Articles

Sialyl Lewis x Liposomes as a Multivalent Ligand and Inhibitor of E-Selectin Mediated Cellular Adhesion

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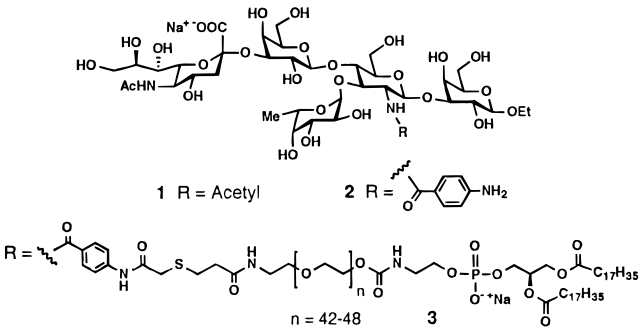
Received December 7, 1995[⊗]

Abstract: A sialyl Lewis x−PEG−DSPE derivative (**3**) has been prepared using a combined chemical enzymatic approach and incorporated into mPEG−DSPE containing liposomes (PEG, poly(ethylene glycol); mPEG, methoxypoly-(ethylene glycol); DSPE, distearoylphosphatidylethanolamine). Several liposomal formulations of **3** were evaluated as inhibitors of E-selectin mediated cellular adhesion in an ELISA assay and were found to be ∼750-fold more potent than the nonliposomal oligosaccharide (**2**) and greater than 5000-fold more potent than the natural glycotope structure (**1**). The dramatic increase in potency of the liposomal formulations suggests that oligosaccharide multivalency enhances inhibition of E-selectin adhesion and is an effective approach to the design of more potent selectin cell adhesion inhibitors.

One of the inflammatory processes arising from tissue injury or infection begins with the expression of endothelial E-selectin and the concomitant adhesion of neutrophils through surface oligosaccharides.[1] The ligand recognized by E-selectin has been identified as sialyl Lewis x (SLe^x)[2] although other structural variants can also interact including sialyl Lewis A[2d] and 3′-*O*-sulfate Le^x.[2e] Studies involving divalent[3] SLe^x and SLe^x−protein conjugates[4] have suggested that the interaction of endothelial E-selectin with glycoprotein structures on neutrophils may be multivalent in nature. A powerful tool for the efficient presentation of ligands to cell surface receptors which require multivalent contact has been through the use of liposome technology.[5] Liposomes containing conjugated sialic acid when examined as influenza cell adhesion inhibitors were shown to have an enhanced inhibitory potency of several orders of magnitude over the free sugar.[6] As part of our interest in the development of novel delivery methods for oligosaccharide therapeutics, we describe here the study of a poly(ethylene glycol) (PEG) derived SLe^x glycolipid incorporated into liposomes to provide a multivalent platform capable of inhibiting E-selectin dependent cellular adhesion.

The synthesis of the SLe^x glycolipid (**3**) was accomplished using a chemoenzymatic synthetic approach that provided the amino alcohol (**4**)[7] (Scheme 1). Acylation with *p*-nitrobenzoyl chloride followed by fucosylation and deprotection afforded **2**. Compound **2** was derivatized with bromoacetic anhydride to afford **8**, which was then coupled with the thiol−PEG−DSPE (**9**)[8] in phosphate buffer (pH 7.2) to provide the desired product **3**.



In the present work, we chose a liposomal formulation which incorporated methoxypoly(ethylene glycol) (mPEG) derived phospholipids (mPEG−DSPE)[9] to minimize potential nonspecific interactions, and to provide the SLe^x containing liposomes

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⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1996.

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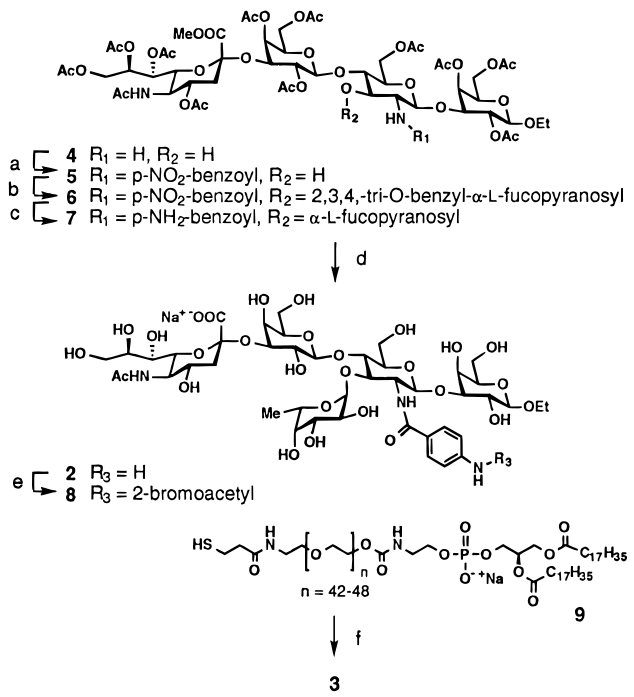
S0002-7863(95)04122-9 CCC: $12.00

Scheme 1[a]



[a] Key: (a) *p*-nitrobenzoyl chloride, $NaHCO_3$, CH_2Cl_2 (90%); (b) tri-*O*-benzyl-α/β-L-fucopyranosyl fluoride, $AgClO_4$, $SnCl_2$, TMU, CH_2Cl_2, 4 Å sieves (68%); (c) Pearlman's catalyst, hydrogen, ethanol, acetic acid (54%); (d) NaOMe, MeOH, water (76%); (e) bromoacetic anhydride, ethanol, water, $NaHCO_3$ (75%); (f) **9**, sodium phosphate buffer (0.1 M, pH = 7.2), EDTA (60%).

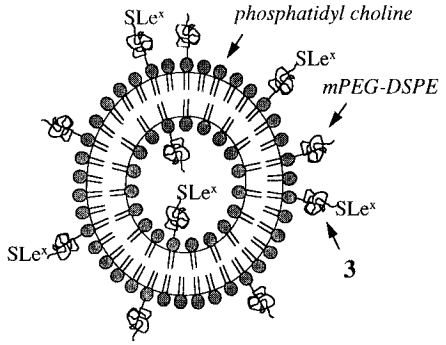


Figure 1. Schematic representation of PEG-derived liposomes containing **3**.

with a prolonged serum half-life (Figure 1).[5b] The SLe^x itself was appended onto the distal end of PEG−DSPE via the GlcN nitrogen[10] in order to extend the glycotope beyond the mPEG liposomal surface which would result after formulation. The liposomes were prepared by hydrating a lipid film of SLe^x−PEG−DSPE (**3**), mPEG−DSPE, phospholipids, and cholesterol and extruding the mixture through a series of pore-size membranes. Previous studies have shown that liposome vesicles prepared using similar conditions formed single bilayer vesicles.[11] This process resulted in the formation of SLe^x liposomes with an average particle diameter of 100 nm (92−111 nm). To ensure that the liposomes were not cleared rapidly from the circulation,[12] the liposomes were formulated to contain varied

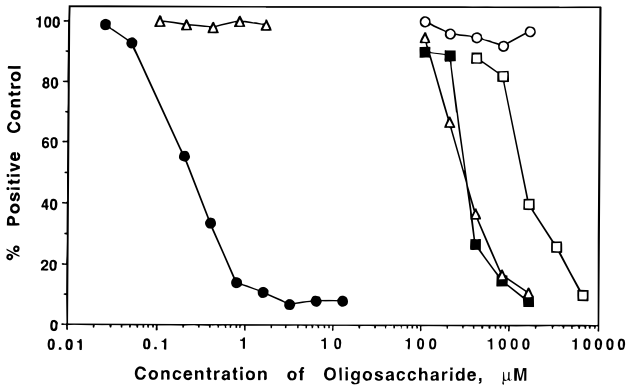


Figure 2. Inhibition of HL-60 binding to rsE-selectin by SLe^x−PEG liposomes.[15] Binding is expressed as the percentage of rsE-selectin binding in the absence of inhibitor. Each point is the average of duplicates, and this figure is the combination of two experiments. The compounds tested were (□) SLe^x pentasaccharide **1**; (■) SLe^x benzamide **2**; (●) SLe^x−PEG liposomes, 2 mol % **3**; (○) mPEG−DSPE control liposomes containing no SLe^x; (△) admixed compound **2** at several concentrations with mPEG−DSPE control liposomes.

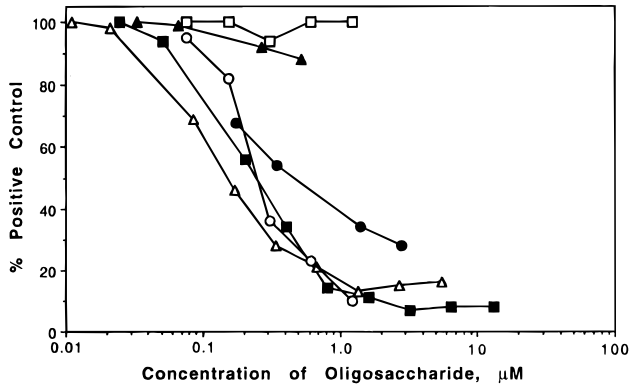


Figure 3. Inhibition of HL-60 binding to rsE-selectin by varied concentrations of **3** incorporated into PEG liposomes. Binding is expressed as the percentage of rsE-selectin binding in the absence of inhibitor. Each point is the average of duplicates. The concentrations tested were (□) 0 mol % **3**; (▲) 0.1 mol % **3**; (●) 0.5 mol % **3**; (△) 1.0 mol % **3**; (■) 2.5 mol % **3**; (○) 5.0 mol % **3**.

concentrations of **3** (0.1−5 mol %)[13] and mPEG−DSPE so that the overall PEG−lipid concentration remained at 5 mol %.

When tested (Figures 2 and 3), liposomal preparations that incorporated 0.5−5 mol % of **3** were all found to be greater than 5000-fold more potent than the native monomeric SLe^x oligosaccharide (**1**) and ∼750-fold more potent than the aglycon (**2**) as inhibitors of E-selectin mediated cellular adhesion (**1**, IC_{50} = 1.0 mM; **2**, IC_{50} = 0.15 mM; **3**, IC_{50} = 0.0002 mM).[3,14,15] The IC_{50} concentration is based on the total amount of SLe^x **2** present as part of **3** incorporated into the liposomes and does not account for the amount of internal SLe^x oligosaccharide unavailable for E-selectin interaction. Control lipo-

(10) In a separate study we found that aromatic substitutions on the GlcN nitrogen of SLe^x were well tolerated by E-selectin and enhanced the inhibitory potency of these compounds. Ramphal, J. Y.; Hiroshige, M.; Lou, B.; Gaudino, J. J.; Hayashi, M.; Chen, S. M.; Chiang, L. C.; Gaeta, F. C. A.; DeFrees, S. A. *J. Med. Chem.* **1996**, *39*, 1357.

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(13) A 5 mol % concentration of **3** is equivalent to a 0.5 mM concentration.

(14) The ELISA assays were carried out according to the procedure described previously.[7] Human soluble recombinant E-selectin was coated on plates, followed by addition of HL-60 cells and carbohydrates. After incubation, the plates were rinsed and the adhesion was determined by the cell lysis and myeloperoxidase method. IC_{50} was the concentration that inhibited cell adhesion by 50%. This method gave consistent results with 10% deviation.

(15) The *in vitro* result for the liposomal preparations of **3** are consistent with the *in vivo* results in which the liposomes were found to be substantially (>40-fold) more potent than the free SLe^x oligosaccharide in a cat myocardial infarction model. Murohara, T.; Margiotta, J.; Phillips, L. M.; Paulson, J. C.; DeFrees, S.; Zalipsky, S.; Guo, L. S. S.; Lefer, A. M. *Cardiovasc. Res.* **1995**, *30*, 965.

somes, liposomes formulated identically to the SLe^x liposomes but lacking **3**, did not inhibit E-selectin mediated cellular adhesion at the highest concentrations tested. Liposomal formulations containing concentrations of **3** below 0.5 mol % (Figure 3) were found to be less potent cell adhesion inhibitors and clearly indicated that, for this vesicle size, a critical amount of surface SLe^x was necessary to provide optimal multivalent inhibitory effects. Admixing the SLe^x benzamide ligand **2** with control mPEG−DSPE liposomes containing no SLe^x provided no increase in potency of the SLe^x ligand, clearly demonstrating that carbohydrate association with the liposome alone was not sufficient to provide an increased inhibitory effect. The amount of surface SLe^x in the 2 mol % liposomal formulations was determined by enzymatic desilylation and HPLC analysis to be about 60% of the total concentration, which would suggest that the IC_{50} for **3** would actually be ~0.0001 mM.

In conclusion, this study provides strong support for the concept that the interaction between E-selectin and SLe^x is multivalent and the multivalent effects require a minimal SLe^x concentration as defined by the liposome particle size and composition. While the exact nature and structure of the neutrophil glycoprotein oligosaccharides responsible for E-selectin adhesion is not known, further studies with defined SLe^x liposomal formulations should clarify the mechanism of E-selectin mediated cellular adhesion and provide insight into the design of potent antiadhesion therapeutics.

Experimental Section

Materials. Partially hydrogenated soybean phosphatidylcholine (PHSPC) was obtained from Lipoid (Ludwigshafen, Germany), and cholesterol was from Croda (Fullerton, CA). All reactions were monitored by thin layer chromatography carried out on 0.25 mm Whatman silica gel plates (60F-254) using UV light, anisaldehyde reagent, Dragendorff's reagent and phenol/H_2SO_4 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 (^{1}H 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. Analytical HPLC was performed on a Waters HPLC system using a Symmetry C-8 column (3.9−50 mm) and a mobile phase of 90% aqueous methanol containing 20 mM ammonium acetate (pH 5.5) with monitoring at 272 nm.

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranuronate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(6-*O*-acetyl-2-deoxy-2-(4-nitrobenzamido)-*β*-D-glucopyranosyl)-(1−3)-*O*-2,4,6-tri-*O*-*β*-D-galactopyranoside (5). The *p*-nitrobenzoyl chloride was added to a suspension of **4** (2.0 g, 1.5 mmol), $NaHCO_3$ (0.64 g, 7.6 mmol), and CH_2Cl_2 (20 mL) and stirred overnight. Additional $NaHCO_3$ (0.64 g, 7.6 mmol) and *p*-nitrobenzoyl chloride (0.25 g) was then added and stirred for 6 h. The mixture was filtered and the filtrate poured into saturated $NaHCO_3$ (30 mL) and water (10 mL) and extracted with CH_2Cl_2 (50 mL). The organic solution was dried (Na_2SO_4), concentrated, and chromatographed (silica, 35% acetone/CH_2Cl_2) to afford 1.95 g (90%) of **5**, R_f = 0.50 (silica, 35% acetone/CH_2Cl_2): MS (FAB) $C_{61}H_{81}N_3O_{37}$ calcd 1447, found 1580 (M + Cs^+). This product was used directly for the next step.

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranuronate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1−3)-*O*-)-[6-*O*-acetyl-2-deoxy-2-(4-nitrobenzamido)-*β*-D-glucopyranosyl]-(1−3)-*O*-2,4,6-tri-*O*-*β*-D-galactopyranoside (6). A suspension of 4 Å sieves (1.0 g), compound **5** (1.75 g, 1.22 mmol), 2,3,4-tri-*O*-benzyl-α/*β*-L-fucosyl fluoride[16] (3.18 g, 7.27 mmol), tetramethylurea (0.868 mL, 7.27 mmol), and CH_2Cl_2 (30 mL) was stirred for 4 h. The silver perchlorate (1.01 g, 4.88 mmol) and tin dichloride (0.922 g, 4.88 mmol) were then added and the reaction mixture stirred for 18 h. The reaction mixture was filtered and the filtrate washed with saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted again with CH_2Cl_2 (20 mL), and the combined organic layers were dried (Na_2SO_4), concentrated, and chromatographed (silica, 5% acetone/ethyl acetate) to afford 1.37 g (68%) of a white solid, R_f = 0.47 (silica, 5% acetone/ethyl acetate): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 8.6 Hz, 2 H, aromatic), 7.52 (d, J = 8.6 Hz, 2 H, aromatic), 7.16−7.33 (m, 15 H, aromatic), 6.60 (d, J = 6.8 Hz, 1 H, NH), 5.50−5.54 (m, 1 H), 5.43 (dd, J = 2.5, 8.4 Hz, 1 H), 5.33 (d, J = 7.9 Hz, 1 H), 5.09 (d, J = 10.2 Hz, 1 H), 5.03 (dd, J = 8.3, 10.2 Hz, 1 H, H-2 Gal), 4.55 (dd, J = 3.3, 10.0 Hz, 1 H, H-3 Gal), 4.43 (brt, J = 8.9 Hz, 1 H), 3.84 (s, 3 H, COOMe), 2.55 (dd, 1H, J = 4.4, 12.5 Hz, 1 H, H-3_{eq} sialic acid (SA)) 2.23 (s, 3 H, OAc), 2.17 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.07 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.86 (s, 3 H, OAc), 1.81 (s, 3 H, NHAc), 1.68 (dd, J = 12.5, 12.5Hz, 1 H, H-3_{ax} SA), 1.23(t, J = 7.1 Hz, 3 H, OCH_2CH_3), 1.18 (d, J = 6.4 Hz, 3 H, H-6 Fuc).

Ethyl (Methyl 5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-α-D-*glycero*-D-*galacto*-2-nonulopyranuronate)-(2−3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1−4)-*O*-(α-L-fucopyranosyl-(1−3)-*O*-)-[6-*O*-acetyl-2-deoxy-2-(4-nitrobenzamido)-*β*-D-glucopyranosyl]-(1−3)-*O*-2,4,6-tri-*O*-*β*-D-galactopyranoside (7). A solution of compound **6** (1.2 g, 0.643 mmol), ethanol (5 mL), and acetic acid (36 μL, 0.643 mmol) was degassed, and Pearlman's catalyst (0.1 g) was added. The suspension was placed under hydrogen (balloon) and stirred for 18 h. Additional Pearlman's catalyst (0.2 g) was then added and the suspension placed under hydrogen (balloon) and stirred for 48 h. The suspension was filtered and the filtrate concentrated and chromatographed (silica, 10% MeOH/CH_2Cl_2) to afford 0.545 g (54%) of a white solid, R_f = 0.19 (silica, 10% MeOH/CH_2Cl_2). The product was used directly for the next step.

Ethyl (Sodium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranuronate)-(2−3)-*O*-(*β*-D-galactopyranosyl)-(1−4)-*O*-(α-L-fucopyranosyl-(1−3)-*O*-)-[2-deoxy-2-(4-aminobenzamido)-*β*-D-glucopyranosyl]-(1−3)-*O*-*β*-D-galactopyranoside (2). A solution of **7** (0.522 g, 0.34 mmol), methanol (3 mL), and 20% NaOMe in methanol (50 μL) was stirred for 24 h. Water (1 mL) was then added and the solution stirred for 24 h. The solution was then neutralized to pH 7.0 with 1 N HCl, concentrated, and chromatographed (Biogel P-2, 20% ethanol/water) to afford 0.294 g (76%) of a white solid, R_f = 0.5 (silica, 1 M NH_4OAc/2-propanol): ^{1}H NMR (300 MHz, D_2O) δ 7.58 (d, J = 8.3 Hz, 2 H, aromatic), 6.81 (d, J = 8.3 Hz, 2 H, aromatic), 5.11 (d, J = 3.5 Hz, 1 H, H-1 Fuc), 4.82 (d, J = 7.8 Hz, 1 H, Glc), 4.55 (d, J = 7.7 Hz, 1 H, H-1 Gal), 4.32 (d, J = 8.0 Hz, 1 H, H-1 Gal), 4.14 (d, J = 3.2 Hz, 1 H, H-4 Gal), 4.10−3.42 (m, 29 H), 2.75 (dd, J = 4.4, 12.5 Hz, 1 H, H-3_{eq} SA), 2.02 (s, 3 H, NHAc), 1.77 (dd, J = 12.5, 12.5 Hz, 1 H, H-3_{ax} SA), 1.16 (t, 3 H, (m, 6H, OCH_2CH_3), 1.14 (d, J = 6.8 Hz, 1 H, H-6 Fuc).

Ethyl (Sodium 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-(2−3)-*O*-(*β*-D-galactopyranosyl)-(1−4)-*O*-[α-L-fucopyranosyl-(1−3)-*O*]-[2-(4-(2-bromoacetamido)benzamido)-2-deoxy-*β*-D-glucopyranosyl]-(1−3)-*O*-*β*-D-galactopyranoside (8). Bromoacetic anhydride (0.515 g, 1.98 mmol) was added to a solution of compound **2** (0.182 g, 0.165 mmol) in ethanol (3 mL), water (3 mL), and saturated $NaHCO_3$ (3 mL). The pH was maintained at 8.5 by addition of solid $NaHCO_3$, and after 30 min, the reaction mixture was concentrated. Chromatography (C-18 silica, water, then 5% MeOH/water) afforded 0.153 g (75%) of a white solid after lyophylization, R_f = 0.27 (silica, 80% 2-propanol/1 M NH_4OAc): ^{1}H NMR (D_2O) δ 7.78 (d, J = 8.6 Hz, 2 H), 7.61 (d, J = 8.7 Hz, 2 H), 5.13 (d, J = 3.9 Hz, 1 H, H-1 Fuc), 4.55 (d, J = 7.9 Hz, 1 H), 4.33 (d, J = 8.0 Hz, 1 H, H-1 Gal), 4.16 (d, J = 3.1 Hz, 1 H), 4.12−3.43 (m, 29 H), 2.76 (dd, J = 12.1, 4.4 Hz, 1 H, H-3 SA), 2.02 (s, 3H, NHAc), 1.79

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(dd, $J = 12.1, 12.1$ Hz, 1 H, H-3 SA), 1.15 (d, $J = 7.0$ Hz, 3 H, H-6 Fuc); MS (FAB) $C_{46}H_{69}N_3O_{29}BrNa$ calcd 1229.309, found 1206 [M $-$ $Na^+]^-$.

Thiol−PEG−DSPE (9). Tributylphosphine (40 μL, 0.16 mmol) was added to a suspension of PDP−PEG−DSPE[8] (150 mg, 0.052 mmol) in 2-propanol/water (1:4, 7 mL, containing 10 mM EDTA) and stirred overnight. The reaction mixture was lyophilized and the yellow solid washed with ether until no further color eluted. This yielded 130 mg (90%) of a white solid, which was used directly for the next step, $R_f = 0.59$ (silica, $CHCl_3/CH_3OH/H_2O$ 90:18:2).

Sialyl Lewis x−PEG−DSPE Conjugate (3). Compound **8** (66 mg, 0.054 mmol) was added to a solution of **9** (130 mg, 0.045 mmol) in sodium phosphate buffer (0.1 M, pH 7.2, containing 10 mM EDTA) and stirred overnight. The solution was dialyzed (25 000 MW cutoff) against water and lyophilized to yield 90 mg (60%) of a white solid, $R_f = 0.37$ (silica, $CHCl_3/CH_3OH/H_2O$, 75:36:6). An analytically pure sample was obtained by silica gel chromatography using an increasing gradient of methanol (from 0 to 80%) in chloroform: ^{1}H NMR (CD_3-OD, 500 MHz) δ 8.09 (bs, 1 H, NH), 7.84 (d, $J = 8.3$ Hz, 2 H, phenyl), 7.70 (d, $J = 8.4$ Hz, 2 H, phenyl), 5.21 (bs, 1 H, NH), 5.06 (d, $J = 3.7$ Hz, 1 H, H-1 Fuc), 4.90 (m, 1 H, H-5 Fuc), 4.54 (d, $J = 7.8$ Hz, 1 H, H-1 GlcN), 4.43 (dd, $J = 3, 12$ Hz, 1 H), 4.21−4.16 (m, 5 H), 4.07−4.04 (m, 4 H), 4.00−3.85 (m, 11 H), 3.78−3.46 (m), 3.63 (bs, PEG), 3.38−3.30 (m), 2.93 (dd, $J = 7.2, 7.2$ Hz, 2 H), 2.87 (dd, $J = 3, 11$ Hz, 1 H, H-3_{eq}-sialic acid), 2.56 (dd, $J = 7.2, 7.2$ Hz, 2 H), 2.31 (m, 4 H), 2.01 (s, 3 H, NHAc), 1.73 (t, $J = 12$ Hz, 1 H, H-3_{ax}-sialic acid), 1.61 (bm, 4 H), 1.29 (bs, 56 H), 1.19 (t, $J = 7$ Hz, 3 H, CH_2CH_3), 1.15 (d, $J = 6.7$ Hz, 3 H, H-6 Fuc), 0.90 (bt, 6 H); HPLC (90% methanol in 20 mM NH_4OAc, pH 5.5) R_t 5.8 min (>98%).

Liposome Preparation. The SLe^x liposomes were formulated to contain 0.1−5 mol % of compound **3** by mixing mPEG−DSPE with SLe^x−PEG−DSPE (**3**) so that the total PEG-derived lipids in the liposomes remained at 5 mol %. All liposomes were prepared by mixing chloroform or methanol solutions of the different lipids (PHSPC/cholesterol/mPEG−DSPE and/or compound **3** in a ratio of 55:40:5) in a round bottom flask and concentrating by rotoevaporation to produce a dried lipid film. Hydration of the film with 10 mM sodium phosphate buffer containing 140 mM NaCl, pH 7.0, produced large multilamellar vesicles, which were passed repeatedly under pressure through 0.4-, 0.1- and 0.05-μm pore size polycarbonate membranes until the average size distribution was approximately 100 nm in diameter. The particle size of the liposomes was determined by dynamic light scattering (Coulter, Model N4 MD) and ranged from 92 to 111 nm with an average particle size of 98 nm. The content of compound **3** was determined by reverse-phase HPLC (Symmetry C-8, Waters Associates) using a mobile phase of 90% aqueous methanol containing 20 mM ammonium acetate, pH 5.5, and monitoring the product of 272 nm. Greater than 97% of the amount of compound **3** used in this formulation process was accounted for by this HPLC analysis (see supporting information).

The control liposomes were composed of PHSPC, a cholesterol, and mPEG−DSPE at a molar ratio of 55:40:5, respectively, and were prepared as described above.

The amount of SLe^x on the surface of the liposomes was determined by treating the liposomes (0.25 mL, 0.4 mg/mL SLe^x, 2 mol % SLe^x−PEG−DSPE) with sialidase (3 units) (vibrio cholera, Oxford Glycosystems) in 50 mM histidine (pH 6.5) containing 4 mM $CaCl_2$ and 0.15 mg/mL BSA and incubating at 37 °C for 18 h. Aliquots were drawn and then analyzed by HPLC (see supporting information) to determine the amount of SLe^x−PEG−DSPE (**3**) that remained. The 2 mol % liposomes were found to contain 60% of the sialic acid from SLe^x on the surface of the liposome.

Acknowledgment. The authors thank Joshua Gittelman, Azar Najafi, and Nasreen Mullah from Sequus Pharmaceuticals for their help with the preparation of the liposomes and William Way from Cytel Corporation for his help with the E-selectin assay.

Supporting Information Available: 1D spectra (^{1}H and ^{13}C NMR) of **3**, COESY of **3**, and physical data (10 pages). Ordering information is given on any current masthead page.

JA954122G