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Ligand Recognition by E- and P-Selectin: Chemoenzymatic Synthesis and Inhibitory Activity of Bivalent Sialyl Lewis x Derivatives and Sialyl Lewis x Carboxylic Acids

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Described is the preparation of five sLe^x dimers and five sLe^x carboxylic acids by coupling chemoenzymatically synthesized amino-substituted sialyl Lewis x (sLe^x) derivative **4** to homobifunctional cross-linkers **20**−**24** of varying chain length. **20**−**24** were obtained by alkylating low-molecular-weight oligoethylene glycols with *tert*-butyl bromoacetate and subsequent transformation of the di-*tert*-butyl esters into disuccinimide esters. The products were assayed for inhibition against binding of a sLe^a-polymer to immobilized E- and P-selectin. In the E-selectin assay all dimers had lower IC$_{50}$ values than the sLe^x monomer. The results show that comparable binding enhancements can be obtained with linkers of completely different length and rigidity. In the P-selectin assay four of the five sLe^x carboxylic acids displayed significantly improved inhibitory potency. The lowest IC$_{50}$ value was observed for the compound with the shortest spacer between the sLe^x moiety and the additional carboxylate, being ca. 20−40 times more potent than unmodified sLe^x. These findings should be of importance for the design of new multivalent forms of sLe^x as well as sLe^x mimetics as high-affinity selectin ligands.

The leukocyte adhesion to vascular endothelium during the inflammatory response is a multistep process. The initial events, the tethering and rolling of leukocytes, are mediated by the selectins, a family of calcium-dependent carbohydrate-binding cell adhesion molecules.[1,2] The tetrasaccharides sialyl Lewis x (sLe^x),[3] sialyl Lewis a (sLe^a),[4] and sulfated derivatives thereof[5] have been identified as minimal carbohydrate epitopes recognized by the selectins. However, the binding of monovalent sLe^x to the selectins is weak with K_D values in the low-millimolar range.[3h,6,7] In contrast, the physiological ligands of the selectins have been shown to bind with high affinity specifically to individual selectins. The molecular details responsible for this multivalent tight binding are not known.

The ligands of L-selectin (GlyCAM-1,[8] CD34,[9] and MAdCAM-1[10]) and P-selectin (PSGL-1[11]) are mucin-like molecules with densely clustered O-linked oligosaccharides. ESL-1,[12] a ligand of E-selectin, carries N-linked oligosaccharides and has five potential glycosylation sites. Possible contributing factor to avidity may be a multivalent interaction of the highly glycosylated glycoprotein ligands with the selectins. Also it might be possible that sLe^x is only part of a bigger recognition system in which additional carbohydrate residues or residues from the protein scaffold either have direct favorable contacts with the selectins or indirectly influence the sLe^x conformation, resulting in a tight and specific binding. Attachment of a reduced disaccharide to the reducing end of a bivalent sLe^x oligosaccharide, for example, appeared to increase the affinity of the glycan to L-selectin.[13] Patel

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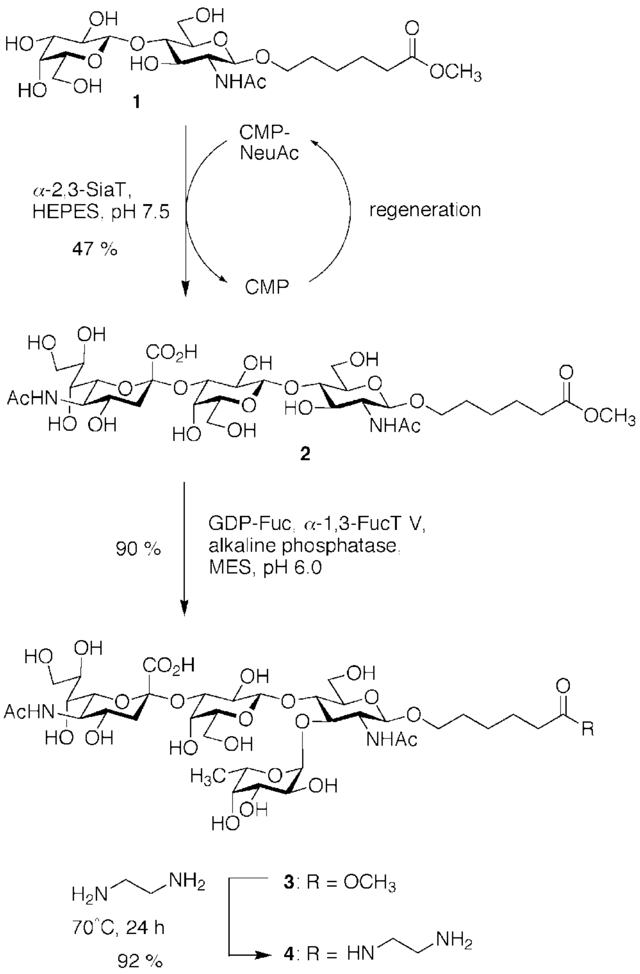
et al. isolated tetraantennary endogenous protein-associated carbohydrates bearing a sialylated dimeric Lewis x epitope on one branch which bind with high affinity to E-selectin even when monovalent, under conditions where sLe^{x} itself does not bind.[14] Sulfation of at least one of three specific tyrosine residues of the mature PSGL-1 protein has been demonstrated to be a requirement for high-avidity binding by P-selectin[15] and led to a two-site model of P-selectin binding to PSGL-1 in which one site recognizes sLe^{x}-like oligosaccharides while the other site interacts with the anionic tyrosine sulfate.[16]

To evaluate the theory of multivalent interactions, several ligands containing two,[13,17−19] three,[18,20,21] four,[22] or many[23−28] sLe^{x} moieties have been synthesized and tested in the past. Bivalent sLe^{x} derivatives are especially suited to explore the required structural arrangement of the sLe^{x} groups for a possible multipoint binding to the selectins. Up to now, only a few studies have been undertaken in which the spacing between two sLe^{x} moieties has been varied systematically.[17b,18−20] The most efficient sLe^{x} dimers thus obtained inhibited the adhesion of HL-60 cells to immobilized E-selectin with IC$_{50}$ values 5−6 times lower than those of monomeric sLe^{x}. In these compounds the sLe^{x} groups were connected to the 3- and 6-positions of galactose[17a,b] and to the hydroxyl groups of 1,3-benzenedimethanol,[19] respectively, thus separating the GlcNAc anomeric carbons of the two sLe^{x} groups by 7−8 bonds. Since a bidentate binding can result in a more distinct decrease of IC$_{50}$ values,[29] we decided to prepare a series of sLe^{x} dimers with longer spacers, thus making a possible cross-linking of adjacent selectin molecules easier. Several reasons were decisive to use oligoethylene glycol-based linkers in the sLe^{x} dimers. Such linkers are water-soluble, are conformationally flexible, and do not tend to form aggregates in polar solvents due to hydrophobic interactions.[30] Furthermore a series of oligoethylene glycols of defined length is available from commercial sources.

Scheme 1



Here we present the synthesis of the bivalent sLe^{x} derivatives **25**, **27**, **29**, **31**, and **33** containing oligoethylene glycol-based linkers with varying length and analysis of their E-selectin inhibition activities. Also described are the sLe^{x} carboxylic acids **26**, **28**, **30**, **32**, and **34**, which were obtained as side products during preparation of the dimers, and their ability to bind to immobilized P-selectin.

Results and Discussion

The sLe^{x} dimers were synthesized following a convergent strategy by coupling the chemoenzymatically prepared amino-functionalized sLe^{x} derivative **4** with a series of bisactivated oligoglycolic acids (**20**−**24**).

Chemoenzymatic Synthesis of SLe^{x} Amine 4. The *N*-acetyllactosamine (LacNAc) derivative **1**[31] was sialylated to trisaccharide **2** catalyzed by α-2,3-sialyltransferase (α-2,3-SiaT) with in situ regeneration of CMP-*N*-acetylneuraminic acid (CMP-NeuAc)[32] (Scheme 1). In the

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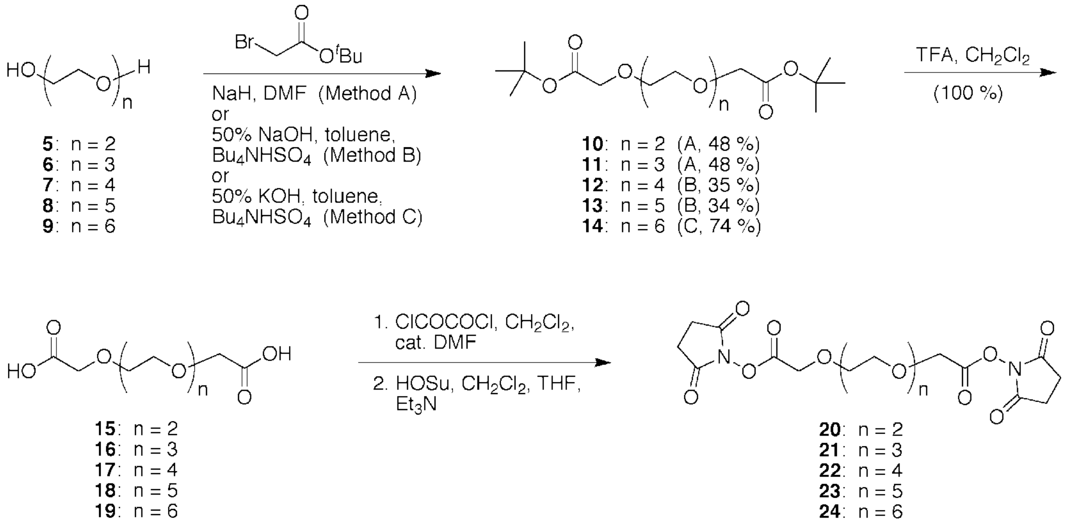
Scheme 2



5: n = 2
6: n = 3
7: n = 4
8: n = 5
9: n = 6

10: n = 2 (A, 48 %)
11: n = 3 (A, 48 %)
12: n = 4 (B, 35 %)
13: n = 5 (B, 34 %)
14: n = 6 (C, 74 %)

15: n = 2
16: n = 3
17: n = 4
18: n = 5
19: n = 6

20: n = 2
21: n = 3
22: n = 4
23: n = 5
24: n = 6

next step a fucose residue was transferred from guanosine 5′-diphospho-β-L-fucose (GDP-Fuc) to **2** under α-1,3-fucosyltransferase V (α-1,3-FucT V)[33] catalysis to give sLe^x derivative **3** in 90% yield after Bio-Gel chromatography. Alkaline phosphatase was added to hydrolyze produced GDP and thus prevent product inhibition.[34] The reaction was carried out at a pH of 6.0. According to studies of our group, this value is close to the pH optimum of the enzyme and GDP-Fuc is still stable enough.[31b] It is also worth mentioning that alkaline phosphatase cleaves GDP efficiently at this pH, whereas it does not affect GDP-Fuc. Acid phosphatase is not suitable because it also degrades GDP-Fuc besides GDP. Finally, it turned out that MES buffer is superior to the widely used cacodylic buffer because enzyme activity is preserved over a longer period of time. Treatment of **3** with ethylenediamine then gave the new building block **4** in high yield.

Synthesis of Homobifunctional Linkers. Starting materials for the synthesis of the bisactivated cross-linkers were the commercially available oligoethylene glycols **5**−**9** (Scheme 2). Succinimide esters were selected for activation to exclusively allow attachment to the amino group of **4**. The conversion of oligoethylene glycols into the corresponding dicarboxylic acids by HNO$_3$[35] and electrochemical[36] oxidation, respectively, has been reported. However, separation of the products from equally polar contaminants was difficult. Therefore we decided to alkylate the diols with *tert*-butyl bromoacetate giving rise to the nonpolar di-*tert*-butyl esters **10**−**14** which were conveniently separated and purified by silica gel column chromatography. Furthermore, this chain elongation procedure makes longer linkers accessible as compared to the oxidation process. Tetra- (**7**) and pentaethylene glycol (**8**) were conveniently alkylated in acceptable yields under phase-transfer catalysis (PTC) (method B, Scheme 2) to give **12** and **13**, respectively. For di- (**5**) and triethylene glycol (**6**) the PTC reaction was not applicable. Here, best results were obtained by deprotonation with sodium hydride in DMF and addition of the resulting mixture to a flask containing *tert*-butyl bromoacetate (method A).[37] However, both methods gave unsatisfactory low yields (<10%) with hexaethylene glycol (**9**). For this chain length it turned out to be crucial to switch over from a sodium salt as base to the corresponding potassium compound. Thus PTC reaction with aqueous KOH gave **14** in a yield of 75%. Treatment of the di-*tert*-butyl esters **10**−**14** with TFA quantitatively gave the dicarboxylic acids **15**−**19** which were further transformed into the disuccinimide esters **20**−**24** via the corresponding diacid chlorides.

Synthesis of SLe^x Dimers and SLe^x Carboxylic Acids. For the preparation of the sLe^x dimers, building block **4** was dissolved in DMF and triethylamine (4 equiv) and the corresponding disuccinimide ester (0.5−0.75 equiv) was slowly added until TLC analysis indicated complete consumption of **4** (Scheme 3). Bio-Gel chromatography gave the dimers **25**, **27**, **29**, **31**, and **33** in 54−68% yield as well as the sLe^x carboxylic acids **26**, **28**, **30**, **32**, and **34** (14−28%), stemming from coupling of **4** to the monohydrolyzed linkers, in pure form. The structures of the sLe^x derivatives were confirmed by ^{1}H and ^{13}C NMR spectroscopy and by mass spectrometry using electrospray ionization (ESIMS). In the ^{1}H and ^{13}C NMR spectra of the dimers, single sets of signals for both sLe^x

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Scheme 3

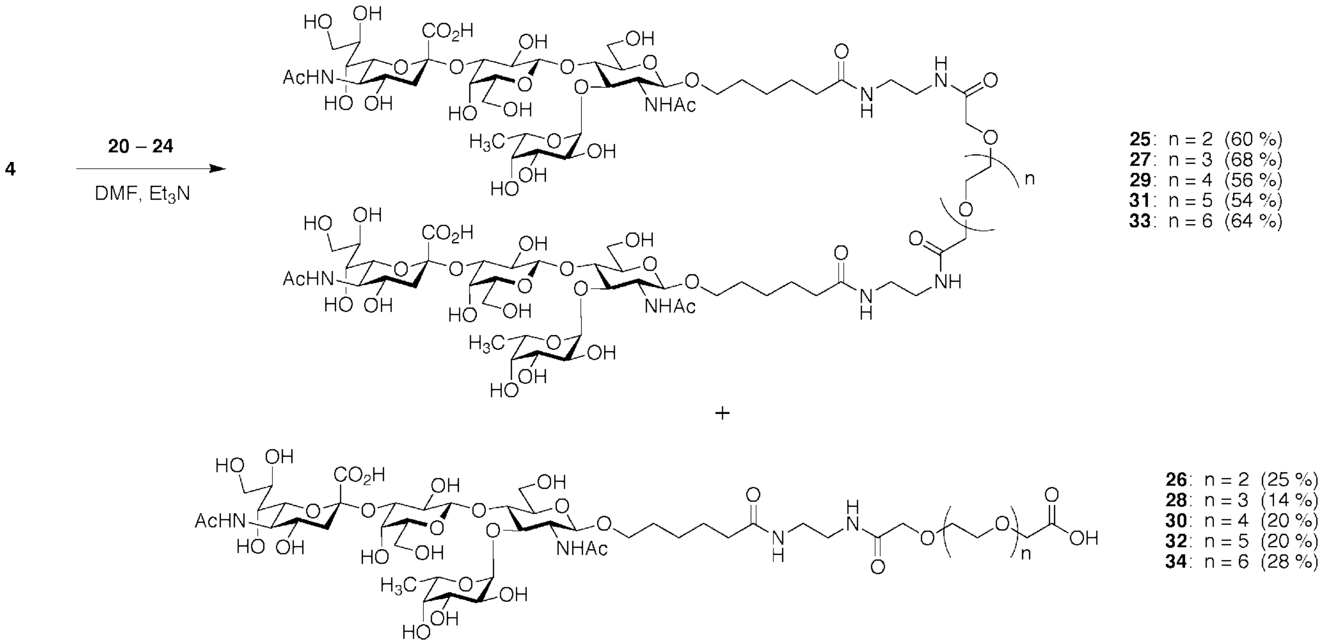

Table 1. Inhibitory Potencies of sLe^x Derivatives on sLe^a-polymer Binding to Immobilized E- and P-Selectin

compd	IC$_{50}$ (μM) E-selectin	IC$_{50}$ (μM) P-selectin
dimers		
25	228	>300
27	70	>300
29	179	
31	176	
33	81	>300
acids		
26		197
28		281
30		232
32		935
34		>1000
sLe^x monomer^a	393	>3000

^{a} 3′-Sialyl Lewis x (sLe^x-OH), obtained from Oxford Glyco Systems.

groups were obtained. The chemical shifts were in excellent agreement with those of the tetrasaccharide moieties of **3**, **4**, and the sLe^x acids suggesting that the conformation of the sLe^x groups is the same in all these compounds.

Inhibition Activities of SLe^x Dimers and SLe^x Carboxylic Acids. The inhibitory potency of the sLe^x dimers and sLe^x carboxylic acids on binding of a biotinylated sLe^a-polyacrylamide glycoconjugate (sLe^a-polymer) to immobilized E- and P-selectin was assayed according to a published procedure.[38] Briefly, selectin mouse Cκ fusion protein was immobilized onto microtiter plates and incubated with a complex of sLe^a-polymer with streptavidin–peroxidase in the presence of different concentrations of the synthetic sLe^x derivatives. Bound complex was detected by the peroxidase reaction. The concentrations of the inhibitors required to block 50% of sLe^a-polymer binding to the selectins (IC$_{50}$) are summarized in Table 1.

With IC$_{50}$ values ranging from 70 to 228 μM, all sLe^x dimers inhibited the sLe^a-polymer binding to immobilized E-selectin more efficiently than monomeric sLe^x (IC$_{50}$ = 393 μM). Dimer **27** (n = 3, IC$_{50}$ = 70 μM) is the most potent of all bivalents (5.6 times more effective than the monomer). Interestingly, **33** (n = 6), having a significantly longer oligoethylene glycol linker, binds with similar affinity (IC$_{50}$ = 81 μM), whereas the dimers **29** and **31** (n = 4 and 5, respectively) are only about 2 times more potent than the sLe^x monomer. Clearly, these results do not allow to identify a specific spacing between two sLe^x groups being optimal for enhanced inhibitory potency. Compared with earlier observations,[17a,b,19] it rather seems to be possible to generate comparable binding improvement with linkers of completely different length and rigidity.

The sLe^x carboxylic acids were assayed for their potency to inhibit sLe^a-polymer binding to immobilized P-selectin. We were interested if an additional negatively charged group connected to sLe^x via a linker of suitable length results in an enhanced binding affinity to P-selectin according to the two-site model.[16] Indeed, four out of the five sLe^x acids (**26**, **28**, **30**, and **32**) displayed significantly lower IC$_{50}$ values than unmodified sLe^x.[39] Interestingly, with decreasing length of the side chain carrying the negative charge the inhibitory potency increased. Assuming a two-point binding of the sLe^x epitope and the carboxylate, this behavior corresponds to the smaller entropy loss upon binding of a short-chain derivative compared to a long-chain derivative. Currently we investigate if sLe^x carboxylic acids with even shorter linkers show a further improved inhibitory potency.

Conclusion

A series of sLe^x dimers and sLe^x carboxylic acids have been prepared by cross-linking chemoenymatically synthesized sLe^x amine **4** with homobifunctionalized oligo-

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[38] Weitz-Schmidt, G.; Stokmaier, D.; Scheel, G.; Nifant′ev, N. E.; Tuzikov, A. B.; Bovin, N. V. *Anal. Biochem.* **1996**, *238*, 184–190.

[39] Unmodified sLe^x is inactive in the P-selectin assay up to a concentration of 3 mM. An equilibrium binding constant (K_D) of 7.8 mM has been determined NMR spectroscopically for the binding of sLe^x methyl glycoside to P-selectin.[7]

ethylene glycols of varying chain length (**20**−**24**). The products were assayed for inhibitory potency on binding of a sLe^a-polymer to immobilized E- and P-selectin. In the E-selectin assay all dimers had lower IC$_{50}$ values than the sLe^x monomer. Interestingly, the highest binding enhancements obtained (5.6 and 4.9 times, respectively) are similar to previous studies in which two sLe^x moieties were connected by significantly shorter and more rigid linkers resulting in 5−6 times more active dimers.[17a,b,19] Obviously it is possible to obtain the same binding enhancement with completely different spacers. Four of the five sLe^x carboxylic acids which were obtained as side products during the cross-linking reaction displayed significantly improved inhibitory potency in the P-selectin assay. The lowest IC$_{50}$ value was observed for the compound with the shortest side chain (**26**, $n = 2$) thus being ca. 20−40 times[39] more potent than unmodified sLe^x. It is noted that the affinity may depend on the assay system. These findings, however, should be of importance for the design of new multivalent forms of sLe^x as well as sLe^x mimetics as high-affinity selectin ligands.

Experimental Section

General Methods. LacNAc derivative **1**[31] and monoammonium GDP-Fuc[40] were prepared according to published procedures. Trisodium phosphoenolpyruvate (PEP·3Na), CTP, ATP, nucleoside monophosphate kinase (NMK), pyruvate kinase (PK), pyrophosphatase (PPase), and alkaline phosphatase (type VII-N, from bovine intestinal mucosa, P-2276) were purchased from Sigma (St. Louis, MO). Anhydrous DMF was purchased from Aldrich (Milwaukee, WI). CMP-NeuAc synthetase, α-2,3-sialyltransferase, and α-1,3-fucosyltransferase V were a kind donation from Cytel (San Diego, CA). Dichloromethane and triethylamine were distilled from calcium hydride. Flash chromatography (FC) was performed on Mallinckrodt silica gel 60 (230−400 mesh). Analytical thin-layer chromatography was performed using silica gel 60 F$_{254}$ precoated glass plates from Merck (Darmstadt, Germany); compound spots were visualized by quenching of fluorescence and/or by charring after treatment with cerium molybdophosphate. Size-exclusion chromatography was performed on Bio-Gel P-2 gel (fine) and Bio-Gel P-4 gel (fine) (Bio-Rad Laboratories, Hercules, CA). NMR spectra were recorded on Bruker AM-250, AMX-400, or AMX-500 spectrometers. ^{1}H NMR chemical shifts are referenced to residual protic solvent (CDCl$_3$, $\delta_H = 7.26$; D$_2$O, $\delta_H = 4.80$) or the internal standard TMS ($\delta_H = 0.00$). ^{13}C NMR chemical shifts are referenced to the solvent signal (CDCl$_3$, $\delta_C = 77.0$) or to DMSO-d_6 ($\delta_C = 39.5$) as external standard. High-resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB) method in a *m*-nitrobenzyl alcohol matrix doped with NaI or CsI.

5-(Methoxycarbonyl)pentyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranoside (2). A solution of the lactosamine derivative **1**[31] (395 mg, 0.77 mmol), NeuAc (255 mg, 0.83 mmol), PEP·3Na (452 mg, 1.92 mmol), MgCl$_2$·6H$_2$O (32 mg, 0.16 mmol), MnCl$_2$·4H$_2$O (5 mg, 0.04 mmol), KCl (12 mg, 0.16 mmol), CTP (40 mg, 0.08 mmol), ATP (4.3 mg, 0.008 mmol), and mercaptoethanol (54 μL) in HEPES buffer (200 mM, pH 7.5) (5.6 mL) was adjusted with 1 N NaOH to pH 7.5, and the enzymes NMK (5 U), PK (200 U), PPase (20 U), CMP-NeuAc synthetase (0.9 U), and α-2,3-sialyltransferase (0.16 U) were added to the solution. The mixture was gently stirred under an argon atmosphere at room temperature for 5 days. The mixture was concentrated, and the residue was chromatographed on silica gel (EtOAc/MeOH/0.02% aqueous CaCl$_2$, 5:2:1) to give recovered **1** (140 mg, 35%) and the title compound (393 mg, 47%): ^{1}H NMR (400 MHz, D$_2$O) δ 4.53 (d, 1H, $J = 7.9$ Hz), 4.49 (d, 1H, $J = 7.5$ Hz), 4.09 (dd, 1H, $J = 3.1$, 9.9 Hz), 3.97 (dd, 1H, $J = 1.9$, 12.0 Hz), 3.93 (d, 1H, $J = 3.1$ Hz), 3.91−3.80 (m, 5H), 3.74−3.52 (m, 13H), 3.67 (s, 3H), 2.73 (dd, 1H, $J = 4.6$, 12.6 Hz), 2.37 (t, 2H, $J = 7.4$ Hz), 2.01 (s, 3H), 2.00 (s, 3H), 1.78 (t, 1H, $J = 12.6$ Hz), 1.60−1.50 (m, 4H), 1.35−1.26 (m, 2H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 177.6, 175.1, 174.4, 174.0, 102.6, 101.2, 99.9, 78.3, 75.5, 75.2, 74.8, 72.9, 72.5, 71.8, 70.3, 69.5, 68.4, 68.1, 67.5, 62.6, 61.1, 60.1, 55.1, 52.1, 51.7, 39.7, 33.7, 28.3, 24.7, 24.0, 23.6, 22.2; HRMS (FAB+, NBA/CsI) calcd for $C_{32}H_{53}N_2O_{21}Cs$ $(M - H + 2Cs^+)$ *m/e* 1067.1249, found 1067.1261.

5-(Methoxycarbonyl)pentyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-*β*-D-glucopyranoside (3). To a mixture of compound **2** (76 mg, 94.7 μmol), monoammonium GDP-Fuc (85 mg, 140 μmol), MnCl$_2$·4H$_2$O (1 M in H$_2$O) (280 μL, 280 μmol), MES buffer (100 mM, pH 6.0) (7 mL), and water (6.4 mL) were added α-1,3-fucosyltransferase V (324 μL, 700 mU) and alkaline phosphatase (20 μL, 100 U). The mixture was gently shaken at 37 °C for 5 days, filtered, and evaporated. Purification by size-exclusion chromatography (Bio-Gel P-2, 2.5 × 70 cm, 50 mM NH$_4$HCO$_3$) gave **3** (81 mg, 90%) as a white solid after lyophilization: R_f 0.25 (*i*-PrOH/1 M NH$_4$OAc, 4:1); ^{1}H NMR (500 MHz, D$_2$O) δ 5.06 (d, 1H, $J = 4.0$ Hz), 4.48 (d, 1H, $J = 7.8$ Hz), 4.47 (d, 1H, $J = 8.3$ Hz), 4.05 (dd, 1H, $J = 3.2$, 9.8 Hz), 3.97 (dd, 1H, $J = 2.3$, 12.3 Hz), 3.92−3.79 (m, 10H), 3.74 (d, 1H, $J = 3.5$ Hz), 3.67−3.51 (m, 10H), 3.65 (s, 3H), 3.50 (dd, 1H, $J = 7.8$, 9.8 Hz), 2.72 (dd, 1H, $J = 4.6$, 12.4 Hz), 2.35 (t, 2H, $J = 7.5$ Hz), 1.99 (s, 3H), 1.98 (s, 3H), 1.76 (t, 1H, $J = 12.2$ Hz), 1.60−1.49 (m, 4H), 1.32−1.25 (m, 2H), 1.13 (d, 3H, $J = 6.7$ Hz); ^{13}C NMR (125 MHz, D$_2$O) δ 178.3, 175.8, 174.9, 174.3, 102.4, 101.7, 100.2, 99.4, 76.4, 76.0, 75.64, 75.59, 74.1, 73.7, 72.7, 72.5, 71.1, 70.0, 69.9, 68.94, 68.87, 68.5, 68.0, 67.4, 63.4, 62.2, 60.4, 56.6, 52.8, 52.4, 40.4, 34.4, 29.0, 25.4, 24.7, 23.0, 22.8, 16.0; HRMS (FAB+, NBA/CsI) calcd for $C_{38}H_{64}N_2O_{25}Cs$ $(M + Cs^+)$ *m/e* 1081.2853, found 1081.2889.

5-[[(2-Aminoethyl)amino]carbonyl]pentyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosylonic acid)-(2→3)-*β*-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-*β*-D-glucopyranoside (4). A mixture of compound **3** (52 mg, 54.8 μmol) and ethylenediamine (3 mL) was stirred under argon at 70 °C for 24 h. The mixture was evaporated, and ethanol (2 × 3 mL) and toluene (2 × 3 mL) were evaporated from the remainder. Size-exclusion chromatography (Bio-Gel P-2, 2.5 × 70 cm, 50 mM NH$_4$HCO$_3$) gave **4** (49.5 mg, 92%) as a white solid after lyophilization: R_f 0.37 (*i*-PrOH/1 M NH$_4$OAc, 2:1); ^{1}H NMR (400 MHz, D$_2$O) δ 5.08 (d, 1H, $J = 3.9$ Hz), 4.50 (d, 1H, $J = 7.8$ Hz), 4.49 (d, 1H, $J = 8.1$ Hz), 4.06 (dd, 1H, $J = 2.9$, 9.8 Hz), 4.01−3.79 (m, 11H), 3.76 (d, 1H, $J = 3.1$ Hz), 3.71−3.49 (m, 11H), 3.45 (t, 2H, $J = 6.1$ Hz), 3.08 (t, 2H, $J = 6.0$ Hz), 2.75 (dd, 1H, $J = 4.6$, 12.5 Hz), 2.26 (t, 2H, $J = 7.4$ Hz), 2.01 (s, 3H), 2.00 (s, 3H), 1.77 (t, 1H, $J = 12.1$ Hz), 1.60−1.50 (m, 4H), 1.34−1.26 (m, 2H), 1.15 (d, 3H, $J = 6.6$ Hz); ^{13}C NMR (100 MHz, D$_2$O) δ 180.3, 177.5, 176.6, 176.3, 104.1, 103.5, 102.1, 101.1, 78.1, 77.7, 77.4, 77.3, 75.8, 75.4, 74.3, 72.9, 71.7, 71.6, 70.8, 70.5, 70.1, 69.8, 69.1, 65.0, 64.0, 62.1, 58.3, 54.1, 42.2, 41.6, 39.7, 38.0, 30.8, 27.2, 24.7, 24.5, 17.7; ESIMS (H$_2$O, neg) calcd for $C_{39}H_{67}N_4O_{24}$ $(M - H^+)$ *m/e* 975, found 975.

General Procedures for the Alkylation of Glycols. Method A. The glycol (5 mmol) was added to a stirred suspension of NaH (95%) (278 mg, 11 mmol) in anhydrous DMF (25 mL). After 2 h at room temperature, the mixture was slowly transferred via syringe to a cooled (0 °C) flask containing *tert*-butyl bromoacetate (2.95 mL, 20 mmol) using an additional 10 mL of DMF to achieve a complete transfer and stirred for 30 min at 0 °C and for 2 h at room temperature. After addition of a few milliliters of saturated NH$_4$Cl solution, the mixture was evaporated and distributed between ethyl acetate and saturated NH$_4$Cl solution. The organic layer was washed with saturated NH$_4$Cl solution, saturated NaHCO$_3$ solution, and brine, dried (Na$_2$SO$_4$), and evaporated.

Method B. A mixture of 50% aqueous NaOH (15 mL), toluene (15 mL), and the glycol (5 mmol) was cooled to 0 °C,

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[40] Wittmann, V.; Wong, C.-H. *J. Org. Chem.* **1997**, *62*, 2144−2147.

and n-Bu_4NHSO_4 (3.73 g, 11 mmol) and *tert*-butyl bromoacetate (2.95 mL, 20 mmol) were added with vigorous stirring. After 10 min, the two-phase system was allowed to warm to room temperature and stirred for another 30 min. Water (100 mL) was added, and the organic layer was washed with saturated NH_4Cl solution, saturated $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and evaporated.

Method C. The procedure was identical to method B except that NaOH was replaced by KOH.

3,6,9-Trioxaundecane-1,11-dioic Acid Di-*tert*-butyl Ester (10). Diethylene glycol (475 μL, 5 mmol) was alkylated according to the general procedure (method A) and purified by FC (120 g of silica, toluene/ethyl acetate, 2:1) to give **10** (808 mg, 48%) as a colorless oil: R_f 0.34 (toluene/ethanol, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (4H, s, 2 $CH_2C(O)$), 3.74−3.70 (8H, m, 2 OCH_2CH_2O), 1.48 (18H, s, 2 t-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.48 (2 C═O), 81.29 (2 $C(CH_3)_3$), 70.52, 70.44, 68.84, 27.92 (2 $C(CH_3)_3$); HRMS (FAB+, NBA/NaI) calcd for $C_{16}H_{30}O_7Na$ (M + Na$^+$) m/e 357.1889, found 357.1898.

3,6,9,12-Tetraoxatetradecane-1,14-dioic Acid Di-*tert*-butyl Ester (11). Triethylene glycol (667 μL, 5 mmol) was alkylated according to the general procedure (method A) and purified by FC (70 g of silica, first toluene/ethyl acetate, 1:1, then ethyl acetate) to give **11** (907 mg, 48%) as a colorless oil: R_f 0.22 (toluene/ethanol, 9:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.02 (4H, s, 2 $CH_2C(O)$), 3.73−3.67 (12H, m, 3 OCH_2CH_2O), 1.48 (18H, s, 2 t-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.60 (2 C═O), 81.43 (2 $C(CH_3)_3$), 70.63, 70.51, 68.96, 28.03 (2 $C(CH_3)_3$); HRMS (FAB+, NBA/NaI) calcd for $C_{18}H_{34}O_8Na$ (M + Na$^+$) m/e 401.2151, found 401.2165.

3,6,9,12,15-Pentaoxaheptadecane-1,17-dioic Acid Di-*tert*-butyl Ester (12). Tetraethylene glycol (863 μL, 5 mmol) was alkylated according to the general procedure (method B) and purified by FC (60 g of silica, first toluene/ethyl acetate, 1:1, then toluene/ethyl acetate/ethanol, 10:10:1) to give **12** (742 mg, 35%) as a colorless oil: R_f 0.26 (toluene/ethanol, 9:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.03 (4H, s, 2 $CH_2C(O)$), 3.75−3.63 (16H, m, 4 OCH_2CH_2O), 1.48 (18H, s, 2 t-Bu); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 169.54 (2 C═O), 81.35 (2 $C(CH_3)_3$), 70.58, 70.47, 68.88, 27.98 (2 $C(CH_3)_3$); HRMS (FAB+, NBA/CsI) calcd for $C_{20}H_{38}O_9Cs$ (M + Cs$^+$) m/e 555.1570, found 555.1581.

3,6,9,12,15,18-Hexaoxaeicosane-1,20-dioic Acid Di-*tert*-butyl Ester (13). Pentaethylene glycol (1.06 mL, 5 mmol) was alkylated according to the general procedure (method B) and purified by FC (65 g of silica, first toluene/ethyl acetate, 1:4, then ethyl acetate) to give **13** (786 mg, 34%) as a colorless oil: R_f 0.22 (toluene/ethyl acetate, 1:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.02 (4H, s, 2 $CH_2C(O)$), 3.73−3.65 (20H, m, 5 OCH_2CH_2O), 1.48 (18H, s, 2 t-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.55 (2 C═O), 81.39 (2 $C(CH_3)_3$), 70.60, 70.48, 70.46, 68.92, 28.00 (2 $C(CH_3)_3$); HRMS (FAB+, NBA/NaI) calcd for $C_{22}H_{42}O_{10}Na$ (M + Na$^+$) m/e 489.2676, found 489.2666.

3,6,9,12,15,18,21-Heptaoxatricosane-1,23-dioic Acid Di-*tert*-butyl Ester (14). Hexaethylene glycol (1.25 mL, 5 mmol) was alkylated according to the general procedure (method C) and purified by FC (110 g of silica, first ethyl acetate, then ethyl acetate/ethanol, 95:5 → 90:10) to give **14** (1.884 g, 74%) as a colorless oil: R_f 0.17 (toluene/ethanol, 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (4H, s, 2 $CH_2C(O)$), 3.73−3.65 (24H, m, 6 OCH_2CH_2O), 1.48 (18H, s, 2 t-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.55 (2 C═O), 81.38 (2 $C(CH_3)_3$), 70.59, 70.48, 70.45, 68.90, 27.99 (2 $C(CH_3)_3$); HRMS (FAB+, NBA/CsI) calcd for $C_{24}H_{46}O_{11}Cs$ (M + Cs$^+$) m/e 643.2094, found 643.2076.

3,6,9-Trioxaundecane-1,11-dioic Acid (15). **10** (577 mg, 1.73 mmol) was treated with TFA (2.5 mL) and CH_2Cl_2 (2.5 mL) for 2 h at room temperature. Removal of the solvent gave **15** (410 mg, quantitative) as a colorless oil: R_f 0.19 (MeCN/H_2O, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (2H, s, 2 CO_2H), 4.21 (4H, s, 2 $CH_2C(O)$), 3.78−3.71 (8H, m, 2 OCH_2CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.58 (2 C═O), 70.87, 70.35, 68.29; HRMS (FAB+, NBA/NaI) calcd for $C_8H_{14}O_7Na$ (M + Na$^+$) m/e 245.0637, found 245.0633.

3,6,9,12-Tetraoxatetradecane-1,14-dioic Acid (16). **11** (575 mg, 1.52 mmol) was treated with TFA (2.5 mL) and CH_2Cl_2 (2.5 mL) for 1 h at room temperature. Removal of the solvent gave **16** (393 mg, 97%) as a colorless oil: R_f 0.14 (MeCN/H_2O, 4:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.70 (2H, s, 2 CO_2H), 4.17 (4H, s, 2 $CH_2C(O)$), 3.74−3.67 (12H, m, 3 OCH_2CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.52 (2 C═O), 70.83, 70.13, 68.15; HRMS (FAB+, NBA/NaI) calcd for $C_{10}H_{18}O_8Na$ (M + Na$^+$) m/e 289.0899, found 289.0905.

3,6,9,12,15-Pentaoxaheptadecane-1,17-dioic Acid (17). **12** (648 mg, 1.53 mmol) was treated with TFA (2.5 mL) and CH_2Cl_2 (2.5 mL) for 1 h at room temperature. Removal of the solvent gave **17** (499 mg, quantitative) as a colorless oil: R_f 0.10 (MeCN/H_2O, 4:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.35 (2H, s, 2 CO_2H), 4.19 (4H, s, 2 $CH_2C(O)$), 3.78−3.66 (16H, m, 4 OCH_2CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.68 (2 C═O), 70.42, 70.28, 70.17, 68.50; HRMS (FAB+, NBA/NaI) calcd for $C_{12}H_{22}O_9Na$ (M + Na$^+$) m/e 333.1162, found 333.1149.

3,6,9,12,15,18-Hexaoxaeicosane-1,20-dioic Acid (18). **13** (446 mg, 0.956 mmol) was treated with TFA (1.5 mL) and CH_2Cl_2 (1.5 mL) for 1.5 h at room temperature. Removal of the solvent gave **18** (367 mg, quantitative) as a colorless oil: R_f 0.18 (MeCN/H_2O, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.66 (2H, s, 2 CO_2H), 4.19 (4H, s, 2 $CH_2C(O)$), 3.77−3.68 (20H, m, 5 OCH_2CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.67 (2 C═O), 70.99, 70.34, 70.31, 70.28, 70.17, 68.44; HRMS (FAB+, NBA/NaI) calcd for $C_{14}H_{26}O_{10}Na$ (M + Na$^+$) m/e 377.1424, found 377.1417.

3,6,9,12,15,18,21-Heptaoxatricosane-1,23-dioic Acid (19). **14** (84 mg, 0.164 mmol) was treated with TFA (1 mL) and CH_2Cl_2 (1 mL) for 2 h at room temperature. Removal of the solvent gave **19** (66.8 mg, quantitative) as a colorless oil: R_f 0.12 (MeCN/H_2O, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (2H, s, 2 CO_2H), 4.19 (4H, s, 2 $CH_2C(O)$), 3.76−3.67 (24H, m, 6 OCH_2CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.20 (2 C═O), 70.83, 70.30, 70.23, 70.19, 70.13, 68.45; HRMS (FAB+, NBA/NaI) calcd for $C_{16}H_{30}O_{11}Na$ (M + Na$^+$) m/e 421.1686, found 421.1676.

General Procedure for the Conversion of the Diacids 15−19 into the Disuccinimidyl Esters 20−24. To a solution of the diacid (0.3 mmol) in dry CH_2Cl_2 (2 mL) were added oxalyl chloride (2 M in CH_2Cl_2) (0.9 mL, 1.8 mmol) and a catalytic amount of DMF (1 drop of a solution of 1 drop of DMF in 1 mL of CH_2Cl_2) at 0 °C. After 5 min, the solution was allowed to warm to room temperature and stirred for 1 h. The solvent was removed by evaporation, and CH_2Cl_2 was evaporated twice from the remaining diacid chloride which was then dissolved in CH_2Cl_2 (2.0 mL). In a separate flask, N-hydroxysuccinimide (76 mg, 0.66 mmol) was coevaporated with dry toluene (3 × 2 mL), dissolved in dry THF (1 mL), and cooled to −10 °C. Then the solution of the diacid chloride and pyridine (53 μL, 0.66 mmol) was added, and the mixture was stirred for 2 h at room temperature. An aliquot of the resulting solution of the disuccinimidyl ester was used directly to prepare the corresponding sLe^x dimer (see the following procedure).

General Procedure for the Preparation of the SLe^x Dimers 25, 27, 29, 31, and 33 and the SLe^x Acids 26, 28, 30, 32, and 34. The sLe^x amine **4** (7 mg, 7.16 μmol) was dissolved in DMF (150 μL). After addition of Et_3N (4 μL, 29 μmol), the mixture was cooled to 0 °C, and the solution of the disuccinimidyl ester **20**, **21**, **22**, **23**, or **24**, respectively (3.58 μmol, 36 μL), was added slowly. The mixture was stirred for 10 min at 0 °C and for 20 min at room temperature. If TLC analysis (i-PrOH/1 M NH_4OAc, 2:1, ninhydrin stain) showed remaining amine **4**, the solution was cooled again to 0 °C and another portion of the disuccinimidyl ester solution (1.79 μmol, 18 μL) was added. After 10 min at 0 °C and 20 min at room temperature, the reaction was quenched by adding a solution of NH_3 in methanol (saturated at 0 °C) (50 μL), evaporated, and purified by size-exclusion chromatography on a Bio-Gel P-4 column (2.5 × 95 cm) using a 50 mM NH_4HCO_3 solution as eluent. The sLe^x dimer eluted first, followed by the sLe^x acid.

Dimer 25 and Acid 26. According to the general procedure, **25** (4.6 mg, 60%) and **26** (2.1 mg, 25%) were obtained as white solids after lyophilization. **25**: R_f 0.21 (i-PrOH/1 M NH_4OAc, 2:1); ^{1}H NMR (400 MHz, D_2O) δ 5.08 (d, 2H, J = 4.0 Hz), 4.51 (d, 2H, J = 7.8 Hz), 4.50 (d, 2H, J = 7.8 Hz), 4.07 (dd, 2H, $J = 3.1$, 9.8 Hz), 4.05 (s, 4H), 3.99 (m, 2H), 3.95−3.79 (m, 20H), 3.76 (d, 2H, $J = 3.3$ Hz), 3.74−3.49 (m, 30H), 3.38−3.31 (m, 8H), 2.75 (dd, 2H, $J = 4.7$, 12.6 Hz), 2.21 (t, 4H, $J = 7.4$ Hz), 2.02 (s, 6H), 2.00 (s, 6H), 1.78 (t, 2H, $J = 12.2$ Hz), 1.60−1.50 (m, 8H), 1.32−1.24 (m, 4H), 1.15 (d, 6H, $J = 6.6$ Hz); ^{13}C NMR (100 MHz, D_2O) δ 179.6, 177.5, 176.5, 176.4, 175.2, 104.1, 103.4, 102.1, 101.1, 78.1, 77.7, 77.34, 77.30, 75.8, 75.3, 74.34, 74.30, 72.8, 72.0, 71.9, 71.7, 71.6, 70.8, 70.5, 70.1, 69.7, 69.1, 65.0, 63.9, 62.1, 58.3, 54.1, 42.2, 40.8, 38.2, 30.7, 27.4, 27.1, 24.7, 24.5, 17.7; ESIMS (H_2O, pos) calcd for $C_{86}H_{146}N_8O_{53}Na$ (M + Na$^+$) *m*/*e* 2162, found 2162. **26**: R_f 0.29 (*i*-PrOH/1 M NH_4OAc, 2:1); ^{1}H NMR (400 MHz, D_2O) δ 5.08 (d, 1H, $J = 4.0$ Hz), 4.51 (d, 1H, $J = 7.9$ Hz), 4.50 (d, 1H, $J = 8.0$ Hz), 4.09−4.05 (m, 1H), 4.08 (s, 2H), 4.05 (s, 2H), 3.99 (m, 1H), 3.95−3.79 (m, 10H), 3.78−3.49 (m, 20H), 3.38−3.31 (m, 4H), 2.75 (dd, 1H, $J = 4.6$, 12.4 Hz), 2.21 (t, 2H, $J = 7.5$ Hz), 2.02 (s, 3H), 2.00 (s, 3H), 1.78 (t, 1H, $J = 12.1$ Hz), 1.60−1.50 (m, 4H), 1.32−1.24 (m, 2H), 1.15 (d, 3H, $J = 6.7$ Hz); ESIMS (H_2O, neg) calcd for $C_{47}H_{79}N_4O_{30}$ (M − H$^+$) *m*/*e* 1179, found 1179.

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Supporting Information Available: Experimental details and characterization of **27**−**34** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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