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Synthesis of sulfated alkyl malto- and laminara-oligosaccharides with potent inhibitory effects on AIDS virus infection

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Abstract

A series of sulfated alkyl oligosaccharides, including a sulfated dodecyl laminarapentaoside and a sulfated octadecyl maltohexaoside with potent anti-human immunodeficiency virus (HIV) activity, has been synthesized. An alkyl oligosaccharide in which a long alkyl group is bonded to the reducing end of the oligosaccharide was first synthesized in high yield. Peracetylated oligosaccharides reacted with such aliphatic alcohols as 1-decyl and 1-dodecyl alcohols with Lewis acids as catalysts. As in the glycosylation of the α and β peracetylated glycosides, the β anomer reacted exclusively, the acetylation was carried out with a sodium acetate–acetic anhydride at high temperatures to maximize the proportion of the β anomer.

1. Introduction

In the search of drugs for acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV), [1], a sulfated dextran (dextran sulfate) was found to have anti-HIV activity [2,3]. Since we have investigated the synthesis of sulfated synthetic polysaccharides with high anticoagulant activities [4], the anti-HIV activities of these sulfated polysaccharides were examined [5,6]. The

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highly anticoagulant sulfated polysaccharides showed anti-HIV activities in vitro. Since AIDS drugs must be administered into the bloodstream where HIVs are located the anticoagulant activity of sulfated polysaccharides was assumed to be a significant side effect when used as an AIDS drug. It was recently reported that administration of dextran sulfate into the bloodstream of HIV carriers caused serious side effects, probably originating from the anticoagulant activity [7].

We successfully synthesized both lentinan and curdlan sulfates with high anti-HIV activity but with low anticoagulant activities [8,9]. Phase I/II tests for the curdlan sulfate have been underway since the end of 1992.

As with the interaction of heparin and antithrombin III in relation to the anticoagulant activity [10], the mechanism of action of sulfated polysaccharides is assumed to originate from the interaction between the negatively charged oligosaccharide portion and the positively charged protein portion [11]. However, it is known that the negatively charged oligosaccharide portion of heparin alone cannot cause the anticoagulant activity [12]. Thus, when medium-molecular-weight sulfated alkyl oligosaccharides having structures and properties resembling surface-active agents were prepared, these compounds were found to exhibit high anti-HIV activities, almost equivalent to those of high-molecular-weight curdlan sulfates [13].

In this study, we report the synthesis of sulfated alkyl oligosaccharides in which the long alkyl group is bonded to the reducing end of the sulfated oligosaccharides, starting from the oligosaccharides. In addition, high anti-HIV activities of sulfated alkyl malto-oligosaccharides and laminara-oligosaccharides are reported.

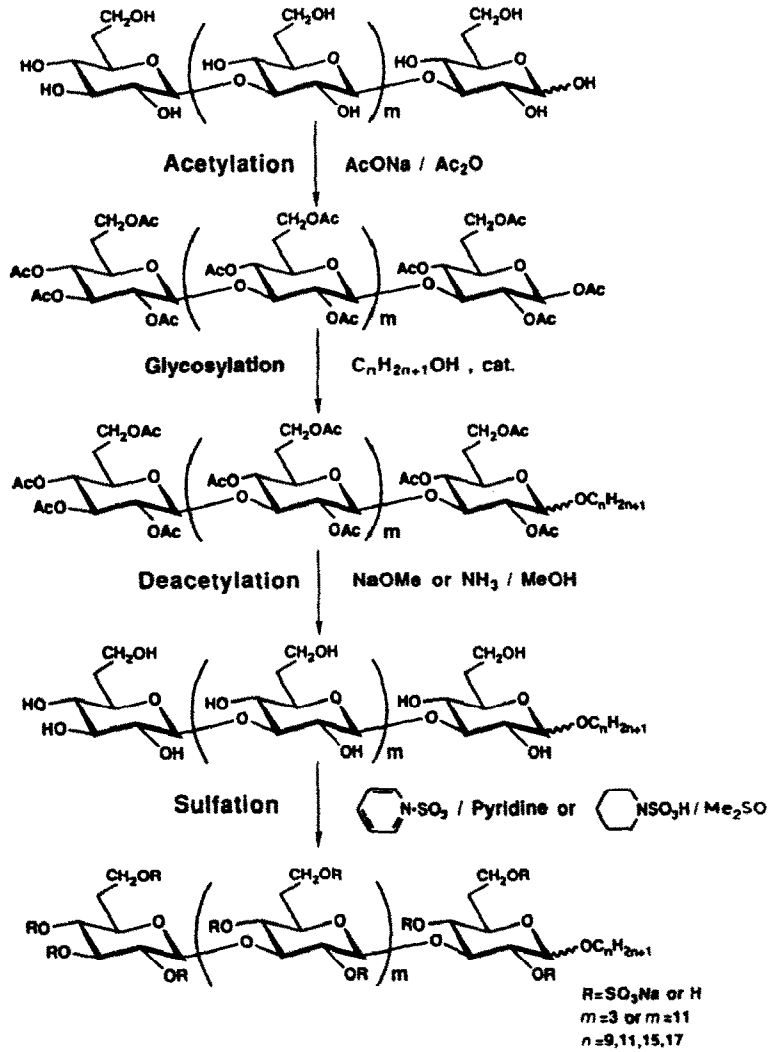
2. Results and discussion

Acetylation of oligosaccharides.—A series of reactions depicted in Scheme 1 were performed to prepare sulfated alkyl oligosaccharides.

Oligosaccharides were acetylated with acetic anhydride and alkali metal acetates to maximize [14] the proportion of β-oligosaccharide peracetate. In the following glycosylation step of the oligosaccharide peracetate, only the β-glycoside reacted. Results of the acetylation of maltohexaose, maltoheptaose, and laminarapentaose are shown in Table 1.

Acetylation of maltoheptaose with sodium acetate gave a β/α ratio in the range of 2.4–4.8, but potassium acetate gave the highest β/α ratio (6.4). As the α anomer does not isomerize in a boiling mixture of acetic anhydride and sodium acetate, it appears that the β/α ratio depends on the kind of alkali metals and it is determined before the hydroxyl group at the reducing end is acetylated.

Glycosylation of oligosaccharides with alcohols.—For the glycosylation of monosaccharides, where main-chain scission is not an issue, acids and acidic ion-change resins are generally used [15]. As the main-chain acetal linkages of oligosaccharides are cleaved by acidic catalysts, the glycosylation of the oligosaccharide peracetate was carried out with a mild Lewis acid as catalyst. The results of the glycosylation of malto-oligosaccharides with ferric chloride as catalyst are shown in Table 2.



Scheme 1. Synthetic route to sulfated laminara-oligosaccharides.

When such aliphatic alcohols as dodecyl, tetradecyl, and octadecyl alcohols were used for the glycosylation of malto-hexaoside and malto-heptaoside peracetates at 45°C, the respective alkyl oligosaccharide peracetates were obtained in 31–66% yields. The latter oligosaccharide gave higher yields than the former. However, no reaction occurred with niobium pentafluoride and tantalum pentachloride as catalysts (Table 2). All of the unreacted maltohexaoside peracetate was the α-peracetate, revealing that exclusively the β anomer reacts under the conditions used. In addition, almost no detectable degradation of the oligosaccharide occurred.

Table 1
Acetylation of oligosaccharides [a]

No.	Sugar [b]		Catalyst		Temperature (°C)	Time (h)	Yield [c]		β/α [d]
	Type	Weight (g)	Type	Weight (g)			(g)	(%)	
1	M6	0.20	NaOAc	0.20	140	1	0.35	95	4.2
2	M6	0.20	KOAc	0.20	140	1	0.35	95	5.6
3	M7	0.20	NaOAc	0.20	60	168	0.31	84	2.3
4	M7	0.20	NaOAc	0.20	90	24	0.34	93	2.9
5	M7	0.20	NaOAc	0.20	140	1	0.36	98	3.9
6	M7	0.10	NaOAc	0.10	170	0.4	0.18	98	4.8
7	M7	0.20	KOAc	0.20	140	1	0.36	98	6.4
8	M7	0.10	RbOAc	0.14	140	1	0.17	92	6.1
9	M7	0.10	CsOAc	0.19	140	1	0.18	98	5.0
10	L5	0.20	NaOAc	0.20	140	1	0.36	98	3.0
11	L5	0.20	KOAc	0.20	140	1	0.36	98	3.8

[a] Acetic anhydride (10 mL/100 mg sugar). [b] M6, maltohexaose; M7, maltoheptasose; L5, laminarapentaose. [c] α and β mixture. [d] Determined by ^{1}H NMR.

For the glycosylation of laminara-oligosaccharide peracetates, the results are summarized in Table 3. When the laminarapentaoside peracetates were reacted with such 1-alkyl alcohols as 1-decyl, 1-dodecyl, and 1-hexadecyl alcohols with tin tetrachloride catalyst at room temperature, the corresponding 1-alkyl laminarapentaoside peracetates were obtained in 47–56% yields. Oligosaccharide derivatives of lower molecular weight were detected by TLC, and thus some degradation of the

Table 2
Glycosylation of malto-oligosaccharides with alcohols [a]

No.	Acetylated malto-oligosaccharide [b]		1-Alkanol		Catalyst		Yield [c]
	glucose unit	g (mmol)	Carbon number of alkyl chain	g (equiv)	Type	mol% to sugar	
1	M6	1.01 (0.55)	12	0.10 (1.0)	$FeCl_3$	35	39
2	M6	1.20 (0.66) [d]	14	0.21 (1.5)	$FeCl_3$	33	31
3	M6	1.01 (0.55)	18	0.30 (2.0)	$FeCl_3$	42	36
4	M7	1.00 (0.47)	12	0.09 (1.0)	$FeCl_3$	30	37
5	M7	1.20 (0.57) [d]	14	0.18 (1.5)	$FeCl_3$	39	42
6	M7	1.20 (0.57)	18	0.30 (2.0)	$FeCl_3$	40	40
7 [e]	M7	0.40 (0.19)	18	0.11 (2.0)	$FeCl_3$	50	63
8 [e]	M7	0.48 (0.23)	18	0.10 (1.6)	$FeCl_3$	80	66
9 [e]	M7	0.20 (0.09)	18	0.05 (2.0)	NbF_5	66	trace
10 [e]	M7	0.20 (0.09)	18	0.05 (2.0)	$TaCl_5$	78	trace

[a] Stirred for 4 h in toluene at 45°C. [b] M6, Maltohexaose M7 Maltoheptaose. [c] After purification by column chromatography. [d] Containing 4A molecular sieves. [e] Under vacuum.

oligosaccharides occurred. Phosphotungstic acid may be used as catalyst, affording somewhat lower yields (23–43%), with a greater extent of degradation than with tin tetrachloride.

The glycosylation of oligosaccharides was carried out under high vacuum using thoroughly dried reagents to ascertain the effects of water and oxygen on the reaction. The results are shown for the maltoheptaoside (Nos. 7–10 in Table 2) and the laminarapentaoside (Nos. 9–11 in Table 3). Comparing No. 6 (atmospheric) with No. 8 (vacuum) for the former saccharide reveals an increase in the yield of the alkyl maltoheptaoside peracetate from 40 to 66%, but with the laminarapentaoside, there was almost no improvement in yield. The crude products obtained under vacuum were less discolored than those obtained under atmospheric conditions, probably attributable to the oxygen-free conditions employed. The β/α ratios of almost all alkyl oligosaccharide peracetates were in the range 3.0–4.6. The alkyl oligosaccharide peracetates were deacetylated to give the OH-free alkyl oligosaccharides in high yields.

Sulfation of alkyl oligosaccharides.—The alkyl oligosaccharides were sulfated with piperidine-*N*-sulfonic acid in dimethyl sulfoxide or with sulfur trioxide–pyridine complex in pyridine. The results are summarized in Table 4.

The NMR spectra described in the experimental section were obtained from the crude sulfated 1-dodecyl laminarapentaoside. The data indicate the homogeneity of the product (aside from differences in the sulfate-group position) because no anomeric-proton peak originating from a cleaved product was detected. Thus, the sulfation by these methods did not cleave the linkage between the sugar units and the glycosidic bond.

Anti-HIV activity and anticoagulant activity.—The anti-HIV activity was assayed using MTT, the MT-4 cell line, and HIV-$1_{HTLV\text{-}III}$ as described elsewhere [16]. The anti-HIV activities of the sulfated alkyl oligosaccharides are summarized in Table 5.

The anti-HIV activity is denoted as EC_{50}, which is defined as the concentration of compound required to decrease HIV-induced cytopathic effects by 50%. Although the anti-HIV activity of free sulfated oligosaccharides was low, with EC_{50} values in the range of 80–630 μg/mL, such sulfated alkyl oligosaccharides as sulfated octadecyl maltoheptaoside, and sulfated dodecyl laminarapentaoside had very high activities, showing EC_{50} values of 0.5, and 0.2 μg/mL, respectively. These high activities were equivalent to the high anti-HIV activity demonstrated by curdlan sulfate [9,13], namely $EC_{50} = 0.4$ μg/mL. The sulfated alkyl oligosaccharides that have long alkyl groups at the reducing end exhibited tens to hundreds times higher anti-HIV activities than those of the corresponding sulfated oligosaccharides without alkyl groups. The increase in activity may be attributable to formation of the same type of structure as that of surface-active agents.

As the anticoagulant activity of sulfated polysaccharides usually depends on the polymer structure [4], the anticoagulant activity of the sulfated alkyl oligosaccharide was measured. Sulfated dodecyl laminarapentaoside and sulfated dodecyl laminara-oligosaccharides ($\overline{dp}_n = 11$) exhibited very low anticoagulant activities of < 1 and 4 units mg, respectively.

Table 3
Glycosylation of peracetylated laminara-oligosaccharide with alcohols

No.	Peracetylated laminara-oligosaccharide			Catalyst		Alcohol		Solvent		Temperature (°C)	Time (h)	Yield [a] (%)
	Type	g	(mmol)	Type	(mol%) [b]	Carbon number	(equiv) [c]	Type	mL			
1	L5	0.50	(0.32)	$SnCl_4$	(100)	10	(1.5)	CH_2Cl_2	20	rt	16	55
2	L5	0.50	(0.32)	$SnCl_4$	(100)	12	(1.5)	CH_2Cl_2	30	rt	4	47
3	L5	0.50	(0.32)	$SnCl_4$	(100)	12	(1.7)	CH_2Cl_2	30	rt	21	56
4	L5	0.50	(0.32)	$SnCl_4$	(100)	16	(2.0)	CH_2Cl_2	30	rt	4.5	53
5	L5	0.15	(0.15)	$SnCl_4$	(200)	18	(2.0)	CH_2Cl_2	4	40	3	49
6	L5	0.50	(0.32)	WPA [d]	(2)	12	(1.7)	PhME	20	95	17	23
7	L5	0.50	(0.32)	WPA [d]	(2)	10	(1.6)	PhME	20	95	7	43
8	LO [e]	0.50	(0.14)	$SnCl_4$	(100)	12	(2.0)	CH_2Cl_2	30	rt	5	80
9 [f]	L5	0.14	(0.10)	$SnCl_4$	(100)	18	(2.0)	CH_2Cl_2	4	40	3	32
10 [f]	L5	0.15	(0.30)	$SnCl_4$	(300)	18	(2.0)	CH_2Cl_2	5	rt	4	40
11 [f]	L5	0.30	(0.19)	$SnCl_4$	(200)	18	(2.0)	CH_2Cl_2	5	rt	20	53

[a] After purification by column chromatography. [b] Equivalent to sugar. [c] Equivalent to sugar. [d] Phosphotungstic acid. [e] Laminara-oligomer; mean number of sugar unit, 11, determined by HPLC. [f] Under vacuum.

Table 4
Sulfation of alkyl oligosaccharides

Sample	Alkyl oligosaccharide			Sulfating agent [a]	Sulfated alkyl-oligosaccharide		Elemental analysis (%)				
	Number of glucose units	Carbon number of alkyl chain	Weight (g)		Yield (mg)	ds [b]	C found and (calcd)	H	N	S	Na
Maltooligosaccharide											
M5C0S	5		0.06	PS	0.24						
M6C0S	6		0.10	PS	0.15	2.3	16.0(14.3)	3.5(1.4)	0(0)	16.7(21.2)	12.0(15.2)
M7C0S	7		0.10	PS	0.26	2.8	13.4(14.4)	3.3(1.4)	0(0)	17.2(21.1)	15.2(15.1)
M5C12S	5	12	0.08	PS	0.16	3.1	16.1(19.2)	3.1(2.3)	0(0)	15.9(19.5)	
M6C12S	6	12	0.10	PS	0.21	2.5	17.4(18.6)	3.9(2.2)	0(0)	14.7(19.7)	12.1(14.1)
M6C14S	6	14	0.09	PS	0.13	2.3	20.1(19.2)	4.4(2.3)	0(0)	14.7(19.5)	10.6(14.0)
M6C18S	6	18	0.10	PS	0.14	2.6	21.4(20.4)	4.4(2.5)	0(0)	16.4(19.2)	11.7(13.7)
M7C12S	7	12	0.10	PS	0.18	2.4	18.3(18.2)	4.0(2.1)	0(0)	14.9(19.8)	12.5(14.2)
M7C14S	7	14	0.10	PS	0.18	2.3	18.8(18.7)	3.9(2.2)	0(0)	14.4(19.6)	12.1(14.1)
M7C14S	7	14	0.10	SO_3–Pyr	0.10	0.3	37.5(18.7)	6.6(2.2)	0(0)	3.8(19.6)	3.3(14.1)
M7C18S	7	18	0.10	PS	0.19	2.3	21.3(19.7)	4.7(2.4)	0(0)	15.2(19.3)	10.5(13.9)
Laminaraoligosaccharide											
L5C0S	5		0.15	PS	0.32						
LOC0S	11 [c]		0.10	PS	0.30	3.1	10.5(17.2)	1.8(1.9)	0(0)	14.6(20.0)	17.5(14.4)
L5C10S	5	10	0.07	PS	0.19						
L5C12S	5	12	0.10	PS	0.19	3.0	17.2(19.2)	3.1(2.3)	0(0)	16.2(19.5)	
L5C12S	5	12	0.10	SO_3–Pyr	0.25	3.0	17.8(19.2)	3.0(2.3)	0(0)	17.0(19.5)	12.2(14.0)
L5C16S	5	16	0.10	PS	0.25						
LOC12S	11 [c]	12	0.10	SO_3–Pyr	0.25	2.2	16.5(17.2)	3.3(4.9)	0(0)	15.8(20.0)	11.4(14.4)

[a] PS: Piperidine-*N*-sulfonic acid 3 equiv at 85°C for 80 min in dry Me_2SO, SO_3–Pyr: sulfur trioxide–pyridine complex (2.2 equiv) at 80°C for 90 min in dry pyridine. [b] Degree of sulfation (ds) designates the number of sulfate groups per glucose residue. [c] Determined by HPLC.

Table 5
The anti-HIV activity of sulfated alkyl oligosaccharides and oligosaccharides

Samples	Sulfated alkyl oligosaccharide		Anti-HIV activity [a] EC_{50} (μg/ml)	Cytotoxic effect [b] CC_{50} (μg/ml)	SI [c] (CC_{50}/EC_{50})
	Number of glucose units	Carbon number of alkyl chain			
M6C0S [e]	6	0	207	> 1000	> 5
M6C12S [e]	6	12	13	770	59
M6C14S [e]	6	14	9.8	810	82
M6C18S [e]	6	18	1.0	820	820
M7C0S [e]	7	0	80	> 1000	> 13
M7C12S [e]	7	12	10	820	79
M7C14S [e]	7	14	9.2	820	89
M7C18S [e]	7	18	0.5	810	1600
L5C0S [f]	5	0	160	> 1000	> 4
L5C10S [f]	5	10	46	> 1000	> 15
L5C12S [f]	5	12	0.2	> 1000	> 5000
LOC0S [f]	11 [d]	0	630	> 1000	> 1
LOC12S [f,g]	11 [d]	12	0.1	> 2000	> 22000
Curdlan sulfate [h]		0	0.4	> 1000	> 2300
Dextran sulfate		0	7.9	> 1000	> 126

[a] Drug concentration effective for 50% inhibition of virus infection in 5 day HIV-infected MT-4 cell culture. [b] Drug concentration for 50% cytotoxicity in 5 days MT-4 cell culture. [c] Selectivity index. [d] Determined by HPLC. [e] Sulfated by piperidine-*N*-sulfonic acid. [f] Sulfated with sulfur trioxide–pyridine complex. [g] Ref 13. [h] Curdlan sulfate with molecular weight of 79×10^3 used for measurement of anti-HIV activity as reference.

In conclusion, sulfated alkyl oligosaccharides composed of 5–11 glucose residues and alkyl groups ranging from 10 to 18 at the reducing-terminal carbon atoms were synthesized. These compounds showed high anti-HIV activities, almost equivalent to those of highly anti-HIV-active sulfated polysaccharides.

3. Experimental

General methods.—NMR spectra were measured on Jeol GX-270 and GSX-400 spectrometers in $CDCl_3$ using Me_4Si or D_2O using sodium 4,4-dimethyl-4-sila-

Table 6
Anticoagulant activity of sulfated alkyl laminara-oligosaccharides

Sample	Number of glucose units	Carbon number of alkyl chain	Anticoagulant activity [a] (unit/mg)
Sulfated dodecyl laminara pentaoside	5	12	0 [b]
Sulfated dodecyl laminara oligosaccharide	11 [c]	12	4

[a] Dextran sulfate with molecular weight of 5.8×10^3 used for measurement of anti-HIV activity as reference (19.3 unit/mg). [b] Below the limit of determination by this method. [c] Determined by HPLC.

pentanoate as the internal standard. Mass spectra were measured on a Shimadzu mass spectrometer GCMS9100-MK using the FD and FAB methods. For column chromatography, silica gel (Kiesel-gel 60, 70–230 mesh ASTM, Merck) was used. Malto-oligosaccharides were kindly supplied by Nihon Shokuhin Kako Co., Ltd. Laminaraoligosaccharides ($\overline{dp}_n = 11$) and laminarapentaose were produced enzymically by using enzymes obtained from a new strain, *Streptomyces sp.* DIC L-108 [17]. Piperidine-*N*-sulfonic acid was prepared according to Nagasawa's method [18]. Sulfur trioxide–pyridine complex (Aldrich Chemical Co.) was used without further purification.

Acetylation.—Acetylation of maltohexaose is described as a representative reaction. To Ac_2O (20 mL) at boiling point (140°C) in a three-necked flask was added 200 mg of NaOAc. Then, 200 mg of maltohexaose was added gradually with vigorous stirring. The solution was kept for 1 h at 140°C, and then cooled to room temperature. After conventional work-up, 350 mg (95%) of peracetylated maltohexaoside was obtained. The acetylation at 170°C was performed in a glass ampoule sealed under vacuum. A suspension of 100 mg of NaOAc in 10 mL of Ac_2O was sealed in a glass ampoule to which a glass graft containing 100 mg of maltoheptaose was connected through a breakable seal. When the temperature of the contents reached 170°C, the two reactants were mixed under vigorous stirring.

Glycosylation of peracetylated malto-oligosaccharide with long chain 1-alkanols.—To a flask containing 10 mL of dry toluene heated at 45°C, 1.01 g of peracetylated maltohexaose and 102 mg of 1-dodecyl alcohol were added. After the mixture became a clear solution, 31 mg of $FeCl_3$ was added. The reaction was kept at 45°C for 4.5 h and then it was diluted with 50 mL of $CHCl_3$ and successively washed with aq $NaHCO_3$ twice and with satd NaCl solution twice. The solution was dried over Na_2SO_4, and then the solvent was evaporated under diminished pressure, the residue was chromatographed on silica gel using 5 : 1–1 : 3 hexane–EtOH as eluent to give an off-white amorphous solid in 39% yield. The glycosylation of other malto-oligosaccharides was carried out similarly.

Glycosylation of peracetylated laminara-oligosaccharide with 1-alkanols.—Peracetylated laminarapentaose (500 mg) and 90 mg of 1-dodecyl alcohol were added to 30 mL of dry CH_2Cl_2 at room temperature, and then, 96 mg of $SnCl_4$ was added. The mixture was stirred for 5 h, and then diluted with 50 mL of CH_2Cl_2. After conventional work-up, dodecyl laminarapentaoside peracetate was obtained as a white, amorphous solid; yield 0.30 mg (56%); MS: *m/z* 1692 (M + Na); ^{1}H NMR: δ 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1 of 1st sugar), 4.46 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.39 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1), 4.37 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.34 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1); ^{13}C NMR: δ 101.05 (C-1), 100.80 (C-1), 100.74 (C-1), 100.62 (C-1), 100.52 (C-1), 69.92 (OCH_2), 62.28 (C-6), 62.10 (C-6), 62.05 (C-6), 61.93 (C-6), and 61.66 (C-6).

Deacetylation of alkyl oligosaccharide peracetate.—The alkyl oligosaccharide peracetate at room temperature (400 mg) was stirred in MeOH saturated with NH_3 for ~40 h, and then the solvent was removed under diminished pressure. The residue was dissolved in small amount of MeOH, and the product precipitated with acetone, affording the off-white alkyl oligosaccharide glycoside in 75–88%

yield. In an alternative method, NaOMe was used. Peracetylated alkyl oligosaccharide (100 mg) was stirred in MeOH containing 0.2 equiv of NaOMe to the acetyl group at room temperature for 5 h, with subsequent neutralization with an H^+ type ion-exchange resin (Daia Ion SK-1B) to pH 6.0. The colorless alkyl oligosaccharide glycoside was obtained in quantitative yield. 1-Dodecyl laminarapentaoside MS: m/z 1019 (M + Na).

Sulfation of 1-octadecyl maltohexaosides.—1-Octadecyl maltohexaoside (100 mg) was dissolved in Me_2SO at 85°C, and 460 mg (3.0 equiv to hydroxyl groups) of piperidine-*N*-sulfonic acid was added, and the mixture was stirred for 80 min. After cooling at room temperature, the solution was adjusted with satd $Ba(OH)_2$ to pH 8.0 in an ice bath. The $BaSO_4$ was separated by centrifugation, and water was evaporated off at 35°C. The residual Me_2SO solution was passed through an Na^+ type ion-exchange resin column. The crude product was precipitated with acetone, washed with acetone, and freeze-dried from water. The yield of white sulfated 1-octadecyl maltohexaoside was 141 mg.

Sulfation of 1-dodecyl laminarapentaoside.—1-Dodecyl laminarapentaoside (100 mg) was dissolved in pyridine at 85°C, and 340 mg (2.2 equiv to hydroxyl groups) of SO_3–pyridine complex was added, with subsequent stirring for 90 min. The work-up procedure was the same as that for 1-octadecyl maltohexaoside. The precipitate was freeze-dried from water to give 141 mg of an off-white product; ^1H NMR (D_2O): δ 5.26 (d, 0.14 H, $J_{1,2}$ 3.6 Hz, H-1 of α anomer), 5.19 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 5.16 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 5.14 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 5.13 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 4.86 (d, 0.86 H, $J_{1,2}$ 5.2 Hz, H-1 of β anomer); ^{13}C NMR δ 102.7 (C-1 of 1st sugar), 102.2 (C-1 of 2nd sugar), 101.9 (C-1 of 3rd and 4th sugar), 101.5 (C-1 of 5th sugar), 71.1, 70.8, 70.8, 70.6 (2 C, C-6 of each sugar), and 73.3 ($-OCH_2-$).

Anti-HIV assay.—The anti-HIV activity of a series of sulfated alkyl oligosaccharides in fresh cell-free HIV infection was determined by the protection from HIV-induced cytopathic effects (CPE). MT-4 cells were infected with HTLV-IIIB at the multiplicity of infection (MOI) of 0.01. HIV- or mock-infected MT-4 cells (1.5×10^5 cells/mL, 200 mL) were incubated in the presence of various concentrations of the test compounds. The cell viability was quantified by a colorimetric assay which monitors the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue-colored formazan product according to Pauwels et al. [16] The absorbances were determined in a microcomputer-controlled photometer (Titertek Multiskan, Labsystem Oy, Helsinki, Finland). These values were translated into percentage cytotoxcity and percentage antiviral protection, from which 50% cytotoxic concentration (CC_{50}), 50% effective concentration (EC_{50}) which denotes the drug concentration for 50% protection of HIV-induced cytopathic effects, and selectivity index ($SI = CC_{50}/EC_{50}$) were calculated.

Anticoagulant activity.—Anticoagulant activity was measured according to a modification of the United States Pharmacopoeia using bovine plasma. Dextran sulfate with a molecular weight of 5800 was used as a reference sample (19.3 units/mg).

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