation of glycoside-primed glycans altered the formation of glycoproteins on the cell surface and inhibited cell adhesion dependent on E-selectin.

Keywords: Glycoside primers; Cell adhesion; Inhibitors; Sialyl Lewis X

1. Introduction

Sialyl Lewis X (sLe^X), a common carbohydrate antigen expressed on leukocytes, mediates cell adhesion to selectin receptors on endothelial cells and platelets (reviewed in [1]). The interaction initiates a cascade of events that result in the eventual extravasation of leukocytes from the circulation to lymph nodes and inflamed tissue. Similar interactions occur amongst circulating tumor cells, platelets and endothelial cells, which may contribute to metastasis [2,3]. Great interest exists in finding inhibitors that block selectin–carbohydrate interactions since these compounds might prove useful in treating pathological conditions characterized by unwanted cellular extravasation, as in chronic inflammatory diseases, acute ischemic injury or tumor metastasis [4].

Although the precise structure of the functional carbohydrate ligand varies amongst the different selectins and depends on the tissue and organism under study [5], it typically consists of one or more fucosylated and sialylated glycans like those found on cell surface and

* Corresponding author. Tel.: +1-858-8221100; fax: +1-858-5345611.

E-mail address: jesko@ucsd.edu (J.D. Esko).

[1] Present address: North American Vaccine, Inc., 10150 Old Columbia Road, Columbia, MD 21046, USA.

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secreted mucins (Fig. 1). Attempts to interfere with selectin-dependent adhesion have involved the use of competitive ligands containing these sugars or analogs that bind the carbohydrate recognition domain (see [6] and references therein). Another approach is to interfere with the biosynthesis of the oligosaccharide chains. Towards this end, we have demonstrated that per-O-acetylated disaccharides such as 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**6**) and 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**14**) are taken up by a variety of cells [6,7]. Endogenous carboxylesterases remove the acetyl groups, generating compounds that resemble intermediates in the biosynthetic pathway (Galβ1-4GlcNAc-R and GlcNAcβ1-3Gal-R). The glycosides give rise to tri, tetra and pentasaccharides that are secreted into the growth medium. The formation of these compounds diverts the assembly of oligosaccharides away from endogenous glycoproteins, thereby reducing the expression of Lewis determinants on the cell surface. Thus, these disaccharides inhibit adhesion mediated by selectin–sLe^X interactions [6,7].

To extend these studies, we have now investigated three more per-O-acetylated 2-naphthylmethyl disaccharides that represent other substructures found in vertebrate mucins. Here, we report the synthesis of all five synthetic disaccharides, their relative efficacy towards priming oligosaccharide chains in U937 cells and their effect on selectin-dependent cell adhesion.

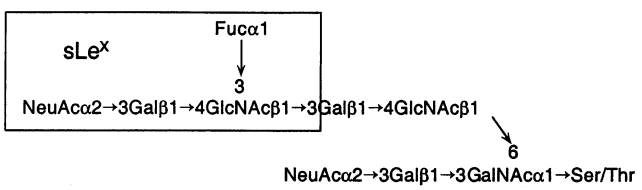
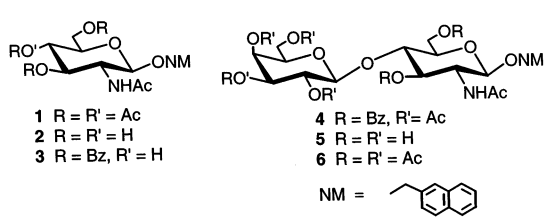


Fig. 1. Sialyl Lewis X (sLe^X) occurs on the non-reducing end of Ser/Thr-linked oligosaccharides.

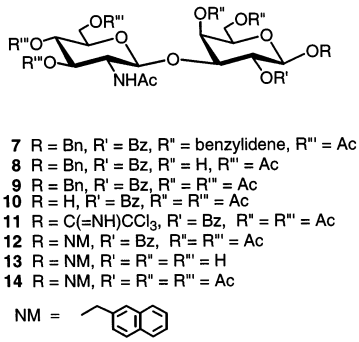


Scheme 1.

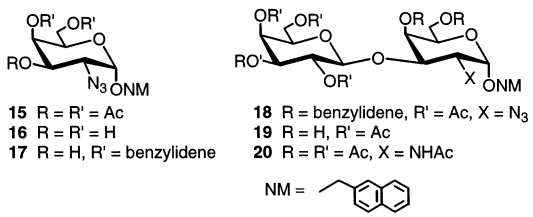
2. Results and discussion

Synthesis of N-*acetylglucosamine analogues.*—Reaction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl chloride with 2-naphthylmethanol [8] gave derivative **1**, and subsequent deprotection with 0.05 M sodium methoxide afforded 2-naphthylmethyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**). In order to selectively glycosylate **2** at the 4-position, the 3- and 6-position were selectively benzoylated using benzoyl chloride at low temperature [9], yielding **3** in 70% yield. Coupling of **3** with ethyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside as the glycosyl donor [10–14] utilizing NIS-triflic acid mediated activation gave low yields (5–10%). In contrast, condensation in the presence of bromine and silver trifluoromethanesulfonate [15] afforded disaccharide, **4** in 32% yield. After the removal of benzoate and acetate groups by mild alkali treatment and subsequent per-O-acetylation, the target disaccharide, 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**6**) was obtained in 84% yield (Scheme 1). Peaks at δ 4.50 (J 8.75 Hz) and 4.47 (J 7.25 Hz) in the ^{1}H NMR of **6** confirmed the β-glycosidic linkage between the two sugars, and a peak at δ 5.44 (J 9.35 Hz) confirmed the presence of the *N*-acetyl group of the GlcNAc residue in the structure. ^{13}C NMR signals at δ 104.93 (C-1′), 99.41 (C-1) and 75.59 (C-4) supported that the sugars were β-(1→4)-linked.

Reaction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride with known benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside [8,16,17] afforded benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzylidene-β-D-galactopyranoside (**7**). The removal of the benzylidene protecting group under acidic conditions and purification of the product by column chromatography



Scheme 2.



Scheme 3.

afforded **8**. Subsequent peracetylation of **8** afforded benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranoside (**9**). Catalytic hydrogenation with palladium-on-charcoal furnished **10**, which was subsequently converted to the trichloroacetamidate derivative **11** by treatment with 1,8-diazabicyclo[5.4.0]-undec-7-ene and trichloroacetonitrile. Reaction of **11** with 2-naphthylmethanol [13,18] gave 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranoside (**12**). Compound **12** was treated with 0.1 M sodium methoxide to furnish **13** and subsequently peracetylated with pyridine and acetic anhydride to afford the fully acetylated target disaccharide, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**14**) (Scheme 2). ^{1}H and ^{13}C NMR spectra were in good accordance with the proposed structure of compound **14**.

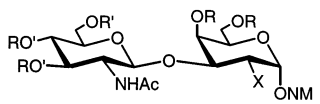
Synthesis of N-*acetylgalactosamine analogs*. —For the synthesis of 2-naphthylmethyl glycosides of *N*-acetylgalactosamine, we followed the procedure described by Lemieux and Ratcliffe [19]. The reaction of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl trichloroacetamidate [20] with 2-naphthylmethanol in presence of trimethylsilyltrifluoromethanesulfonate (TMS triflate) in hexane–dichloromethane afforded 2-naphthylmethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranoside (**15**) in 61% yield. O-Deacetylation of **15** with 0.05 M sodium methoxide afforded **16**. Compound **16** was subsequently converted to 2-naphthylmethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**17**) by treatment with benzaldehyde dimethylacetal in the presence of *p*-toluenesulfonic acid. Reaction of **17** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetamidate in the presence of TMS triflate [21] gave 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**18**) in 71% yield. The benzylidene group from **18** was removed with 80% acetic acid at high temperature to afford **19**. We adapted the reaction scheme developed by Malik and co-workers [22] to reduce the azide to an amine (palladium-on-charcoal under refluxing conditions in methanol). Subsequent acetylation with pyridine and acetic anhydride afforded the fully acetylated target disaccharide, 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**20**) in 70% yield (Scheme 3). Peaks at δ 5.09 (J 4 Hz) and 4.57 (J 8 Hz) in the ^{1}H NMR spectrum of **20** confirmed the α-glycosidic linkage between the GalNAc residue and the aglycone and the β-glycosidic linkage between the two sugars, respectively. A peak at δ 5.64 (J 9 Hz) in the ^{1}H NMR spectrum confirmed the presence of the *N*-acetyl group of the GalNAc residue, thereby confirming conversion of N_3 to NHAc. The ^{13}C NMR spectrum was in good accordance with the proposed structure of compound **20**.

Disaccharide derivative, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**21**) was obtained by treating **17** with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride in the presence of mercuric cyanide and mercuric chloride in 1:1 nitromethane–benzene. The benzylidene group was removed

by treatment with 80% acetic acid at high temperature to afford 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-azido-2-deoxy-α-D-galactopyranoside (**22**). Treatment of **22** with palladium-on-charcoal and subsequent acetylation with pyridine and acetic anhydride furnished the desired target disaccharide, 2-naphthylmethy 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**23**) in 70% yield (Scheme 4). Peaks at δ 5.09 (J 3.5 Hz) and 4.96 (J 8.5 Hz) in the ^{1}H NMR spectrum confirmed the α-glycosidic linkage between the GalNAc residue and the aglycone and the β-glycosidic linkage between the two sugars, respectively. Two peaks at δ 6.04 (J 8.5 Hz) and 5.80 (J 8 Hz) in the ^{1}H NMR spectrum confirmed the presence of the *N*-acetyl group of the GalNAc and GlcNAc residues, respectively. ^{13}C NMR signals at δ 99.05 (C-1′), 96.97 (C-1) and 72.46 (C-3) support the proposed structure.

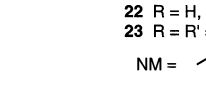
The disaccharide derivative, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside (**25**) was obtained by first reacting **16** with 2,2-dimethoxypropane in presence of camphorsulfonic acid to afford 2-naphthylmethyl 2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside (**24**). Subsequently, **24** was reacted with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride in the presence of mercuric cyanide and mercuric chloride to afford **25**. The isopropylidene group from **25** was removed with 70% acetic acid at high temperature to afford **26**. Compound **26** was reacted with palladium-on-charcoal and acetylated using pyridine and acetic anhydride to furnish the fully acetylated target disaccharide, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**27**) in 71% yield (Scheme 5). Peaks at δ 5.00 (J 3.5 Hz) and 4.72 (J 8 Hz) in the ^{1}H NMR spectrum confirmed the α-glycosidic linkage between the GalNAc residue and the aglycone and the β-glycosidic linkage between the two sugars, respectively. Two peaks at δ 5.61 (J 9.5 Hz) and 5.50 (J 8.5 Hz) in the ^{1}H NMR spectrum confirmed the presence of the *N*-acetyl group of the GalNAc and GlcNAc residues, respectively. The ^{13}C NMR spectrum was in good accordance with the proposed structure.

Biological activity of acetylated disaccharides.—We have shown that cells take up and rapidly deacetylate per-O-acetylated forms of **6** and **14** [6]. These disaccharides resemble intermediates in the pathway and therefore act as substrates, giving rise to fucosylated and sialylated oligosaccharides. The priming of oligosaccharides by per-O-acetylated forms of **20**, **23** and **27** was therefore measured in comparison to **6** and **14**. As shown in Table 1 and previously [6], **6** stimulated the incorporation of [6-^{3}H]Fuc into oligosaccharides. Previous studies showed that the glycan primed on **6** consisted entirely of Le^X (Galβ1-4(Fucα1-3)GlcNAcβ-R. In contrast, **14** primed oligosaccharides containing Gal, Fuc and sialic acid, including sLe^X [6], and therefore stimulated the incorporation of both [6-^{3}H]Fuc and [6-^{3}H]Gal. The large increase in incorporation of all three radioactive sugars into oligosaccharides primed on **20** suggests that a complex set of products were formed, including GlcNAc-containing oligosaccharides. Dennis and co-workers have shown that several fucosylated and sialylated oligosaccharides are assembled on benzyl α-GalNAc*p*

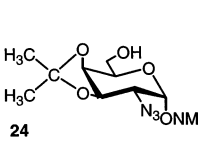


21 R = benzylidene, R' = Ac, X = N_3
22 R = H, R' = Ac, X = N_3
23 R = R' = Ac, X = NHAc

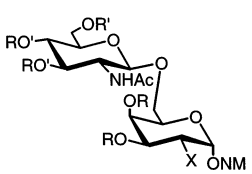
NM =



Scheme 4.

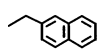


24



25 R = ispr., R' = Ac, X = N_3
26 R = H, R' = Ac, X = N_3
27 R = R' = Ac, X = NHAc

NM =



Scheme 5.

Table 1
Oligosaccharide synthesis on acetylated primers in U937 cells [a]

Glycosides	[^{3}H]Fuc	[^{3}H]Gal	[^{3}H]GlcN
	cpm incorporated $\times 10^{-3}$		
Experiment I			
None	4	14	84
6	9 [b]	16	77
14	20 [b]	53 [b]	72
Experiment II			
None	10	19	68
20	35 [b]	52 [b]	160 [b]
23	12	21	78
27	11	25	93

[a] U937 cells (2×10^5 cells) were incubated with the indicated glycoside for 48 h in growth medium supplemented with 10 μCi/mL [6-^{3}H]Fuc, 10 μCi/mL [6-^{3}H]Gal, or 10 μCi/mL [6-^{3}H]GlcN. Radioactive oligosaccharides were isolated by Sep-Pak C_{18} reversed-phase chromatography and quantitated (see Section 3). The data represents the average of duplicate determinations that varied by $<20\%$.

[b] Indicates values that were significantly different from the controls in which no compound was added.

Table 2
Glycoside inhibition of glycosylation of endogenous glycoproteins in U937 cells [a]

Glycoside	[^{3}H]Thy	[^{3}H]Fuc	[^{3}H]Gal	[^{3}H]GlcN
	cpm $\times 10^{-3}$			
Experiment I				
None	235	38	370	nd
6	222	22 [b]	240 [b]	nd
14	240	24 [b]	235 [b]	nd
Experiment II				
None	192	47	291	194
20	192	26 [b]	164 [b]	147 [b]
23	189	43	272	194
27	178	44	277	186

[a] U937 cells (2×10^5 cells) were incubated with or without glycosides (50 μM) for 28 h, and then the cells were labeled with 10 μCi/mL [6-^{3}H]Fuc, 10 μCi/mL [^{3}H]Gal, 20 μCi/mL [6-^{3}H]GlcN, or 1 μCi/mL [^{3}H-methyl]thymidine for another 20 h. Cellular glycoconjugates were precipitated with 10% trichloroAcOH, washed once with 2% trichloroAcOH, and dissolved in a small volume of 0.1 M sodium hydroxide. A portion of each sample was counted by liquid scintillation spectrometry. Each experiment was performed in duplicate, and the average value varied by $\leq 10\%$; nd, not determined.

[b] Indicates values that were significantly different from controls.

when the compound was fed to Colo 205 cells [23]. Although detailed studies have not yet been done on the oligosaccharides produced on **20** in U937 cells, it seems likely that some would be related to those primed on benzyl α-GalNAc*p* since **20** resembles the next intermediate in the pathway (Galβ1-3GalNAcα-R). Neither **23** nor **27** stimulated the incorporation of precursors into oligosaccharides. The failure of **23** to prime oligosaccharides supports the idea that extension of the β1-6 branch depends on prior formation of the core structure (Galβ1-3GalNAcα-R) [24].

To test whether the extent of priming by **20** was sufficient to inhibit the glycosylation of endogenous glycoproteins, samples of radioactively labeled cells were treated with trichloroacetic acid to precipitate glycoconjugates and others macromolecules. As shown in Table 2, **20** inhibited the incorporation of labeled sugars into precipitated material with potencies similar to that observed for **6** and **14**. In contrast, **23** and **27** had no effect, consistent with their negligible priming activity (Table 1). None of the compounds showed any toxicity under these conditions, based on the level of incorporation of [6-^{3}H]thymidine into DNA.

To test if inhibiting glycosylation had any effect on cell adhesion, treated U937 cells were challenged to adhere to E-selectin expressed on TNF-α-activated human umbilical vein endothelial cells. As shown in Fig. 2, adhesion was dependent on prior stimulation of the endothelial cells with cytokine and on sialic acids expressed on the surface of U937 cells. Treatment of U937 cells with 50 μM of **6**, **14** or **20** showed reduction in adhesion and the effect was dose dependent [6]. As expected from the results shown in Tables 1 and 2, **23** and **27** had no significant effect on adhesion.

These data support the idea that per-O-acetylated disaccharides provide a rich source of potential inhibitors by acting as biosynthetic decoys. One advantage is that these compounds have inhibitory effects in the micromolar range, whereas other compounds such as aryl *N*-acetyl-galactosaminides require millimolar amounts to observe comparable effects [23,25,26]. This difference in effectiveness is not related to the aglycone, as the per-O-

acetylated or free GalNAcα-napthalenemethanol derivatives have the same dose profile as GalNAcα-benzyl (data not shown). Presumably the enhanced efficacy of the disaccharides reflects their greater similarity to natural intermediates in the pathway and higher affinities for the relevant transferases. The fact that some compounds do not act as primers (e.g., **23**) presumably reflects the restricted order of reactions during the assembly process. Thus, disaccharides like the ones reported here allow one to probe the biosynthetic pathway in vivo and to make correlations with substrate specificity studies of the individual enzymes in vitro. In other cases (e.g., **27**), the failure to prime oligosaccharides may merely reflect the restricted repertoire of glycosyltransferases expressed by U937. Further studies of the compounds in other cell lines should provide insight into this problem.

3. Experimental

General methods.—Optical rotations were measured at ambient temperature with an Autopol III automatic polarimeter. NMR experiments were carried out on a Varian Unity Inova 500 spectrometer, at 25 °C in $CDCl_3$ (sample volumes of 0.6–0.7 mL in 5-mm tubes, Wilmad 528-PP). A Varian 5-mm pulsed-field-gradient inverse-detection (PFG-ID) probe was used for all experiments. A SUN Microsystems Ultra-10 computer running Varian's VNMR software (version 6.1B) controlled data acquisition. Chloroform was used as internal standard for chemical shift calibration; the ^1H chemical shift of $CHCl_3$ was set to 7.262 ppm, the ^{13}C chemical shift of $CDCl_3$ to 77.0 ppm. Two-dimensional (2D) ^1H double-quantum-filtered COSY data sets were collected in phase-sensitive mode. In each of the experiments, 256 FIDs of 2048 complex data points were acquired. The data were processed with a squared sine bell function applied in the t_2 dimension and a shifted squared sine bell function and zero-filling applied in the t_1 dimension. Two-dimensional heteronuclear single-quantum coherence (HSQC) data sets, providing one-bond ^1H, ^{13}C correlation maps, were collected in phase-sensitive mode, with BIRD nulling of ^1H–^{12}C signal components during the preparation period and ^{13}C GARP decoupling during acquisition. In each of the experiments, 512 FIDs of 2048 complex data points were acquired, with 32 or 64 scans per t_1 increment. HSQC data were processed with a squared sine bell function applied in the t_2 dimension and a line broadening function and zero-filling applied in the t_1 dimension. ^1H NMR spectra (500 MHz) of intermediates were recorded at 25 °C on a Bruker AM 500 spectrometer. Selected NMR data for intermediates are reported in Table 3, and the spectra were interpreted by comparison of the chemical shifts to those obtained with the parent compound. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ). All reaction products were analyzed by analytical thin-layer chromatography (TLC) on Silica Gel 60-F_{254} (E. Merck) plates and detection by UV light or by charring with 5% (v/v) H_2SO_4 in EtOH. Column chromatography was performed on Silica Gel 60-F_{254} (Aldrich). Molecular sieves (4 Å) were heated to 180 °C for 24 h prior to use. All solvents were distilled prior to use and stored over molecular sieves. All evaporations were carried out at 40–50 °C under reduced pressure, unless otherwise noted. Solvents used for TLC A, 2:1 EtOAc–

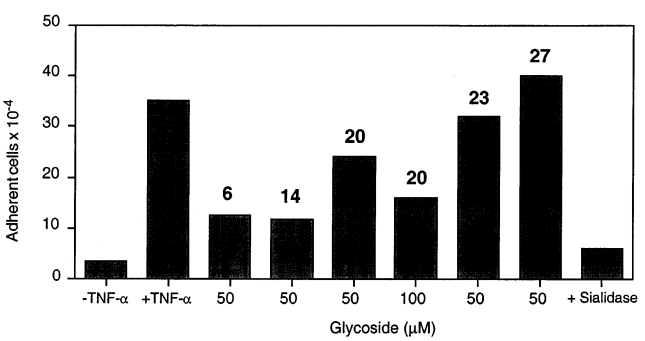


Fig. 2. Adhesion of U937 cells to activated HUVEC. U937 cells (5 × 105 cells) were radiolabeled for 48 h at 37 °C with [6-^{3}H]thymidine and the per-O-acetylated disaccharides at the indicated concentrations. The cells were then challenged to adhere to TNF-α-activated HUVEC, and the total number of adherent cells were measured (see Section 3). Control incubations with untreated U937 cells were carried out in the absence of TNF-α (−) or with TNF-α (+) to demonstrate the dependence on E-selectin activation. Another sample of U937 cells was treated with sialidase to destroy Lewis antigens on the cell surface. Each measurement is the average of duplicate determinations that varied by ≤15%.

Table 3
Selected ^{1}H NMR data of synthetic intermediates

Compounds	Chemical shifts (δ)
1	7.80–7.40 (m, 7 H, Ar*H*), 5.32 (d, 1 H, $J_{2,NH}$ 8.9 Hz, N–*H*), 4.67 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 2.10–1.90 (12 H, CH_3CO)
2	7.80–7.40 (m, 7 H, Ar*H*), 5.0 (1 H, N–*H*), 4.41 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 1.84 (s, 3 H, CH_3CO)
3	8.10–7.39 (m, 17 H, Ar*H*), 5.5 (d, 1 H, $J_{2,NH}$ 9.2 Hz, N–*H*), 4.76 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 1.79 (s, 3 H, CH_3CO)
4	8.08–7.36 (m, 17 H, Ar*H*), 5.62 (d, 1 H, $J_{2,NH}$ 9.5 Hz, N–*H*), 4.57 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.62 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 2.00–1.90 (5 s, 15 H, CH_3CO)
8	8.00–7.32 (m, 10 H, Ar*H*), 5.32 (d, 1 H, $J_{2,NH}$ 9.2 Hz, N–*H*), 5.04–4.89 (m, 2 H, H-1′ and H-4′), 4.40 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 2.12–1.89 (12 H, CH_3CO)
12	8.01–7.26 (m, 12 H, Ar*H*), 5.51 (d, 1 H, $J_{2,NH}$ 9.5 Hz, N–*H*), 4.99 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.42 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 2.10–1.92 (18 H, CH_3CO)
16	7.8–7.4 (m, 7 H, Ar*H*), 5.01 (1 H, $J_{1,2}$ 3.1 Hz, H-1), 3.75 (dd, 1 H, H-2)
17	7.86–7.37 (m, 12 H, Ar*H*), 5.56 (s, 1 H, benzylidene), 5.16 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1)
18	7.86–7.32 (m, 12 H, Ar*H*), 5.54 (s, 1 H, benzylidene), 5.18 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.78 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 2.14–1.98 (4 s, 12 H, CH_3CO)
19	7.84–7.32 (m, 7 H, Ar*H*), 5.16 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.78 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 2.14–1.96 (4 s, 12 H, CH_3CO)
21	7.86–7.46 (m, 12 H, Ar*H*), 5.52 (s, 1 H, benzylidene), 5.46 (d, 1 H, $J_{2,NH}$ 9.1 Hz, N–*H*), 5.04 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 4.78 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 2.1–1.9 (4 s, 12 H, CH_3CO)
22	7.82–7.42 (m, 7 H, Ar*H*), 5.65 (d, 1 H, N–*H*), 5.03 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.87 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 2.05–1.98 (4 s, 12 H, CH_3CO)
24	7.84–7.48 (m, 7 H, Ar*H*), 5.05 (d, 1 H, $J_{1,2}$ 3.25 Hz, H-1), 1.36, 1.22 (2s, 6 H, 2-Pr)
25	7.86–7.48 (m, 7 H, Ar*H*), 5.46 (d, 1 H, $J_{2,NH}$ 8.4 Hz, N–*H*), 5.06 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.62 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 2.12–1.98 (4 s, 12 H, CH_3CO), 1.36 and 1.22 (2 s, 6 H, 2-Pr)
26	7.86–7.49 (m, 7 H, Ar*H*), 5.44 (d, 1 H, $J_{2,NH}$ 8.8 Hz, N–*H*), 5.04 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.64 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 2.11–1.88 (4 s, 12 H, CH_3CO)

hexane; B, 3:1 EtOAc–hexane; C, 9:1 $CHCl_3$–MeOH; D, 13:6:1 $CHCl_3$–MeOH–water. [6-^{3}H]fucose (83 Ci/mmol), [6-^{3}H]galactose (32 Ci/mmol), [6-^{3}H]glucosamine (33.3 Ci/mmol), or 1 μCi/mL [^{3}H-methyl]thymidine (83 Ci/mmol) (NEN Life Science Products, Inc., Boston, MA.).

2-Naphthylmethyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranoside* (**6**). —To a suspension of molecular sieves (4 Å, 5 g) in CH_2Cl_2 (100 mL) was added silver trifluoromethanesulfonate (16 g, 62 mmol). The mixture was stirred for 1 h at rt in the dark under argon. The reaction mixture was cooled to 0 °C, and 2-naphthylmethanol (12 g, 76 mmol) was added with stirring, followed by the addition of a soln of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (10 g, 27 mmol) in anhyd CH_2Cl_2 (50 mL) dropwise during 1 h. Stirring was continued for 2 h at 0 °C and the reaction was stopped and filtered through a bed of Celite. Dichloromethane was added (150 mL), and the organic layer washed successively with a soln of 5% sodium thiosulfate, water and cold satd $NaHCO_3$ soln. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness. Precipitation of the reaction with ether yielded the monosaccharide, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**1**) (9.5 g, 71%). R_f 0.14 (solvent A); $[\alpha]_D$ −5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{25}H_{29}NO_9$: C, 61.59; H, 6.00; N, 2.87. Found: C, 61.52; H, 6.15; N, 2.64.

Compound **1** (5 g, 10 mmol) was stirred at rt with 0.05 M CH_3ONa in MeOH (50 mL) for 3 h. The reaction mixture was neutralized with AcOH and concd to dryness. The solid residue was resuspended in MeOH (25 mL) and kept in the cold for 2 h, whereby the precipitate, 2-naphthylmethyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**2**) (3 g, 81%) was collected by filtration. R_f 0.4 (solvent C); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{19}H_{23}NO_6$: C, 63.15; H, 6.41; N, 3.88. Found: C, 63.12; H, 6.24; N, 3.65.

Compound **2** (2 g, 6 mmol) was dissolved in pyridine (40 mL), and then cooled to −40 °C. Benzoyl chloride (1.4 mL) was diluted with pyridine (4 mL), and the mixture was added to

the soln of compound **2** dropwise during 2 h with stirring, maintaining the temperature at −40 °C. The reaction mixture was stirred for a further 1 h at −40 °C and then allowed to warm up to rt while stirring for 2 h. The excess benzoyl chloride was decomposed by the addition of MeOH (0.4 mL). The reaction mixture was concd to dryness and purified by column chromatography ($CHCl_3$ and 5% acetone in $CHCl_3$) yielding the monosaccharide, 2-naphthylmethyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**3**) (2.2 g, 70%); R_f 0.35 (solvent A); $[\alpha]_D$ −1° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{33}H_{31}NO_8$: C, 69.58; H, 5.49; N, 2.46. Found: C, 69.57; H, 5.42; N, 2.33.

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (1.3 g, 3.2 mmol) and molecular sieves (4 Å, 2.5 g) were added to a soln of compound **3** (1 g, 2 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was allowed to stir at rt for 2 h under argon. Silver trifluoromethanesulfonate (1.8 g, 6.8 mmol) was added, and stirring was continued for 15 min, then Br_2 (0.1 mL) was added, and the reaction mixture was stirred for a further 2 h under argon. Triethylamine (0.5 mL) was added to quench the reaction, and the reaction mixture was filtered through a bed of Celite. Dichloromethane was added (100 mL), and the organic layer was washed successively with cold 5% sodium thiosulfate, cold satd $NaHCO_3$ and cold water. The reaction was dried over Na_2SO_4, filtered and concd to dryness. The residue was purified by column chromatography (increasing gradient of EtOAc in hexane 30 → 50 → 60%) yielding the disaccharide intermediate, 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**4**) (0.5 g, 32%); R_f 0.29 (solvent A); $[\alpha]_D$ −1° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{47}H_{49}NO_{17}$: C, 62.73; H, 5.49; N, 1.56. Found: C, 62.44; H, 5.67; N, 1.46.

Compound **4** (0.45 g, 0.50 mmol) was stirred with 0.1 M CH_3ONa in MeOH (15 mL) for 8 h, after which time the reaction was neutralized with IR-120 (H^+) cation-exchange resin. The resin was filtered, and the mixture was concd to dryness to give the fully deprotected disaccharide, 2-naphthylmethyl β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**5**) (0.22 g, 84%); R_f 0.37 (solvent D); $[\alpha]_D$ −5° (*c* 0.2, MeOH). Anal. Calcd for $C_{25}H_{33}NO_{11}$: C, 57.35; H, 6.35; N, 2.68. Found: C, 57.07; H, 6.55; N, 2.44.

Compound **5** (0.1 g, 0.2 mmol) was per-*O*-acetylated by stirring with 1:1 pyridine–Ac_2O (1 mL) for 16 h at rt. The reaction mixture was concd to dryness, and the residue was purified by column chromatography (9:1 EtOAc–hexane) to yield the fully acetylated target disaccharide, 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**6**) (0.127 g, 84%). R_f 0.24 (solvent B); $[\alpha]_D$ −2° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.84–7.39 (m, 7 H, Ar*H*), 5.44 (d, 1 H, $J_{2,NH}$ 9.35, N-*H*), 5.35 (d, 1 H, H-3′), 5.12–5.08 (dd, 1 H, H-2′), 5.03–4.95 (m, 3 H, H-3, H-3′ and CH_2), 4.74 (dd, 1 H, CH_2), 4.56 (d, 1 H, H-6a/b), 4.50 (d, 1 H, $J_{1',2'}$ 8.75 Hz, H-1′), 4.47 (d, 1 H, $J_{1,2}$ 7.25 Hz, H-1), 4.19–4.13 (m, 2 H, H-2 and H-6a/b), 4.12–4.09 (m, 2 H, H-6′a and H-6′b), 3.88 (dd, 1 H, H-5′), 3.83 (dd, 1 H, H-4), 3.62–3.59 (m, 1 H, H-5), 2.14, 2.13, 2.05, 2.04, 2.03, 1.96, 1.94 (7s, 21 H, CH_3CO); ^{13}C NMR ($CDCl_3$): δ 169.32–170.66 (*C*=O), 134.25, 133.13, 133.03, 128.29, 127.83, 127.12, 126.87, 126.35, 126.19, 125.65 (10 C, Ar*C*), 100.93 (C-1′), 99.41 (C-1), 75.59 (C-4), 72.70 (C-5), 72.33, 70.82 (2 C), 70.50, 69.00 (C-2′), 66.55 (C-4′), 62.36 (C-6), 60.75 (C-6′), 53.04 (C-2), 29.68 (*C*H_3CON), 20.92–20.28 (6 C, *C*H_3COO). Anal. Calcd for $C_{37}H_{45}NO_{17}$: C, 57.29; H, 5.85; N, 1.81. Found: C, 57.11; H, 5.68; N, 1.62.

2-Naphthylmethyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri*-O-*acetyl-β-D-galactopyranoside* (**14**). —Benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (1.3 g, 2.7 mmol) was dissolved in 1:1 nitromethane–benzene (45 mL), and approximately one-third of the solvent was distilled off. The reaction mixture was cooled to 35–40 °C, molecular sieves (4 Å, 2 g) were added, and the reaction mixture was stirred. Mercuric cyanide (1.4 g, 5.5 mmol) and mercuric bromide (1.8 g, 5.0 mmol) were added, and the reaction mixture was stirred

for 1 h at 35–40 °C under argon and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (1.5 g, 4.1 mmol) was added. The reaction mixture was stirred for 16 h at 35–40 °C under argon, and then additional mercuric cyanide (0.6 g, 2.4 mmol), mercuric bromide (0.8 g, 2.2 mmol) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (0.8 g, 2.1 mmol) were added and stirred for another 20 h at 35–40 °C. The reaction mixture was filtered and diluted with benzene (100 mL), and the organic layer was washed with satd $NaHCO_3$, satd KI, and water. The organic layer was dried over Na_2SO_4, filtered and concd to dryness. The crude reaction mixture of disaccharide **7** was used directly in the next step, whereby the reaction mixture was treated with 80% AcOH at 80 °C for 2 h. The solvent was concd to dryness and precipitation with ether yielded benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactopyranoside (**8**) (0.77 g, 41%); R_f 0.25 (Solvent B); $[\alpha]_D$ −5° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{34}H_{41}NO_{15}$: C, 58.03; H, 5.87; N, 1.99. Found: C, 57.71; H, 5.72; N, 2.05.

Compound **8** (0.8 g, 1.1 mmol) was acetylated by stirring in 1:1 pyridine–Ac_2O (10 mL) at rt overnight. The reaction mixture was evaporated to dryness, and the resulting residue was subjected to column chromatography (19:1 $CHCl_3$–MeOH) to yield the disaccharide, benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranoside (**9**) (0.75 g, 89%); R_f 0.24 (solvent A), $[\alpha]_D$ 0° (*c* 0.2, $CHCl_3$); Anal. Calcd for $C_{38}H_{45}NO_{17}$: C, 57.93; H, 5.76; N, 1.78. Found: C, 58.23; H, 6.04; N, 1.78.

A soln of **9** (0.75 g, 0.95 mmol) in AcOH (10 mL) was hydrogenated in presence of 10 wt.% Pd–C (0.3 g) by shaking under H_2 (52 psi) overnight at rt. The reaction mixture was filtered and concd to dryness to give 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-D-galactopyranoside (**10**). Crude compound **10** (0.5 g, 0.7 mmol) was used directly in the next step. It was dissolved in anhyd CH_2Cl_2 (10 mL), and 1,8-diazabicyclo[5.4.0]-undec-7-ene (0.15 mL) and CCl_3CN (0.4 mL) were added. The reaction mixture was stirred at rt for 1 h, concd to dryness and subjected to column chromatography (2:1 EtOAc–hexane containing 0.1% Et_3N) to yield 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-galactopyranosyl trichloroacetamidate (**11**) (0.5 g, 82%). To a soln of **11** (0.5 g, 0.6 mmol) and 2-naphthylmethanol (0.4 g, 2.5 mmol) in anhyd CH_2Cl_2 (10 mL) was added toluene (10 mL). The reaction mixture was stirred with molecular sieves (4 Å, 2.5 g) for 1 h under argon at rt. The reaction mixture was cooled to −10 °C, and a soln of trimethylsilyl trifluoromethanesulfonate (TMS triflate) (0.5 M soln in toluene, 0.5 mL) was added with stirring at −10 °C for 1 h. The reaction mixture was then neutralized with Et_3N, filtered and concd to dryness. The residue was purified by column chromatography (increasing gradient of EtOAc in hexane 33→50→66→75%) to yield 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranoside (**12**) (0.4 g, 81% from **11**): R_f 0.25 (solvent B); $[\alpha]_D$ −2° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{42}H_{47}NO_{17}$: C, 60.21; H, 5.65; N, 1.67. Found: C, 60.28; H, 6.08; N, 1.72.

Compound **12** (0.3 g, 0.4 mmol) was O-deacetylated using 0.1 M CH_3ONa in MeOH (5 mL), and after standard work up yielded 2-naphthylmethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**13**) (0.18 g, 94%); R_f 0.4 (solvent D), $[\alpha]_D$ −2° (*c* 0.4, 0.05% Me_2SO in MeOH). Anal. Calcd for $C_{25}H_{33}NO_{11}$: C, 57.35; H, 6.35; N, 2.68. Found: C, 57.08; H, 6.59; N, 2.41.

Compound **13** (0.15 g, 0.29 mmol) was acetylated with 1:1 pyridine–Ac_2O (5 mL), after which the solvent was concd to dryness. Column chromatography (4:1 EtOAc–hexane) yielded the fully acetylated target disaccharide, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**14**) (0.2 g, 90%); R_f 0.18 (solvent C), $[\alpha]_D$ −2° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.85–7.39 (m, 7 H, Ar*H*), 5.50 (dd, 1 H, H-3′), 5.41 (d, 1 H, $J_{2,NH}$ 9.6 Hz, N-*H*), 5.36

(dd, 1 H, H-4), 5.24 (dd, 1 H, H-2), 5.04–4.99 (m, 3 H, H-1′, H-4′, CH_2), 4.79 (d, 1 H, CH_2), 4.44 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.30 (dd, 1 H, H-6′a/b), 4.17–4.12 (m, 2 H, H-6a and H-6b), 4.07–4.04 (m, 1 H, H-6′a/b), 3.79–3.77 (m, 2 H, H-3 and H-5), 3.64–3.60 (dd, 1 H, H-5′), 3.31–3.25 (dd, 1 H, H-2′), 2.13, 2.10, 2.08, 2.05, 2.04, 2.00, 1.89 (7s, 21 H, CH_3CO). ^{13}C NMR ($CDCl_3$): δ 170.72–169.48 (*C*=O), 134.18, 133.12, 133.03, 128.28, 127.82, 127.73, 126.87, 126.33, 126.17, 125.69 (10 C, Ar*C*), 99.59 (C-1′), 99.22 (C-1), 75.98, 71.58 (C-5′), 71.25 (C-3′), 71.10, 70.85 (C-2), 70.46, 68.74, 69.22 (C-4), 62.09 (C-6), 61.24 (C-6′), 56.17 (C-2′), 23.30 (*C*H_3CON), 21.07–20.64 (6 C, *C*H_3COO). Anal. Calcd for $C_{37}H_{45}NO_{17}$: C, 57.29; H, 5.85; N, 1.81. Found: C, 57.28; H, 6.08; N, 1.72.

2-Naphthylmethyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl*-*(1→3)*-*2*-*acetamido*-*4,6*-*di*-O-*acetyl*-*2*-*deoxy*-α-D-*galactopyranoside* (**20**).—To a soln of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl trichloroacetamidate (4.0 g, 8.4 mmol) in CH_2Cl_2 (70 mL) was added 2-naphthylmethanol (2.7 g, 17.5 mmol). The reaction mixture was cooled to −20 °C, and a soln of TMS triflate in CH_2Cl_2 (to a final concentration of 0.05 M in the reaction mixture) was added. The reaction mixture was stirred for 30 min at −20 °C, neutralized with Et_3N, and concd to dryness. Column chromatography (9:1 toluene–acetone) gave 2-naphthylmethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranoside (**15**) (2.4 g, 61%); R_f 0.72 (solvent C). Compound **15** (2.2 g, 4.7 mmol) was used directly in the next step, whereby the reaction mixture was O-deacetylated with 0.02 M CH_3ONa in MeOH (25 mL). After standard work-up procedure, the reaction mixture was concd to dryness, and precipitation from ice-cold MeOH gave 2-naphthylmethyl 2-azido-2-deoxy-α-D-galactopyranoside (**16**) (1 g, 62%). R_f 0.4 (solvent C), $[\alpha]_D$ +24° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{17}H_{19}N_3O_5$: C, 59.12; H, 5.55; N, 12.17. Found: C, 58.41, H, 5.61; N, 11.77.

To a soln of compound **16** (1 g, 3 mmol) in *N*,*N*-dimethylformamide (DMF, 15 mL) was added benzaldehyde dimethylacetal (1 mL) and *p*-toluenesulfonic acid (0.2 g, 1.1 mmol) with stirring. The reaction was allowed to stir for 5 h at rt. Triethylamine (0.5 mL) was added to quench the reaction, the solvent was concd to dryness, and the residue was subjected to column chromatography (1:1 EtOAc–hexane) to yield 2-naphthylmethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**17**) (1.05 g, 83%); R_f 0.68 (solvent B); $[\alpha]_D$ +16° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{24}H_{25}N_3O_5$: C, 66.19; H, 5.79; N, 9.65. Found: C, 66.51; H, 5.53; N, 9.68.Compound **17** (0.4 g, 0.9 mmol) was dissolved in anhyd CH_2Cl_2 (15 mL) and stirred with molecular sieves (4 Å, 2.5 g) under argon for 30 min. 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyrosyl trichloroacetamidate (0.6 g, 1.1 mmol) was added, followed by toluene (10 mL). After stirring for 10 min at rt, the reaction mixture was cooled to −10 °C, and a 0.5 M soln of TMS triflate (final concentration of TMS triflate in the reaction mixture was 0.01 M) was added. The reaction mixture was stirred for a further 1 h at −10 °C under argon. Triethylamine (0.5 mL) was added to quench the reaction, and it was filtered through Celite and concd to dryness. The residue was purified by column chromatography using an increasing gradient of EtOAc in hexane (25→33→50%) to yield 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**18**) (0.5 g, 71%); R_f 0.61 (solvent B), $[\alpha]_D$ +5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{38}H_{43}N_3O_4$: C, 59.60; H, 5.66; N, 5.49. Found: C, 58.96; H, 5.56; N, 5.33.

Compound **18** (0.4 g, 0.5 mmol) was treated with 80% AcOH for 2 h at 80 °C. The solvent was evaporated off, and the resulting immobile oil residue was subjected to column chromatography ($CHCl_3$) to give 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl-(1→3)-2-azido-2-deoxy-α-D-galactopyranoside (**19**) (0.29 g, 83%); R_f 0.37 (solvent B), $[\alpha]_D$ +5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{31}H_{37}N_3O_4$: C, 55.11; H, 5.52; N, 6.22. Found: C, 55.21; H, 5.92; N, 5.98.

To a soln of compound **19** (0.2 g, 0.3 mmol) in MeOH (5 mL) was added 20 wt.% palla-

dium hydroxide–charcoal (0.04 g) and hydrazine (0.15 mL). The reaction mixture was heated under gentle reflux for 2 h, after which time the solvent was concd to dryness and the residue was per-O-acetylated with 1:1 pyridine–Ac_2O (2 mL). The reaction mixture was concd to dryness and purified by column chromatography with increasing gradient of EtOAc in hexane (33 → 50 → 66 → 75%) to give the fully acetylated target disaccharide, 2-naphthylmethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**20**) (0.16 g, 70%); R_f 0.30 (solvent B), $[\alpha]_D$ 0° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.89–7.43 (m, 7 H, Ar*H*), 5.64 (d, 1 H, $J_{2,NH}$ 9 Hz, N-*H*), 5.40 (dd, 1 H, H-4), 5.33 (dd, 1 H, H-4′), 5.12 (dd, 1 H, H-2′), 5.09 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.92 (dd, 1 H, H-3′), 4.85 (d, 1 H, CH_2), 4.61 (d, 1 H, CH_2), 4.57 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 3.54 (dd, 1 H, H-2), 4.25–4.18 (m, 2 H, H-5, H-6′a/b), 4.15–4.12 (m, 2 H, H-6a, H-6b), 4.07–4.04 (m, 1 H, H-6′a, H-6′b), 3.95 (dd, 1 H, H-3), 3.85 (m, 1 H, H-5), 2.18–1.95 (7s, 21 H, CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.68–169.72(*C*=O), 134.12, 133.24. 133.21, 128.64, 127.90, 127.81, 127.68, 126.65, 126.55, 126.06 (10 C, Ar*C*), 100.51 (C-1′), 97.24 (C-1), 72.76 (C-3), 70.81 (C-5/C-3′), 70.72 (C-5/C-3′), 70.33 (*C*H_2), 68.79 (C-4), 68.49 (C-2′), 67.60 (C-5), 66.69 (C-4′), 62.67 (C-6′), 61.09 (C-6), 48.77 (C-2), 23.23 (*C*H_3CON), 20.68–20.41 (6 C, *C*H_3COO). Anal. Calcd for $C_{37}H_{45}NO_{17}$: C, 57.29; H, 5.85; N, 1.81. Found: C, 57.02; H, 6.01; N, 1.61.

2-Naphthylmethyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1 → 3)-2-acetamido-4,6-di*-O-*acetyl-2-deoxy-α*-D-*galactopyranoside* (**23**).—A 1:1 mixture of nitromethane and benzene (40 mL) was distilled off until one-third of the solvent was removed from the reaction flask. The mixture was then cooled down to 40 °C and molecular sieves (4 Å, 4 g) were added followed, by mercuric cyanide (0.3 g, 1.2 mmol) and mercuric bromide (0.4 g, 1.1 mmol) with stirring. Compound **17** (0.2 g, 0.5 mmol) was added, and the reaction mixture was stirred for a further 30 min under argon. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (0.4 g, 1.0 mmol) was added, and stirring was continued overnight at 40 °C under argon. The reaction mixture was filtered through Celite and washed successively with cold satd $NaHCO_3$, satd KI, and water. The organic layer was concd to dryness, and column chromatography (1:1 EtOAc–hexane) gave 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**21**) (0.17 g, 48%); R_f 0.52 (solvent B); $[\alpha]_D$ + 2° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{38}H_{44}N_4O_{13}$: C, 59.68; H, 5.80; N, 7.33. Found: C, 59.92; H, 5.56; N, 7.55.

Compound **21** (0.1 g, 0.2 mmol) was treated with 80% AcOH at 80 °C for 2 h. The solvent was concd to dryness, and the residue was subjected to column chromatography (19:1 $CHCl_3$–MeOH) to give 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1 → 3)-2-azido-2-deoxy-α-D-galactopyranoside (**22**) (0.093 g, 88%); R_f 0.32 (solvent B); $[\alpha]_D$ + 4° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{31}H_{38}N_4O_{13}$: C, 55.19; H, 5.68; N, 8.30. Found: C, 54.95; H, 6.01; N, 8.25.

Compound **22** (0.1 g, 0.1 mmol) in MeOH (5 mL) was treated with 20 wt.% palladium hydroxide–charcoal (0.03 g) and hydrazine (0.1 mL) under refluxing conditions for 2 h. The solvent was evaporated to dryness, and the residue was per-O-acetylated using 1:1 pyridine–Ac_2O. The solvent was concd to dryness, and the residue was purified by column chromatography (EtOAc in hexane 50 → 66 → 75%) to yield the fully acetylated target disaccharide, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**23**) (0.055 g, 70%); R_f 0.57 (solvent C); $[\alpha]_D$ + 8° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.88–7.43 (m, 7 H, Ar*H*), 6.04 (d, 1 H, $J_{2,NH}$ 8.5 Hz, N-*H*), 5.80 (d, 1 H, $J_{2,NH}$ 8.0 Hz, N-*H*), 5.39 (dd, 1 H, H-4), 5.35 (dd, 1 H, H-3′), 5.09 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.04 (dd, 1 H, H-4′), 4.96 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1′), 4.85 (d, 1 H, CH_2), 4.66 (d, 1 H, CH_2), 4.46–4.42 (m, 1 H, H-2), 4.33–4.30 (m, 1 H, H-6′a/b), 4.23–4.21 (m, 1 H, H-5), 4.17–4.14 (m, 1 H, H-6a/b), 4.09–4.04 (m, 2 H, H-6′a/b, H-6a/b), 3.98 (dd, 1 H, H-3), 3.68–3.64 (m, 1

H, H-5′), 3.58–3.52 (m, 1 H, H-2′), 2.12–1.91 (7s, 21 H, C*H*$_3$CO). ^{13}C NMR ($CDCl_3$): 171.13–169.36 (*C*=O), 134.03, 133.15, 128.55, 127.84, 127.75, 127.53, 126.53 (2 C), 126.42, 125.95 (10 C, Ar*C*), 99.05 (C-1′), 96.97 (C-1), 72.46 (C-3), 71.97 (C-3′, C-5′), 70.18 (*C*H$_2$), 68.83 (C-4), 68.31 (C-4′), 67.44 (C-5), 62.52 (C-6), 61.28 (C-6′), 55.22 (C-2′), 48.95 (C-2), 23.29 (*C*H$_3$CON), 20.69 (6 C, *C*H$_3$COO). Anal. Calcd for $C_{37}H_{46}N_2O_{16}$: C, 57.28; H, 5.98; N, 3.62. Found: C, 56.99; H, 6.15; N, 3.72.

*2-Naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-*D*-galactopyranoside* (**27**).—To a suspension of compound **16** (0.1 g, 0.3 mmol) in 2,2-dimethoxypropane (5 mL) was added 10-camphorsulfonic acid (0.04 g, 0.17 mmol), and the mixture was stirred for 24 h at rt. Triethylamine (0.1 mL) was added to quench the reaction, and the mixture was concd to dryness. The residue was treated with 9:1 MeOH–water (10 mL) and heated for 24 h under gentle reflux. The reaction mixture was again concd to dryness, and the residue was purified by column chromatography (1:2 EtOAc–hexane) to give 2-naphthylmethyl 2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside (**24**) (0.096 g, 86%); R_f 0.62 (solvent B), $[\alpha]_D$ +20° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{20}H_{23}N_3O_5$: C, 62.33; H, 6.01; N, 10.90. Found: C, 61.88; H, 6.45; N, 11.02.

Compound **24** (0.09 g, 0.234 mmol) was reacted with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (0.16 g, 0.44 mmol) in a similar manner as previously described for compound **21** to give 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside (**25**) (0.087 g, 52%); R_f 0.62 (solvent B), $[\alpha]_D$ +10° (*c* 0.2, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): Table 3; Anal. Calcd for $C_{34}H_{42}N_4O_{13}$: C, 57.14; H, 5.92; N, 7.84. Found: C, 57.21; H, 6.02; 7.82.

Compound **25** (0.08 g, 0.11 mmol) was treated with 70% AcOH (5 mL) at 80 °C for 2 h, after which time the solvent was concd to dryness and the residue was subjected to column chromatography (19:1 $CHCl_3$–MeOH) to give 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-azido-2-deoxy-α-D-galactopyranoside (**26**) (0.062 g, 82%); R_f 0.52 (solvent B); $[\alpha]_D$ +12° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): Table 3; Anal. Calcd for $C_{31}H_{38}N_4O_{13}$: C, 55.19; H, 5.68; N, 8.30. Found: C, 54.89; H, 5.72; N, 8.30.

Compound **26** (0.05 g, 0.07 mmol) was treated with hydrazine (0.02 g) and 20 wt.% Pd–C (0.015 g), followed by acetylation with 1:1 pyridine–Ac_2O to give the fully acetylated target disaccharide, 2-naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**27**) (0.041 g, 71%). R_f 0.52 (solvent C), $[\alpha]_D$ +8° (*c* 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.88–7.45 (m, 7 H, Ar*H*), 5.61 (d, 1 H, $J_{2,NH}$ 9.5 Hz, N*H*), 5.50 (d, 1 H, $J_{2,NH}$ 8.5 Hz, N*H*), 5.37 (dd, 1 H, H-4), 5.33 (dd, 1 H, H-3′), 5.18 (dd, 1 H, H-3), 5.03 (dd, 1 H, H-4′), 5.00 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.88 (d, 1 H, C*H*$_2$), 4.72 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.66 (d, 1 H, C*H*$_2$), 4.58 (m, 1 H, H-2), 4.27–4.08 (m, 2 H, H-6′a/b, H-6a/b), 3.83 (m, 1 H, H-6a/b), 3.70 (dd, 1 H, H-2′), 3.64 (m, 1 H, H-5′), 3.55 (m, 1 H, H-5), 2.15, 2.07, 2.03, 2.02, 1.98, 1.91, 1.90 (7s, 21 H, C*H*$_3$CO); ^{13}C NMR ($CDCl_3$): δ 170.88–168.32 (*C*=O), 134.14, 133.16, 133.06, 128.49, 127.93, 127.73, 127.28, 126.46, 126.33, 125.90 (10 C, Ar*C*), 100.37 (C-1′), 99.91 (C-1), 71.89 (C-5′), 71.56 (C-3′), 70.05 (*C*H$_2$), 68.52 (2 C), 67.85, 67.64, 67.45, 61.90 (C-6′), 55.07 (C-2′), 47.80 (C-2), 23.90 (*C*H$_3$CON), 20.72 (6 C, *C*H$_3$COO). Anal. Calcd for $C_{37}H_{46}N_2O_{16}$: C, 57.36; H, 5.98; N, 3.62. Found: C, 57.16; H, 6.21; N, 3.67.

Cell culture.—U937 human histiocytic lymphoma cells were from the American Type Culture Collection (CRL 1593.2) and were grown in RPMI 1640 medium (GIBCO) supplemented with 10% (v/v) fetal bovine serum (Hyclone), glutamine (0.3 g/L), streptomycin sulfate (100 μg/mL), and penicillin (100 Units/mL) [27,28]. Human umbilical vein endothelial cells (HUVEC) were from Clonetics Corporation (CC-2519) and were grown in Medium 199 (GIBCO) containing 20% (v/v) fetal bovine serum, heparin (100 μg/mL,

Sigma) and endothelial cell growth supplement (100 μg/mL, Collaborative Biomedical). All cell lines were maintained at 37 °C in a humidified incubator containing 5% CO_2 and 95% air.

Adhesion assays.—E-selectin expression on human umbilical vein endothelial cells (HUVEC) was stimulated with 20 ng/mL TNF-α (R & D Systems) [29]. After 5 h, the monolayers were washed three times with Minimal Essential Medium (MEM, GIBCO) and used for adhesion assays. U937 cells (2×10^5/well) in RPMI 1640 medium were incubated with and without acetylated disaccharide and [^{3}H-methyl]thymidine (1 μCi/mL) for 2 days. Washed cells in 250 μL of PBS (containing Ca^{2+} and Mg^{2+}) were then added to TNF-α activated HUVEC and incubated for 35 min at 4 °C. The plates were washed gently three times with 1 mL of cold medium and the cells were solubilized with 0.1 M NaOH. Radioactivity was measured after neutralizing the soln with AcOH. The values were normalized to the radiospecific activity of the cells determined on a separate aliquot. Cell adhesion was $>80\%$ dependent on E-selectin under these conditions based on the inhibitory activity of anti-ELAM-1 mAb (R&D Systems).

Labeling studies.—The per-O-acetylated disaccharides were dissolved in Me_2SO and added to the growth media to achieve the concentrations indicated in Tables 1 and 2 and Fig. 2. For the labeling studies, cells were incubated with 10 μCi/mL [6-^{3}H]fucose (83 Ci/mmol), 10 μCi/mL [6-^{3}H]galactose (32 Ci/mmol), 10 μCi/mL [6-^{3}H]glucosamine (33.3 Ci/mmol) or 1 μCi/mL [^{3}H-methyl]thymidine (83 Ci/mmol). After ~ 24 h, the cells were sedimented by centrifugation, and the amount of radioactivity that was incorporated into cellular glycoproteins was determined by precipitation with 10% trichloroAcOH (TCA). After centrifugation, the pellets were washed with 2% TCA, dissolved in 0.1 M NaOH, and counted by liquid scintillation spectrometry using UltimaGold (Packard) cocktail.

To measure priming of oligosaccharides, the conditioned medium was adjusted to 0.5 M NaCl and centrifuged. The supernatants were then applied to 0.2 mL Sep-Pak Vac RC C_{18} Cartridges (Waters), which had been pre-washed with 100% MeOH, water, and 0.5 M NaCl. The columns were washed sequentially with 0.5 M NaCl (2.5 mL) and water (25 mL). Radioactive oligosaccharides were eluted with 40% MeOH in water (2.5 mL), dried and counted by liquid scintillation.

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