Synthesis of the Sialyl Lewis X Epitope Attached to Glycolipids with Different Core Structures and their Selectin-Binding Characteristics in a Dynamic Test System

Christian Gege,[a] Jan Vogel,[b] Gerd Bendas,[b] Ulrich Rothe,[c] and Richard R. Schmidt*[a]

Abstract: Sialyl Lewis X (sLe^X)/selectin-mediated leukocyte rolling along endothelial cells has recently gained wide interest. In this paper the influence of the spacer length of laterally clustered neoglycolipids **1a–d** on cell rolling in a dynamic test system is investigated. The required di-*O*-hexadecyl glycerols with none, and with three, six, or nine ethylene glycol units as spacer groups (compounds **4a–d**) could be readily obtained. The synthesis of 1-*O*-thexyldimethylsilyl-protected sLe^X **24** was based on sialylation of 2,3,4-*O*-unprotected galactose derivative **11** with sialyl phosphite **8** as donor; this afforded the desired disaccharide **12**, which was transformed into trichloroacetimidate **14** as disaccharide donor. Reaction of 3-*O*-unprotected glucosamine derivative **18** with fucosyl donor **20** afforded disaccharide **21**, which was transformed into the 4-*O*-unprotected derivative **23**. Reaction of **14** with **23** furnished the desired tetrasaccharide **24** in good yield. Transformation of **24** into the trichloroacetimidate **26** as donor, followed by the reaction with **4a–d** as acceptor gave, after deprotection, the target molecules **1a–d**. For comparison, **4d** was also connected with a sialyl residue (→**31**) and with an *N*-acetylglucosamine residue (→**34**). Compounds **1c** and **1d** with a hexaethylene glycol and a nonaethylene glycol spacer, respectively, were much more efficient in mediating selectin-dependent cell rolling in the dynamic test system than compounds **1a** and **1b**, which had no spacer (**1a**), or only a triethylene glycol spacer (**1b**).

Keywords: cell adhesion · glycolipids · membranes · molecular recognition · selectin

Introduction

The recruitment of leukocytes to sites of injury or infection is a receptor-mediated process essential for the immune response. Leukocyte rolling along endothelial cells under the shear force in postcapillary venules represents the first step in a sequence of adhesive interactions that lead to firm attachment and subsequent emigration through the venular wall.[1] The selectins, a family of three adhesion molecules on both the leukocyte and endothelial surfaces, are thought to mediate rolling by binding carbohydrate-presenting ligands with rapid association and dissociation rate constants.[2] The identified natural selectin ligands are extended mucin-like glycoproteins that present a series of sialylated and fucosylated polylactosamines as binding epitopes; these are glycosidically linked to the peptide backbone.[3–5] The tetrasaccharide sialyl Lewis X (sLe^X) (Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc) is of key importance. The selectins bind these carbohydrates at their N-terminal lectin domain. Although numerous studies performed under static conditions have confirmed fundamental findings about selectin-binding characteristics and the structure–activity relationships of their binding epitopes,[6–9] they do not provide information on how ligands interact with selectins under dynamic conditions. Additional binding studies under the simulated shear force conditions of the vasculature confirmed that tethering, rolling velocity, and shear force resistance of neutrophils are regulated by the kinetics of bond formation and dissociation, and are different for each of the individual selectins.[10–12]

However, several questions about the essential structure of the carbohydrate ligands for the mediation of rolling remain unanswered. For instance, the molecular basis of the high affinity of the natural sialomucins to the selectins could not be fully elucidated, since single binding epitopes like sLe^X exhibit only low affinity.[13] Multiple protein–carbohydrate interactions are therefore thought to be the reason for a higher binding efficiency mediated by a special molecular arrangement of binding epitopes.[14] Therefore, various research groups have prepared synthetic sLe^X clusters, but these

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  0947-6539/00/0601-0111 $ 17.50+.50/0

studies could only in part support this hypothesis of increased affinity.[15, 16]

Furthermore, the extended flexible structure of the sialomucins is thought to be essential for the mediation of cell rolling. However, lipid based sLe^X molecules (glycosphingolipids) at the leukocyte surface, as well as selectin ligands, have been postulated to act in the rolling process.[17, 18]

In our previous study we introduced a dynamic model system for the investigation of ligand characteristics that are fundamental for the rolling process.[19] The adhesion and rolling of selectin-presenting cells along a ligand-containing supported membrane were analyzed; this reflects molecular recognition requirements like ligand structure, concentration, and lateral distribution. It was shown that sLe^X-glycolipids mediate a selectin-dependent cell rolling when they are arranged in lateral clusters in the model membrane.[19] This study supports the hypothesis of multivalent binding and extends its applicability to glycolipids.

The present study focuses on the molecular features of glycolipid ligands that are important for the mediation of rolling. To this end, four sLe^X glycolipids were synthesized that differ in their spacer length between the hydrophobic moiety and the carbohydrate headgroup. The resulting differences in flexibility and accessibility of the sLe^X in these compounds, when incorporated into the model membrane of the flow chamber system, should give new insights into the cell-rolling process.

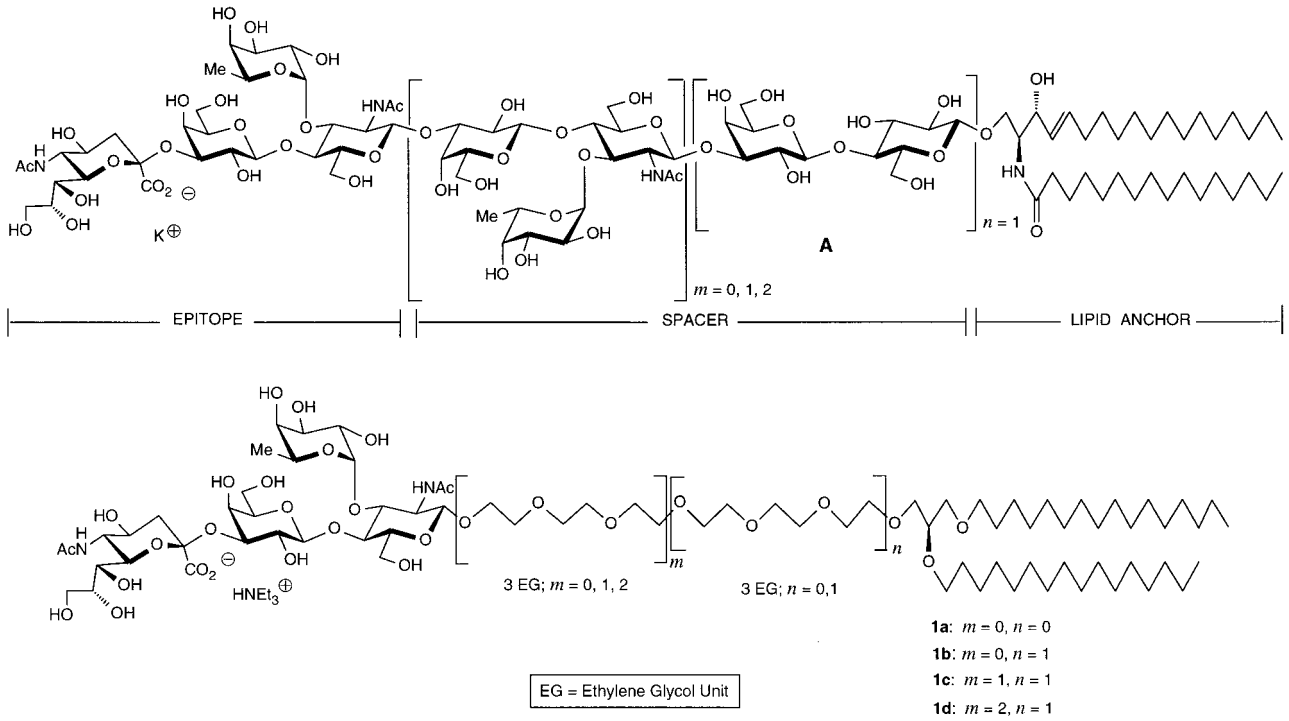
For the synthesis of the sLe^X tetrasaccharide moiety a highly convergent strategy was designed, which differed in the protective-group pattern of the required glucosamine building block and in the sequence of the connection of the four sugar residues from previous strategies.[20]

In order to minimize the influence of the carbohydrate backbone, to which the sLe^X moiety is generally attached in nature (Scheme 1, **A**), an oligoethylene glycol spacer was selected instead. Its length was chosen in order to mimic β(1-3)-linked lactose and/or lactosamine residues, that is, triethylene glycol units were combined. Thus, **1a** ($m=n=0$) corresponds to a compound in which the sLe^X moiety is directly linked to the ceramide moiety, which has not been found in nature, **1b** ($m=0, n=1$) corresponds to natural sLe^X, **1c** ($m=n=1$) to dimer sLe^X, and **1d** ($m=2, n=1$) to trimer sLe^X. In addition, in **1a–d** the ceramide residue is replaced by the 1,2-di-*O*-hexadecyl glycerol moiety to avoid physical demixing.[21]

Results and Discussion

Synthesis of sLe^X neoglycolipids 1a–1d: The starting material for the molecules with 1,2-di-*O*-hexadecylglycerol was the readily available 1,2-di-*O*-isopropylidene-*sn*-glycerol (Scheme 2). Its transformation into known 1-*O*-benzyl-*sn*-glycerol (**2a**) followed standard procedures.[22] Transformation of **2a** into the di-*O*-hexadecyl intermediate **3a** and then hydrogenolytic *O*-debenzylation (→**4a**) was performed with some modification of known procedures.[23]

For the attachment of one, two, or three triethylene glycol units to the glycerol residue, commercially available triethylene glycol monochlorohydrin **5** was chosen; this was treated with benzyl bromide in the presence of NaH as base to afford *O*-benzyl-protected derivative **6**. Reaction of 1,2-di-*O*-isopropylidene-*sn*-glycerol with **6** in the presence of NaH as base and tetrabutylammonium iodide (TBAI) as activator gave alkylation product **7**. Acid catalyzed de-*O*-isopropylidenation (→**2b**), then *O*-alkylation with hexadecyl bromide under the above described conditions (→**3b**), and finally hydrogenolytic *O*-debenzylation afforded glycerol derivative **4b**[24] with one



Scheme 1.

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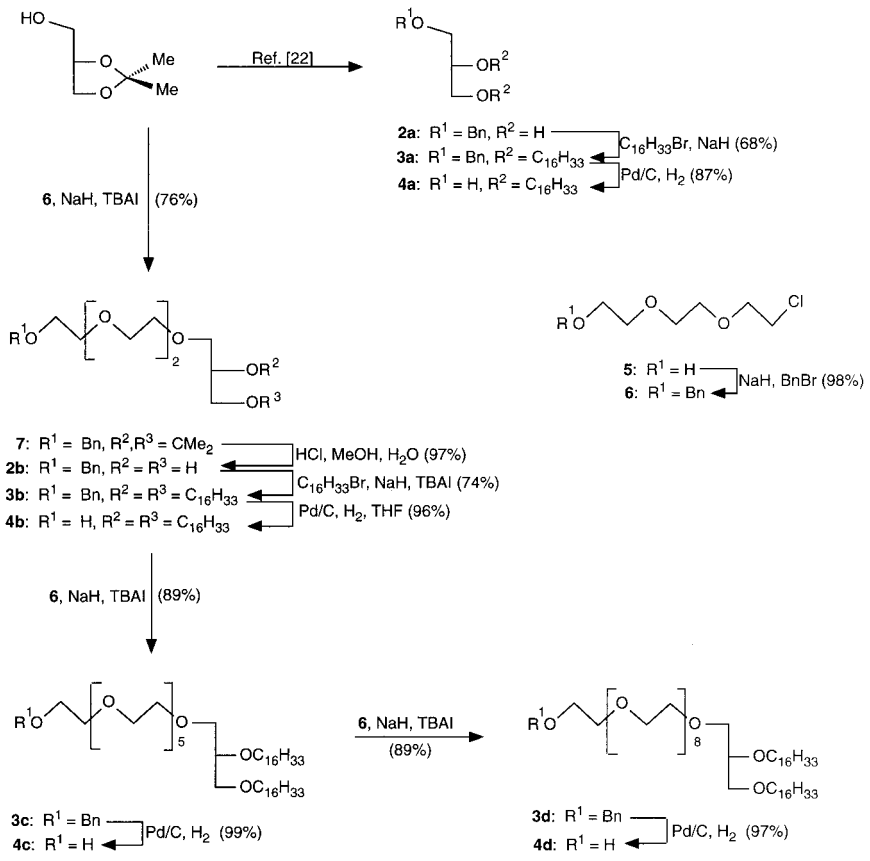


Scheme 2.

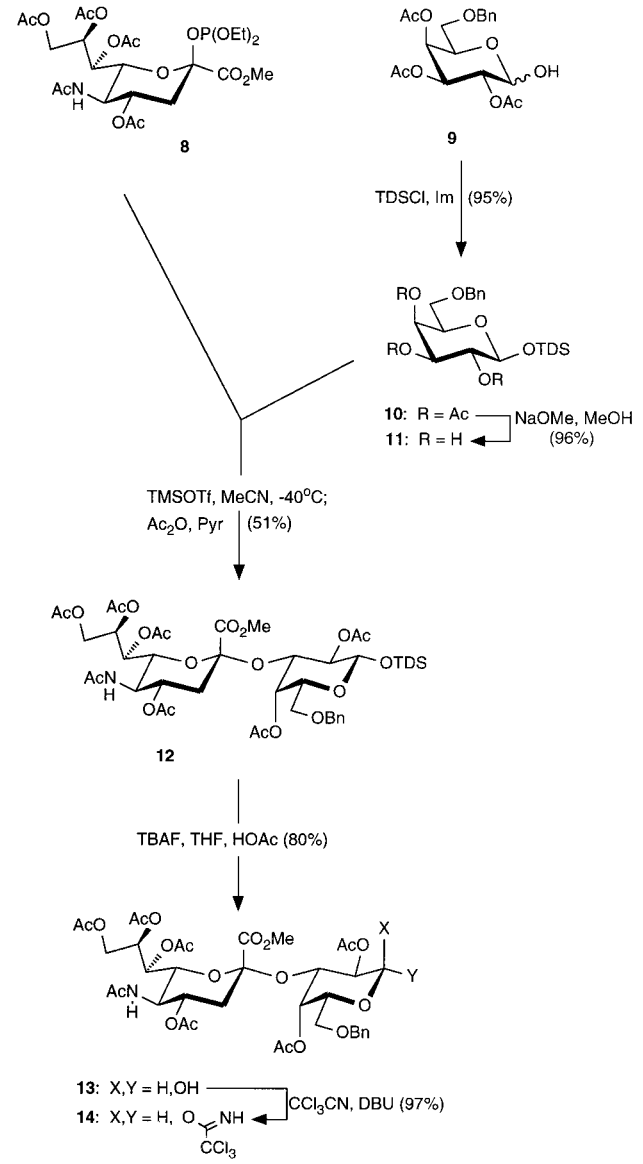
O-unprotected triethylene glycol unit. Compound **4b** served also for the chain extension in the synthesis of **4c** and **4d**. Reaction of **4b** with alkylating agent **6** gave **3c** under the above described conditions, which afforded on hydrogenolytic *O*-debenzylation compound **4c**. Repetition of the same two steps with **4c** furnished via **3d** compound **4d**. Thus, all four compounds **4a–d** are available that are required for the synthesis of **1a–d**.

For the synthesis of the sLe^X epitope, proper ligation of *N*-acetylneuraminic acid, D-galactose, L-fucose, and *N*-acetyl-D-glucosamine is required. Several successful approaches to solving this problem have already been reported.[9, 20] We selected here a convergent strategy that seemed to be particularly appropriate; we employed sialylation of a galactose-derived acceptor and fucosylation of a glucosamine-derived acceptor and finally ligation of the two disaccharides to the tetrasaccharide. As the sialyl donor the known phosphite derivative **8**[25] (Scheme 3) was chosen. The high acceptor reactivity of 2,3,4-*O*-unprotected galactose residues in sialylation reactions[20, 25, 26] was reason to transform known 6-*O*-benzyl derivative **9** into thexyldimethylsilyl (TDS)-protected compound **10**, which on *O*-deacetylation at low temperatures afforded acceptor **11** without silyl group migration.[27] Sialylation of **11** with **8** in acetonitrile at $-40\,^{\circ}C$ and in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the catalyst and then immediate *O*-acetylation of the product mixture afforded the desired α-linked disaccharide **12**, as derived from the NMR data, in 51% overall yield (gated proton-decoupled ^{13}C NMR:[28] $J_{C\text{-}1,H\text{-}3ax} = 6.0$ Hz). Removal of the 1a-*O*-TDS group by treatment with tetrabutylammonium fluoride (TBAF) in THF/HOAc afforded 1a-*O*-unprotected **13**, which on reaction with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base afforded trichloroacetimidate **14** in almost quantitative yield.

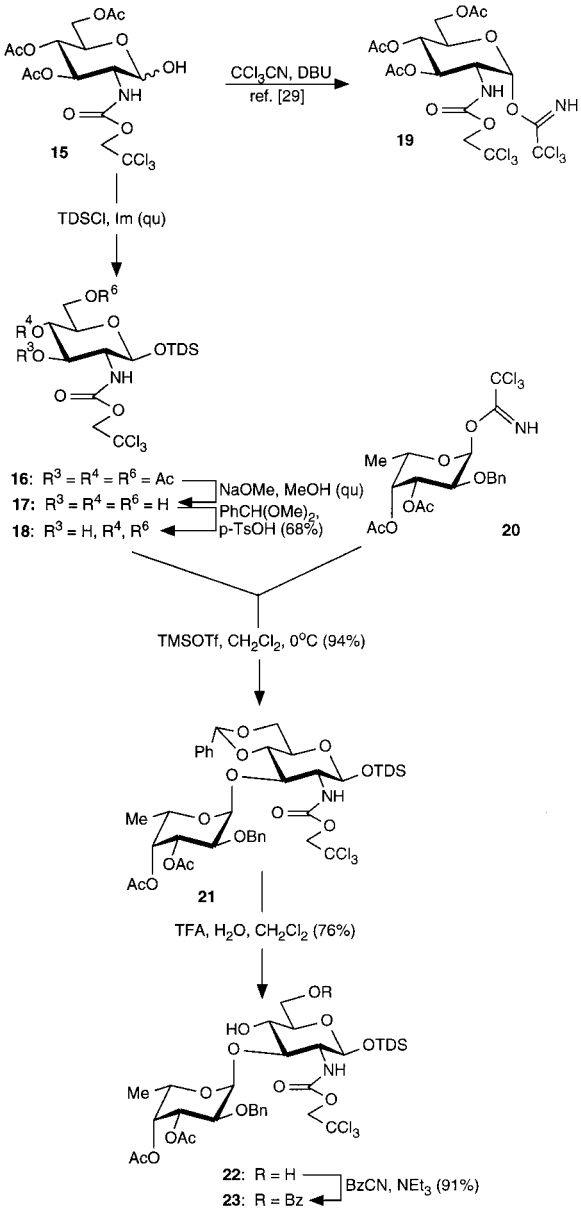
For the glucosamine-derived acceptor, *N*-trichloroethoxycarbonyl (*N*-Teoc) protection was selected because it is compatible with standard protecting group manipulations required in oligosaccharide synthesis; as a neighboring group it supports β-glycoside bond formation, and convenient replacement by the *N*-acetyl group has also been reported.[29] Thus, the known compound **15** (Scheme 4) was transformed into TDS-protected derivative **16**, which on *O*-deacetylation ($\rightarrow$**17**) and ensuing 4,6-*O*-benzylidenation gave 3-*O*-unprotected derivative **18**. Additionally, known trichloroacetimidate **19** was obtained from **15**; this was required at a later stage of the synthetic studies. As fucosyl donor the trichloroacetimidate **20** of 2-*O*-benzyl-3,4-di-*O*-acetylfucose was chosen as it has been shown to give α-fucopyranosides in high yield.[30] Fucosylation of **18** with donor **20** in the presence of TMSOTf as catalyst afforded α-linked disaccharide **21** in almost quantitative yield (^1H NMR: $J_{1b,2b}$=3.6 Hz). Acid-catalyzed cleavage of the 4a,6a-*O*-benzylidene group ($\rightarrow$**22**) and then regioselective 6a-*O*-benzoylation afforded 4a-*O*-unprotected **23**. All yields could be improved over previously published results.[31]

Glycosylation of acceptor **23** with disaccharide donor **14** (Scheme 5) was performed in dichloromethane, and TMSOTf again served as catalyst, to afford the desired sLe^X intermediate **24** in 66% yield (^1H NMR: $J_{1c,2c} \approx 8.3$ Hz). 1a-*O*-Desilylation with TBAF in THF/HOAc afforded 1a-*O*-unprotected **25**; treatment with trichloroacetonitrile in the presence of DBU as base furnished trichloroacetimidate **26**, which served as tetrasaccharide donor for the acceptors **4a–d**.

The glycosylation reactions of **4a–d** with **26** were performed in dichloromethane as solvent and with TMSOTf as the catalyst; they afforded the desired β-glycosides **27a–d** in 56–88% yields (**27d**: ^1H NMR: $J_{1a,2a} \approx 8.0$ Hz, ^{13}C NMR: C-1a $\delta = 100.22$). Treatment of **27a–d** with Zn in acetic anhydride[29] led to replacement of the *N*-Teoc group by the *N*-acetyl group; immediate hydrogenolytic *O*-debenzylation



Scheme 3.



Scheme 4.

afforded partly *O*-acylated intermediates **28a–d**, which on treatment with NaOMe in MeOH and then with KOH (0.2N) gave target molecules **1a–d**; they were isolated as triethylammonium salts after chromatography with chloroform/MeOH/H_2O/NEt_3 as eluents.

In order to differentiate molecular recognition between selectins on CHO-E cells and the sLe^X epitope from unspecified interactions, ligation of **4d** directly to the sialyl residue and to the *N*-acetylglucosamine residue was performed for comparison. To this end, glycosylation of **4d** with **8** was carried out; this gave a 1:1 α/β-mixture of glycosides **29**α,β (Scheme 6). De-*O*-acetylation ($\rightarrow$**30**α,β) and then saponification of the ester moiety gave the desired **31**α and also **31**β. Similarly, reaction of **4d** with donor **19** led to β-linked glycoside **32** (^{1}H NMR: $J_{1,2}$=8.6 Hz) in high yield. Treatment of **32** with Zn in acetic anhydride led to the *N*-acetyl derivative **33**, which furnished on treatment with NaOMe in MeOH the desired *N*-acetylglucosamine derivative **34**.

Cell-rolling investigations: In order to investigate the ability of the compounds **1a–d, 31**α, **31**β, and **34** to mediate selectin-induced cell rolling, the previously described dynamic model system was employed.[19] By means of the Langmuir–Blodgett technique, the glycolipids were incorporated into a well defined support-fixed model membrane and assembled into a flow chamber. In contrast to the established static binding assays, which solely focus on quantifying cell-binding events,[9] this flow chamber assay considers the physiological function of selectins and selectin ligands in the mediation of rolling of fluorescently labeled selectin-presenting cells under physiological shear force conditions; this process can be directly followed by microscopic means. It was shown that selectin-mediated cell rolling, as a special form of cell adhesion with a much lower velocity than free flowing cells, is sensitively balanced between firm adhesion and detachment by the ligand densities in the model membrane. Whereas in static

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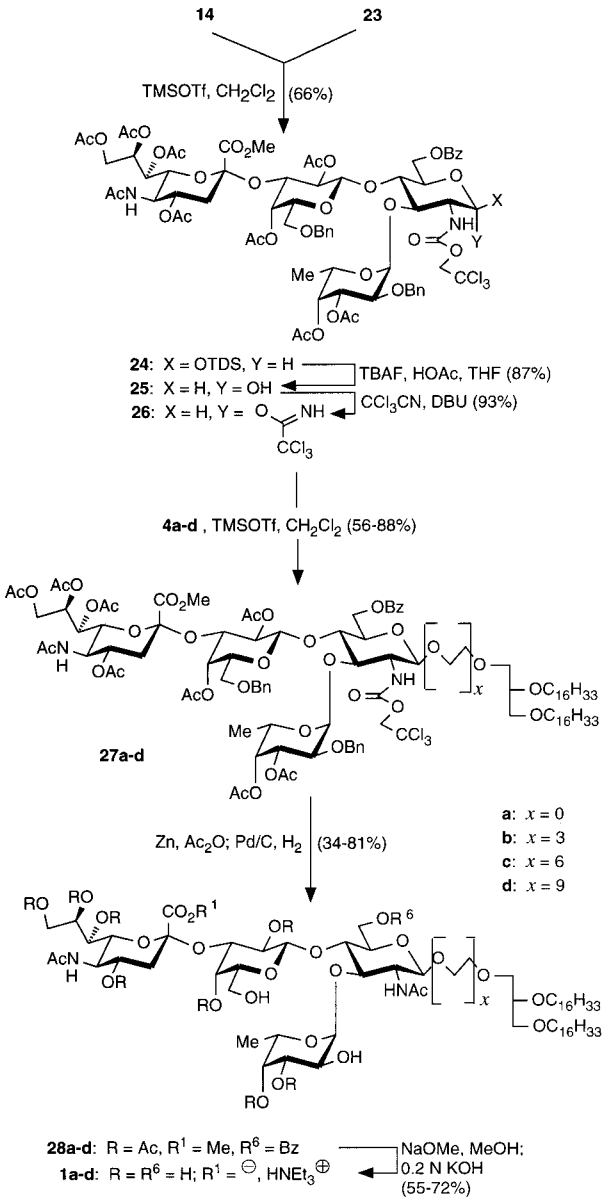


Scheme 5.

binding assays relatively high ligand concentrations or pure ligand layers were used, in the dynamic system cell rolling could be detected within a relatively low concentration range (around 0.05% glycolipid incorporated in a phospholipid matrix), which corresponds better to the physiological conditions.

Furthermore, it could be proven that the phospholipid matrix is of great importance because of its ligand organizing effects. A clustering of glycolipid ligands within the matrix was regarded to be essential for effective selectin recognition.[19] The compounds **1a–d**, **31*α*,*β***, and **34**, with spacers of different lengths, were investigated in order to focus on the influence of sLe^X mobility and accessibility at the membrane surface on the rolling movement.

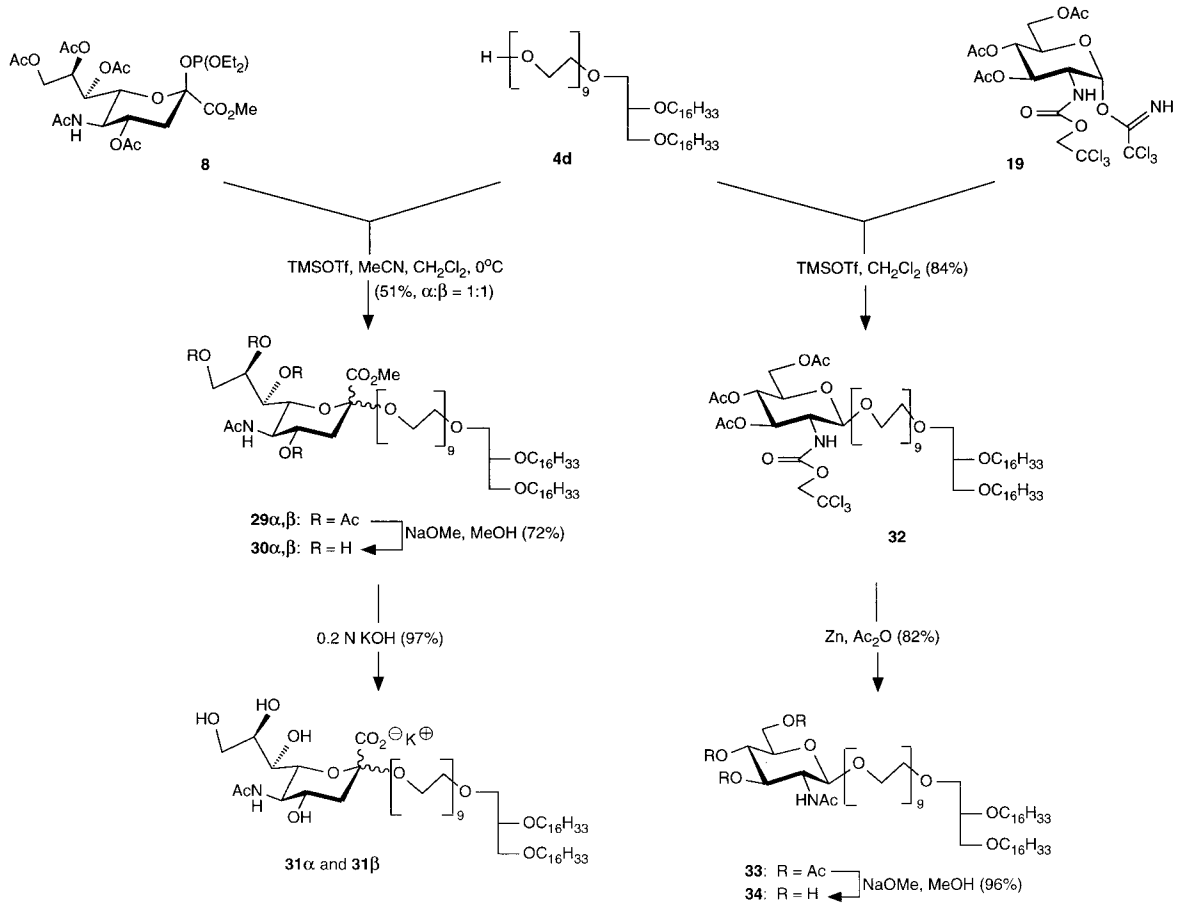
Model membranes were prepared by incorporating different concentrations of compounds **1a–d** into a 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) matrix and, as a prerequisite for the following investigations, their clustered arrangement was proven (data not shown). These membranes were mounted into the flow chamber, and Chinese hamster ovarian cells that stably express E-selectin (CHO-E) were allowed to interact with the model membrane under static conditions for five minutes. After that time, the cell adhesion and rolling were analyzed at a shear rate of 200 s^{-1}, as shown in Figure 1. At concentrations above 0.1% of each compound in the membranes, all cells in the analyzed area stay adhered and did not show any mobility. Reduction of the ligand concentrations led to great differences in cell-membrane interactions. Whereas the spacerless compound **1a** was no longer able to mediate cell binding and all cells were detached under the flow conditions, lower concentrations of the spacer-containing compounds **1b–d** induced a cell movement that could be defined as rolling (rolling velocity of about 5–25 μm s^{-1} versus 1–2 mm s^{-1} of freely flowing cells).

Furthermore, spacer elongation, which corresponds to an increased headgroup flexibility, is of crucial importance in supporting effective cell rolling. The compounds **1c** and **1d** with long spacers mediated a rolling at lower concentrations down to 0.0025% in the lipid matrix compared with compound **1b**, which has a shorter spacer. To focus on the influence of spacer length and structure on rolling, a glycolipid with a naturally occurring, relatively stiff lactose spacer between the sLe^X headgroup and a ceramide moiety [i.e. sLe^X glycosphingolipid **A** ($m = 0$, $n = 1$)[20, 35], Scheme 1] was used. Membranes containing this substance were nearly as effective as those with compound **1b**; this should be attributed to the similar spacer length of these two compounds, illustrated in Scheme 1. Because of the higher flexibility of the ethylene glycol spacer in **1b**, this substance seems to be slightly more efficient in cell binding, as reflected in the reduced cell-rolling velocity. All these cell-binding events were of a specific nature, since preincubation of the cells with a blocking E-selectin antibody prevented any cell-membrane interactions. Compounds **31*α***, **31*β***, and **34** were not able to interact with selectin-containing cells, since nearly all cells were removed from these model membranes. This demonstrates that a complete sLe^X structure is essential for selectin binding.

Thus, it could be demonstrated that sLe^X glycolipids are able to mediate a selectin-dependent rolling when they are laterally clustered in a model phospholipid matrix, and minimal flexibility and accessibility of the sLe^X moiety is sufficient to maintain cell rolling. Therefore, it can be concluded that the extended silaomucin structures of the natural ligands, which are extended and not well defined with respect to structural requirements for mediating cell rolling, are not ultimately necessary for rolling; however, the sialomucin structures with their high flexibility offer optimal conditions for a rolling event.

Conclusion

In the present study a series of sLe^X glycolipids were successfully synthesized which differed in their ethylene glycol spacer length. Thus, the influence of the carbohydrate headgroup mobility in glycolipids on the biological recogni-



Scheme 6.

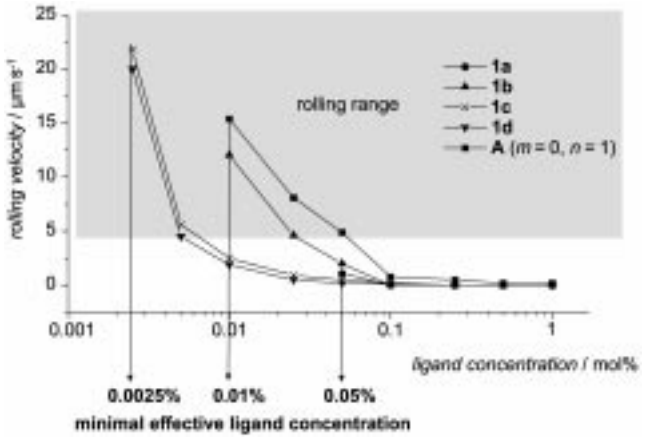


Figure 1. Selectin-mediated cell rolling along model membranes containing different ligand structures (see Scheme 1).

tion phenomena of selectin-dependent cell rolling could be analyzed for the first time.

It was demonstrated that a minimal headgroup mobility is an important factor for mediating cell rolling, since sLe^X-lipids with longer spacers were much more effective than those with shorter spacers. The study gives an insight into the mechanism of leukocyte rolling along the endothelium and it provides structural information on natural selectin ligands.

Experimental Section

General techniques: Solvents were purified according to the standard procedures. Flash chromatography was performed on J.T. Baker silica gel 60 (0.040 – 0.063 mm) at a pressure of 0.4 bar. Thin-layer chromatography was performed on Merck silica gel plastic plates, $60F_{254}$ or Merck silica gel glass plates, HPTLC $60F_{254}$; compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL) and heating at 150 °C. Optical rotations were measured on a Perkin – Elmer polarimeter 241 in a 1 dm cell at 22 °C. NMR measurements were recorded at 22 °C on a Bruker AC250 Cryospec or a Bruker DRX600. TMS or the resonance of the deuterated solvent was used as internal standard; solvents: $CDCl_3$, $\delta = 7.24$; C_6D_6, $\delta = 7.15$; D_2O, $\delta = 4.63$. Target molecules **1a – d** (max. 6 mg) were measured in a 320 mmolar solution of $[D_{25}]$sodiumdodecyl sulfate (SDS) in 0.5 mL D_2O. MALDI-mass spectra were recorded on a Kratos Kompact MALDI I instrument using a 2,5-dihydroxybenzoic acid matrix.

3-*O*-Benzyl-1,2-di-*O*-hexadecyl-*sn*-glycerol (3a): Sodium hydride (50 mg, 21 mmol) was added in small portions to a solution of **2a**[22] (750 mg, 4.12 mmol), hexadecyl bromide (3.80 mL, 12.4 mmol), and a catalytic amount of tetrabutylammonium iodide in dry DMF (15 mL) at room temperature. After stirring for 36 h at room temperature, the mixture was diluted with ethyl acetate (200 mL) and washed several times with brine. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. Flash chromatography (petroleum ether to petroleum ether/ethyl acetate 25:1) afforded **3a** (1.77 g, 68%) as a colorless oil. The physical properties found for **3a** are in full accordance with those described in reference [23].

1,2-Di-*O*-hexadecyl-*sn*-glycerol (4a): Compound **3a** (1.72 g, 2.73 mmol) was dissolved in THF/methanol (1:1, 20 mL) and palladium on charcoal (0.17 g, 10% Pd) was added. The mixture was stirred vigorously under a hydrogen atmosphere at normal pressure. After 16 h the catalyst was

0947-6539/00/0601-0116 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 1

filtered off and washed with THF. Evaporation of the solvent and flash chromatography (petroleum ether/ethyl acetate 19:1 to 9:1) furnished **4a** (1.28 g, 87%) as a colorless solid. The physical properties found for **3b** are in full accordance with those described in reference [23].

8-Benzyloxy-1-chloro-3,6-dioxaoctane (6): Sodium hydride (7.92 g, 330 mmol) was added in small portions to a solution of triethylene glycol monochlorohydrin **5**[32] (43.6 mL, 300 mmol) and benzyl bromide (107 mL, 900 mmol) in dry DMF (100 mL) at 0 °C. After stirring overnight at room temperature, the mixture was filtered and concentrated under reduced pressure. Flash chromatography (toluene to toluene/ethyl acetate 1:1) afforded **6** (76.2 g, 98%) as a colorless oil. The physical properties found for **6** are in full accordance with those described in reference [33].

3-*O*-[8-Benzyloxy-3,6-dioxaoctyl]-1,2-*O*-isopropylidene-*sn*-glycerol (7): Sodium hydride (3.22 g, 134 mmol) was added to a solution of 1,2-di-*O*-isopropylidene-*sn*-glycerol[32, 34] (11.8 g, 89.3 mmol), compound **6** (34.6 g, 134 mmol), and tetrabutylammonium iodide (1.7 g, 4.6 mmol) in dry THF (150 mL). After stirring for 15 h under reflux, additional **6** (11.5 g, 44.5 mmol) and sodium hydride (1.07 g, 44.6 mmol) were added. After 6 h under reflux the solution was concentrated under reduced pressure, and brine (500 mL) was slowly added. The solution was extracted with ethyl acetate (3 × 500 mL), the combined organic layers were dried over magnesium sulfate and concentrated in vacuo. Flash chromatography (toluene/ethyl acetate 2:1 to 1:1) yielded **7** (24.2 g, 76%) as a colorless oil. $R_f = 0.64$ ($CHCl_3$/methanol 15:1); $[\alpha]_D = +8.9$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.36$, 1.42 (2s, 6H; $2CH_3$), 3.49, 3.58 (2dd, $^2J = 10.0$ Hz, $^3J = 5.6$ Hz, 2H; CH_2 glycerol), 3.61–3.71 (m, 12H; $3OCH_2$-CH_2O), 3.72, 4.04 (2dd, $^2J = 10.0$ Hz, $^3J = 5.6$ Hz, 2H; CH_2 glycerol), 4.28 (tt, $^3J = 5.8$ Hz and 6.2 Hz, 1H; CH glycerol), 4.57 (s, 2H; CH_2Ph), 7.25–7.35 (m, 5H; C_6H_5); $C_{19}H_{30}O_6$ (354.4): calcd C 64.39, H 8.53; found C 63.94, H 8.43%.

3-*O*-[8-Benzyloxy-3,6-dioxaoctyl)-*sn*-glycerol (2b): A solution of **7** (19.7 g, 55.6 mmol) in methanol (80 mL) was treated with a solution of hydrochloric acid (1M, 50 mL). After stirring for 1 h at room temperature, the solution was concentrated in vacuo and coevaporated with toluene. The residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **2b** (16.5 g, 97%) as a colorless oil. $R_f = 0.40$ ($CHCl_3$/methanol 15:1); $[\alpha]_D = -3.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 2.48$ (br s, 2H; 2OH), 3.56–3.67 (m, 16H; $8CH_2O$), 3.85 (m, 1H; CH of glycerol), 4.57 (s, 2H; CH_2Ph), 7.26–7.35 (m, 5H; C_6H_5); $C_{16}H_{26}O_6$ (314.4): calcd C 61.13, H 8.34; found C 60.92, H 8.35%.

3-*O*-[8-Benzyloxy-3,6-dioxaoctyl)-1,2-di-*O*-hexadecyl-*sn*-glycerol (3b): Sodium hydride (4.38 g, 183 mmol) was added to a solution of **2b** (16.4 g, 52.2 mmol), hexadecyl bromide (64 mL, 0.21 mol), and tetrabutylammonium iodide (0.96 g, 2.6 mmol) in dry THF (250 mL). After stirring for 20 h under reflux, methanol (50 mL) was added carefully, and the solution was concentrated in vacuo. The residue was diluted with water (50 mL) and subsequently extracted with ethyl acetate (2 × 400 mL). The combined organic layers were dried over magnesium sulfate, and the solvent was evaporated. Purification was accomplished by flash chromatography (toluene/ethyl acetate 9:1), which furnished pure **3b** (29.4 g, 74%) as a colorless solid. $R_f = 0.43$ (petroleum ether/ethyl acetate 4:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J = 6.6$ Hz, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.50–1.59 (m, 4H; $2OCH_2CH_2$), 3.40–3.71 (m, 21H; 21HCO), 4.57 (s, 2H; CH_2Ph), 7.26–7.35 (m, 5H; C_6H_5); $C_{48}H_{90}O_6$ (763.2): calcd C 75.54, H 11.89; found C 75.82, H 11.86%.

1,2-Di-*O*-hexadecyl-3-*O*-[8-hydroxy-3,6-dioxaoctyl)-*sn*-glycerol (4b): Compound **3b** (27.1 g, 35.5 mmol) was dissolved in THF (400 mL), and palladium on charcoal (1.3 g, 10% Pd) was added. The mixture was stirred vigorously under a hydrogen atmosphere at normal pressure. After 16 h the catalyst was filtered off and washed. Evaporation of the solvent and flash chromatography (toluene/ethyl acetate 4:1) furnished **4b** (23.0 g, 96%) as a colorless solid. $R_f = 0.42$ (petroleum ether/ethyl acetate 1:2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J = 6.6$ Hz, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.51–1.59 (m, 4H; $2OCH_2CH_2$), 2.26–2.31 (m, 1H; OH), 3.40–3.75 (m, 21H; 21HCO); $C_{41}H_{84}O_6$ (673.1): calcd C 73.16, H 12.58; found C 72.74, H 12.68%.

3-*O*-[17-Benzyloxy-3,6,9,12,15-pentaoxaheptadecyl]-1,2-di-*O*-hexadecyl-*sn*-glycerol (3c): Sodium hydride (1.0 g, 42 mmol) was added to a solution of compound **4b** (18.8 g, 27.9 mmol), compound **6** (10.8 g, 41.9 mmol), and tetrabutylammonium iodide (0.52 g, 1.4 mmol) in dry THF (200 mL). After stirring for 15 h under reflux, additional **6** (7.20 g, 27.8 mmol) and sodium hydride (0.67 g, 28 mmol) were added. After a further night under reflux the mixture was filtered and washed with THF. Evaporation of the solvent and flash chromatography (toluene/ethyl acetate 4:1 to 1:1) furnished pure **3c** (22.2 g, 89%) as a colorless solid. $R_f = 0.38$ (petroleum ether/ethyl acetate 1:2); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J = 6.7$ Hz, 6H; $2CH_3$), 1.25 (br s, 52H; 26 CH_2), 1.52–1.58 (m, 4H; $2OCH_2CH_2$), 3.40–3.66 (m, 33H; 33HCO), 4.57 (s, 2H; CH_2Ph), 7.26–7.34 (m, 5H; C_6H_5); $C_{54}H_{102}O_9$ (895.4): calcd C 72.44, H 11.48; found C 72.23, H 11.28%.

1,2-Di-*O*-hexadecyl-3-*O*-[17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl]-*sn*-glycerol (4c): Compound **3c** (21.5 g, 24.0 mmol) was dissolved in THF (300 mL), and palladium on charcoal (1.9 g, 10% Pd) was added. The mixture was stirred vigorously under a hydrogen atmosphere at normal pressure. After 16 h the catalyst was filtered off and washed. Evaporation of the solvent furnished **4c** (19.2 g, 99%) as a colorless solid. $R_f = 0.67$ ($CHCl_3$/methanol 15:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.50–1.57 (m, 4H; $2OCH_2CH_2$), 2.28 (br s, 1H; OH), 3.39–3.75 (m, 33H; 33HCO); $C_{47}H_{96}O_9$ (805.3): calcd C 70.10, H 12.02; found C 70.03, H 11.95%.

3-*O*-[26-Benzyloxy-3,6,9,12,15,18,21,24-octaoxahexacosyl]-1,2-di-*O*-hexadecyl-*sn*-glycerol (3d): Sodium hydride (130 mg, 5.3 mmol) was added to a solution of compound **4c** (2.86 g, 3.55 mmol), compound **6** (1.38 g, 5.33 mmol), and tetrabutylammonium iodide (70 mg, 0.19 mmol) in dry THF (100 mL). After stirring for 15 h under reflux additional **6** (0.92 g, 3.6 mmol) and sodium hydride (85 mg, 3.5 mmol) were added. After a further night under reflux the mixture was filtered and washed with THF. Evaporation of the solvent and flash chromatography (toluene/ethyl acetate 4:1 to 2:1) furnished pure **3d** (3.25 g, 89%) as a colorless solid. $R_f = 0.51$ ($CHCl_3$/methanol 15:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.51–1.57 (m, 4H; $2OCH_2CH_2$), 3.36–3.70 (m, 45H; 45HCO), 4.57 (s, 2H; CH_2Ph), 7.26–7.34 (m, 5H; C_6H_5); $C_{60}H_{114}O_{12}$ (1027.6): calcd C 70.13, H 11.18; found C 70.03, H 11.00%.

1,2-Di-*O*-hexadecyl-3-*O*-[26-hydroxy-3,6,9,12,15,18,21,24-octaoxahexacosyl]-*sn*-glycerol (4d): Compound **3d** (2.35 g, 2.29 mmol) was dissolved in THF (60 mL), and palladium on charcoal (0.24 g, 10% Pd) was added. The mixture was stirred vigorously under a hydrogen atmosphere at normal pressure. After 16 h the catalyst was filtered off and washed. Evaporation of the solvent and flash chromatography (toluene/acetone 1:1) furnished **4d** (2.08 g, 97%) as a colorless solid. $R_f = 0.36$ (toluene/acetone 1:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.50–1.57 (m, 4H; $2OCH_2CH_2$), 2.5 (br s, 1H; OH), 3.40–3.75 (m, 45H; 45HCO); $C_{53}H_{108}O_{12}$ (937.4): calcd C 67.91, H 11.61; found C 67.73, H 11.53%.

Thexyldimethylsilyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranoside (10): Compound **9**[35] (28.7 g, 72.4 mmol) and imidazole (8.38 g, 123 mmol) were dissolved in dry DMF (250 mL). Thexyldimethylsilyl chloride (21.4 mL, 109 mmol) was added at room temperature to the mixture. After stirring overnight, methanol (50 mL) was added. The solution was concentrated in vacuo and then diluted with ethyl acetate (1000 mL). The organic layer was washed with brine (3 × 300 mL), dried over magnesium sulfate, and evaporated. Flash chromatography (toluene/ethyl acetate 2:1) gave compound **10** (37.1 g, 95%) as a colorless oil. $R_f = 0.41$ (petroleum ether/ethyl acetate 4:1); $[\alpha]_D = -15.8$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.15$, 0.18 (2s, 6H; $Si(CH_3)_2$), 0.83, 0.83, 0.84, 0.87 (4s, 12H; $4CH_3$), 1.55–1.66 (m, 1H; CH), 1.98, 2.02, 2.06 (3s, 9H; $3COCH_3$), 3.46–3.58 (m, 2H; 6-, 6′-H), 3.81–3.87 (m, 1H; 5-H), 4.43, 4.56 (2d, $^2J = 12.0$ Hz, 2H; CH_2Ph), 4.69 (d, $J(1,2) = 7.5$ Hz, 1H; 1-H), 4.99 (dd, $J(2,3) = 10.5$ Hz, $J(3,4) = 3.4$ Hz, 1H; 3-H), 5.14 (dd, $J(1,2) = 7.5$ Hz, $J(2,3) = 10.5$ Hz, 1H; 2-H), 5.44 (dd, $J(3,4) = 3.4$ Hz, $J(4,5) = 1.1$ Hz, 1H; 4-H), 7.26–7.37 (m, 5H; C_6H_5); $C_{27}H_{42}O_9Si$ (538.7): calcd C 60.20, H 7.86; found C 60.56, H 7.92%.

Thexyldimethylsilyl 6-*O*-benzyl-β-D-galactopyranoside (11): A solution of **10** (38.9 g, 72.2 mmol) in dry methanol (220 mL) was treated at −25 °C with a solution of sodium methoxide (1M) in methanol (1.5 mL). After stirring overnight at −15 °C the solution was neutralized with Amberlite IR120 (H^+), filtered, and evaporated. Flash chromatography (ethyl acetate) yielded **11** (28.6 g, 96%) as a colorless oil. $R_f = 0.53$ (ethyl acetate); $[\alpha]_D = -4.5$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.18$, 0.19 (2s, 6H; $Si(CH_3)_2$), 0.87, 0.87, 0.89, 0.90 (4s, 12H; $4CH_3$), 1.59–1.70 (m, 1H;

CH), 3.55–3.57 (m, 2H; 2-, 3-H), 3.62 (td, $J(4,5)=1.0$ Hz, $J(5,6)=J(5,6')=5.5$ Hz, 1H; 5-H), 3.71 (dd, $^2J=10.0$ Hz, $J(5,6)=5.4$ Hz, 1H; 6-H), 3.78 (dd, $^2J=10.0$ Hz, $J(5,6')=5.6$ Hz, 1H; 6′-H), 3.98 (dd, $J(3,4)=2.7$ Hz, $J(4,5)=1.1$ Hz, 1H; 4-H), 4.45 (d, $J(1,2)=7.3$ Hz, 1H; 1-H), 4.57 (s, 2H; CH_2Ph), 7.25–7.39 (m, 5H; C_6H_5); $C_{21}H_{36}O_6Si$ (412.6): calcd C 61.13, H 8.79; found C 60.82, H 8.79%.

Thexyldimethylsilyl *O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranoside (12): Acceptor **11** (5.11 g, 12.4 mmol) was dissolved in dry acetonitrile (80 mL) over molecular sieves (4 Å). After one hour the solution was added to **8**[25] (12.7 g, 20.8 mmol) and cooled to −40 °C. After addition of trimethylsilyl trifluoromethanesulfonate (500 μL, 0.28 mmol), the solution was stirred for 45 min and then neutralized with triethylamine (500 μL) and concentrated in vacuo. The residue was dissolved in chloroform (200 mL) and washed with hydrochloric acid (1M). The organic layer was neutralized with sodium bicarbonate and dried over magnesium sulfate. The remaining foam was dissolved in pyridine (80 mL), and acetic anhydride (80 mL) was added. After 2 days the solution was evaporated, and the residue was purified by flash chromatography (toluene/acetone 3:1) to give **12** (6.16 g, 51%) as a pale yellow foam. $R_f=0.54$ (toluene/acetone 1:1); $[\alpha]_D=-11.4$ ($c=1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=0.15$, 0.16 (2s, 6H; $Si(CH_3)_2$), 0.83–0.87 (m, 12H; $2C(CH_3)_2$), 1.53–1.64 (m, 1H; CH), 1.71 (dd, $^2J=J(3_{ax},4)=12.4$ Hz, 1H; $3b_{ax}$-H), 1.83, 1.98, 2.01, 2.03, 2.05, 2.13, 2.16 (7s, 21H; $7COCH_3$), 2.56 (dd, 1H; $3b_{eq}$-H), 3.45 (dd, $^2J=9.9$ Hz, $J(5,6)=6.1$ Hz, 1H; 6a-H), 3.47 (dd, $^2J=10.0$ Hz, $J(5,6')=6.2$ Hz, 1H; 6′a-H), 3.62 (dd, $J(5,6)=10.7$ Hz, $J(6,7)=2.8$ Hz, 1H; 6b-CH3), 3.78–3.81 (m, 1H; 5a-H), 3.82 (s, 3H; $COOCH_3$), 4.00 (dd, $^2J=12.4$ Hz, $J(8,9)=5.8$ Hz, 1H; 9b-H), 4.04 (ddd, $J(4,5)=J(5,6)=J(5,N)=10.5$ Hz, 1H; 5b-H), 4.33 (dd, $^2J=12.4$ Hz, $J(8,9')=2.7$ Hz, 1H; 9′b-H), 4.42 (d, $^2J=11.8$ Hz, 1H; C*H*HPh), 4.47 (dd, $J(2,3)=10.2$ Hz, $J(3,4)=3.4$ Hz, 1H; 3a-H), 4.51 (d, $^2J=11.8$ Hz, 1H; C*H*HPh), 4.83 (d, $J(1,2)=7.7$ Hz, 1H; 1a-H), 4.85 (ddd, $J(3_{ax},4)=12.1$ Hz, $J(3_{eq},4)=4.6$ Hz, $J(4,5)=10.3$ Hz, 1H; 4b-H), 4.93–4.96 (m, 2H; 2a-, 4a-H), 5.05 (d, $J(5,N)=10.3$ Hz, 1H; NH), 5.34 (dd, $J(6,7)=2.8$ Hz, $J(7,8)\approx 8.9$ Hz, 1H; 7b-H), 5.53 (ddd, $J(7,8)\approx 8.9$ Hz, $J(8,9)=5.8$ Hz, $J(8,9')=2.7$ Hz, 1H; 8b-H), 7.25–7.31 (m, 5H; C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta=-3.32$ ($SiCH_3$), −1.99 ($SiCH_3$), 18.47, 18.51, 19.90, 19.97 ($4CH_3$), 20.73, 20.76, 20.85, 21.07, 21.39, 23.16, 24.82 ($7CO\mathit{C}H_3$), 33.91 ($CHMe_2$), 37.54 (3b-C), 49.10 (5b-C), 53.12 (OCH_3), 62.36 (9b-C), 67.20 (7b-C), 67.90 (8b-C), 68.43 (4a-C), 68.49 (6a-C), 69.42 (4b-C), 71.76 (3a-C), 71.84 (2a-C), 72.01 (6b-C), 72.09 (5a-C), 73.37 (CH_2Ph), 95.72 (2b-C), 96.90 (1a-C), 127.59–138.04 (phenyl-C), 167.87 (1b-C), 169.61, 169.67, 170.20, 170.33, 170.41, 170.46, 170.89 (7COMe); $C_{45}H_{67}NO_{20}Si$ (970.1): calcd C 55.71, H 6.96, N 1.44; found C 55.27, H 6.80, N 1.28%.

O*-(Methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzyl-α/β-D-galactopyranose (13)**: Compound **12** (766 mg, 790 μmol) was dissolved in dry THF (10 mL). Acetic acid (90 μL, 1.7 mmol) and then a solution of tetrabutylammonium fluoride (1M) in THF (1.7 mL, 1.7 mmol) were added at −25 °C. The mixture was slowly warmed to room temperature and stirred for 3 days. After addition of water (100 mL) the solution was washed with diethyl ether (5 × 100 mL). The combined organic layers were dried over magnesium sulfate and concentrated in vacuo. Purification was accomplished by flash chromatography (toluene/acetone 3:1 to 2:1) to furnish pure **13** (523 mg, 80%) as a colorless foam in a ratio of $\alpha/\beta=4:3$. $R_f=0.39$ and 0.42 ($CHCl_3$/methanol 15:1); ^{1}H NMR (600 MHz, $CDCl_3$): **13*α: $\delta=1.65-1.75$ (m, 1H; $3b_{ax}$-H), 1.83 (s, 3H; $NCOCH_3$), 1.99–2.22 (m, 21H; $7COCH_3$), 2.57 (dd, $^2J=12.6$ Hz, $J(3_{eq},4)=4.5$ Hz, 1H; $3b_{eq}$-H), 3.38 (dd, $^2J=9.7$ Hz, $J(5,6)=6.7$ Hz, 1H; 6a-H), 3.48 (dd, $^2J=9.7$ Hz, $J(5,6')=5.9$ Hz, 1H; 6′a-H), 3.63–3.65 (m, 1H; 6b-CH_3), 3.83 or 3.84 (s, 3H; $COOCH_3$), 3.95–3.99 (m, 1H; 9b-H), 4.06 (ddd, $J(4,5)=J(5,6)=J(5,N)=10.4$ Hz, 1H; 5b-H), 4.19 (dd, $J(5,6)=J(5,6')=6.3$ Hz, 1H; 5a-H), 4.36 (d, $^2J=12.3$ Hz, 1H; 9′b-H), 4.41, 4.51 (2d, $^2J=11.8$ Hz, 2H; CH_2Ph), 4.62 (dd, $J(2,3)=10.7$ Hz, $J(3,4)=3.5$ Hz, 1H; 3a-H), 4.87 (m, 1H; 4b-H), 5.04 (d, $J(3,4)=3.4$ Hz, 1H; 4a-H), 5.11–5.15 (m, 2H; 2a-H, NH), 5.28–5.30 (m, 2H; 1a-, 7b-H), 5.62 (ddd, $J(7,8)=9.1$ Hz, $J(8,9)=6.7$ Hz, $J(8,9')=2.5$ Hz, 1H; 8b-H), 7.24–7.31 (m, 5H; C_6H_5). **13*β***: $\delta=1.65-1.75$ (m, 1H; $3b_{ax}$-H), 1.83 (s, 3H; $NCOCH_3$), 1.99–2.22 (m, 21H; $7COCH_3$), 2.57 (dd, $^2J=12.6$ Hz, $J(3_{eq},4)=4.5$ Hz, 1H; $3b_{eq}$-H), 3.41(dd, $^2J=9.8$ Hz, $J(5,6)=6.3$ Hz, 1H; 6a-H), 3.51 (dd, $^2J=9.2$ Hz, $J(5,6')=6.2$ Hz, 1H; 6′a-H), 3.63–3.65 (m, 1H; 6b-CH_3), 3.83 or 3.84 (2s, 3H; $COOCH_3$), 3.85 (dd, $J(5,6)=J(5,6')=6.3$ Hz, 1H; 5a-H), 3.95–3.99 (m, 1H; 9b-H), 4.04 (ddd, $J(4,5)=J(5,6)=J(5,N)=10.4$ Hz, 1H; 5b-H), 4.36 (d, $^2J=12.3$ Hz, 1H; 9′b-H), 4.41, 4.51 (d, $^2J=11.8$ Hz, 2H; CH_2Ph), 4.59 (dd, $J(2,3)=10.1$ Hz, $J(3,4)=3.4$ Hz, 1H; 3a-H), 4.82 (d, $J(1,2)=8.0$ Hz, 1H; 1a-H), 4.87 (m, 1H; 4b-H), 4.89 (dd, 1H; 2a-H), 5.00 (d, $J(3,4)=3.4$ Hz, 1H; 4a-H), 5.13 (d, $J(N,5)=10.4$ Hz, 1H; NH), 5.35 (dd, $J(6,7)=2.7$ Hz, $J(7,8)=9.0$ Hz, 1H; 7b-H), 5.53 (ddd, $J(7,8)=8.7$ Hz, $J(8,9)=2.7$ Hz, $J(8,9')=5.9$ Hz, 1H; 8b-H), 7.24–7.31 (m, 5H; C_6H_5); MALDI: m/z: 851 $[M+Na]^+$, 868 $[M+K]^+$; $C_{37}H_{49}NO_{20}\cdot H_2O$ (845.8): calcd C 52.54, H 6.08, N 1.66; found C 52.59, H 6.24, N 1.59%.

***O*-(Methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,4-di-*O*-acetyl-6-*O*-benzyl-α/β-D-galactopyranosyl trichloroacetimidate (14)**: Trichloroacetonitrile (3.6 mL, 3.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (27 μL, 0.18 mmol) were added to a solution of **13** (2.95 g, 3.56 mmol) in dry dichloromethane (100 mL). After 1 h the mixture was concentrated in vacuo. Flash chromatography (toluene/acetone 2:1 + 1% triethylamine) furnished **14** (3.34 g, 97%) as a colorless foam in a ratio of $\alpha/\beta\approx 1:9$. $R_f=0.59$ (toluene/acetone 1:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=1.74$ (dd, $^2J=J(3_{ax},4)=12.4$ Hz, 1H; $3b_{ax}$-H), 2.01, 2.02, 2.04, 2.07, 2.07, 2.17, 2.18 (7s, 21H; $7COCH_3$), 2.59 (dd, $^2J=12.6$ Hz, $J(3_{eq},4)=4.6$ Hz, 1H; $3b_{eq}$-H), 3.46 (dd, $^2J=10.0$ Hz, $J(5,6)=6.9$ Hz, 1H; 6a-H), 3.57 (dd, $^2J=9.9$ Hz, $J(5,6')=5.7$ Hz, 1H; 6′a-H), 3.65 (dd, $J(5,6)=10.7$ Hz, $J(6,7)=2.7$ Hz, 1H; 6b-CH_3), 3.88 (s, 3H; $COOCH_3$), 3.98 (dd, $^2J=12.4$ Hz, $J(8,9)=5.9$ Hz, 1H; 9b-H), 3.98–4.09 (m, 2H; 5a-, 5b-H), 4.32–4.46 (m, 3H; 3a-, 9′b-H, C*H*HPh), 4.55 (d, $^2J=11.9$ Hz, 1H; C*H*HPh), 4.72 (dd, $J(2,3)=10.2$ Hz, $J(3,4)=3.4$ Hz, 1H; 3a-H), 4.90 (ddd, $J(3_{ax},4)=12.1$ Hz, $J(3_{eq},4)=4.6$ Hz, $J(4,5)=10.3$ Hz, 1H; 4b-H), 5.04 (d, $J(5,N)=10.1$ Hz, 1H; NH), 5.11 (d, $J(3,4)=2.7$ Hz, 1H; 4a-H), 5.30 (dd, $J(1,2)=8.3$ Hz, $J(2,3)=10.1$ Hz, 1H; 2a-H), 5.37 (dd, $J(6,7)=2.7$ Hz, $J(7,8)=8.9$ Hz, 1H; 7b-H), 5.58 (ddd, $J(7,8)\approx 8.6$ Hz, $J(8,9)=5.9$ Hz, $J(8,9')=2.5$ Hz, 1H; 8b-H), 5.95 (d, $J(1,2)=8.3$ Hz, 1H; 1a-H), 7.23–7.35 (m, 5H; C_6H_5), 8.67 (s, 1H; =NH); $C_{39}H_{49}Cl_3N_2O_{20}$ (972.2): calcd C 48.18, H 5.08, N 2.88; found C 47.99, H 5.24, N 2.84%.

Thexyldimethylsilyl *O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (24): A solution of **14** (860 mg, 885 μmol) and **23**[31] (1.51 g, 1.64 mmol) in dry dichloromethane (10 mL) was treated at room temperature with trimethylsilyl trifluoromethanesulfonate (16 μL, 88 μmol). After stirring for 15 min, the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 2:1) to give starting material **23** and **24** (1.01 g, 66%) as a colorless foam. $R_f=0.64$ (toluene/acetone 1:1); $[\alpha]_D=-24.7$ ($c=1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta=0.01$, 0.03 (2s, 6H; $Si(CH_3)_2$), 0.73 (2s, 6H; $SiC(CH_3)_2$), 0.77, 0.78 (2d, $^3J=6.7$ Hz, 6H; $CH(CH_3)_2$), 1.18 (d, $J(5,6)=6.4$ Hz, 3H; 6b-CH_3), 1.54–1.62 (m, 1H; CH), 1.67 (dd, $^2J=J(3_{ax},4)\approx 12.4$ Hz, 1H; $3d_{ax}$-H), 1.81 (2s, 6H; $2NCOCH_3$), 1.92, 1.97, 1.98, 2.03, 2.06, 2.10, 2.21 (7s, 21H, $7COCH_3$), 2.54 (dd, $^2J=12.6$ Hz, $J(3_{eq},4)=4.3$ Hz, 1H; $3d_{eq}$-H), 2.93 (m, 1H; 2a-H), 3.58 (dd, $J(5,6)=10.7$ Hz, $J(6,7)=2.8$ Hz, 1H; 6d-H), 3.63–3.66 (m, 2H; 5a-, 6c-H), 3.76 (t, $J(5,6)=6.4$ Hz, 1H; 5c-H), 3.79 (s, 3H; $COOCH_3$), 3.84–3.86 (m, 2H; 2b-, 6c-H), 3.92 (dd, $J(3,4)=J(4,5)=9.5$ Hz, 1H; 4a-H), 4.01 (ddd, $J(4,5)=J(5,6)=J(5,N)=10.4$ Hz, 1H; 5d-H), 4.13 (dd, $^2J=12.8$ Hz, $J(8,9)=4.0$ Hz, 1H; 9d-H), 4.16 (dd, $^2J=11.9$ Hz, $J(5,6)=5.8$ Hz, 1H; 6a-H), 4.26 (dd, $^2J=12.8$ Hz, $J(8,9')=2.1$ Hz, 1H; 9′d-H), 4.30 (dd, $J(2,3)=J(3,4)=9.6$ Hz, 1H; 3a-H), 4.49–4.53 (m, 3H, 3c-H, C*H*HPh, C*H*HCCl$_3$), 4.62 (d, $^2J=11.7$ Hz, 1H; C*H*HPh), 4.67 (s, 2H; CH_2Ph), 4.72 (d, $^2J=11.9$ Hz, 1H; C*H*HCCl$_3$), 4.83 (ddd, $J(3_{ax},4)=J(4,5)=10.7$ Hz, $J(3_{eq},4)=4.6$ Hz, 1H; 4d-H), 4.88 (d, $J(1,2)=8.2$ Hz, 1H; 1c-H), 4.95 (dd, $J(1,2)\approx 8.2$ Hz, $J(2,3)=9.1$ Hz, 1H; 2c-H), 4.99–5.02 (m, 3H; 4c-H, 2NH), 5.04–5.06 (m, 2H; 6a-, 5b-H), 5.12 (d, $J(1,2)=7.7$ Hz, 1H; 1a-H), 5.20 (d, $J(1,2)=3.6$ Hz, 1H; 1b-H), 5.26–5.28 (m, 2H; 3b-, 4b-H), 5.38 (dd, $J(6,7)=2.7$ Hz, $J(7,8)=9.4$ Hz, 1H; 7d-H), 5.56 (ddd, $J(7,8)=9.3$ Hz, $J(8,9)=J(8,9')=3.3$ Hz, 1H; 8d-H), 7.15–7.97 (m, 15H; $3C_6H_5$); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta=15.89$ (6b-C), 18.41–24.64 ($9CO\mathit{C}H_3$, $4CH_3$), 34.04 ($CHMe_2$), 37.39 (3d-C), 49.08 (5d-C), 53.14 (OCH_3), 61.89 (2a-C), 62.01 (9d-C), 62.55 (6a-C), 64.19 (5b-C), 66.65 (7d-C), 67.64 (8d-C), 67.97 (6c-C), 68.11 (4c-C), 69.45 (4d-C), 69.91 (2c-C), 70.26 (3b-C), 71.59

0947-6539/00/0601-0118 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 1

(3c-C), 71.94 (6d-C), 72.06 (4b-C), 72.88 (5c-C), 73.13 ($PhCH_2$), 73.27 (5a-C), 73.33 ($PhCH_2$), 73.58 (3a-C), 74.24 (2b-C), 74.65 (CH_2CCl_3), 75.09 (4a-C), 93.86 (1a-C), 95.15 (CH_2CCl_3), 96.94 (2d-C), 97.22 (1b-C), 99.84 (1c-C), 127.36–138.66 (phenyl-C), 153.39 ($COCH_2CCl_3$), 165.62 (COPh), 167.71 (1d-C), 169.41, 169.53, 169.56, 170.01, 170.18, 170.36, 170.58, 170.76, 170.81 (9 COMe); $C_{78}H_{103}Cl_3N_2O_{33}Si$ (1731.1): calcd C 54.12, H 6.00, N 1.62; found C 54.10, H 6.00, N 2.01%.

(Methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*α*-D-glucopyranose (25): Compound **24** (1.42 g, 820 μmol) was dissolved in dry THF (10 mL). Acetic acid (320 μL, 5.6 mmol) and then a solution of tetrabutylammonium fluoride (1M) in THF (1.1 mL, 1.1 mmol) were added at room temperature, and the mixture was stirred for 5 days. An additional solution of tetrabutylammonium fluoride (1M) in THF (0.1 mL, 0.1 mmol) was added, and the mixture was stirred for a further 4 days. After the addition of a saturated sodium bicarbonate solution (20 mL), the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried over magnesium sulfate and concentrated in vacuo. Purification was accomplished by flash chromatography (toluene/acetone 3:2), which furnished pure **25** (1.14 g, 87%) as a colorless foam. $R_f = 0.41$ ($CHCl_3$/methanol 15:1); ^1H NMR (600 MHz, $CDCl_3$): $\delta =$ 1.12 (d, $J(5,6) = 6.2$ Hz, 3H; 6b-CH_3), 1.53 (s, 3H; $COCH_3$), 1.71 (dd, $^2J = J(3_{ax},4) \approx 12.4$ Hz, 1H; 3d_{ax}-H), 1.79, 1.81, 1.96, 2.00, 2.08, 2.08, 2.10, 2.20 (8s, 24H; 8 $COCH_3$), 2.52 (dd, $^2J = 12.6$ Hz, $J(3_{eq},4) = 4.3$ Hz, 1H; 3d_{eq}-H), 3.55 (dd, $J(5,6) = 10.7$ Hz, $J(6,7) = 2.8$ Hz, 1H; 6d-H), 3.60 (m, 1H; 5c-H), 3.73 (dd, $^2J = 10.4$ Hz, $J(5,6) = 6.4$ Hz, 1H; 6c-H), 3.76 (s, 3H; $COOCH_3$), 3.80 (dd, $J(2,3) = 10.5$ Hz, $J(1,2) = 3.3$ Hz, 1H; 2b-H), 3.91–4.05 [m, 5H; H,H-COSY: 3.91 (d, 9d-H), 3.95 (dd, 6c-H), 3.98 (dd, 5d-H), 4.00 (d, C*H*HCl$_3$), 4.05 (br d, 5a-H)], 4.13–4.14 (m, 2H; 2a-, 3a-H), 4.31–4.36 [m, 3H; H,H-COSY: 4.32 (s, 4c-H), 4.33 (dd, 4a-H), 4.36 (d, 6a-H)], 4.52–4.66 [m, 5H; H,H-COSY: 4.53 (d, $^2J = 12.3$ Hz, C*H*HPh), 4.54 (d, $^2J = 11.6$ Hz, C*H*HPh), 4.62 (d, 9d-H), 4.64 (d, C*H*HPh), 4.65 (d, C*H*HCl$_3$)], 4.75 (d, $^2J = 12.3$ Hz, 1H; C*H*HPh), 4.81 (m, 1H; 4d-H), 4.84 (d, $^2J = 11.1$ Hz, 1H; 6a-H), 4.89 (m, 1H; 4c-H), 4.93–4.98 [m, 3H; H,H-COSY: 4.94 (d, 1c-H), 4.94 (N_d-H), 4.97 (dd, 2c-H)], 5.05 (m, 1H; 5b-H), 5.24–5.28 [m, 3H; H,H-COSY: 5.24 (s, 1a-H), 5.25 (d, 3b-H), 5.28 (s, 4b-H)], 5.42 (dd, $J(6,7) = 2.6$ Hz, $J(7,8) = 10.1$ Hz, 1H; 7d-H), 5.45 (d, $J(1,2) = 2.9$ Hz, 1H; 1b-H), 5.61 (d, $J(7,8) = 10.0$ Hz, 1H; 8d-H), 6.36 (d, $J(2,N) = 5.7$ Hz, 1H; N_a-H), 7.21–7.94 (m, 15H; 3 C_6H_5); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 16.09$ (6b-C), 20.42–23.21 (9 $COCH_3$), 37.24 (3d-C), 49.01 (5d-C), 53.11 (OCH_3), 56.55 (2a-C), 62.04 (9d-C), 63.05 (6a-C), 64.06 (5b-C), 65.69 (7d-C), 67.30 (8d-C), 67.98 (4c-C), 68.17 (6c-C), 69.43 (5a-C), 69.52 (4d-C), 70.11 (3b-C), 70.20 (2c-C), 71.57 (6d-C), 71.68 (3c-C), 72.05 (4b-C), 72.11 (3a-C), 72.17 (CH_2Ph), 73.02 (2b-C), 73.36 (5c-C), 73.63 (CH_2Ph), 73.93 (4a-C), 74.91 (CH_2CCl_3), 92.27 (1a-C), 96.91 (2d-C), 97.41 (1b-C), 99.67 (1c-C), 127.36–138.77 (phenyl-C), 154.75 ($COCH_2CCl_3$), 167.36–171.78 (1d-C, 10 COMe/Ph); $C_{70}H_{85}Cl_3N_2O_{33}$ (1588.8): calcd C 52.92, H 5.39, N 1.76; found C 53.24, H 5.62, N 2.05%.

***O*-(Methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*α*-D-glucopyranosyl trichloroacetimidate (26)**: Trichloroacetonitrile (520 μL, 5.2 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (4 μL) was added to a solution of **25** (817 mg, 514 μmol) in dry dichloromethane (10 mL). After 1 h the mixture was concentrated in vacuo. Flash chromatography (toluene/acetone 3:1+1% triethylamine) furnished **26** (828 mg, 93%) as a colorless foam. $R_f = 0.56$ (toluene/acetone 1:1); $[\alpha]_D = -6.5$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.20$ (d, $J(5,6) =$ 6.5 Hz, 3H; 6b-CH_3), 1.6–1.7 (m, 1H; 3d_{ax}-H), 1.75–2.21 (m, 27H; 9 $COCH_3$), 2.56 (dd, $^2J = 12.6$ Hz, $J(3ex,4) = 4.6$ Hz, 1H; 3d_{eq}-H), 3.59 (dd, $J(5,6) = 10.7$ Hz, $J(6,7) = 2.9$ Hz, 1H; 6d-H), 3.66–5.60 (m, 32H; 2 1-H, 3 2-H, 3 3-H, 4 4-H; 4 5-H, 4 6-H, 7-H, 8-H, 2 9-H, 2 NH, 2 CH_2Ph, CH_2CCl_3), 6.35 (d, $J(1,2) = 3.6$ Hz, 1H; 1a-H), 7.23–7.97 (m, 15H; 3 C_6H_5), 8.69 (s, 1H; =NH); $C_{72}H_{85}Cl_6N_3O_{33}$ (1733.2): calcd C 49.90, H 4.94, N 2.42; found C 49.96, H 5.14, N 2.64%.

(1,2-Di-*O*-hexadecyl-*sn*-3-glyceryl)-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (27a): A solution of **4a** (192 mg, 355 μmol) and **26** (410 mg, 237 μmol) in dry dichloromethane (4 mL) was treated at room temperature with a solution of trimethylsilyl trifluoromethanesulfonate (0.25M) in dichloromethane (95 μL, 24 μmol). After stirring for 30 min, the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 4:1) to give **27a** (400 mg, 80%) as a colorless foam. $R_f = 0.23$ (toluene/acetone 3:1); $[\alpha]_D = -20.3$ ($c = 1.0$ in $CHCl_3$); $C_{105}H_{155}Cl_3N_2O_{35}$ (2111.7): calcd C 59.72, H 7.40, N 1.33; found C 59.46, H 7.52, N 1.69%.

[8-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6-dioxaoct-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (27b): A solution of **4b** (220 mg, 327 μmol) and **26** (330 mg, 190 μmol) in dry dichloromethane (5 mL) was treated at room temperature with a solution of trimethylsilyl trifluoromethanesulfonate (0.25M) in dichloromethane (75 μL, 19 μmol). After stirring for 30 min, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 5:1 to 3:1) to give **27b** (375 mg, 88%) as a colorless foam. $R_f = 0.64$ (toluene/acetone 1:1); $[\alpha]_D = -19.4$ ($c = 1.0$ in $CHCl_3$); $C_{111}H_{167}Cl_3N_2O_{38}$ (2243.9): calcd C 59.42, H 7.50, N 1.25; found C 59.68, H 7.26, N 1.80%.

[17-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15-pentaoxaheptadec-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (27c): A solution of **4c** (326 mg, 405 μmol) and **26** (420 mg, 242 μmol) in dry dichloromethane (8 mL) was treated at room temperature with a solution of trimethylsilyl trifluoromethanesulfonate (0.25M) in dichloromethane (100 μL, 25 μmol). After stirring for 30 min, the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 3:1 to 2:1) to give **27c** (321 mg, 56%) as a colorless foam. $R_f = 0.28$ (toluene/acetone 2:1); $[\alpha]_D = -15.6$ ($c = 1.0$ in $CHCl_3$); $C_{117}H_{179}Cl_3N_2O_{41}$ (2376.1): calcd C 59.14, H 7.59, N 1.18; found C 59.40, H 7.75, N 1.31%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-6-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-*α*-L-fucopyranosyl)-(1→3)]-6-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (27d): A solution of **4d** (487 mg, 520 μmol) and **26** (450 mg, 260 μmol) in dry dichloromethane (10 mL) was treated at room temperature with a solution of trimethylsilyl trifluoromethanesulfonate (0.25M) in dichloromethane (100 μL, 25 μmol). After stirring for 30 min, the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **27d** (380 mg, 58%) as a colorless foam. $R_f = 0.48$ (ethyl acetate/methanol 9:1); $[\alpha]_D = -13.8$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J \approx 7.0$ Hz, 6H; 2 CH_3), 1.20 (d, $J(5,6) = 6.9$ Hz, 3H; 6b-CH_3), 1.25 (br s, 52H; 26 CH_2), 1.55 (quintet, $^3J \approx 6.8$ Hz, 4H; 2 OCH_2CH_2), 1.69–1.71 (m, 1H; 3d_{ax}-H), 1.70, 1.83, 1.90, 1.99, 2.02, 2.05, 2.08, 2.10, 2.22 (9s, 27H; 9 $COCH_3$), 2.56 (dd, $^2J =$ 12.6 Hz, $J(3_{eq},4) = 4.5$ Hz, 1H; 3d_{eq}-H), 3.40–3.90 [m, 57H; H,H-COSY: 3.39 (2a-H), 3.59 (5c-H), 3.59 (6d-H), 3.64 (5a-H), 3.70 (6c-H), 3.70 (C*H*HPh), 3.79 (s, 3H; $COOCH_3$), 3.84 (2b-H), 3.84 (6′c-H), 3.90 (C*H*HPh), 18 CH_2 spacer, 5H glycerol, 2 OCH_2CH_2], 4.02 (ddd, $J(4,5) = J(5,6) = J(5,N) = 10.4$ Hz, 1H; 5d-H), 4.11 (dd, $J(3,4) = J(4,5) \approx 9.0$ Hz, 1H; 4a-H), 4.18–4.25 [m, 3H; H,H-COSY: 4.18 (dd, $^2J = 12.9$ Hz, $J(8,9) =$ 3.7 Hz, 9d-H), 4.20 (dd, 3a-H), 4.23 (dd, $^2J = 12.1$ Hz, $J(5,6) = 4.3$ Hz, 6a-H)], 4.29 (dd, $^2J = 12.9$ Hz, $J(8,9') = 2.6$ Hz, 1H; 9′d-H), 4.48 (dd, $J(2,3) \approx$ 9.0 Hz, $J(3,4) \approx 3.4$ Hz, 1H; 3c-H), 4.53 (d, $^2J = 11.6$ Hz, 1H; C*H*HPh), 4.58, 4.74 (2d, $^2J = 11.8$ Hz; 1H; CH_2CCl_3 conformational exchange), 4.60, 4.78 (2d, $^2J = 12.0$ Hz, 1H; CH_2CCl_3 conformational exchange), 4.61 (d, $^2J = 11.6$ Hz, 1H; C*H*HPh), 4.84 (ddd, $J(3_{ax},4) = 11.9$ Hz, $J(3_{eq},4) = 4.6$ Hz, $J(4,5) = 10.5$ Hz, 1H; 4d-H), 4.95–5.05 [m, 6H; H,H-COSY: 4.95 (d, $J(1,2) \approx 8.0$ Hz; 1a-H), 4.96 (d, 1c-H), 4.96 (dd, 2c-H), 4.99 (s, 4c-H), 5.02 (d, N_dH), 5.03 (d, 6a-H)], 5.08 (q, $J(5,6) = 6.6$ Hz, 1H; 5b-H), 5.28–5.32 [m,

3H; H,H-COSY: 5.29 (d, 3b-H), 5.32 (s, 4b-H), 5.34 (s, 1b-H)], 5.41 (dd, $J(6,7)=2.8$ Hz, $J(7,8)=9.5$ Hz, 1H; 7d-H), 5.57 (ddd, $J(7,8)=9.5$ Hz, $J(8,9)=J(8,9')=3.2$ Hz, 1H; 8d-H), 5.76 (brd, 1H; N_aH), 7.23–7.98 (m, 15H; $3C_6H_5$); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta=14.09$ ($2CH_3$), 15.91 (6b-C), 20.72–23.17 ($9COCH_3$), 20.07–31.89 (CH_2 alkyl), 37.40 (3d-C), 49.04 (5d-C), 53.11 (OCH_3), 59.18 (2a-C), 61.96 (9d-C), 62.32 (6a-C), 64.16 (5b-C), 66.58 (7d-C), 67.45 (8d-C), 67.92 (CH_2Ph), 68.03 (4c-C), 68.70 (6c-C), 69.46 (4d-C), 70.07 (2c-C), 70.24 (3b-C), 70.37–71.39 (C spacer, C glycerol, 5a-C, 5c-C, 6d-C), 71.54 (3c-C), 72.02 (4b-C), 72.94, 74.49 (CH_2CCl_3 conformational exchange), 73.32 (CH_2Ph), 73.70 (2b-C), 74.06 (3a-C), 74.26 (4a-C), 95.71 (CH_2CCl_3), 96.96 (2d-C), 97.09 (1b-C), 99.56 (1c-C), 100.22 (1a-C), 127.32–138.69 (phenyl-C), 154.01 ($COCH_2CH_3$), 165.63 (COPh), 167.65 (1d-C), 169.33, 169.41, 169.44, 169.83, 170.20, 170.37, 170.62, 170.72, 170.76 ($9COMe$); $C_{123}H_{191}Cl_3N_2O_{44}\cdot 2H_2O$ (2544.3): calcd C 58.07, H 7.72, N 1.10; found C 58.00, H 7.71, N 1.04%.

(1,2-Di-*O*-hexadecyl-*sn*-3-glyceryl)-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (28a): A solution of **27a** (360 mg, 242 μmol) in THF/acetic anhydride/acetic acid (3:2:1, 12 mL) was treated with activated zinc powder (420 mg, activation with 2% $CuSO_4$ in water for 5 min). The mixture was stirred overnight at room temperature and then filtered and washed with THF. The solvent was evaporated under reduced pressure, and the residue was separated from zinc salts by flash chromatography (toluene/acetone 2:1). The product was dissolved in THF (8 mL) and acetic acid (2 drops). Palladium on charcoal (150 mg, 10% Pd) was added, and the solution was stirred vigorously under a hydrogen atmosphere for 2 days. The catalyst was filtered off and washed with THF. Evaporation and purification by flash chromatography (toluene/acetone 2:1) gave **28a** (233 mg, 76%) as a colorless foam. $R_f=0.33$ (toluene/acetone 1:1, HPTLC); $[\alpha]_D=-28.8$ ($c=1.0$ in $CHCl_3$); $C_{90}H_{144}N_2O_{34}\cdot H_2O$ (1816.2): calcd C 59.52, H 8.10, N 1.54; found C 59.60, H 8.32, N 1.92%.

[8-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6-dioxaoct-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (28b): Compound **27b** (346 mg, 154 μmol) was treated as described above to furnish after flash chromatography (toluene/acetone 1:1) pure **28b** (102 mg, 34%) as a colorless foam. $R_f=0.20$ (toluene/acetone 1:1, HPTLC); $C_{96}H_{156}N_2O_{37}$ (1930.3).

[17-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15-pentaoxaheptadec-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (28c): Compound **27c** (259 mg, 109 μmol) was treated as described above to furnish after flash chromatography (toluene/acetone 2:3) pure **28c** (182 mg, 81%) as a colorless foam. $R_f=0.15$ (toluene/acetone 2:3, HPTLC); $C_{102}H_{168}N_2O_{40}$ (2062.4).

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,4-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (28d): Compound **27d** (310 mg, 124 μmol) was treated as described above to furnish after flash chromatography (ethyl acetate to ethyl acetate/methanol 9:1) pure **28d** (140 mg, 51%) as a colorless foam. $R_f=0.32$ (ethyl acetate/methanol 5:1); MALDI: m/z: 2218 $[M+Na]^+$; $C_{108}H_{180}N_2O_{43}$ (2194.6).

(1,2-Di-*O*-hexadecyl-*sn*-3-glyceryl)-*O*-(triethylammonium-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1a): Compound **28a** (213 mg, 118 μmol) was dissolved in dry methanol, and a solution of sodium methoxide (1M) in methanol (200 μL) was added. After stirring overnight at room temperature, the solution was neutralized with Amberlite IR120 (H^+), filtered, and evaporated. The residue was dissolved in dioxane/water 1:1 (10 mL), and an aqueous solution of potassium hydroxide (0.2M, 1 mL) was added. After stirring overnight, carbon dioxide was added and the solution was lyophilized. Flash chromatography ($CHCl_3$/methanol/water/triethylamine 80:20:2:0.1 to 70:30:5:0.1) yielded **1a** (110 mg, 65%) as a colorless powder after lyophilization from water. $R_f=0.23$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 70:30:5, HPTLC); $[\alpha]_D=-30.1$ ($c=1.0$ in $CHCl_3$); ^1H NMR (600 MHz, SDS/D_2O): $\delta=0.84$ (m, 6H; $2CH_3$), 1.20 (d, $J(5,6)=6.3$ Hz, 3H; 6b-CH_3), 1.25–1.36 (m, 52H; $26CH_2$), 1.41 (t, 9H; $N(CH_2CH_3)_3$), 1.60 (brs, 4H; $2OCH_2CH_2$), 1.85 (t, 1H; $3d_{ax}$-H), 2.06, 2.07 (2s, 6H; $2COCH_3$), 2.78 (dd, 1H; $3d_{eq}$-H), 3.24 (q, $^3J=7.3$ Hz, 6H; $N(CH_2Me)_3$), 3.40–4.15 (m, 30H), 4.53 (d, $J(1,2)=7.5$ Hz, 1H; 1c-H), 4.59 (brs, 1H; 1a-H), 4.84 (q, $J(5,6)=6.5$ Hz, 1H; 5b-H), 5.13 (brd, 1H; 1b-H); $C_{72}H_{137}N_3O_{25}\cdot 4H_2O$ (1564.9): calcd C 55.26, H 9.34, N 2.69; found C 55.33, H 9.68, N 3.08%.

[8-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6-dioxaoct-1-yl]-*O*-(triethylammonium-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1b): Compound **28b** (100 mg, 52 μmol) was treated as described above to furnish **1b** (45.4 mg, 55%) as a colorless powder. $R_f=0.24$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 70:30:5, HPTLC); $[\alpha]_D=-27.2$ ($c=1.0$ in $CHCl_3$); ^1H NMR (600 MHz, SDS/D_2O): $\delta=0.80$ (m, 6H; $2CH_3$), 1.17 (d, $J(5,6)=6.3$ Hz, 3H; 6b-CH_3), 1.21–1.32 (m, 61H; $26CH_2$, $N(CH_2CH_3)_3$), 1.57 (brs, 4H; $2OCH_2CH_2$), 1.81 (t, 1H; $3d_{ax}$-H), 2.02, 2.04 (2s, 6H; $2COCH_3$), 2.76 (dd, 1H; $3d_{eq}$-H), 3.20 (q, $^3J=7.3$ Hz, 6H; $N(CH_2Me)_3$), 3.43–4.13 (m, 42H), 4.53 (d, $J(1,2)=7.7$ Hz, 1H; 1c-H), 4.61 (d, $J(1,2)=6.4$ Hz, 1H; 1a-H), 4.83 (q, $J(5,6)=6.3$ Hz, 1H; 5b-H), 5.12 (d, $J(1,2)=3.6$ Hz, 1H; 1b-H); $C_{78}H_{149}N_3O_{28}\cdot 5H_2O$ (1667.1): calcd C 56.20, H 9.61, N 2.52; found C 56.00, H 9.63, N 2.14%.

[17-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15-pentaoxaheptadec-1-yl]-*O*-(triethylammonium-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1c): Compound **28c** (182 mg, 88 μmol) was treated as described above to furnish **1c** (107 mg, 71%) as a colorless powder. $R_f=0.23$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 70:30:5, HPTLC); $[\alpha]_D=-20.2$ ($c=1.0$ in $CHCl_3$); ^1H NMR (600 MHz, SDS/D_2O): $\delta=0.81$ (m, 6H; $2CH_3$), 1.17 (d, $J(5,6)=6.3$ Hz, 3H; 6b-CH_3), 1.19–1.32 (m, 61H; $26CH_2$, $N(CH_2CH_3)_3$), 1.56 (brs, 4H; $2OCH_2CH_2$), 1.80 (t, 1H; $3d_{ax}$-H), 2.02, 2.04 (2s, 6H; $2COCH_3$), 2.75 (dd, 1H; $3d_{eq}$-H), 3.20 (q, $^3J=7.3$ Hz, 6H; $N(CH_2Me)_3$), 3.44–4.10 (m, 54H), 4.52 (d, $J(1,2)=7.7$ Hz, 1H; 1c-H), 4.61 (d, $J(1,2)=8.3$ Hz, 1H; 1a-H), 4.83 (q, $J(5,6)=6.5$ Hz, 1H; 5b-H), 5.12 (d, $J(1,2)=3.4$ Hz, 1H; 1b-H); $C_{84}H_{161}N_3O_{31}\cdot 5H_2O$ (1799.3): calcd C 56.07, H 9.58, N 2.34; found C 56.08, H 9.64, N 2.10%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl]-*O*-(triethylammonium-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(β-D-galactopyranosyl)-(1→4)-[α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1d): Compound **28d** (135 mg, 61.5 μmol) was treated as described above to furnish pure **1d** (92 mg, 77%) as a colorless powder. $R_f=0.25$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 70:30:5, HPTLC); $[\alpha]_D=-19.7$ ($c=1.0$ in $CHCl_3$); ^1H NMR (600 MHz, SDS/D_2O): $\delta=0.81$ (m, 6H; $2CH_3$), 1.17 (d, $J(5,6)=6.4$ Hz, 3H; 6b-CH_3), 1.22–1.28 (m, 52H; $26CH_2$), 1.31 (t, 9H; $N(CH_2CH_3)_3$), 1.56 (brs, 4H; $2OCH_2CH_2$), 1.80 (t, 1H; $3d_{ax}$-H), 2.02, 2.04 (2s, 6H; $2COCH_3$), 2.76 (dd, 1H; $3d_{eq}$-H), 3.21 (q, $^3J=7.3$ Hz, 6H; $N(CH_2Me)_3$), 3.44–4.09 [m, 66H; H,H-COSY: 3.52 (2c-H), 3.58 (5a-H), 3.59 (5c-, 7d-H), 3.64 (9d-H), 3.67 (4d-H), 3.68 (6d-H), 3.70 (2b-H), 3.76 (4b-H), 3.77 (3a-H), 3.85 (5d-H), 3.88 (6a-, 3b-, 6′c-, 6c-H), 3.89 (9′d-H), 3.90 (4a-, 8d-H), 3.91 (2a-H), 3.93 (4c-H), 4.00 (6′a-H), $18CH_2$ spacer, 5H glycerol, $2OCH_2CH_2$], 4.09 (dd, $J(2,3)=9.6$ Hz, $J(3,4)=2.1$ Hz, 1H; 3c-H), 4.52 (d, $J(1,2)=7.7$ Hz, 1H; 1c-H), 4.61 (d, $J(1,2)=7.8$ Hz, 1H; 1a-H), 4.83 (q, $J(5,6)=6.5$ Hz, 1H; 5b-H), 5.11 (d, $J(1,2)=3.5$ Hz, 1H; 1b-H); $C_{90}H_{173}N_3O_{34}\cdot 5H_2O$ (1931.4): calcd C 55.97, H 9.55, N 2.18; found C 55.94, H 9.45, N 2.39%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl]-*O*-(methyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosyl)onate (29α/β): A solution of **8**[25] (550 mg, 900 μmol) and acceptor **4d** (550 mg, 580 μmol) in dry acetonitrile/dichloromethane (1:1, 25 mL) was treated at 0°C with trimethylsilyl trifluoromethanesulfonate (50 μL, 270 μmol). After stirring for 45 min, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (ethyl acetate/methanol 1:0 to 30:1) to give **29α/β** (420 mg, 51%) as a colorless foam in a ratio of $\alpha/\beta=1:1$. $R_f=0.56$ and 0.63 ($CHCl_3$/methanol 15:1); ^1H NMR (600 MHz, C_6D_6): **29α**: $\delta=0.88$ (t, 6H; $2CH_3$), 1.32 (brs, 52H; $26CH_2$),

0947-6539/00/0601-0120 $ 17.50+.50/0 Chem. Eur. J. 2000, 6, No. 1

1.51–1.57 (m, 4H; $2OCH_2CH_2$), 1.57 (s, 3H; $COCH_3$), 1.60 (m, 1H; 3_{ax}-H), 1.68–2.13 (4s, 12H; $4COCH_3$), 2.81 (dd, $^2J = 12.7$ Hz, $J(3_{eq},4) = 4.5$ Hz, 1H; 3_{eq}-H), 3.23–4.14 (m, 48H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, $COOCH_3$), 4.48 (dd, $J(8,9) = 8.9$ Hz, $^2J = 12.2$ Hz, 1H; 9-H), 4.68 (m, 1H; 5-H), 4.95 (dd, $J(5.6) = 10.7$ Hz, $J(6,7) < 1$ Hz, 1H; 6-H), 5.46–5.49 (m, 1H; 9′-H), 5.53 (ddd, $J(3_{ax},4) = J(4,5) \approx 10.9$ Hz, $J(3_{ax},4) = 4.9$ Hz, 1H; 4-H), 5.80 (br d, $J(8,9) = 8.8$ Hz, 1H; 8-H), 5.87 (s, 1H; 7-H), 6.48 (d, $J(5,N) = 10.2$ Hz, 1H; NH); **29β**: $\delta = 0.88$ (t, 6H; $2CH_3$), 1.32 (br s, 52H; $26CH_2$), 1.51–1.57 (m, 4H; $2OCH_2CH_2$), 1.57 (s, 3H; $COCH_3$), 1.65 (m, 1H; 3_{ax}-H), 1.68–2.13 (4s, 12H; $4COCH_3$), 2.62 (dd, $^2J = 12.7$ Hz, $J(3_{eq},4) = 4.9$ Hz, 1H; 3_{eq}-H), 3.23–4.14 [m, 50H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, $COOCH_3$, H,H-COSY: 4.11–4.14 (6-H, NH)], 4.31 (dd, $J(8,9) = 6.3$ Hz, $^2J = 12.4$ Hz, 1H; 9-H), 4.41 (ddd, $J(4,5) = J(5,6) = J(5,N) \approx 10.5$ Hz, 1H; 5-H), 4.65–4.68 (m, 1H; 9′-H), 4.84 (ddd, $J(3_{ax},4) = J(4,5) \approx 10.9$ Hz, $J(3_{eq},4) = 4.9$ Hz, 1H; 4-H), 5.47–5.49 (m, 1H; 7-H), 5.84 (m, 1H; 8-H); $C_{73}H_{135}NO_{24}$ (1410.9): calcd C 62.15, H 9.64, N 0.99; found C 61.90, H 9.52, N 0.82%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl]-*O*-(methyl-5-acetamido-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosyl)onate (30α/β): A solution of sodium methoxide (0.5 M) in methanol (110 μL) was added to a solution of **29α/β** (387 mg, 274 μmol) in dry methanol (10 mL). After 3 hours the solution was neutralized with Amberlite IR120 (H^+), filtered, and evaporated. Flash chromatography ($CHCl_3$/methanol = 9:1) yielded **30α/β** (250 mg, 72%) as a colorless foam in a ratio of $\alpha/\beta = 1{:}1$. $R_f = 0.35$ ($CHCl_3$/methanol 9:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H; $2CH_3$), 1.26 (br s, 52H; $26CH_2$), 1.52–1.57 (m, 4H; $2OCH_2CH_2$), 1.60 (m, 1H; 3_{ax}-H), 2.03, 2.05 (2s, 3H; $NCOCH_3$), 2.43, 2.78 (2dd, 1H; 3_{eq}-H), 3.38–4.15 (m, 55.5H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, $COOCH_3$, 4-, 5-, 6-, 7-, 8-, 9-, 9′-H, 0.5HN), 6.43 (d, $J(5,N) = 8.4$ Hz, 0.5H; 0.5NH); $C_{65}H_{127}NO_{20} \cdot 1.5H_2O$ (1269.74): calcd C 61.49, H 10.32, N 1.10; found C 61.56, H 10.33, N 1.05%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl]-*O*-(potassium-5-acetamido-3,5-dideoxy-D-glycero-α/β-D-galacto-2-nonulopyranosyl)onate (31α) and (31β): Aqueous potassium hydroxide (0.2 M, 680 μL) was added to a solution of **30α/β** (115 mg, 90.6 μmol) in water/dioxane (1:1; 6 mL). After stirring for 2 hours, carbon dioxide was added and the solution was lyophilized. The two anomers were separated by flash chromatography ($CHCl_3$/methanol/water 85:15:1 to 80:20:2) to give **31α** (58 mg, 49%) and **31β** (58 mg, 48%) as colorless solids.

Compound ***31α***: $R_f = 0.48$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 80:20:2, HPTLC); $[\alpha]_D = -5.3$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD/D_2O 65:25:4): $\delta = 0.89$ (t, 6H; $2CH_3$), 1.27 (br s, 52H; $26CH_2$), 1.55–1.58 (m, 4H; $2OCH_2CH_2$), 1.62 (dd, $^2J = J(3_{ax},4) = 11.4$ Hz, 1H; 3_{ax}-H), 2.04 (s, 3H; $COCH_3$), 2.77 (dd, $^2J = 12.7$ Hz, $J(3_{eq},4) = 3.8$ Hz, 1H; 3_{eq}-H), 3.45–3.90 (m, 53H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, NH, 4-, 5-, 6-, 7-, 8-, 9-, 9′-H); $C_{64}H_{124}KNO_{20} \cdot 1.5H_2O$ (1293.8): calcd C 59.41, H 9.89, N 1.08; found C 59.36, H 9.88, N 0.69%.

Compound ***31β***: $R_f = 0.34$ ($CHCl_3$/methanol/0.2% aqueous $CaCl_2$ 80:20:2, HPTLC); $[\alpha]_D = -9.1$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD/D_2O 65:25:4): $\delta = 0.89$ (t, 6H; $2CH_3$), 1.27 (br s, 52H; $26CH_2$), 1.55–1.58 (m, 4H; $2OCH_2CH_2$), 1.77 (t, 1H; 3_{ax}-H), 2.04 (s, 3H; $COCH_3$), 2.34 (dd, 1H; 3_{eq}-H), 3.46–3.97 (m, 53H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, NH, 4-, 5-, 6-, 7-, 8-, 9-, 9′-H); $C_{64}H_{124}KNO_{20} \cdot 4H_2O$ (1338.8): calcd C 57.27, H 9.94, N 1.05; found C 57.18, H 9.68, N 0.79%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl] 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (32): A solution of **19**[29] (523 mg, 837 μmol) and acceptor **4d** (523 mg, 558 μmol) in dry dichloromethane (10 mL) was treated at room temperature with a solution of trimethylsilyl trifluoromethanesulfonate (0.24 M) in dichloromethane (82 μL, 20 μmol). After stirring for 30 min, the reaction mixture was neutralized with triethylamine (100 μL) and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone 4:1) to give **32** (654 mg, 84%) as a colorless foam. $R_f = 0.68$ (toluene/acetone 1:1); $[\alpha]_D = -2.9$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J = 6.6$ Hz, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.51–1.58 (m, 4H; $2OCH_2CH_2$), 2.00, 2.01, 2.09 (3s, 9H; $3COCH_3$), 3.40–3.89 (m, 47H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, 2-, 5-H), 4.12 (dd, $J(5,6) = 2.3$ Hz, $^2J = 12.3$ Hz, 1H; 6-H), 4.27 (dd, $J(5,6') = 4.7$ Hz, $^2J = 12.3$ Hz, 1H; 6′-H), 4.73 (s, 2H; CH_2CCl_3), 4.83 (d, $J(1,2) = 8.6$ Hz, 1H; 1-H), 5.02–5.15 (m, 2H; 3-, 4-H), 6.46 (d, $J(2,N) = 9.5$ Hz, 1H; NH); $C_{68}H_{126}Cl_3NO_{21}$ (1400.1): calcd C 58.33, H 9.07, N 1.00; found C 58.44, H 9.32, N 1.28%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl] 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (33): A solution of **32** (252 mg, 180 μmol) in THF/acetic anhydride/acetic acid (4:2:1; 14 mL) was treated with activated zinc powder (200 mg; activation with 2% $CuSO_4$ in water for 5 min). The mixture was stirred for 18 h at room temperature, then diluted with toluene, filtered, and washed with toluene. Evaporation and flash chromatography (toluene/acetone 1:1 to 2:3) furnished **33** (186 mg, 82%) as a colorless solid. $R_f = 0.25$ (toluene/acetone 1:1); $[\alpha]_D = -7.6$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J = 6.5$ Hz, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.51–1.57 (m, 4H; $2OCH_2CH_2$), 2.01, 2.02, 2.06, 2.08 (4s, 12H; $4COCH_3$), 3.40–3.92 (m, 47H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, 5-H), 4.06–4.17 (m, 2H; 2-, 6-H), 4.27 (dd, $^2J = 12.2$ Hz, $J(5,6') = 4.5$ Hz, 1H; 6′-H), 4.87 (d, $J(1,2) = 8.4$ Hz, 1H; 1-H), 5.06 (dd, $J(3,4) = J(4,5) = 9.4$ Hz, 1H; 4-H), 5.15 (dd, $J(2,3) = J(3,4) = 10.0$ Hz, 1H; 3-H), 6.94 (br s, 1H; NH); $C_{67}H_{127}NO_{20} \cdot 2H_2O$ (1266.74): calcd C 61.77, H 10.13, N 1.08; found C 61.80, H 10.22, N 1.08%.

[26-(1,2-Di-*O*-hexadecyl-*sn*-glycer-3-oxy)-3,6,9,12,15,18,21,24-octaoxahexacos-1-yl] 2-acetamido-2-deoxy-β-D-glucopyranoside (34): A catalytic quantity of sodium methoxide was added to a solution of **33** (186 mg, 147 μmol) in dry methanol (9 mL). After 3 hours the solution was neutralized with Amberlite IR120 (H^+), filtered, and evaporated. Flash chromatography ($CHCl_3$/methanol = 15:1) yielded **34** (160 mg, 96%) as a colorless solid. $R_f = 0.32$ ($CHCl_3$/methanol 9:1); $[\alpha]_D = -15.7$ ($c = 0.30$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.88$ (t, $^3J = 6.6$ Hz, 6H; $2CH_3$), 1.25 (br s, 52H; $26CH_2$), 1.51–1.57 (m, 4H; $2OCH_2CH_2$), 2.07 (s, 3H; $COCH_3$), 3.40–3.96 (m, 51H; $18CH_2$ spacer, $2OCH_2CH_2CH_2$, 5H glycerol, 2-, 3-, 4-, 5-, 6-, 6′-H), 4.64 (d, $J(1,2) = 8.4$ Hz, 1H; 1-H), 7.16 (d, 1H; NH); $C_{61}H_{121}NO_{17}$ (1140.6): calcd C 64.22, H 10.70, N 1.23; found C 64.11, H 10.52, N 1.60%.

Preparation of supported planar bilayers: Supported planar bilayers were prepared by using the Langmuir–Blodgett technique. Microscope slides (glass, diameter of 18 mm, thickness of 0.2 mm) were used as transparent supports. Slides were first cleaned to achieve a highly homogeneous surface by the following procedure. Slides were treated with a conc. H_2SO_4/H_2O_2 mixture (7:3) at 80 °C for 30 minutes under ultrasonic conditions and were then rinsed with ultrapure water for 30 minutes. To increase the density of silanole groups at the surface, a cleaning procedure with $NH_3/H_2O_2/H_2O$ (1:1:5) followed, before finally rinsing with ultrapure water and drying the slides. The first step in forming a supported bilayer is the covalent binding of monochlorodimethyloctadecylsilane (Sigma, Deisenhofen, Germany) at 50 °C for 30 minutes to produce the first monolayer on the slide. The bilayer was completed by transferring the preformed lipid film at the Langmuir trough. The lipid mixtures were transferred at a lateral pressure of 38 mN m^{-1} and a speed of 0.5 mm min^{-1} to hydrophobic substrates as a X-type monolayer. The transfer ratios were between 0.95 and 1. Freshly prepared supported bilayers were immediately used for experiments in the flow chamber.

Laminar flow experiments: The parallel-plate flow chamber used in these studies has been described in detail in our previous investigations.[36] The flow apparatus was mounted onto an inverted fluorescent microscope Axiovert 135 of a laser scanning microscope (LSM 410 invert, Carl Zeiss, Germany).

Adhesion experiments were performed at 25 °C in a temperature-controlled manner to maintain the lateral structure of the model membrane. MEM-α medium was used as flow medium at shear rate of 200 s^{-1} powered by hydrostatic pressure. For the flow experiments, 10^6 fluorescently labeled CHO-E cells in 100 μL medium were injected into the streaming medium without dilution. Either, cell adhesion or rolling was analyzed immediately, or the flow was stopped for 5 minutes to allow cells to interact with the supported membrane. After this time, flow with the desired shear force was continued and the adhesion behavior of the cells was monitored by a sequence of images taken every 2 seconds. To characterize the cell movement, 50 to 150 cells within an area of 630 × 630 μm were analyzed over a period of 20 seconds. Only those cells observed to directly contact the membrane in absence of prior contacts with adherent cells were counted and analyzed. The experiments for the presented data were repeated four times under similar conditions.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 197 and Stu 17/1) and the Fonds der Chemischen Industrie. We are grateful to Dr. A. Geyer for his help in the structural assignments by NMR experiments.

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Received: April 28, 1999 [F1752]

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