Ligand Interactions with E-Selectin. Identification of a New Binding Site for Recognition of *N*-Acyl Aromatic Glucosamine Substituents of Sialyl Lewis X

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Several *N*-acylglucosamine derivatives of sialyl Lewis X (**1**−**3**) were prepared using a combined chemical enzymatic approach and evaluated as an inhibitor of E-selectin-mediated cellular adhesion. Compounds with aromatic functionality, **1** and **2**, were found to be 3−10 times more potent than the *N*-acetyl derivative (**14**) in an ELISA E-selectin cell adhesion assay. Conformational analysis with NMR indicated that the sialyl Lewis x domain of **1** retained the conformation of the *N*-acetyl derivative (**14**) despite the presence of the *N*-naphthamido group. The dramatic order of magnitude increase in potency of these monovalent structures can be utilized to design more potent selectin-based cell adhesion inhibitors.

One process of leukocyte trafficking during an inflammatory response begins with the carbohydrate mediated cell adhesion of leukocytes to endothelial cells involving E-, P-, and L-selectin.[1] These oligosaccharides have been shown to be composed of the 3-O-sialylated and sulfated structures of Lewis x[2] and Lewis a[2d] as well as 6-*O*-sulfate sialyl Lewis x[3] for L-selectin. Many groups have investigated derivatizing these structures in attempts to increase the potency of the native oligosaccharide and include such sialyl Lewis x (SLe^x) analogs as lactose (NANAα2-3Galβ1-4(Fucα1-3)Glc)[4a] and glucosamine *N*-acyl alkyl[4b] structures. With the exception of multivalent SLe^x constructs,[5] however, analogs of monovalent SLe^x have thus far failed to significantly increase the inhibitory potency of this class of compounds over the natural structures.

During our studies of the structure−activity relationships of SLe^x and its interaction with E-selectin, we have found that aromatic *N*-acyl substitutions on the glucosamine of sialyl Lewis x (**1** and **2**) increased the inhibitory potency of this class of oligosaccharide when tested as inhibitors of E-selectin-mediated cell adhesion.[6] Compound **1** was found to be 10-fold more potent than *N*-acetyl SLe^x (**14**)[7] (**1**, IC_{50} = 0.08 mM verses **14**, IC_{50} = 1.0 mM), and **2** was found to be ∼3-fold more potent (IC_{50} = 0.3 mM) (Figure 1).

One possible explanation for the increased inhibitory potencies of **1** and **2** may be the result of topostructural changes in the orientation of the neighboring Gal's and Fuc due to steric interactions with the aryl substituent. If this were to occur, the steric energies resulting from acyl substitution must exceed the structural stabilizing *exo*-anomeric effects[8] of Gal and Fuc which normally fix the glycosidic torsion angles into a specific topographic orientation. Alternatively, the increase in potency observed with **1** and **2** may be the result of a new complementary binding site for SLe^x on E-selectin which is not accounted for in current models of oligosaccharide binding. Conformational analysis of compound **1** using NMR[9] has indicated that the topographic orientation is essentially identical to those described for other *N*-acetamido SLe^x's (Table 1).[5] These findings suggest that the increase in potency of **1** and **2** results from the direct interaction of the aromatic acyl group with E-selectin presumably through a hydrophobic mechanism.

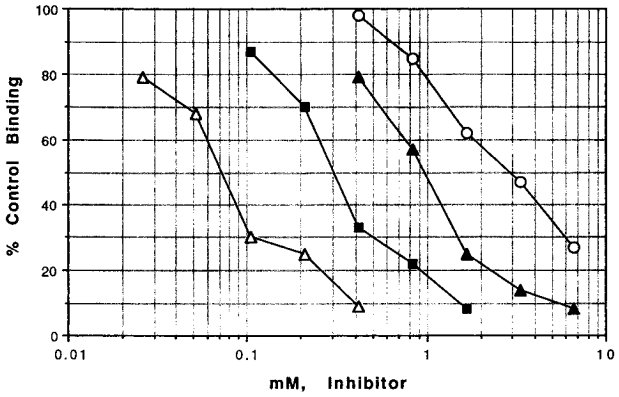

Figure 1. Inhibition of HL-60 cell binding to rsE-selectin by SLe^x analogs. Each data point is the average of duplicates. Binding is expressed as the percentage of rsE-selectin binding in the absence of inhibitor. The SLe^x analogs: (△) **1**; (■) **2**; (○) **3**; (▲) **14**.

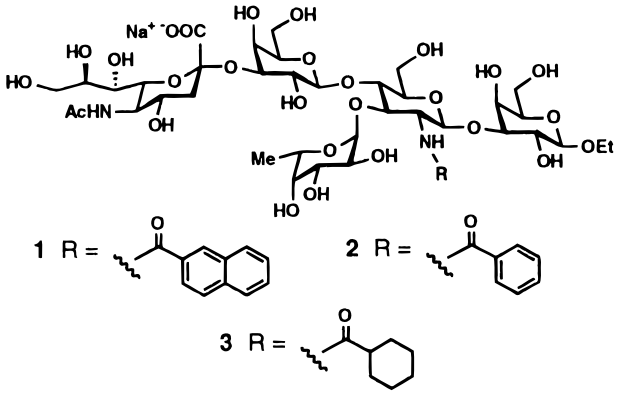

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Table 1. ^1H and ^{13}C Chemical Shift Assignments (ppm) of **1**

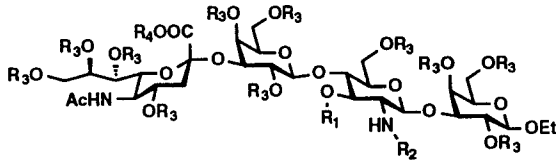
	Neu5Ac		Gal		Fuc		GlcN		Gal	
carbon	H	C	H	C	H	C	H	C	H	C
C1		177.6	4.57	105.3	5.19	102.2	4.96	105.8	4.32	106.1
C2		103.4	3.53	73.0	3.60	78.6	4.26	60.4	3.48	73.3
C3	1.78, 2.72	43.5	4.09	79.4	3.90	73.0	4.11	78.4	3.72	86.1
C4	3.67	72.0	3.92	71.0	3.75	75.6	4.04	76.8	4.17	72.0
C5	3.82	55.3	3.59	71.6	4.84	70.4	3.63	76.6	3.63	78.5
C6	3.66	78.9	3.68	65.1	1.15	18.8	3.93, 4.02	63.2	3.72	64.6
C7	3.57	71.4								
C8	3.89	75.6								
C9	3.63, 3.87	66.3								
CH_3	2.05	25.6							1.13	17.5
CH_2									3.59, 3.86	69.8
C=O		178.7								

The importance of the aromatic character of the *N*-acyl GlcN substituent of **1** and **2** for selectin inhibition is exemplified by the inhibition results of **3**, which contains a cyclohexane ring in place of the benzene ring of **2**. Compound **3** (IC_{50} = 2.9 mM) was found to be less potent than the *N*-acetyl SLe^x derivative (**14**) (IC_{50} = 1 mM). Recent reports have also shown that fatty acyl GlcN substituents of SLe^x with varying alkyl lengths were no more potent than *N*-acetyl SLe^x as E-selectin adhesion inhibitors.[4b,10] These findings suggest that the observed increased inhibitory potencies of **1** and **2** resulted from strong (potent) E-selectin interactions with the aromatic substituents on the GlcN nitrogen and may be due to $\pi-\pi$ interactions. When **1** and **2** were incorporated into several proposed SLe^x–E-selectin binding models,[11,12] specific interactions could not be identified although these models are speculative and SLe^x or these analogs could well bind at different sites or in different ways.

The synthesis of **1**–**3** was accomplished with a combined chemical and enzymatic approach in which the starting tetrasaccharide (**4**) was prepared by enzymatic sialylation and galactosylation which was then acetylated as reported previously (Scheme 1).[7] The GlcN hydroxyl to be fucosylated was easily and selectively deprotected using the 2-amino group of GlcN to direct the hydrolysis.[13] Acylation with the appropriate acyl chlorides provided **6**, **8**, or **10** which were subsequently fucosylated using tri-*O*-benzyl fucosyl halide[7] (Br or F) to provide the protected pentasaccharides (**7**, **9**, and **11**). Compounds **7** and **9** were then deprotected using hydrogenation[14] and deacetylation to provide **1** and **2**. The Cbz-protected pentasaccharide (**1**) was hydrogenated and then acylated with cyclohexanecarbonyl chloride to provide **12**. Compound **3** was obtained after hydrolysis of the acetyl and ester groups.

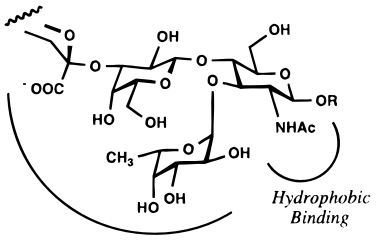
In summary, this study suggests that the interaction of these SLe^x analogs with E-selectin encompasses both the proposed topostructure of SLe^x containing the carboxylate, Gal, and Fuc functionalities as well as an additional complementary hydrophobic binding site on E-selectin which is adjacent to the glucosamine nitrogen as seen in **15**. Although the exact nature of this additional binding site and its role in ligand adhesion to E-selectin has not been fully characterized, further studies with additional *N*-acylglucosamine substituents of SLe^x should clarify the mechanism of this E-selectin interaction and suggest new routes for the design of more potent antiadhesion molecules.

Scheme 1[a]



a **4** R_1 = Ac; R_2 = Alloc
a–e **5** R_1 = H; R_2 = H; R_3 = Ac; R_4 = Me
b **6** R_1 = H; R_2 = 2-naphthoyl; R_3 = Ac; R_4 = Me
c **7** R_1 = 2,3,4-tri-O-benzyl-α-L-fucopyranosyl; R_2 = 2-naphthoyl; R_3 = Ac; R_4 = Me
d **1** R_1 = α-L-fucopyranosyl; R_2 = 2-naphthoyl; R_3 = H; R_4 = Na^+
f **8** R_1 = H; R_2 = benzoyl chloride; R_3 = Ac; R_4 = Me
f–g **9** R_1 = 2,3,4-tri-O-benzyl-α-L-fucopyranosyl; R_2 = benzoyl; R_3 = Ac; R_4 = Me
g **2** R_1 = α-L-fucopyranosyl; R_2 = benzoyl; R_3 = H; R_4 = Na^+
h **10** R_1 = H; R_2 = Cbz; R_3 = Ac; R_4 = Me
i **11** R_1 = 2,3,4-tri-O-benzyl-α-L-fucopyranosyl; R_2 = Cbz; R_3 = Ac; R_4 = Me
j **12** R_1 = α-L-fucopyranosyl; R_2 = H; R_3 = Ac; R_4 = Me
j **3** R_1 = α-L-fucopyranosyl; R_2 = cyclohexanecarbonyl; R_3 = H; R_4 = NH_4^+

[a] Key; (a) (i) $Pd(Ph_3P)_4$, polymethylsiloxane, THF (68%); (ii) AcOH, MeOH, H_2O, 55 °C (72%); (b) $NaHCO_3$, CH_2Cl_2, 2-naphthoyl chloride (77%); (c) (i) tri-*O*-benzyl-α/β-L-fucopyranosyl fluoride, $AgClO_4$, $SnCl_2$, TMU, dichloromethane, 4 Å sieves (57%); (d) (i) cyclohexene, 5% Pd/$BaSO_4$, ethanol, 80 °C (75%); (ii) NaOMe, MeOH, H_2O (95%); (e) $NaHCO_3$, CH_2Cl_2, acyl chloride (**8**, benzoyl chloride, 80%; **10**, Cbz chloride, 65%); (f) tri-*O*-benzyl-α-L-fucopyranosyl bromide, Et_4NBr, DMF, dichloromethane, 4 Å sieves (62%); (g) (i) NaOMe, MeOH, water (89%); (ii) Pearlman's catalyst, hydrogen, water, ethanol (97%); (h) tri-*O*-benzyl-α/β-L-fucopyranosyl fluoride, $AgClO_4$, $SnCl_2$, TMU, dichloromethane, 4 Å sieves (73%); (i) 10% Pd/C, ethanol, NH_4CO_3, reflux (96%); (j) (i) cyclohexanecarbonyl chloride, $NaHCO_3$, CH_2Cl_2, (ii) NaOMe, MeOH, water (93%).



15 Binding Site Interactions

Experimental Section

All reactions were monitored by thin layer chromatography carried out on 0.25 mm Whatman silica gel plates (60F-254) using UV light and anisaldehyde reagent[1] as developing agent. E. Merck silica gel (60, particle size 0.040–0.063 mm) and Bakerbond octadecyl silica gel (C_{18}, particle size 40 μm) were used for flash chromatography.

All reactions were carried out under an argon atmosphere with anhydrous solvents from Aldrich unless otherwise noted. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials unless otherwise stated. NMR spectra were recorded on a 300 MHz General Electric QE-300 NMR and a Bruker AM-500 NMR spectrometer. The FAB

mass spectra and exact mass calculations were acquired on a VG Fisons ZAB 2SE mass spectrometer.

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2,3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1,4)-*O*-(6-*O*-acetyl-2-amino-2-deoxy-*β*-D-glucopyranosyl)-(1,3)-*O*-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside (5). Tetrakis-(triphenylphosphine)palladium (1.29 g, 1.12 mmol) was added to a solution of polymethylsiloxane (2.98 mL, 8.95 mmol), ethyl (methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2,3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1,4)-*O*-(3,6-di-*O*-acetyl-2-(allyloxycarbamoyl)-2-deoxy-*β*-D-glucopyranosyl-(1,3)-*O*-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside[7] (**4**) (30 g, 22.37 mmol), and THF (250 mL). The solution was stirred overnight and diluted with ethyl acetate (1.2 L). The solution was washed with water (400 mL) and brine (400 mL), and the combined aqueous layers were extracted again with ethyl acetate (2 × 250 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and chromatographed (silica, 20% acetone/ethyl acetate) to yield 19.3 g (68%) of a yellow solid: R_f = 0.31 (silica, 20% acetone/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.47 (m, 1 H), 5.42 (d, J = 2.6 Hz, 1 H), 5.38 (d, J = 3.0 Hz, 1 H), 5.06−5.01 (m, 2 H), 4.95−4.84 (m, 3 H), 4.66 (d, J = 8.1 Hz, 1 H, β-anomer), 4.63 (d, J = 12.4 Hz, 1 H), 4.50 (dd, J = 3.3, 10.1 Hz, 1 H), 4.41−4.34 (m, 3 H), 4.15−3.93 (m, 7 H), 3.90−3.80 (m, 4 H), 3.83 (s, 3 H, OMe), 3.77−3.51 (m, 5 H), 2.56 (dd, J = 4.4, 12.7 Hz, 1 H, H-3$_{eq}$ SA), 2.21 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 2.07 (s, 6 H, OAc), 2.05 (s, 9 H, OAc), 2.03 (s, 3 H, OAc), 1.99 (s, 6 H, OAc), 1.84 (s, 3 H, NHAc), 1.67 (dd, J = 12.4, 12.4 Hz, 1 H, H-3$_{ax}$ SA), 1.17 (t, J = 7.0 Hz, 3 H, Me).

A solution of the above compound (19.2 g, 14.33 mmol), acetic acid (847 μL, 14.79 mmol), methanol (1.26 L), and water (312 mL) was heated to 55 °C for 24 h. The mixture was concentrated and chromatographed (silica, ethyl acetate/ether/acetone, 6/1/3) to afford 13.54 g (72%) of a yellow solid: R_f = 0.30 (silica, 20% acetone/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.53−5.49 (bm, 1 H), 5.41−5.37 (m, 2 H), 5.16 (dd, J = 8.1, 10.0 Hz, 1 H), 5.10−4.96 (m, 2 H), 4.95−4.84 (m, 2 H), 4.67−4.49 (m, 3H), 4.42−4.24 (m, 3 H), 4.21−3.42 (m, 16 H), 3.84 (s, 3 H, OMe), 2.70 (bs, 1 H, OH), 2.56 (dd, J = 4.4, 12.4 Hz, 1 H, H-3$_{eq}$ SA), 2.25 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 2.10 (s, 6 H, OAc), 2.08 (s, 6 H, OAc), 2.06 (s, 6 H, OAc), 2.05 (s, 6 H, OAc), 2.00 (s, 3 H, OAc), 1.85 (s, 3 H, NHAc), 1.67 (dd, J = 12.4, 12.4 Hz, 1 H, H-3$_{ax}$ SA), 1.20 (t, 3 H, Me).

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2,3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1,4)-*O*-[6-*O*-acetyl-2-(2-naphthamido)-2-deoxy-*β*-D-glucopyranosyl]-(1,3)-*O*-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside (6). The 2-naphthoyl chloride (1.28 g, 6.72 mmol) was added to a suspension of compound **5** (5.81 g, 4.48 mmol) and sodium bicarbonate (3.01 g, 35.8 mmol) in CH_2Cl_2 (80 mL). The mixture was stirred overnight and filtered. The filtrate was washed with saturated $NaHCO_3$ (10 mL) and dried (Na_2SO_4). Concentration and chromatography (silica, 35% acetone/CH_2Cl_2) afforded 5.03 g (77%) of a white solid: R_f = 0.45 (10% acetone/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1 H, H-1 naphthalene), 7.88 (m, 4 H, aromatic), 7.57 (m, 2 H, aromatic), 6.58 (d, J = 5.3 Hz, 1 H, NH), 5.53 (m, 1 H), 5.44 (d, J = 2.9 Hz, 1 H, H-4 Gal), 5.39−5.23 (m, 3 H), 5.17−5.01 (m, 3 H), 4.89 (d, J = 3 Hz, 1 H), 4.68 (d, J = 8 Hz, 1 H, H-1 Gal), 4.57 (dd, J = 3, 7 Hz, 1 H), 4.42−3.28 (m, 19 H), 3.81 (s, 3 H, OMe), 3.25 (q, J = 6 Hz, 1 H, CH_2), 2.57 (dd, J = 4.3, 12.5 Hz, 1 H, H-3$_{eq}$ SA), 2.27 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.08 (s, 6 H, OAc), 2.07 (s, 6 H, OAc), 2.03 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.92 (s, 3 H, OAc), 1.84 (s, 3 H, NHAc), 1.68 (dd, J = 12.4, 12.4 Hz, 1 H, H-3$_{ax}$ SA), 1.12 (t, J = 6 Hz, 3 H, Me). Anal. ($C_{65}H_{84}N_2O_{35}$·2.5H_2O) Calcd: C, 52.10; H, 5.98; N, 1.86. Found: C, 52.18; H, 5.81; N, 1.75.

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2,3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactoypyranosyl)-(1,4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1,3)-*O*-[6-*O*-acetyl-2-(2-naphthamido)-2-deoxy-*β*-D-glucopyranosyl]-(1,3)-*O*-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside (7). A solution of compound **6** (135 mg, 0.093 mmol), tri-*O*-benzyl-α/*β*-L-fucosyl fluoride[2] (283 mg, 0.65 mmol), 4 Å sieves (100 mg), tetramethylurea (122 μL, 1.02 mmol), and dichloroethane (10 mL) was stirred for 4 h. Silver perchlorate (67 mg, 0.33 mmol) followed by $SnCl_2$ (61 mg, 0.33 mmol) was then added, and the reaction mixture was stirred for 2 days. Ethyl acetate (300 mL) was then added and the suspension filtered. The filtrate was washed with water (100 mL) and dried ($MgSO_4$). Concentration and chromatography (silica, 8% acetone/ethyl acetate) afforded 99 mg (57%) of white solid: R_f = 0.39 (8% acetone/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1 H, H-1 naphthalene), 7.74 (d, J = 7 Hz, 1 H, aromatic), 7.68 (d, J = 7 Hz, 1 H, aromatic), 7.47 (m, 2 H, aromatic), 7.37−7.11 (m, 16 H, aromatic), 6.98 (d, J = 7 Hz, 1 H, aromatic), 6.35 (d, J = 6.4 Hz, 1 H, NH), 5.57−5.35 (m, 3 H), 5.12−5.03 (m, 3 H), 4.97−4.45 (m, 8 H), 4.32−4.25 (m, 2 H), 4.14−3.72 (m, 22 H), 3.81 (s, 3 H, OMe), 3.71−3.60 (m, 2 H), 3.48−3.43 (m, 1 H), 3.25 (m, 1 H), 2.55 (dd, J = 4.5, 12.4 Hz, 1 H, H-3$_{eq}$ SA), 2.24 (s, 3 H, OAc), 2.22 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 2.09 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 1.85 (s, 3 H, NHAc), 1.68 (dd, J = 12.4, 12.4 Hz, 1 H, H-3$_{ax}$ SA), 1.18 (d, J = 6.5 Hz, 3 H, H-6 Fuc), 1.08 (t, 3 H, Me). Anal. ($C_{92}H_{112}N_2O_{39}$·2H_2O) Calcd: C, 57.97; H, 6.12; N, 1.47. Found: C, 57.89; H, 5.98; N, 1.37.

Ethyl (Sodium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyronosylonate)-(2,3)-*O*-(*β*-D-galactopyranosyl)-(1,4)-*O*-(α-L-fucopyranosyl)-(1,3)-*O*-[2-(2-naphthamido)-2-deoxy-*β*-D-glucopyranosyl]-(1,3)-*O*-*β*-D-galactopyranoside (1). Compound **7** (14 mg, 7.49 μmol) was dissolved in ethanol (5 mL) and degassed under vacuum. Cyclohexene (50 μL) followed by 5% Pd/$BaSO_4$ (30 mg) was then added and suspension heated at 80 °C for 18 h. The reaction mixture was filtered, concentrated, and chromatographed (silica, hexane/ethyl acetate/ethanol, 2/2/1) to afford 9 mg (75%) of a white solid: R_f = 0.28 (silica, hexane/ethyl acetate/ethanol, 2/2/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1 H, H-1 naphthalene), 7.94 (d, J = 7.1 Hz, 1 H, aromatic), 7.86 (s, 2 H, aromatic), 7.84 (d, J = 10.2 Hz, 1 H, aromatic), 7.54 (m, 2 H, aromatic), 6.94 (bd, J = 7.2 Hz, 1 H, NH), 5.55−5.51 (m, 1 H), 5.45−5.41 (m, 2 H), 5.17−5.07 (m, 4 H), 4.96−4.85 (m, 3 H), 4.74 (m, 2 H), 4.57 (m, 2 H), 4.38−4.33 (m, 3 H), 4.30−3.99 (m, 6 H), 3.95−3.71 (m, 9 H), 3.84 (s, 3 H, OMe), 3.66−3.59 (m, 4 H), 3.50 (m, 1 H), 2.58 (dd, J = 4.4, 12.4 Hz, 1 H, H-3$_{eq}$ SA), 2.24 (s, 3 H, OAc), 2.17 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.07 (s, 3 H, OAc), 2.04 (s, 6 H, OAc), 2.03 (s, 6 H, OAc), 2.00 (s, 3 H, OAc), 1.86 (s, 3 H, NHAc), 1.70 (dd, J = 12.4, 12.4 Hz, 1 H, H-3$_{ax}$ SA), 1.29 (d, J = 6.6 Hz, 3 H, H-6 Fuc), 1.12 (t, 3 H, Me). Anal. ($C_{71}H_{94}N_2O_{39}$) Calcd: C, 53.31; H, 5.92; N, 1.75. Found: C, 53.57; H, 6.30; N, 1.72.

This compound (1.34 g, 0.839 mmol) was then dissolved in MeOH (30 mL), and a solution of 20% NaOMe in MeOH (1.0 mL) was added. The solution was stirred for 18 h and water (5 mL) added. After 24 h, the solution pH was adjusted to 7.0 with acetic acid and the solution concentrated. Chromatography (Bakerbond C-18, water then 10% MeOH in water) afforded 0.92 g (95%) of a white solid after lyophilization: R_f = 0.52 (1 M NH_4OAc/2-propanol, 1/3); ^{1}H NMR (500 MHz, D_2O) δ 8.38 (s, 1H, H-1 naphth), 8.10−7.99 (m, 3 H, H-4, 5 and 8 naphth), 7.81 (dd, J = 1.6, 8.5 Hz, 1 H, H-3 naphth), 7.72−7.63 (m, 2 H, H-6 and 7 naphth), 5.19 (d, J = 4.0 Hz, 1 H, H-1 Fuc), 4.96 (d, J = 8.4 Hz, 1 H, H-1 GlcN), 4.84 (m, 1 H, H-5 Fuc), 4.57 (d, J = 7.9 Hz, 1 H, H-1 Gal), 4.32 (d, J = 8.0 Hz, 1 H, H-1, Gal′), 4.26 (bt, 1 H, H-2 GlcN), 4.17 (d, J = 3.3 Hz, 1 H, H-4, Gal′), 4.12−4.02 (m, 4 H, H-3 and 4 GlcN, H-3 Gal, H-6′ GlcN), 3.96−3.84 (m, 7 H), 3.78−3.54 (m, 14 H), 3.48 (dd, J = 2.1, 9.2 Hz, 1 H, H-2 Gal′), 2.76 (dd, J = 4.6, 12.4 Hz, 1 H, H-3 SA), 2.03 (s, 3 H, NHAc SA), 1.78 (dd, J = 12.2, 12.2 Hz, 1 H, H-3 SA), 1.17 (d, J = 6.6 Hz, 3 H, H-6 Fuc), 1.13 (dd, J = 7.9, 7.9 Hz, 3 H, CH_2CH_3); ^{13}C NMR (120 MHz, D_2O) see Table 1. Anal. ($C_{48}H_{69}N_2O_{28}Na$·6H_2O) Calcd: C, 46.00; H, 6.51; N, 2.23. Found: C, 45.97; H, 6.47; N, 2.23.

NMR Experiments. Proton and carbon NMR experiments were conducted in D_2O at 295 K using a Bruker AM-500 NMR

spectrometer equipped with an X-32 computer and an ASPECT-3000 process controller. The sample was not spun. ^{1}H chemical shifts were referenced to internal HOD at 4.76 ppm, and ^{13}C chemical shifts were referenced to external DMSO at 39.5 ppm. All NMR data were processed and analyzed with the Felix program run on a Sun SPARC station or a Silicon Graphics Indigo workstation. For further experimental details, see ref 5a.

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Supporting Information Available: 1D spectra (^{1}H and ^{13}C NMR) of **1**, Roesy of **1**, procedures for the synthesis of **2** and **3**, and physical data (12 pages). Ordering information is given on any current masthead page.

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(14) Hydrogenation of **7** using hydrogen and palladium catalysts such as Pd/C, Pearlman's catalyst, and Pd/$BaSO_4$ produced varying amounts of an overreduced naphthamide product. The catalytic phase transfer hydrogenation conditions were found to produce no detectable amounts of the overreduced product.

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