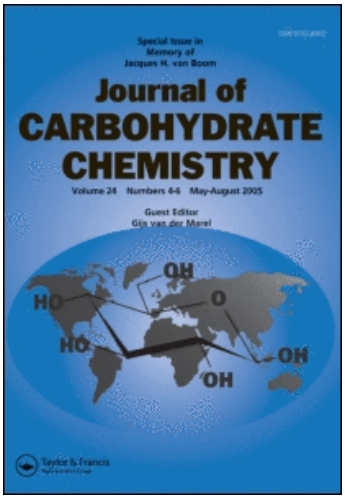

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Synthesis and Selectin-Blocking Activity of a Novel Analog of Sulfatide: 3-*C*-Carboxymethylgalactosyl Lipid[†,#]

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ABSTRACT

The 3-*C*-carboxymethylgalactose derivative carrying the 2-(tetradecyl)hexadecyl residue, which was designed as a novel analog of sulfatide, was synthesized. A key intermediate, 3-*C*-carboxymethylgalactose, was prepared from the suitably protected galactose by Swern oxidation and Wittig-Horner carboxymethylenation, followed by stereoselective reduction of the double bond. The compound obtained showed much more potent activity as a selectin blocker than the 3-*O*-sulfogalactose derivative with 2-(tetradecyl)hexadecyl residue.

Key Words: Selectin; Sulfatide; Inflammation; 3-*C*-carboxymethylgalactose.

[†]This paper is dedicated to Professor Gérard Descotes on the occasion of his 70th birthday.
[#]Synthetic studies on sialoglycoconjugates, Part 131. For Part 130, see Ref. [1].
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INTRODUCTION

Cell adhesion molecules (CAMs) play a crucial role during inflammatory conditions in several immune disorders by recruiting leukocytes to the injured area.[2] The selectins are a family of CAMs composed of three structurally related carbohydrate-binding proteins (E-, L-, and P-selectin).[3–6] E-and P-selectin are induced on the endothelial surface in response to inflammatory signals, whereas L-selectin is constitutively expressed on all circulating leukocytes and interacts with cognate ligands on endothelial cells. It is now well-accepted that all three selectins can efficiently recognize sialyl Le^X [Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc].[7] However, sulfatide (**I**, Figure 1), one of the major acidic glycosphingolipids in mammalian tissues, was found to be a good ligand for L-and P-selectin.[8] It shows highly protective effects against selectin-dependent inflammatory lung injury.[9,10]

In view of these facts, we describe herein the synthesis and selectin-blocking activity of a novel sulfatide analog containing 3-*C*-carboxymethylgalactose as part of our search for selectin inhibitors.[11]

RESULTS AND DISCUSSION

The target compound (**III**) (Figure 1) was designed based on previous results indicating that the 2-(tetradecyl)hexadecyl group could serve as a ceramide substitute,[12,13] and that 3-*C*-carboxymethyl Le^x was much more potent than the corresponding sialyl Le^x derivative in the ligand-selectin competitive binding assay.[14]

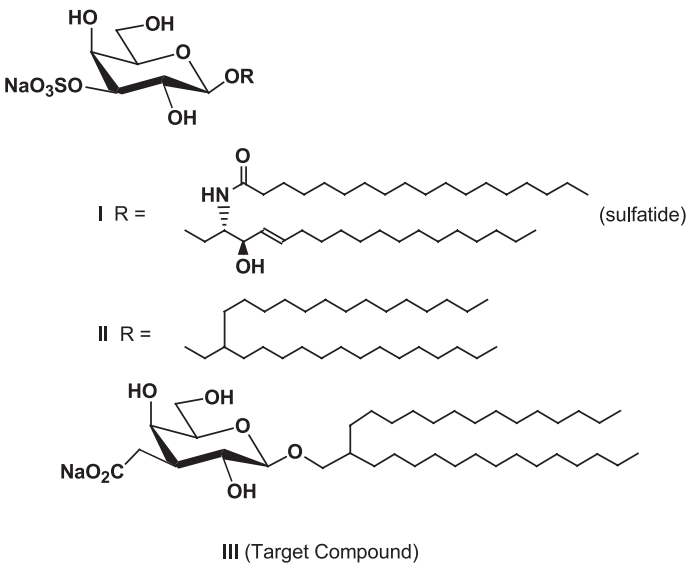

Figure 1. Structures of sulfatide and its analogs.

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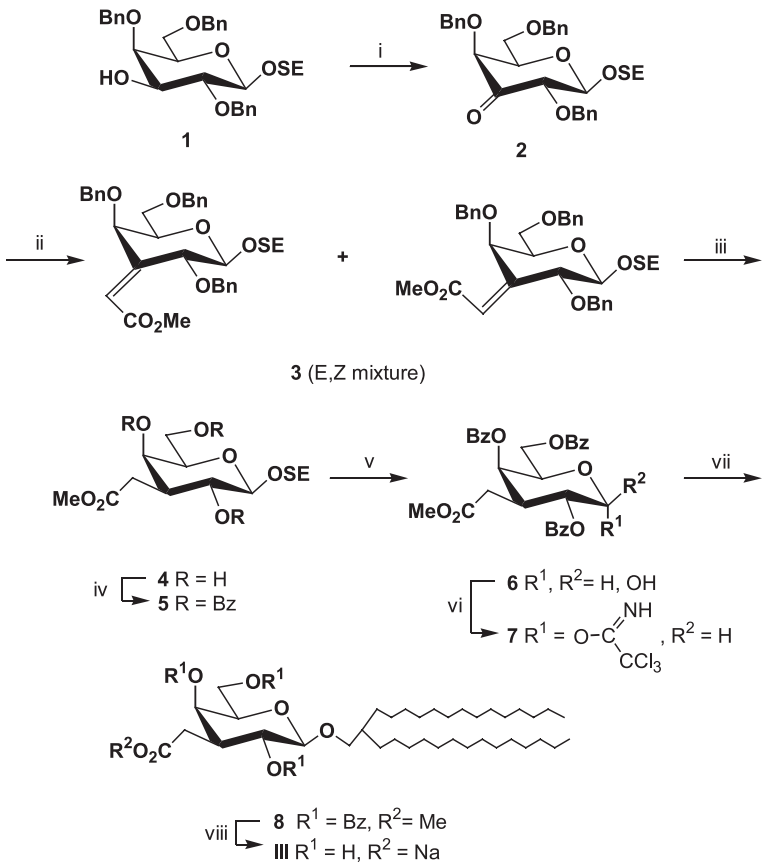

Scheme 1. I: $COCl_2$, DMSO, Et_3N; ii: diethylphosphonoacetate, MeONa, iii: H_2, Pd-C; iv: benzoyl chloride, pyr.; v: TFA; vi: CCl_3CN, DBU; vii: 2-(tetradecyl)hexadecan-1-ol, TMSOTf; viii: MeONa and then H_2O (NaOH).

For the synthesis of the required 3-*C*-carboxymethylgalactose moiety, 2-(trimethylsilyl)ethyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**1**)[15] was employed as a suitable starting material (Scheme 1). Oxidation[16] of **1** with oxalyl chloride and dimethyl sulfoxide gave the 3-ulose derivative **2**, which, on treatment[17] with ethyl diethylphosphonoacetate in the presence of sodium methoxide, afforded the methyl ester of the 3-*C*-carboxymethylene derivative **3** (83% in two steps) as a 1:1 mixture of *E*,*Z*-isomers. Transesterification from ethyl to methyl was observed during the course of the reaction. Catalytic hydrogenation of **3** over 10% palladium-on-charcoal in 4:1 methanol-acetic acid at rt gave the desired 3-*C*-carboxymethylgalactoside **4** stereoselectively. Benzoylation of **4** gave **5** (73% from **3**). In the ^1H NMR spectrum of **5**, H-2 (dd, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 11.2 Hz) was observed at δ 5.54, indicating the configuration of the substituent at C-3 to be equatorial. Compound **5** was converted into the α-trichloroacetimidate donor **7** by selective removal of 2-(trimethylsilyl)ethyl group with

Table 1. Selectin-blocking activity of sulfatide analogs.

	% inhibition at 0.3 mM		
Compound	P-selectin	L-selectin	E-selectin
III	79 ± 3	92 ± 3	12 ± 8
II	6 ± 3	13 ± 3	0 ± 5
sLe^x	3 ± 6	0 ± 5	0 ± 4

Each value represents the mean ± S.E. of 5 wells.

trifluoroacetic acid (98%), and subsequent imidate formation (88%).[18–20] Glycosylation of 2-(tetradecyl)hexadecanol with **7** in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate gave only the desired β-glycoside **8** in 95% yield, as indicated by the one-proton doublet at δ 4.66 ppm (d, $J_{1,2}$ 7.7 Hz) assigned to H-1 in the ^{1}H NMR spectrum of **8**. *O*-Debenzoylation of **8** with sodium methoxide in methanol, and subsequent saponification of the methyl ester group by addition of water, yielded the desired 3-*C*-carboxymethylgalactosyl lipid **III** in 85% yield.

The activity of the target compound in vitro was measured in adhesion assays in terms of the inhibition of the binding of sialyl Le^x expressing HL-60 cells to recombinant human selectin-IgG fusion proteins.[21,22] The synthesized glycolipid (**III**) was significantly more potent than the sialyl Le^x tetrasaccharide and 3-*O*-sulfogalactosyl lipid (**II**) to block the adhesion to P-and L-selectin (Table 1). Taking into consideration that the 3-*C*-carboxymethylgalactose residue is expected to be more stable to both chemical and enzymatic degradations than the corresponding sialyl- or sulfo derivatives, the glycolipid synthesized here is viewed as a promising therapeutic agent against selectin-dependent inflammation.

EXPERIMENTAL

General procedures. Optical rotations were determined with a Union PM-201 polarimeter at 25°C, and IR spectra were recorded with a Jasco IRA-100 spectrophotometer. ^{1}H NMR spectra were recorded at 400 or 200 MHz with a Varian Inova 400 or Varian Gemini-2000 spectrometer using deuterated solvents ($CDCl_3$) with TMS as the internal standard. TLC was performed on Silica Gel 60 (E. Merck), and column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was accomplished with the specified solvent systems (v/v). Concentrations were conducted in vacuo.

2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-deoxy-3-*C*-(methoxycarbonylmethyl)-β-D-galactopyranoside (5). Oxalyl chloride (174 μL, 2.0 mmol) and dimethyl sulfoxide (283 μL, 3.99 mmol) were added to dichloromethane (3 mL) at −50°C, and stirred under a nitrogen atmosphere for 10 min at −50°C. After the addition of **1** (1.0 g, 1.82 mmol), the mixture was stirred for a further 30 min at −50°C. Triethylamine (1.3 mL, 9.07 mmol) was added, and the mixture was washed with brine. The organic layer was dried over Na_2SO_4, and concentrated to give the corresponding ketone **2**; IR (film): 3075−2850 (CH), 1725 (C═O) and 710 (phenyl) cm^{-1}.

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A solution of ethyl diethylphosphonoacetate (329 μL, 2.36 mmol) and methanolic sodium methoxide freshly prepared from sodium (200 mg, 8.9 mmol) and methanol (4.4 mL), was stirred for 10 min at rt. After addition of **2**, the mixture was stirred for 1 h at rt and washed with water. The organic layer was dried over Na_2SO_4 and concentrated to a syrup, which was chromatographed on a column of silica gel with 1:10 ethyl acetate-hexane to give **3** (911 mg, 83% in two steps) as a mixture of *E*,*Z*-isomers.

A solution of **3** (500 mg, 827 μmmol) in methanol (164 mL) and acetic acid (36 mL) was treated with hydrogen over 10% Pd-C (500 mg) for 6 h at rt. The insoluble materials were removed by filtration and the filtrate was concentrated to give **4**. The residue was dissolved in pyridine (3 mL) and treated with benzoyl chloride (346 μL, 2.98 mmol) for 12 h at rt. The mixture was diluted with chloroform and washed with 2M HCl and water, dried over Na_2SO_4, and concentrated. The obtained syrup was chromatographed on a column of silica gel with 1:4 ethyl acetate-hexane to give **5** (390 mg, 73%) as an amorphous mass; $[\alpha]_D$ + 16 (*c* 4.5, $CHCl_3$); IR (film): 3075−2850 (CH), 1750 (methyl ester), 1740 and 1240 (benzoate) and 710 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (m, 2H, $(CH_3)_3Si\mathit{CH_2}CH_2O$), 2.55 (m, 2H, $\mathit{CH_2}COOCH_3$), 3.03 (m, 1H, H-3), 3.52 (s, 3H, $COO\mathit{CH_3}$), 3.66 (m, 1H, $(CH_3)_3SiCH_2\mathit{CH_2}O$), 4.15 (m, 1H, $(CH_3)_3SiCH_2\mathit{CH_2}O$), 4.40 (t, 1H, $J_{5,6b}$ = 5.6 Hz, H-5), 4.53 (dd, 1H, J_{gem} = 11.3 Hz, H-6a), 4.64 (dd, 1H, H-6b), 4.90 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 5.54 (dd, 1H, $J_{2,3}$ = 11.2 Hz, H-2), 5.87 (d, 1H, H-4), 7.34−8.24 (m, 15H, 3Ph).

Anal. Calcd for $C_{35}H_{40}O_{10}Si$: C, 64.80; H, 6.21. Found: C, 64.56; H, 5.98.

2,4,6-Tri-*O*-benzoyl-3-deoxy-3-*C*-(methoxycarbonylmethyl)-D-galactopyranose (6). To a solution of **5** (342 mg, 527 μmol) in dichloromethane, cooled to 0°C, was added trifluoroacetic acid (5 mL), and the mixture was stirred for 1 h at rt. Ethyl acetate was added at 0°C, and the mixture was concentrated at 20°C. The residue was diluted with chloroform, washed with sat aq Na_2CO_3 and H_2O, dried over Na_2SO_4, and concentrated. The obtained syrup was chromatographed on a column of silica gel with 1:2 ethyl acetate-hexane to give **6** (284 mg, 98%) as an amorphous mass; $[\alpha]_D$ + 67° (*c* 5.7, $CHCl_3$); IR (film): 3600−3300 (OH), 3075−2850 (CH), 1750 (methyl ester), 1740 and 1240 (benzoate) and 710 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.78 (m, 2H, $\mathit{CH_2}COOCH_3$), 3.67 (m, 1H, H-3), 3.85 (s, 3H, $COO\mathit{CH_3}$), 4.68 (m, 2H, H-5, H-6a), 5.07 (t, 1H, $J_{5,6b}$ = 6.4 Hz, H-6b), 5.57 (t, $J_{1\beta,2}$ = 8.2 Hz, H-2β), 5.69 (dd, H-2α), 6.02 (d, 1H, H-4), 7.67−8.46 (m, 15H, 3Ph).

Anal. Calcd for $C_{30}H_{28}O_{10}$: C, 65.69; H, 5.15. Found: C, 65.69; H, 4.95.

2,4,6-Tri-*O*-benzoyl-3-deoxy-3-*C*-(methoxycarbonylmethyl)-α-D-galactopyranosyl trichloroacetimidate (7). To a solution of **6** (282 mg, 514 μmol) in dichloromethane (1 mL) were added trichloroacetonitrile (0.158 mL, 1.54 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 40 μL, 257 μmol) at 0°C, and the mixture was stirred for 3 h at 0°C. Column chromatography (1:2 ethyl acetate-hexane) of the mixture on silica gel afforded **7** (314 mg, 88%); $[\alpha]_D$ + 50° (*c* 1.1, $CHCl_3$); IR (film): 3350 (NH), 3075−2850 (CH), 1750 (methyl ester), 1740 and 1240 (benzoate), and 710 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.74 (m, 2H, $\mathit{CH_2}COOCH_3$), 3.71 (m, 1H, H-3), 3.86 (s, 3H, $COO\mathit{CH_3}$), 4.78 (m, 2H, H-6a,6b), 5.07 (t, 1H, H-5), 5.93 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 11.9 Hz, H-2), 6.02 (d, 1H, H-4), 7.04 (d, 1H, H-1), 7.67−8.46 (m, 15H, 3Ph), 8.92 (s, 1H, NH).

Anal. Calcd for $C_{32}H_{28}NO_{10}CCl_3$: C, 55.47; H, 4.07. Found: C, 55.42; H, 4.02.

2-(Tetradecyl)hexadecyl 2,4,6-Tri-*O*-benzoyl-3-deoxy-3-*C*-(methoxycarbonylmethyl)-β-D-galactopyranoside (8). To a solution of imidate **7** (111 mg, 163 μmol) and 2-(tetradecyl)hexadecan-1-ol (113 mg, 244 μmol) in dichloromethane (4 mL) was added powdered MS 4Å (200 mg), and the mixture was stirred for 5 h at rt under a nitrogen atmosphere, and then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (2.6 μL, 13 μmol) was added and stirring was continued for 12 h at 0°C. Solids were filtered off through a pad of Celite, and washed with chloroform. The combined filtrate and washings were washed with M aq Na_2CO_3 and water, dried over Na_2SO_4, and concentrated. Column chromatography (1:10 ethyl acetate-hexane) of the residue on silica gel gave **8** (158 mg, 95%); $[\alpha]_D$ + 14.0° (*c* 0.9, $CHCl_3$); IR (film): 3075−2850 (CH), 1750 (methyl ester), 1740 and 1240 (benzoate), and 710 (phenyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, $2CH_3$), 1.23 (s, 52H, $26CH_2$), 2.50 (m, 2H, CH_2COOCH_3), 2.86 (m, 1H, H-3), 3.26 (dd, 1H, OCH_2CH), 3.44 (s, 3H, CH_2COOCH_3), 4.23 (m, 1H, H-5), 4.36 (t, 1H, J_{gem} = 11.2 Hz, H-6a), 4.53 (dd, 1H, H-6b), 4.66 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 5.53 (dd, 1H, $J_{2,3}$ = 11.5 Hz, H-2), 5.72 (d, 1H, H-4), 7.27−8.30 (m, 15H, 3Ph).

Anal. Calcd for $C_{60}H_{80}O_{10}$: C, 74.35; H, 9.15. Found: C, 74.16; H, 9.10.

2-(Tetradecyl)hexadecyl 3-*C*-(carboxylmethyl)-3-deoxy-β-D-galactopyranoside (III). To a solution of **8** (170 mg, 175 μmol) in methanol (5 mL) were added 3 drops of 28% sodium methoxide in methanol, the reaction mixture was stirred for 24 h at 40°C, then water (a few drops) was added. After stirring for 7 h at rt, the mixture was treated with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with methanol. The combined filtrate and washings were concentrated to a residue, which was chromatographed on a column of Sephadex LH-20 (5:4:0.7 chloroform-methanol-water) to give the title compound **III** (95 mg, 85%); $[\alpha]_D$ + 61° (*c* 1.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.80 (t, 6H, $2CH_3$), 1.19 (s, 52H, $26CH_2$), 1.49 (m, 1H, CH), 3.24 (t, 1H, $J_{1,2} = J_{2,3}$ = 7.7 Hz, H-2), 3.29, 3.60 (2dd, 2H, J_{gem} = 9.6, J_{vic} = 6.0 Hz, OCH_2 CH), 4.12 (d, 1H, H-1).

Anal. Calcd for $C_{38}H_{74}O_7$: C, 70.98; H, 11.60. Found: C, 70.92; H, 11.49.

Biological Assay. In vitro inhibitory activity: The activity of the compounds in vitro was measured in adhesion assays as the inhibition of binding promyelocytic leukemia HL-60 cells to immobilized recombinant selectin-IgG proteins. Briefly, P-selectin-IgG was immobilized onto microtiter plate wells (96 wells; Nunc Maxisorp) by adding 20 ng of the purified protein to each well in a final volume of 100 μL PBS (+), and incubated overnight at 4°C. The excess coating solution was removed by aspirating, and non-specific binding sites were blocked by a 1 h incubation with PBS (+) containing 1% BSA (w/v) at rt. After aspirating the blocking solution, 100 μL of the test compound was dissolved in RPMI 1640, then 100 μL of HL-60 cells (10^6 cell/mL suspended in the binding buffer) were added to each well. The plate was centrifuged at 500 rpm for 2 min at rt and the wells were carefully filled with the binding buffer. The plate was sealed with acetate sealing tape, without trapping any air bubbles. Non-adherent HL-60 cells were removed by inverting the plate, centrifuging at 500 rpm for 10 min, removing the acetate film, and aspirating the binding buffer. The amount of bound cells was quantified by the WST-1 assay method (Dojin Chemicals, Japan). Inhibition of L-or E-selectin binding was carried out as described above using immobilized L-(100 ng) or E-selectin-IgG (40 ng).

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