Synthesis and Biological Evaluation of a Potent E-Selectin Antagonist

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An early step of the inflammatory response—the rolling of leukocytes on activated endothelial cells—is mediated by selectin/carbohydrate interactions. The tetrasaccharide sialyl Lewis^x (sLe^x) **1** is a ligand for E-, P-, and L-selectin and, therefore, serves as a lead structure to develop analogues which allow the control of acute and chronic inflammation. Here we describe the efficient synthesis (10 linear steps) of the potent sLe^x mimetic **2**. Compared to sLe^x, compound **2** showed a 30-fold improved affinity in a static, cell-free E-selectin-ligand binding assay (IC_{50} = 36 μM). These data were confirmed by a marked inhibition in an in vitro cell−cell rolling assay which simulates in vivo conditions ($IC_{50} \approx 40$ μM). The assays are predictive for the in vivo efficacy of test compounds as indicated by a marked inhibitory effect of **2** in a thioglycollate induced peritonitis model of acute inflammation in mice ($ED_{50} \approx 15$ mg/kg).

Introduction

Leukocyte influx from blood vessels into the surrounding tissues causing inflammation is a beneficial response of the body to control infections and injuries.[1] Excessive leukocyte influx, however, may result in acute or chronic reactions as observed in reperfusion injuries, stroke, psoriasis, rheumatoid arthritis, or respiratory diseases.[2] An early step in the cascade of events which finally leads to the extravasation of leukocytes is their rolling on the endothelial cells which cover the inner surface of the blood vessel wall.[3] It has been shown that a set of inducible adhesion molecules, the so-called selectins, are involved in this step.[4] A possible strategy for preventing the adverse effects of an excessive leukocyte influx is the inhibition of the leukocyte/selectin interactions. The sialyl Lewis^x tetrasaccharide **1** (Chart 1) appears to be a common epitope of the physiological selectin ligands and is recognized by the three known selectins (E-, P-, and L-selectin).[4] Therefore, it became a lead structure to identify simplified but more potent inhibitors. Extensive work elucidated that the essential pharmacophores required for binding to E-selectin are the three hydroxyl groups of the L-fucose, the 4- and the 6-hydroxyl group of the D-galactose, and the carboxylic acid function. On the basis of this knowledge, a multitude of E-selectin antagonists have been prepared.[5]

In this paper we report the synthesis of the novel, potent E-selectin antagonist **2** which can be prepared from commercially available starting materials in 10 linear steps in an overall yield of 18%. Sialic acid and *N*-acetyl glucosamine of the lead structure sialyl Lewis^x **1** have been replaced by *S*-cyclohexyllactic acid and a D-glucal-derived building block, respectively. The combination of these two modifications led to a significantly improved E-selectin inhibition of **2** compared to sLe^x.

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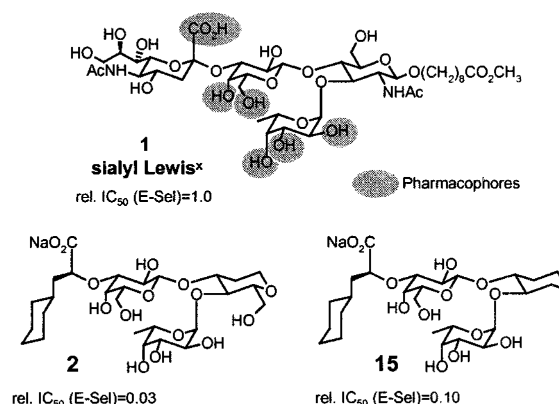
Chart 1



Preparation of E-Selectin Antagonist 2

The sialyl Lewis^x mimic **2** contains D-galactose and L-fucose to provide all the essential hydroxyl groups required for E-selectin binding. Instead of neuraminic acid we incorporated *S*-cyclohexyllactic acid, a modification which has already been shown to improve selectin inhibition.[6] *N*-Acetyl-D-glucosamine was replaced by a glucal-derived building block.[7]

Commercially available triacetyl-D-glucal **3** was used as starting material (Scheme 1). Hydrogenation in THF[8] followed by deacetylation of the crude intermediate and benzylidene protection of the 4- and the 6-hydroxyl groups gave the required building block **4** which could be isolated either by chromatography (88%) or, alternatively, by precipitation with hexane (78%). D-Galactose was introduced using thioglycoside **5**[9] which was activated with NIS/trifluorosulfonic acid. As expected the glycosylation was completely stereoselective leading to β-linked disaccharide **6** in 71% yield. The stereochemistry was deduced from the large coupling constant of 8.0 Hz between H-1 Gal and H-2 Gal. No α-anomer could be detected.[10] Reductive opening of the benzylidene acetal of **6** with $NaCNBH_3$/HCl gave regioselectively compound **7** (72%) with an unprotected hydroxyl group in the 4-position. Fucosylation of **7** was

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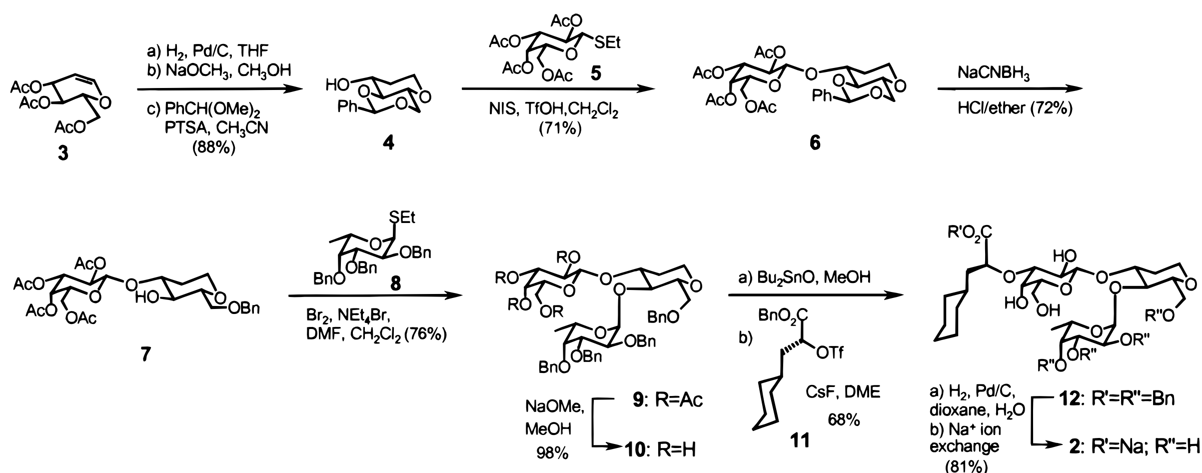
Scheme 1



Scheme 2

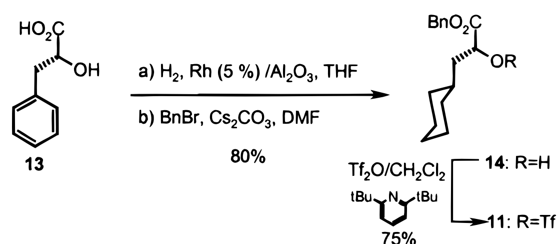


Table 1. E-Selectin Inhibition by Compound **2** Compared to sLe^x (Static, Cell-Free Ligand Binding Assay)

compound **2**		sLe^x (**1**)
IC_{50} [μM]	rel IC_{50}[a]	IC_{50} [μM]
33	0.028	1179
24	0.027	889
51	0.039	1308
36 ± 13	0.031 ± 0.007	1125 ± 214

[a] Rel IC_{50} = IC_{50}(compound **2**)/IC_{50}(sLe^x).

achieved using thioglycoside **8**.[11] In situ generation of the corresponding bromide followed by treatment with Et_4NBr gave α-glycoside **9** in 76% yield. The small coupling constant of 4.0 Hz between H-1 Fuc and H-2 Fuc proved the stereochemistry. The corresponding β-epimer could not be isolated. Removal of the acetates in the presence of catalytic amounts of sodium methylate furnished tetraol **10** in 98% yield. Activation of the 3-hydroxyl group of the galactose with dibutyltinoxide[12] followed by treatment with triflate **11** in the presence of CsF gave trisaccharide **12** in 68% yield. Compound **12** is not very stable (probably due to lactone formation involving the 2- or the 4-hydroxyl group of galactose). The isolated material contained small quantities (~5%) of impuries and was immediately used in the final hydrogenation. The desired selectin inhibitor was transformed into its sodium salt **2** by passage through a column with ion exchange resin and further purified by reverse phase chromatography (82%).[13]

The triflate **11** was prepared from commercially available D(+)-3-phenyllactic acid **13** (Scheme 2). Hydrogenation followed by benzyl ester formation using the crude intermediate gave α-hydroxy ester **14** in 80% yield. Treatment with trifluoromethanesulfonic anhydride in the presence of di-*tert*-butyl pyridine furnished triflate **11** in 75% yield. Compound **11** is surprisingly stable and can be purified by chromatography on silica gel. It can be stored in the deep freezer for months without decomposition.

Biological Evaluation of 2

As a primary screen for E-selectin activity a cell-free, competitive ligand binding assay has been developed using a polylysine/sialyl Lewis^a conjugate as a multivalent ligand.[14] Immobilized E-selectin is incubated with the polymer and then treated with test compounds. The measurement of the dose-dependent displacement of the polymer by potential inhibitors allows the determination of their IC_{50} values (concentration to achieve an inhibitory effect of 50%). Test compounds are always compared to sialyl Lewis^x **1** tetrasaccharide (Chart 1) which is assayed as a reference on each test plate. To compare the test results for compounds which have been tested on different plates, we calculated the relative IC_{50} value which is defined by IC_{50}(test compound)/IC_{50}(sLe^x). In three separate assays analogue **2** (IC_{50} = 36 μM, rel IC_{50} = 0.03; Table 1) showed approximately 30-fold improvement of activity compared to sLe^x (IC_{50} = 1125 μM, rel IC_{50} = 1.00).

Advantages of this static cell free assay are the good reproducibility and the high throughput. On the other hand it has to be noted that rolling of leukocytes on activated endothelium mediated by selectin/oligosaccharide interactions is a nonequilibrium process which occurs under hydrodynamic flow. Therefore, static in vitro assays which measure inhibition under equilibrium conditions could generate data which are not predictive of the in vivo behavior of test compounds under flow conditions. To overcome this, a cell-based in vitro flow assay has been developed which mimics the nonequilibrium in vivo conditions and allows one to monitor E-selectin-dependent rolling under shear stress.[15] A solution containing human polymorphonuclear neutrophils (PMNs) is pumped through a parallel plate flow chamber containing a monolayer of human umbilical vein endothelial cells (HUVECs) which have been stimulated with TNF-α. Rolling is recorded by video microscopy. The reduction of the number of interacting cells (NIC) in the presence of a selectin antagonist is a measure for its inhibitory effect. Sialyl Lewis^x was inactive in this assay up to 1000 μM. Antagonist **2**, however, led to 94% inhibition of the NIC at 200 μM,

60% at 50 μM, and 10% (not significant) at 10 μM. The IC_{50} value of **2** for the flow assay can be estimated to 30−40 μM. The closely related E-selectin inhibitor **15** (Chart 1)[6] which showed 10-fold improved E-selectin inhibition compared to sLe^x in the static ligand binding assay was used as a reference compound in the flow assay. Compound **15** reduced the NIC by only 45% when directly compared at 200 μM with **2** (94% reduction) on the same test day using the same cells. Thus, the inhibitory effects of E-selectin antagonists found in the static equilibrium assay were confirmed by the non-equilibrium flow assay. Our in vitro E-selectin assays seem to be predictive for the in vivo efficacy of test compounds. Thus we observed a significant inhibitory effect of **2** (ED_{50} = 15 mg/kg) in the thioglycollate induced peritonitis reaction in mouse whereas sLe^x was inactive in this model of acute inflammation at doses up to 100 mg/kg.[16]

Conclusion

The E-selectin antagonist **2** is a simplified analogue of sLe^x which can be prepared in 10 linear steps in an overall yield of 18%. The substantially increased inhibitory effect of compound **2** compared to sLe^x was demonstrated in both static and dynamic in vitro assays. The in vitro data were confirmed in an in vivo mouse model of acute inflammation. An explanation for the improved activity of **2** compared to **15** could be an additional interaction between E-selectin and the extra hydroxymethylene group in **2**. An alternative explanation for the good activity of **2** could be an improved preorganization of the bioactive conformation in solution due to beneficial conformational restraints in **2** compared to **15**. We are currently investigating these hypotheses in our laboratory.

Experimental Section

General. All reactions were carried out under an atmosphere of dry argon. Commercially available absolute solvents were used. The NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer. The signal assignments are based on two-dimensional $^1H/^1H$-correlation (COSY) and $^1H/^{13}C$-correlation spectroscopy (HSQC). The MS spectra were obtained on a Finnigan MAT 90 mass spectrometer.

2: A suspension of **12** (700 mg, 0.660 mmol) and Pd (10%) on charcoal (250 mg) in dioxane/water (18 mL, 5:1) was hydrogenated at 20 °C for 56 h using a balloon filled with H_2. After filtration the solvent was removed and the residue subjected to chromatography (silica gel, $CHCl_3/MeOH/H_2O$ 5:5:0.25 → 2:5:2). The product was dissolved in water and passed through a column with Na ion exchange resin to give compound **12** which was further purified by reverse phase chromatography (RP18, water/methanol 1:0 → 1:0.3). Lyophilization gave compound **12** as a colorless solid (340 mg, 81%). ^1H NMR (400 MHz, D_2O) δ = 0.78−1.75 (14 H, m, *H*-2ax, -C*H*$_2$-cC_6*H*$_{11}$), 1.12 (3 H, d, 6.5 Hz, *H*-6 Fuc), 2.17 (1 H, dd (br), 12.0/5.0 Hz, *H*-2eq), 3.32 (1 H, m, *H*-5), 3.33 (1 H, dd, 9.5/3.0 Hz, *H*-3 Gal), 3.42 (1 H, t (br), 11.5 Hz, *H*-1ax), 3.51 (1 H, t, 9.0 Hz, *H*-4), 3.55 (1 H, t, 6.0 Hz, *H*-5 Gal), 3.56 (1 H, t, 9.0 Hz, *H*-2 Gal), 3.67 (2 H, d, 6.0 Hz, *H*-6a Gal, *H*-6b Gal), 3.71 (1 H, dd, 10.5/4.0 Hz, *H*-2 Fuc), 3.75 (1 H, d (br), 3.0 Hz, *H*-4 Fuc), 3.76 (1 H, dd, 12.0/5.0 Hz, *H*-6a), 3.81 (1 H, dd, 10.5/3.0 Hz, *H*-3 Fuc), 3.84 (1 H, d, 3.0 Hz, *H*-4 Gal), 3.86 (1 H, dd, 12.0/2.5 Hz, *H*-6b), 3.88−4.01 (3 H, m, O-C*H*-CO_2Na, *H*-1eq, *H*-3), 4.45 (1 H, d, 8.0 Hz, *H*-1 Gal), 4.71 (1 H, q, 6.5 Hz, *H*-5 Fuc), 4.89 (1 H, d, 4.0 Hz, H-1 Fuc). ^{13}C NMR (100 MHz, D_2O) δ = 15.9 (p), 26.1 (s), 26.3 (s), 26.5 (s), 30.6 (s), 32.2 (s), 33.7 (t), 34.0 (s), 41.6 (s), 60.4 (s), 62.0 (s), 65.5 (s), 66.7 (t), 67.3 (t), 68.4 (t), 69.6 (t), 70.2 (t), 72.3 (t), 74.6 (t), 75.1 (t), 76.0 (t), 79.6 (t), 80.5 (t), 83.1 (t), 99.1 (t), 99.5 (t), 183.0 (q). MS/HR calcd for $C_{27}H_{45}O_{15}Na$ $(M - Na)^-$ 609.2758, found 609.2753.

4: A suspension of **3** (11.0 g, 40.3 mmol) and Pd (10%) on charcoal in THF (200 mL) was hydrogenated at 20 °C using a balloon filled with H_2 for 16 h. The catalyst was filtered off and the solvent removed. The remaining oil was dissolved in methanol (75 mL) and freshly prepared NaOMe (2.2 mmol) was added and stirred at 20 °C for 6 h. Then, 1 g of acidic ion exchange resin (Dowex Wx8) was added, the resin filtered off, and the solvent removed. The crude material was evaporated with CH_3CN (2 × 50 mL) and suspended in CH_3CN (300 mL). Benzaldehyde dimethylacetal (12.25 g, 80.6 mmol) and PTSA (0.43 g, 2.5 mmol) were added and stirred for 2 h at 20 °C. $NaHCO_3$ (1.65 g, 20 mmol) was added to the clear solution. After 10 min the solids were filtered off, the solvent was removed, and the residue was subjected to flash chromatography (silica gel, toluene/ethyl acetate 2:1 → 1:1). Compound **4** (8.52 g, 89%) was isolated as a colorless solid. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.80 (1 H, tdd, 13.0/11.0/5.0 Hz, *H*-2ax), 2.02 (1 H, ddt, 13.0/5.0/1.5 Hz, *H*-2eq), 3.33 (1 H, td, 9.5/5.0 Hz, *H*-5), 3.43 (1 H, t, 9.0 Hz, *H*-4), 3.56 (1 H, td, 12.5/2.0 Hz, *H*-1ax), 3.70 (1 H, t, 10.0 Hz, *H*-6a), 3.88 (1 H, dddd, 11.0/9.0/5.0/1.5, *H*-3), 3.99 (1 H, ddd, 12.0/5.0/1.5 Hz, *H*-1eq), 4.28 (1 H, dd, 10.0/5.0 Hz, *H*-6b), 5.56 (1 H, s, Ar-C*H*-), 7.35−7.50 (5 H, m, Ar-*H*). MS/HR calcd for $C_{13}H_{17}O_4$ $(M + H)^+$ 237.1127, found 237.1124.

6: To a solution of **4** (4.50 g, 19.11 mmol), **5**[9] (5.00 g, 12.74 mmol), and NIS (3.58 g, 15.93 mmol) in CH_2Cl_2 (50 mL) was added droppwise at 0 °C a solution of trifluoromethanesulfonic acid (0.15 M). When the color changed from dark red to dark brown the addition was stopped. After complete consumption of **4** (TLC, toluene/ethyl acetate 2:1) saturated $NaHCO_3$ solution was added (30 mL) and the organic phase separated. The aqueous phase was extracted with CH_2Cl_2 (50 mL), and the combined organic phases were washed with $Na_2S_2O_3$ solution (2 × 25 mL) and dried with Na_2SO_4. Following filtration the solvent was removed and the residue subjected to chromatography (silica gel, hexane/ether 1:1 → 1:3. Compound **6** was isolated as a colorless solid (5.23 g, 72%). ^1H NMR (400 MHz, $CDCl_3$) δ = 1.85 (1 H, m, *H*-2ax), 1.96 (3 H, s, -C(O)-C*H*$_3$), 1.97 (1 H, m, *H*-2eq), 1.98 (3 H, s, -C(O)C*H*$_3$), 2.03 (3 H, s, -C(O)C*H*$_3$), 2.13 (3 H, s, -C(O)C*H*$_3$), 3.34 (1 H, td, 9.5/5.0 Hz, *H*-5), 3.56 (1 H, td, 12.5/2.5 Hz, *H*-1ax), 3.64 (1 H, t, 9.0 Hz, *H*-4), 3.71 (1 H, ddd, 8.0/5.5/1.0 Hz, *H*-5 Gal), 3.74 (1 H, t, 10.0 Hz, *H*-6a), 3.91 (1 H, m, *H*-3), 3.94 (1 H, dd, 11.0/5.5 Hz, *H*-6a Gal), 4.01 (1 H, ddd, 11.5/5.0/2.0 Hz, *H*-1eq), 4.09 (1 H, dd, 11.0/8.0 Hz, *H*-6b Gal), 4.29 (1 H, dd, 10.5/5.0 Hz, *H*-6b), 4.69 (1 H, d, 8.0 Hz, *H*-1 Gal), 4.98 (1 H, dd, 10.5/3.5 Hz, *H*-3 Gal), 5.22 (1 H, dd, 10.5/8.0 Hz, *H*-2 Gal), 5.33 (1 H, dd, 3.5/1.0 Hz, *H*-4 Gal), 5.59 (1 H, s, Ar-C*H*-), 7.35−7.52 (5 H, m, Ar-*H*). MS/HR calcd for $C_{27}H_{34}O_{13}$ $(M + Na)^+$ 589.1897, found 589.1896.

7: At 0 °C a freshly prepared saturated solution of HCl in diethyl ether is added dropwise to a suspension of **6** (2.00 g, 3.53 mmol) and $NaCNBH_3$ (2.22 g, 35.3 mmol) in THF (60 mL). After complete consumption of **6** (TLC ethyl acetate/hexane 3:1), solid $NaHCO_3$ (1.5 g) was added and stirred for 5 min. The mixture was diluted with ethyl acetate (300 mL), extracted with $NaHCO_3$ solution (2 × 150 mL) and brine (2 × 150 mL), and dried with Na_2SO_4. Following filtration the solvent was removed and the residue subjected to chromatography (silica gel, toluene/acetone 3:1 → 2:1). Compound **7** was isolated as a colorless foam (1.45 g, 72%). ^1H NMR (400 MHz, $CDCl_3$) δ = 1.72−1.86 (2 H, m, *H*-2ax, *H*-2eq), 2.00 (3 H, s, -C(O)C*H*$_3$), 2.01 (3 H, s, -C(O)C*H*$_3$), 2.07 (3 H, s, -C(O)C*H*$_3$), 2.17 (3 H, s, -C(O)-C*H*$_3$), 3.32 (1 H, ddd, 9.0/6.0/2.0 Hz, *H*-5), 3.40−3.47 (2 H, m, *H*-1ax, *H*-4), 3.52 (1 H, ddd, 14.0/8.0/5.5 Hz, *H*-3), 3.63 (1 H, dd, 10.5/6.0 Hz, *H*-6a), 3.75 (1 H, d, 1.5 Hz, O*H*), 3.82 (1 H, dd, 10.5/2.0 Hz, *H*-6b), 4.00 (1 H, td, 6.5/1.0 Hz, *H*-5 Gal), 4.02 (1 H, m, *H*-1eq), 4.14 (2 H, d, 6.5 Hz, *H*-6a Gal, *H*-6b Gal), 4.56 (1 H, d, 8.0 Hz, *H*-1 Gal), 4.58 (1 H, d, 12.0 Hz, OBn), 4.62 (1 H, d, 12.0 Hz, OBn), 5.03 (1 H, dd, 10.5/3.5 Hz, *H*-3 Gal), 5.24 (1 H, dd, 10.5/8.0 Hz, *H*-2 Gal), 5.40 (1 H, dd, 3.5/

1.0 Hz, *H*-4 Gal), 7.16−7.37 (5 H, m, Ar-*H*). MS/HR calcd for $C_{27}H_{36}O_{13}$ (M + Na)$^+$ 591.1054, found 591.2051.

9: A solution of Br_2 (298 mg, 1.86 mmol) in CH_2Cl_2 (2 mL) was added dropwise at 0 °C to a solution of **8**[11] (808 mg, 1.69 mmol) in CH_2Cl_2 (2 mL). After stirring for 30 min at 0 °C, cyclohexene (0.2 mL) was added to consume excessive Br_2. The mixture was added within 10 min to a solution of **7** (800 mg, 1.41 mmol) and Et_4NBr (353 mg, 1.69 mmol) in DMF/CH_2Cl_2 (10 mL, 1:1). The mixture was stirred for 44 h at 20 °C. Ethyl acetate (100 mL) was added followed by washings with NaS_2O_3 solution (2 × 50 mL), water (2 × 50 mL), and brine (2 × 50 mL). The resulting solution was dried with Na_2SO_4 and filtered, the solvent removed, and the residue subjected to chromatography (silica gel, toluene/ethyl acetate 1.5:1). Compound **9** was isolated as a colorless solid (1.05 g, 76%). In addition a small amount of starting material **7** (100 mg, 12%) was recovered. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.23 (3 H, d, 6.5 Hz, *H*-6 Fuc), 1.60 (1 H, m, *H*-2ax), 1.87 (3 H, s, -C(O)-C*H*$_3$), 1.97 (3 H, s, -C(O)C*H*$_3$), 2.01 (1 H, m, *H*-2eq), 2.04 (3 H, s, -C(O)C*H*$_3$), 2.05 (3 H, s, -C(O)C*H*$_3$), 3.32 (1 H, m, *H*-5), 3.36 (td, 11.5/1.5 Hz, *H*-1ax), 3.63 (1 H, dd, 10.5/2.0 Hz, *H*-6a), 3.68 (1 H, d (br), 2.5 Hz, *H*-4 Fuc), 3.78−3.85 (3 H, m, *H*-3, *H*-4, *H*-6b), 3.86 (1 H, td, 7.0/1.0 Hz, *H*-5 Gal), 3.94 (1 H, dd, 10.0/2.5 Hz, *H*-3 Fuc), 3.99 (1 H, m, *H*-1eq), 4.02 (2 H, d, 7.0 Hz, *H*-6a Gal, *H*-6b Gal), 4.09 (1 H, dd, 10.0/4.0 Hz, *H*-2 Fuc), 4.39 (1 H, d, 12.0 Hz, OBn), 4.45 (1 H, d, 12.0 Hz, OBn), 4.55 (1 H, d, 8.0 Hz, *H*-1 Gal), 4.61 (1 H, d, 11.5 Hz, OBn), 4.66 (1 H, q (br), 6.5 Hz, *H*-5 Fuc), 4.73 (1 H, d, 12.0 Hz, OBn), 4.78 (2 H, s, 2 × OBn), 4.82 (1 H, d, 11.5 Hz, OBn), 4.97 (1 H, dd, 10.5/4.0 Hz, *H*-3 Gal), 4.98 (1 H, d, 12.0 Hz, OBn), 5.04 (1 H, d, 4.0 Hz, *H*-1 Fuc), 5.11 (1 H, dd, 10.5/8.0 Hz, *H*-2 Gal), 5.35 (1 H, dd, 3.5/1.0 Hz, *H*-4 Gal), 7.24−7.44 (20 H, m, Ar-*H*). MS/HR calcd for $C_{54}H_{64}O_{17}$ (M + Na)$^+$ 1007.4041, found 1007.4048.

10: A solution of **9** (1.00 g, 1.02 mmol) and freshly prepared NaOMe (0.10 mmol) in methanol (10 mL) was stirred at 20 °C for 3 h. Then, 2 drops of acetic acid was added, the solvent removed, and the residue subjected to chromatography (silica gel, ethyl acetate/2-propanol 9:1 → 5:1). Compound **10** was isolated as a colorless solid (820 mg, 98%). ^{1}H NMR (400 MHz, CD_3OD) δ = 1.25 (3 H, d, 6.5 Hz, *H*-6 Fuc), 1.68 (1 H, qd, 12.5/5.0 Hz, *H*-2ax), 2.13 (1 H, dd (br), 12.5/5.0 Hz, *H*-2eq), 3.27 (1 H, ddd, 9.0/4.4/1.5 Hz, *H*-5), 3.41 (t (br), 12.5 Hz, *H*-1ax), 3.48 (1 H, m, *H*-5 Gal), 3.48 (1 H, dd, 9.5/3.0 Hz, *H*-3 Gal), 3.57 (1 H, dd, 9.5/7.5 Hz, *H*-2 Gal), 3.62 (1 H, dd, 11.0/1.5 Hz, *H*-6a), 3.70 (1 H, dd, 11.0/4.5 Hz, *H*-6a Gal,), 3.74 (1 H, t, 9.0 Hz, *H*-4), 3.83 (1 H, dd, 11.0/7.0 Hz, *H*-6b Gal), 3.84 (1 H, d (br), 3.0 Hz, *H*-4 Gal), 3.89 (1 H, dd, 11.0/3.0 Hz, *H*-6b), 3.90−3.96 (3 H, m, *H*-1eq, *H*-3, *H*-4 Fuc), 3.95 (1 H, dd, 10.5/3.5 Hz, *H*-2 Fuc), 3.94 (1 H, dd, 10.5/2.5 Hz, *H*-3 Fuc), 4.35 (1 H, d, 7.5 Hz, *H*-1 Gal), 4.36 (1 H, d, 12.0 Hz, OBn), 4.50 (1 H, d, 11.5 Hz, OBn), 4.53 (1 H, d, 12.0 Hz, OBn), 4.64 (1 H, d, 11.5 Hz, OBn), 4.73 (2 H, d, 11.5 Hz, 2 × OBn), 4.82 (1 H, d, 11.5 Hz, OBn), 4.87 (1 H, m, *H*-5 Fuc), 4.93 (1 H, d, 11.5 Hz, OBn), 5.04 (1 H, d, 3.5 Hz, *H*-1 Fuc), 7.23−7.43 (20 H, m, Ar-*H*). MS/HR calcd for $C_{46}H_{56}O_{13}$ (M + Na)$^+$ 839.3619, found 839.3624.

11: Compound **14** (265 g, 1.01 mol) was dissolved in CH_2Cl_2 (3000 mL) followed by the addition of 2,6-di-*tert*-butyl pyridine (251.3 g, 1.27 mol). The solution was cooled to −20 °C, trifluoromethane sulfonic acid anhydride (334.9 g, 1.16 mol) was added within 10 min, and the mixture was stirred for 4 h. The mixture was diluted with CH_2Cl_2 (1000 mL) and added to a 1 M solution of K_2HPO_4 (5400 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 1000 mL). After the mixture was dried with Na_2SO_4, the solvent was removed (bath temp < 35 °C) and the residue subjected to chromatography (silica gel, hexane/ethyl acetate 10:1). Compound **11** was isolated as a colorless oil (298 g, 75%). $[\alpha]_D$ = 38.6 ± 0.4° ($CHCl_3$, 20 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87−1.77 (11 H, m, CH-CH$_2$-cC$_6$H$_{11}$), 1.80 (1 H, ddd, 15.0/9.0/4.0 Hz, CH-C*H*aHb-cC$_6$H$_{11}$), 1.91 (1 H, ddd, 15.0/9.0/5.0 Hz, CH-CHa*H*b-cC$_6$H$_{11}$), 5.21 (1 H, dd, 9.0/4.0 Hz, C*H*-CH$_2$-cC$_6$H$_{11}$), 5.23 (1 H, d, 12.0 Hz, OBn), 5.27 (1 H, d, 12.0 Hz, OBn), 7.35−7.37 (5 H, m, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 25.7 (s), 26.0 (s), 26.1 (s), 30.1 (q), 32.0 (s), 33.2 (s), 33.4 (s), 39.3 (t), 68.2 (s), 81.9 (t), 128.6 (2 × t), 128.7 (2 × t), 128.9 (t), 134.4 (q), 167.6 (q). MS/CI 394 (M)$^+$.

12: A suspension of **10** (800 mg, 0.980 mmol) and Bu_2SnO (268 mg, 1.08 mmol) in methanol (15 mL) was heated under reflux for 2 h. The solvent was removed and the resulting colorless foam dried in a vacuum for 60 h. The residue was dissolved in DME (15 mL) followed by the addition of **11** (778 mg, 1.96 mmol) and CsF (179 mg, 1.18 mmol). The resulting suspension was stirred at 20 °C for 8 h. Water (150 mL) was added followed by extraction with ethyl acetate (3 × 50 mL). The organic phase was washed with brine (2 × 50 mL) and dried with Na_2SO_4, the solvent was removed, and the residue was subjected to chromatography (silica gel, toluene/acetone 2.5:1). Compound **12** was isolated as a colorless foam (710 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.84−1.86 (14 H, m, *H*-2ax, -C*H*$_2$-cC$_6$*H*$_{11}$), 1.17 (3 H, d, 6.5 Hz, *H*-6 Fuc), 2.09 (1 H, dd (br), 13.0/5.0 Hz, *H*-2eq), 2.63 (1 H, m, Gal-6 O*H*), 2.73 (1 H, m (br), Gal-2 O*H*), 3.25 (1 H, s, Gal-4 O*H*), 3.26 (1 H, dd, 10.0/3.0 Hz, *H*-3 Gal), 3.32−3.42 (3 H, m, *H*-1ax, *H*-5, *H*-5 Gal), 3.67 (1 H, dd, 11.0/1.5 Hz, *H*-6a), 3.68−4.00 (8 H, m, H-1eq, H-3, H-4, H-6b, H-2 Gal, H-4 Gal, H-6a Gal, H-6b Gal), 3.78 (1 H, s (br), *H*-4 Fuc), 4.01 (1 H, dd, 10.0/2.5 Hz, *H*-3 Fuc), 4.05 (1 H, dd, 10.0/3.0 Hz, *H*-2 Fuc), 4.32 (1 H, d, 8.0 Hz, *H*-1 Gal), 4.35 (1 H, m, O-C*H*-CH$_2$-cC$_6$H$_{11}$), 4.37 (1 H, d, 12.0 Hz, OBn), 4.45 (1 H, d, 12.0 Hz, OBn), 4.61 (1 H, d, 11.5 Hz, OBn), 4.63 (1 H, q, 6.5 Hz, *H*-5 Fuc), 4.64 (1 H, d, 11.5 Hz, OBn), 4.78 (2 H, m, 2 × OBn), 4.83 (1 H, d, 11.5 Hz, OBn), 4.97 (1 H, d, 11.5 Hz, OBn), 5.06 (1 H, d, 3.0 Hz, *H*-1 Fuc), 5.17 (1 H, d, 12.0 Hz, OBn), 5.21 (1 H, d, 12.0 Hz, OBn), 7.20−7.42 (25 H, m, Ar-*H*). MS (ESI) 1083 (M + Na)$^+$.

14: A suspension of **13** (200 g, 1.20 mol) and Rh (5%) on activated Al_2O_3 (6.0 g) in THF/H_2O (2000 mL, 1:1) was hydrogenated at 20 °C for 6 h. The catalyst was filtered off, and the solvents were removed in a vacuum. The residue was suspended in CH_3OH/H_2O (1250 mL, 9:1) and the pH adjusted to 8.1 by the addition of an aqueous solution of cesium carbonate. The solvents were removed, and the residue was repeatedly evaporated with ethanol and then with hexane. The gray material was dissolved in DMF (1300 mL). Benzylbromide (219.3 g, 1.20 mol) was added at 20 °C within 20 min. After the mixture was stirred for 16 h, CH_2Cl_2 (200 mL) was added, the layers separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 250 mL). The solvents were removed in a vacuum, and the residue was subjected to chromatography (silica gel, hexane/ethyl acetate 7:1). Compound **14** was isolated as a colorless solid (265 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.85−1.86 (13 H, m, CH-C*H*$_2$-cC$_6$*H*$_{11}$), 2.70 (1 H, M, 3.5 Hz, O*H*), 4.27 (1 H, m, C*H*-CH$_2$-cC$_6$H$_{11}$), 5.20 (2 H, M, OBn), 7.34−7.43 (5 H, m, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 27.0 (s), 27.2 (s), 27.4 (s), 33.3 (s), 34.6 (t), 34.8 (s), 43.0 (s), 68.1 (s), 69.6 (t), 129.2 (2 × t), 129.4 (t), 129,5 (2 × t), 136.2 (q), 176.7 (q). MS/EI 262 (M)$^+$.

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