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Short Synthesis of Sulfatide- and SQDG-Mimetics as Small Molecular Weight Selectin Inhibitors

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Abstract: Small molecular weight sulfatide analogs were synthesised and tested in cell-based selectin mediated adhesion assays. The ceramide moiety of sulfatides could be replaced by simple glycerol ethers to obtain potent mimetics. The specific activity of these inhibitors towards P-selectin is illustrated by analogy with other polyanionic systems forming polyvalent arrays antagonising the polyanionic N-terminal binding sites of the dimeric PSGL-1 ligand.

Introduction

The binding of sialyl Lewis^X (sLe^X) epitopes and related structures found on the termini of glycolipids and glycoproteins is considered to mediate the initial adhesion of several groups of leukocytes to areas of inflammation in the vascular system.[1] The selectins of types E, P, and L are surface lectins with distinct carbohydrate recognition domains (CRD) involved in this process.[2] SLe^X-derived antagonists targeted against the CRDs are therefore potential agents to prevent leukocyte adhesion and

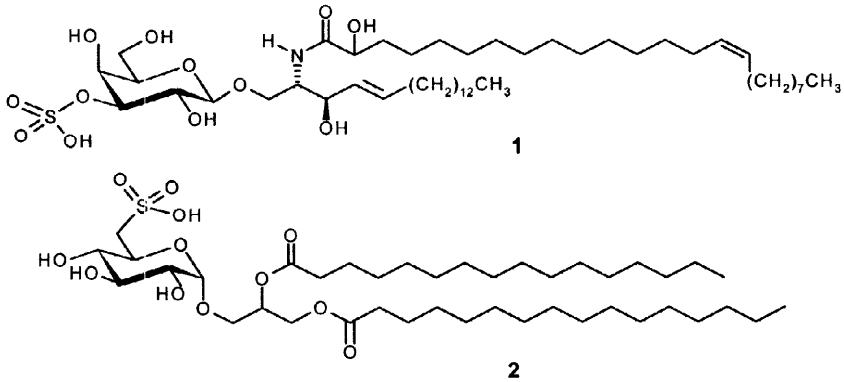

Figure 1: The major component of sulfatide **1** and the sulfonoquinovosyl dipalmitoyl glyceride (SQDG) **2**, isolated i.a. from the marine *alga Dictyochloris fragrans*, were taken as natural product lead structures for selectin antagonists.

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their subsequent migration into the affected tissues in several acute and chronic inflammatory diseases.[3] Considerable effort has been spent on designing glycomimetics and potential new anti-inflammatory drug leads.[4] These were mostly designed to replace the complex tetrasaccharide sLe^X, based on the knowledge about structure-activity relationships which have been obtained by variation of functional groups of sLe^X. However, most of these structures are much too complex to allow for the production of relevant drug-like molecules from an industrial point of view. Therefore we describe here another approach straightforwardly leading to potent glycomimetics (Fig. 2) which represent small molecular weight selectin inhibitors derived from the sulfatides **1** and sulfonoquinovosyl dipalmitoyl glycerides **2** as the natural product leads (Fig. 1).

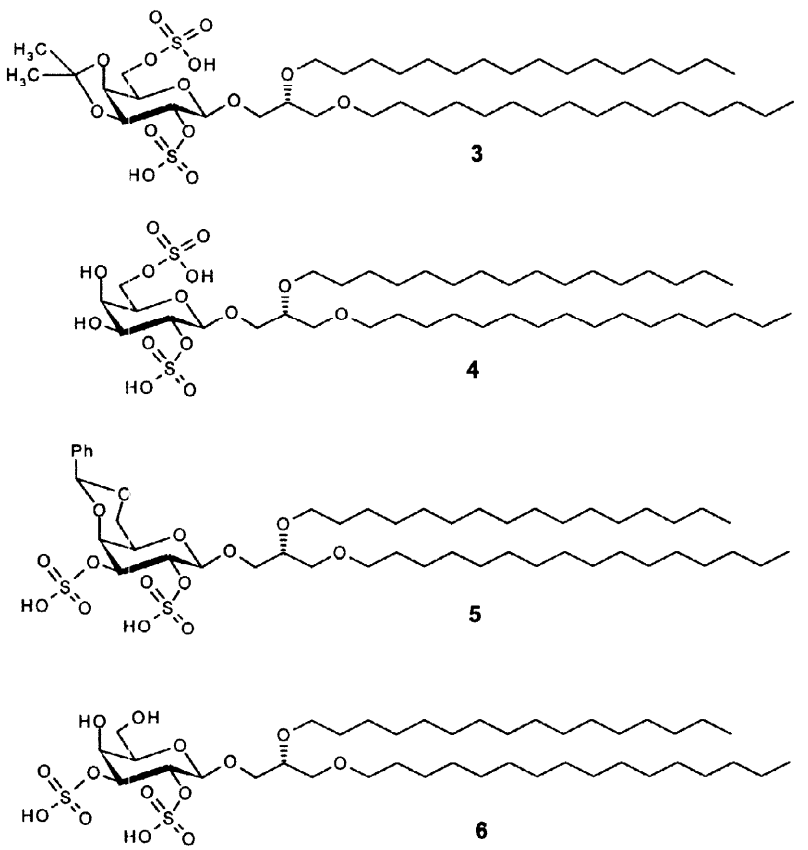


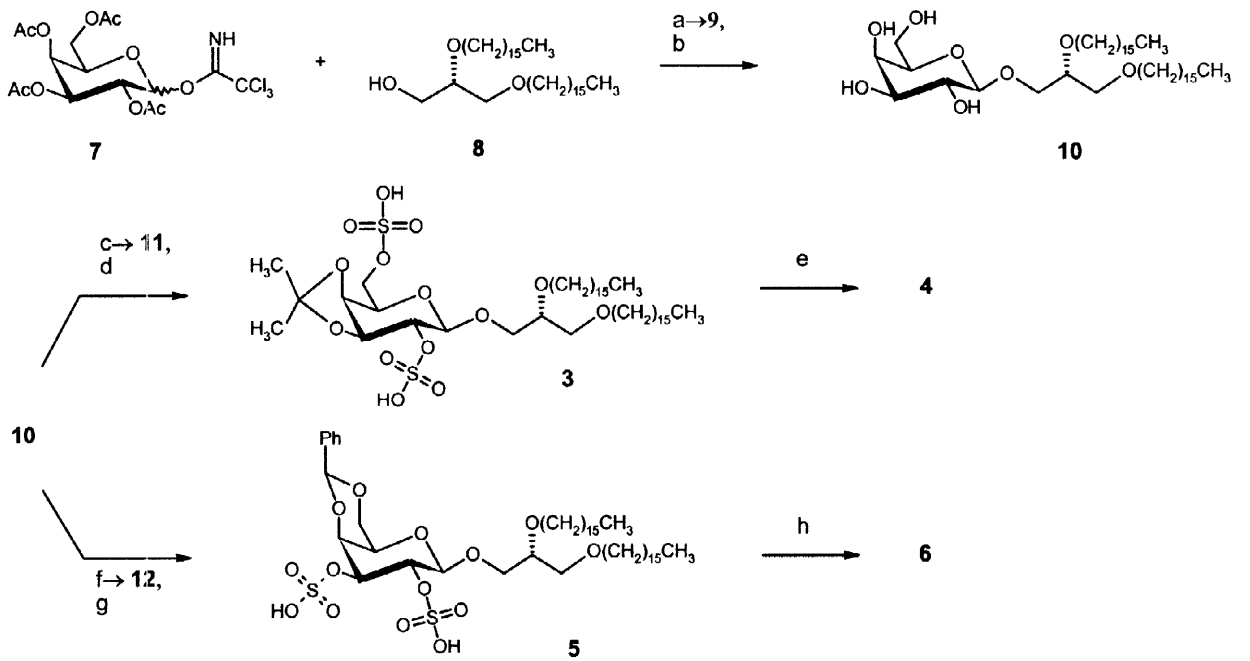
Figure 2: Glycolipid mimetics **3-6** synthesised and evaluated in cell-based adhesion assays with E- and P-selectins (see Table 1)

The sulfatide **1** is abundant in mammalian tissues and it has been shown to bind to recombinant P-selectin IgG chimera.[5a] Furthermore, **1** has exhibited some therapeutic potential in the treatment of certain acute inflammatory reactions by blocking neutrophil recruitment and immune complex deposition.[5b] The 1,2-di-O-acylglycerol-3-yl-(6-deoxy-6-sulfono)-α-D-glucopyranoside **2** (sulfonoquinovosyl diacylglycerol) is a major membrane lipid of carbon dioxide fixing organelles in plants and in photosynthetic bacteria which fix carbon dioxide and it was recently identified as a metabolite in *Rhizobium*, a non-photosynthetic organism.[6] Interestingly, compound **2** was identified in extracts from the marine alga *Dictyochloris fragrans* using a P-selectin assay in a high-throughput screening.[7] The SQDGs have already a history of pharmacological

effects as ingredients in certain tropical barks used in folkloric medicine to treat coughs, diarrhoea and snake bites[8] and they seem to have some anti-tumour promoting activity.[9]

Preparative and Biological Results

We devised a rewarding new approach to provide simplified selectin antagonists derived from the structural features present in either of the natural product leads **1** or **2**. The configuration part of the sugar was overtaken from **1**, whereas the synthetically complex ceramide moiety of **1** was exchanged for the simpler aglycone present in **2**. The acyl groups in **2** were conveniently replaced by the metabolically more stable ether bonds maintaining the C16 aliphatic chains. The sulfate group in **1** was preferred over the sulfono group in **2**, leaving its position variable on the sugar part of the molecule. According to this design, the syntheses of the mimetics **3-6** were easily performed as outlined in Scheme 1:



Scheme 1: Synthesis of the mimetics **3-6**: a) (2R)-3-hydroxy-1,2-di-hexadecyloxypropane, CH_2Cl_2, cat. TMS-OTf, 20°C, 30 min (78% **9**); b) 1M NaOMe in MeOH, 2 h , 20°C, then 1 M HOAc, quant.; c) dimethoxypropane, cat. p-TosOH, 18 h, 20°C, then H_2O, cat. TosOH, 2 h, 20°C (86% **11**); d) pyridine, pyridine-SO_3 complex, 3h, 20°C, (70%); e) CF_3CO_2H/H_2O, 40°C, 4h (88%); f) benzaldehyde-dimethylacetal, cat. p-TosOH, 5h, 50°C (68% **12**); g) pyridine, pyridine-SO_3 complex, 3h, 20°C, (74%); h) HOAc/MeOH, H_2, 10%-Pd/C, 20°C, 18 h (76%).

The glycerol derivative **8** was obtained by two standard reaction steps (alkylation of commercially available (R)-(+)-3-benzyloxy-1,2-propandiol with 1-hexadecyl bromide, then hydrogenation) in 81% overall yield. Galactosylation of **8** with the known trichloroacetimidate **7**[10] catalysed by trimethylsilyl-trifluoromethane sulfonate and subsequent *Zemplen* deacetylation afforded the β-galactoside **10** in 78% yield. This key intermediate was protected either by 3,4-isopropylidenation to **11** or to the 4,6-benzylidene derivative **12**.

The compounds **11** and **12** gave the disulfates **3** and **5**, respectively, upon reaction with the pyridine-SO_3-complex. Acid-catalysed removal of the isopropylidene protecting group in **3** and catalytic hydrogenation of **5**, afforded the 2,6- and the 2,3-disulfate **4** and **6**, respectively, in good yields. Very importantly, all these reactions and the work-up procedures were carried out by strictly avoiding any contact with ion exchange resins.[11-14] The compounds **3-6** were then examined for their inhibitory potency towards the E- and P-selectin receptors. The bioassays for cell binding to immobilised selectin receptor globulins were carried out as previously described.[15] The concentrations of the inhibitors required to block adhesion of 50% of the HL60 cells are given in Table 1:

	Selectin IC_{50} [mM]	
	E	P
reference compound		
sLe^X-1β–$O(CH_2)_6NH_2$	1.5	3.0
1	0.075-0.092	0.006-0.007
2	n.d.[7a]	0.04 [7a]
3	0.64-2.08	0.058-0.063
4	1.60-2.05	0.04-0.08
5	0.16-1.30	0.12-0.80
6	1.11-2.00	2.26-2.50

Table 1: Inhibition of HL60 cell adhesion to recombinant E- and P-selectin-IgG fusion proteins by a sLe^X conjugate, sulfatide **1**, SQDG **2**, and the mimetics **3-6**. IC_{50} values are concentrations of inhibitors required to block adhesion of 50% of the cells compared with the negative control. Assays were repeated several times to afford the values varying within the given limits.

All the simple glycomimetics **3-6** proved to be E-selectin inhibitors with about equivalent potency as a complex sLe^X tetrasaccharide conjugate with IC_{50}'s in the low millimolar range. The deprotected disulfates **4** and **6** showed a higher potency in the P-selectin assay than the reference tetrasaccharide, particularly the 6-sulfated compound **4** was up to 75 times more potent and comparable with the lead compound **2** in this assay. Surprisingly, the protected precursors **3** and **5** likewise were quite good P-selectin inhibitors, indicating that the OH-groups of the galactose or even some distinct recognition for this specific sugar epitope may not be required to block the binding of this receptor to its ligands on the tumour cells. More likely, the combination of the anionic head groups in **3-6** with the hydrophobic tails seems to govern the influence of these compounds on the respective receptors present in the P-selectin assay system. However, the compounds **3-6** investigated here share common structural features with surface-active compounds (detergents) forming micelles, thus presenting their anionic head groups in a polyvalent, polyanionic fashion. This comfortable explanation for the observed data in Table 1 immediately suggests that other very high molecular weight, highly charged aggregates[17] may act in a similar manner dependent on dosage, molecular weight and negative charge distribution/density as was recently discovered for the simple polyanionic oligoacrylates.[12a] At least in the cell-based assay systems which were used here, some inhibitory activity -

in particular in the E-selectin assay - may be attributed to unspecific interactions with the cell membranes.[18] However, the IC_{50}s in both assays make a great difference suggesting that such effects may be rather small here. To illustrate the common nature of this polyanionic inhibitory effect, several structurally unrelated synthetic approaches were compared over a wide range of IC_{50}s suggesting that these polyanionic compounds may have a size/charge-related recognition differing from the generally anticipated sugar-specific binding to the carbohydrate recognition domain (CRD) presented by P-selectin (Figure 3).

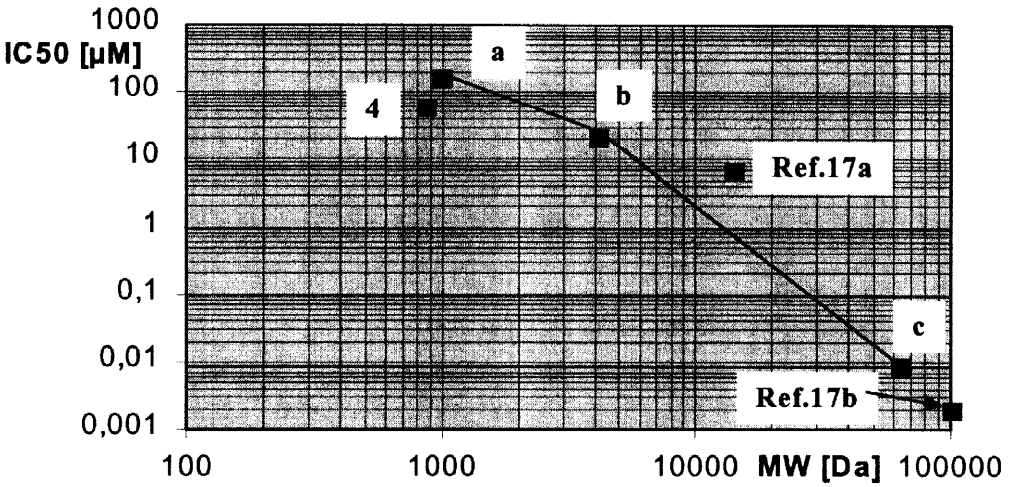


Figure 3: Comparison of the IC_{50} values obtained in HL60/P-selectin assays using structurally different polyanionic inhibitors. The data points a-c refer to oligoacrylates of different size[12a] and ref.[17a] describes a *ROMP*-(*R*ing *O*pening *M*etathesis *P*olymerisation) derived, sulfated glycopolymer. Ref.[17b] assigns to the most potent polymerised glycoliposome containing 5% of a sLe^X-analog. Although the molecular weight of this construct is not given in this publication, it is tentatively assumed to be at least 100 kDa. Point 4 refers to the respective sulfatide mimetic in the present publication. The structures of these inhibitors are shown in Figure 4.

During their evaluation of sialylated and sulfated oligosaccharides, the results of another working group suggested that the binding sites for sialic acid and sulfate may be very close, if not identical, and that the O-sulfate esters mostly improved P- (and L-) selectin recognition dependent upon their position and number.[16] The results reported here strongly confirm this view, suggesting moreover that carbohydrate-derived structures may not be required to achieve the same functional effects in inhibiting selectin-dependent cell adhesion. This does not stand in contrast to the generally accepted stronger binding of ligands to the selectins by multivalent binding sites. However, the hypothesised clustering of the selectins[17c] may not be required to account for the enhanced binding of these highly charged ligands. The major high affinity ligand for P-selectin on human leukocytes is P-selectin glycoprotein ligand-1 (PSGL-1). In support of our hypothesis

about potential modes of selectin-antagonism performed by polyanions,[12a] the requirements of full-lenght PSGL-1 for binding to P-selectin have recently been established.[22] Accordingly, P-selectin seems to bind to the small N-terminal region of dimeric PSGL-1[23] which involves a stretch of anionic amino acids including

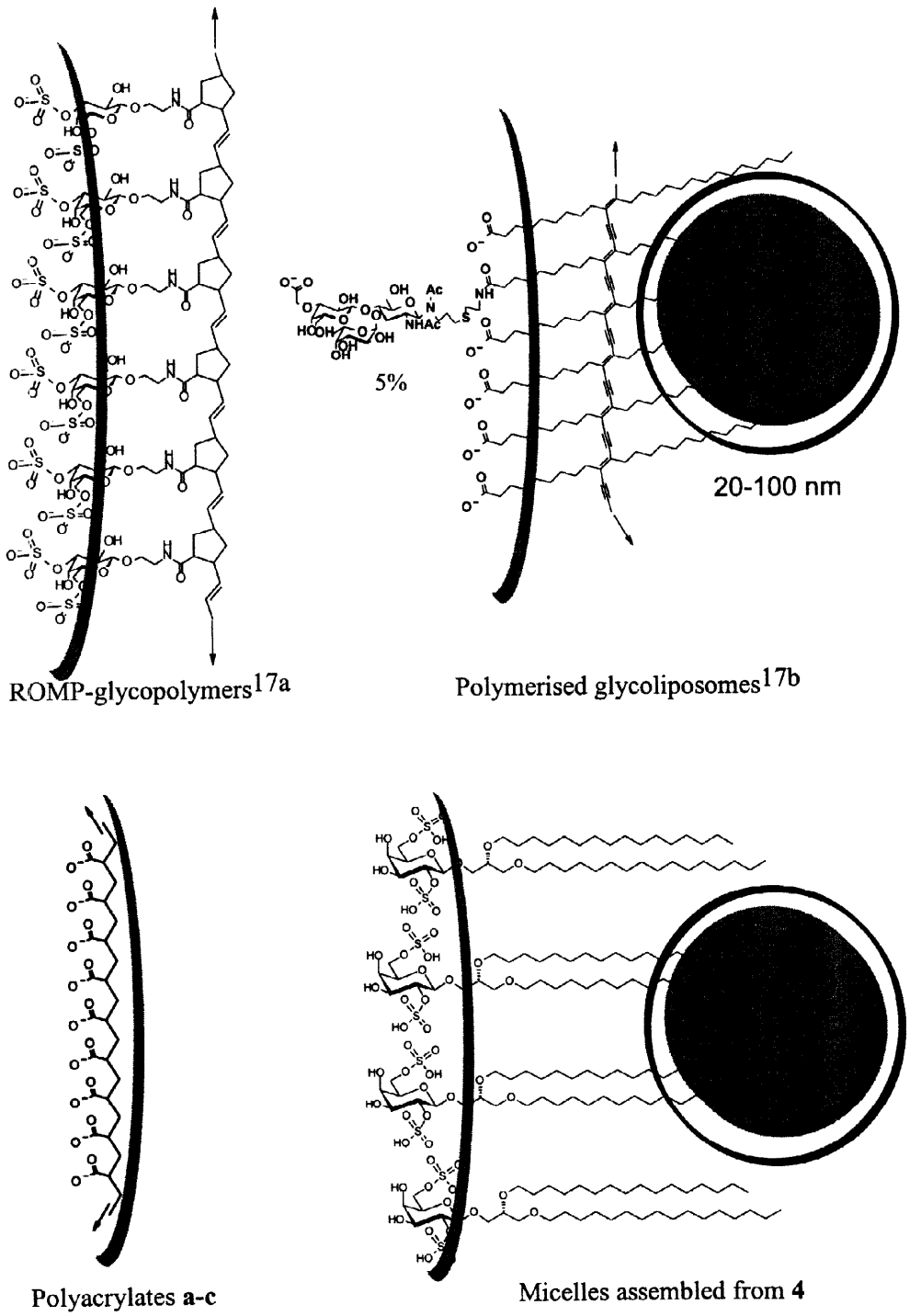


Figure 4: Simplified structures of polyanions as specified in Figure 3 schematically depicting the common acidic multivalent arrays assumed to be responsible for their selective P-selectin inhibition.

at least one sulfated tyrosine in the positions 46,48,51 plus seven acidic side chain amino acids. The sialylated and fucosylated core-2 O-glycan is probably attached to Thr-57.[22] (Figure 5)

H_2N-Gln(42)-Ala-Thr-**Glu**(45)-**Tyr**(46)-**Glu**(47)-**Tyr**(48)-Leu-**Asp**(50)-**Tyr**(51)-**Asp**(52)-

-Phe-Leu-Pro-**Glu**(56)-Thr(57)-**Glu**(58)-Pro-Pro-**Glu**(61)-Met(62)-

Figure 5: Structure of the polyanionic N-terminal stretches of dimeric PSGL-1 required for binding to P-selectin. The potential anionic binding sites are depicted in **bold**, including the potentially sulfated tyrosines.[22]

Very recently it has been reported that notably P- and L-selectin binding to sialyl Lewis^X and to HL60 cells - which are known to carry the native ligand PSGL-1 - is inhibited by unfractionated pharmaceutical heparin preparations at concentrations 12-50-fold lower than those recommended for effective anticoagulation *in vivo*.[19] Hence it is not surprising that particularly the same selectins can bind to polyanionic heparan sulfate glycosaminoglycan chains (*HS-GAGs*) and to defined heparin fragment pools in a the size-dependent manner. It remains to be investigated if the new simple glycomimetics described here are effective drug candidates *in vivo*, e.g. compared with the less readily available sulfatide **1**, either in myocardial ischemia and reperfusion injury[20] or to block lung injury in rats.[21]

EXPERIMENTAL PART

General: Yields refer to chromatographically (TLC) and spectroscopically (NMR) homogeneous materials. Thin layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} plates (0.25 mm thickness, E. Merck). Spots were visualised by spraying the plates with sulfuric acid/anisaldehyde reagent, followed by heating. E.Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. NMR spectra were recorded on a Bruker WT 300 (300 MHz) and 400 (400 MHz). NMR chemical shifts are given as δ-values with reference to tetramethylsilane (TMS) as internal standard, if not otherwise noted. Mass spectra were recorded on a Finnigan LCQ (ion trap spectrometer) using electrospray ionisation (ESI).

*(2R)-3-Hydroxy-1,2-di-hexadecyloxypropane (**8**)*: (R)-(+)-3-Benzyloxy-1,2-propandiol (9.00 g, 49.4 mmol), NaH (3.55 g, 148.2 mmol) in N,N-dimethylformamide (DMF, 675 ml) were stirred at 20°C for 0.5 h. Then 1-hexadecyl bromide (42.2 ml, 138.3 mmol) was added slowly over 0.5 h. Stirring was continued for 18 h. The reaction was quenched with ice/water and the aqueous phase was extracted with diethyl ether (3 x 300 ml). Combined organic phases were washed with water (3 x 20 ml), dried ($MgSO_4$), filtered and concentrated. The residue was purified by filtration over silica gel 60 in *n*-hexane/ethyl acetate (50:1 → 20:1→ 10:1 v/v) to give (2R)-3-benzyloxy-1,2-di-hexadecyloxypropane (30.8 g, 99%). This intermediate product was hydrogenated over Pd/C (10%, 5.00 g) for 18 h in a solvent mixture of methanol/dioxane/acetic acid (255 ml, 2/3/0.01 v/v) under normal pressure. The filtrate was concentrated and the residue was purified by flash chromatography on silica gel in *n*-hexane/ethyl acetate (6:1 → 5:1 v/v) to give compound **8** (21.4 g, 81%).^{1}H NMR ($CDCl_3$): 0.88 (2 t, 6H, 2 Me), 1.28 (m, 52H, 26 CH_2), 1.58 (m, 4H, 2CH_2), 2.19, (dd, 1H, OH), 3.40-3.77 (m, 9H, 4 OCH_2, 2-OCH).

(2'R)-2',3'-Di-hexadecyloxypropyl-O-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**9**): The mixture of O-(2,3,4,6-tetra-O-acetyl-D-galactopyranosyl)-trichloroacetimidate **7**[10] (3.00 g, 5.55 mmol), **8** (4.10 g, 7.60 mmol) and trimethylsilyl-trifluoromethanesulfonate (1 M TMS-OTf, 0.61 ml) was stirred for 0.5 h. The

reaction was neutralised with $NaHCO_3$ (0.50 g), filtered and concentrated The residue was purified on silica gel in *n*-hexane/ethyl acetate (10:1 → 7:1→ 5:1 v/v) to give the tetraacetate **9** (4.14 g, 78%). ^1H NMR (300 MHz, $CDCl_3$): 0.88 (2t, 6H, 2 Me), 1.26 (m, 52H, 26 CH_2), 1.55 (m, 4H, 2 CH_2), 1.98, 2.05, 2.05, 2.15 (4s, 12H, 4 OAc), 3.36-3.66 (m, 8H, 4 OCH_2), 3.88, 4.16 (2m, 4H, 2'-OCH, 5,6-H_{Gal}), 4.16 (m, 2H), 4.52 (d, 1H, 1-H_{Gal}), 5.02 (dd, 1H, 3-H_{Gal}), 5.19 (dd, 1H, 2-H_{Gal}), 5.38 (dd, 1H, 4-H_{Gal}).

(2'R)-2',3'-Di-hexadecyloxypropyl-O-β-D-galactopyranoside (**10**): Tetraacetate **9** (3.30 g, 3.80 mmol) and NaOMe (1 M solution in methanol, 1.2 ml) were stirred for 2 h in dry methanol (10 ml) at 20°C. The reaction was neutralised with acetic acid (1M) and evaporated to dryness. This crude product [^1H NMR (300 MHz, DMSO-d_6): 0.87 (2t, 6H, 2 Me), 1.25 (m, 52H, 26 CH_2), 1.50 (m, 4H, 2 CH_2), 4.13 (d, 1H, 1-H_{Gal})] was not further purified but could be used as such to prepare the compounds **11** and **12**.

(2'R)-2',3'-Di-hexadecyloxypropyl-O-(3,4-O-isopropylidene)-β-D-galactopyranoside (**11**): Compound **10** (2.03 g, 2.89 mmol), dimethoxypropane (120 ml) and p-toluene sulfonic acid (0.15 g) were stirred 18 h at 20°C. In order to cleave the mixed acetal products, water (75 ml) and p-toluene sulfonic acid (0.15 g) were added. After 2 h, ethyl acetate (1.2 l) and saturated aqueous $NaHCO_3$ (0.40 l) were added. After stirring for 0.5 h, the organic layer was washed with $NaHCO_3$-solution (3 x 150 ml), dried over $MgSO_4$ and concentrated *i.vac.*. Flash chromatography (toluene/ ethyl acetate 2:1 v/v) afforded **11** (1.85 g, 86%). ^1H NMR (300 MHz, $CDCl_3$): 0.88 (2t, J = 7.0 Hz, 6H, 2 $CH_2\underline{CH_3}$), 1.26 (m, 52H, 26 CH_2),1.34, 1.52 (2s, 6H, CMe_2), 1.56 (m, 4H, 2 CH_2), 2.39 (*b*d, 1 H, OH), 2.99 (*b*s, 1 H, OH), 3.40-3.64 (m, 8H, 4 OCH_2), 3.70 (m, 1H, 2'-OCH), 3.76-3.90 (m, 2H, H_{Gal}), 3.92-4.20 (m, 2H, H_{Gal}), 4.60-4.17 (m, 2H, H_{Gal}), 4.22 (d, J = 8.2 Hz, 1H, 1-H_{Gal}).

(2'R)-2',3'-Di-hexadecyloxypropyl-O-[(3,4-O-isopropylidene)-2,6-di-O-sulfonyl]-β-D-galactopyranoside (**3**): A mixture of **11** (0.30 g, 0.40 mmol) and SO_3-pyridine complex (0.64 g, 4.00 mmol) in pyridine (10 ml) was stirred for 3 h at 20°C. The reaction was quenched with water (5 ml) and $NaHCO_3$ (0.50 g). After 15 min the mixture was concentrated *i. vac.*, taken up in methanol (5 ml), filtered and concentrated. Chromatography on silica (dichloromethane/methanol 7:1 v/v) gave **3** (0.25 g, 70%). 1H NMR (300 MHz, $CDCl_3/CD_3OD$, 1:1 v/v): 0.89 (2t, 6H, 2 $CH_2\underline{CH_3}$), 1.28 (m, 52H, 26 CH_2),1.34, 1.51 (2 s, 6H, CMe_2), 1.56 (m, 4H, 2 CH_2), 3.87 (dd, 1 H), 4.35 (dd, 1 H), 4.47 (dd, 1 H), 4.57 (m, 1 H).

(2'R)-2',3'-Di-hexadecyloxypropyl-O-(2,6-di-O-sulfonyl)-β-D-galactopyranoside (**4**): A solution of **3** (1.40 g, 1.55 mmol) trifluoroacetic acid/water (1:1 v/v, 84 ml) was stirred for 4 h at 40°C. The reaction mixture was concentrated and the residue purified by flash chromatography (dichloromethane/methanol 20:1 → 10:1 → 5:1; v/v) to afford the disulfate **4** (1.18 g, 88%). ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 1:1 v/v): 0.89 (2t, 6H, 2 Me), 1.28 (m, 52H, 26 CH_2), 1.57 (m, 4H, 2 CH_2), 3.40-3.70 (m, 8H, 4 OCH_2), 3.70-4.03 (m, 3H, 2'-OCH, 6-H_{Gal}), 4.13-4.35 (m, 4H, H_{Gal}), 4.44 (d, 1 H, 8.0 Hz, 1-H_{Gal}), ^{13}C NMR (DEPT, 100.6 MHz, $CDCl_3/CD_3OD$ 1:1 v/v, δ rel. to CD_3OD 49.1 ppm): 15.00 (2 Me), 69.85, 73.00, 73.50, 78.90, 78.95 (2'-C, 2,3,4,5-C_{Gal}), 66.23, 70.72, 71.73, 71.88, 72.96 (5 $O\underline{C}H_2$), 102.85 (1-C_{Gal}). FAB-MS (MAT 95Q Finnigan) for $C_{41}H_{82}O_{14}S_2$ (863.22): m/z 883.2 ([M-2H+Na]$^+$), 781.3 [M-HSO3]$^+$. The surface-active properties of the disodium salt of **4** in bidistilled water (pH 7.0, 20°C) were investigated using a tensiometer (Lauda TD1). The CMC (critical micelle concentration) was approximately at a concentration of 660 μM and the foam stability was weak, the overall detergent properties therefore being apparent but not excellent to this end. Sodium dodecyl sulfate (SDS) had a CMC of 8.6 mM at the same conditions.

(2'R)-2',3'-Di-hexadecyloxypropyl-O-(4,6-O-benzylidene)-β-D-galactopyranoside (**12**): A mixture of **10** (1.50 g, 2.10 mmol), acetonitrile (250 ml), benzaldehyde-dimethylacetal (2.36 ml, 15.75 mmol) and p-toluene sulfonic acid (0.30 g) was stirred for 5 h at 50°C. After cooling to room temperature, the reaction was neutralised with K_2CO_3 (500 mg), filtered and concentrated *i. vac.*.The residue was subjected to

chromatography on silica (toluene/acetone 4:1 v/v) to afford the intermediate **12** (1.14 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): 0.88 (2t, 6H, 2 Me), 1.25 (m, 52H, 26 CH_2), 1.54 (m, 4H, 2 CH_2), 4.33 (d, J = 8.0 Hz, 1H, 1- H_{Gal}), 5.55 (s, 1H, CHPh), 7.35-7.50 (m, 5H, Ph).

(2'R)-2',3'-Di-hexadecyloxypropyl-O-[(4,6-O-benzylidene)-2,3-di-O-sulfonyl]-β-D-galactopyranoside (**5**): A mixture of **12** (0.32 g, 0.40 mmol) and SO_3-pyridine complex (0.64 g, 4.00 mmol) in pyridine (10 ml) was stirred for 3 h at 20°C. The reaction was quenched with water (5 ml) and $NaHCO_3$ (0.50 g). After 15 min the mixture was concentrated *i. vac.*, the residue taken up in methanol (5 ml), filtered and concentrated *i. vac.*. Chromatography on silica (dichloromethane/methanol 7:1 v/v) gave **5** (0.28 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1 v/v): 0.89 (2t, 6H, 2 Me), 1.28 (m, 52H, 26 CH_2), 1.57 (m, 4H, 2 CH_2), 3.44--3.77 (m, 11H, 2'-CHO, 5 OCH_2), 3.98, 4.15, 4.30, 4.70 (4m, 4H, H_{Gal}), 5.61 (s, 1H, CHPh), 7.34, 7.52 (2m, 5H, Ph). ^{13}C NMR (DEPT, 100.6 MHz, $CDCl_3/CD_3OD$ 1:1 v/v, , δ rel. to $CDCl_3$ 77.0 ppm): 17.55 (2 Me), 69.50 (2'-C), 75.38, 2x74.46, 72.74, 72.63 (5 $O\underline{C}H_2$), 77.50, 78.50, 81.00, 82.05 (2,3,4,5-C_{Gal}), 105.05 (1-C_{Gal}). ESI-MS for $C_{48}H_{86}O_{14}S_2$ (951.34): m/z 949.5 (100%, $[M-H]^-$).

(2'R)-2',3'-Di-hexadecyloxypropyl-O-(2,3-di-O-sulfonyl)-β-D-galactopyranoside (**6**): Compound **5** (157 mg, 0.165 mmol) was hydrogenated in acetic acid /methanol (10 ml, 1:1 v/v) on Pd/ (10%, 0-20 g) for 18 h at normal pressure. After stirring with water (20 ml) and methanol (20 ml) at 40-50 °C, the filtered reaction solution was evaporated to dryness. The residue was taken up in warm methanol (20 ml) and filtered and the filtrate allowed to stand at 0°C. The product **5** precipitated (108 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 1:1 v/v): 0.87 (2t, J = 7.0 Hz, 6H, 2 Me), 1.27 (m, 52H, 26 CH_2), 1.55 (m, 4H, 2 CH_2), 3.40-3.95 (m, 13H, 5 OCH_2, 2'-OCH, 4,5-H_{Gal}), 4.35-4.52 (m, 3H, 1,2,3-H_{Gal}). ^{13}C NMR (DEPT, 100.6 MHz, $CDCl_3/CD_3OD$ 1:1 v/v, δ rel. to $CDCl_3$ 77.0 ppm): 17.59 (2 Me), 65.00 (6-C_{Gal}), 71.27 (2'-C), 72.90, 74.47, 74.58, 75.39 (4 $O\underline{C}H_2$), 78.31, 79.91, 81.57, 83.21 (2,3,4,5-C_{Gal}), 105.79 (1-C_{Gal}). ESI-MS for $C_{41}H_{82}O_{14}S_2$ (863.22): m/z 861.4 (100%, $[M-H]^-$).

Bioassay for cell binding to immobilised selectin receptor globulins: The bioassays for cell binding to immobilised selectin receptor globulins were performed as previously described.[15] Briefly, the soluble recombinant E- and P-selectin-IgG fusion proteins which contain the signal sequence, the lectin-like domain, the EGF (epidermal growth factor) repeat domain and six (E-selectin) and two (P-selectin) of the CR-like (complement regulatory) domains obtained from transfected COS cells were adsorbed on anti-human-IgG-antibodies immobilised on ELISA (enzyme-linked immunosorbent assay) plates. Adhesion of labelled HL60 tumour cells was quantitatively measured in a cytofluorometer, and the specific cell binding in the presence of a potential inhibitor was calculated compared with non-specific binding to the CD4-IgG fusion protein.

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