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Synthesis of sulfated oligosaccharide glycosides having high anti-HIV activity and the relationship between activity and chemical structure

Kaname Katsuraya [a], Hideki Nakashima [b], Naoki Yamamoto [c], Toshiyuki Uryu [a,*]

[a] *Institute of Industrial Science, University of Tokyo, 7-22-1 Roppongi, Minato-ku, Tokyo 106, Japan*
[b] *Kagoshima University, Dental School, 35-1 Sakuragaoka, Kagoshima City, Kagoshima 890, Japan*
[c] *Tokyo Medical and Dental University, School of Medicine, Yushima, Bunkyo-ku, Tokyo 113, Japan*

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Abstract

Sulfated laminara-oligosaccharide glycosides having high anti-human immunodeficiency virus (HIV) activities were synthesized from laminara-tetraose, -pentaose and -hexaose. The oligosaccharide glycosides were synthesized by treating peracetylated β-oligosaccharides with various alcohols and Lewis acid catalysts. The effects of the number of glucose residues and the alkyl chain-length on anti-HIV activity were examined. The anti-HIV activity of sulfated dodecyl laminara-pentaosides and -hexaosides increased with increasing degree of sulfation (DS) and the pentaoside having an almost fully-sulfated saccharide portion had the highest activity, whereas for the hexaoside a somewhat lower DS manifested the highest activity. Sulfated laminara-oligosaccharide glycosides having fluoroalkyl-containing aglycons of high hydrophobicity showed potent inhibitory effects against HIV infection. In contrast, hydrophilic substituents containing oligo(ethyleneoxy) groups as aglycons in the sulfated oligosaccharides did not show high anti-HIV activity.

Keywords: Laminara-oligosaccharides; Sulfated sugars; Anti-HIV activity; Hydrophobicity; Oligosaccharide glycosides

1. Introduction

Among the successors to the first-generation acquired immunodeficiency syndrome (AIDS) drugs [azidothymidine (AZT), dideoxyinosine (DDI) and dideoxycytosine (DDC)] [1–3], several protease inhibitors have shown clinical promise in curing AIDS patients [4]. However, as the use of azidothymidine led to the appearance of drug-resistant viruses [5], it is of concern that protease inhibitor-resistant viruses may likewise appear [6].

De Clercq [7] anticipated that such polyanionic compounds as dextran sulfate and poly(-methacrylic acid) might be possible targets for anti-HIV drugs, and in fact, dextran sulfate [(1→6)-α-glucan] shows anti-HIV activity [8,9]. It has been demonstrated that sulfated synthetic polysaccharides having high anticoagulant activities [10] also possess anti-HIV activities [11].

As with the interaction of the natural anticoagulant polysaccharide heparin with antithrombin III [12], the mechanism of action of sulfated polysaccharides is supposed to originate from the interaction between negatively charged oligosaccharide portions and positively charged portions of the virus envel-

* Corresponding author. Fax: +81-3-34038557.

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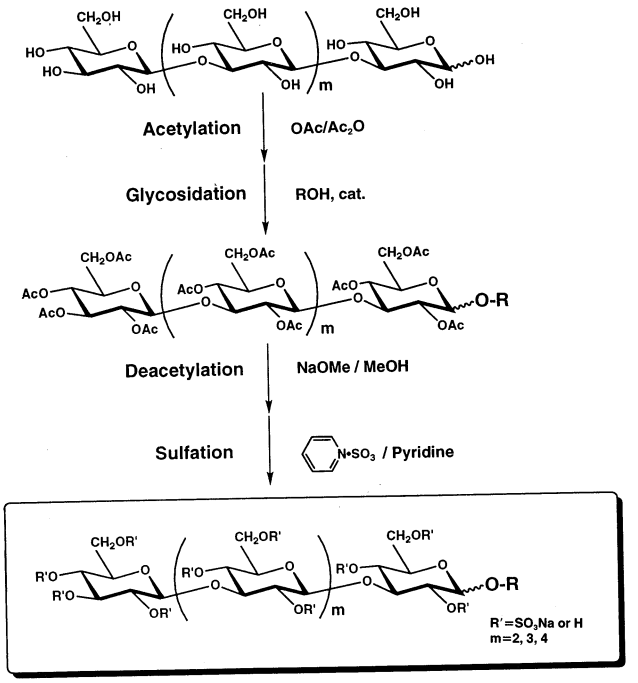
ope protein [13]. Lentinan [a branched (1→3)-β-glucan] sulfate [14,15] and curdlan [(1→3)-β-glucan] sulfate showing potent anti-HIV activity, but very low anticoagulant activity, have been synthesized [16,17]. Curdlan sulfate also showed low toxicity. A phase I/II test of curdlan sulfate was carried out during a 1-year period from December 1992 in the US, and Gordon and co-workers published the results of these tests [18]. Blood anticoagulation effects were not observed upon injecting up to 200 mg of curdlan sulfate per person. It was also reported that there was a large increase in the number of CD4 cells in human blood after injection of curdlan sulfate.

We next synthesized sulfated alkyl oligosaccharide glycosides of high anti-HIV activity having long-chain alkyl groups as the aglycon in the sulfated oligosaccharides [19]. Synthesis and purification of laminara-oligosaccharides containing more than five glucosidic residues has already been reported [20]. It was found that sulfated alkyl laminara-oligosaccharides (pentaose to nonaose) and alkyl malto-oligosaccharides (tetraose to heptaose) showed anti-HIV activities comparable to the very high activity of curdlan sulfate [20–22]. It is suggested that the anti-HIV activity of these sulfated alkyl oligosaccharide glycosides depends to a large extent both on the number of sulfate anions of the oligosaccharide portion and on the length and chemical structure of the alkyl chain.

Here, we have synthesized sulfated alkyl oligosaccharide glycosides having 4–6 glucosidic residues showing high anti-HIV activity. The influence of alkyl chain-length and structure on biological activity was examined to elucidate the mechanism of action of these compounds as AIDS drugs. The effects of degree of sulfation (DS) in sulfated dodecyl laminara-pentaoside and -hexaoside on anti-HIV activity were also evaluated.

2. Results and discussion

Synthesis of sulfated laminara-oligosaccharide glycosides.—Sulfated laminara-oligosaccharide glycosides were prepared according to the route shown in Scheme 1.



Scheme 1. Synthetic route for sulfated laminara-oligosaccharide glycosides.

Laminara-oligosaccharides were acetylated with potassium acetate–acetic anhydride to maximize the yield of peracetylated β-oligosaccharides. Laminara-oligosaccharides (tetraose through hexaose) were individually acetylated to afford the respective peracetylated laminara-oligosaccharides having β:α ratios of 3.2 to 3.6 in high (89–98%) yields.

To examine the effect of alkyl chain-length, the peracetylated laminara-tetraoside and -pentaosides were treated with linear alcohols of various chain-lengths and stannic chloride as a Lewis acid catalyst. The results of this glycosidation are summarized in Table 1.

The yields were in the range of 40–70%, with no clear dependence on the number of sugar residues or the alkyl chain-length. The α:β ratios for representative glycosides were in the range 1:5–1:10. The α- and β-alkyl laminara-oligosaccharide glycosides were not separated, because there was no significant difference in the anti-HIV activity between the final sulfated alkyl α- and β-laminara-oligosaccharide glycosides. These glycosides were conventionally deacetylated in almost quantitative yields. Sulfation was effected by using 2.2–2.5 equiv of sulfur trioxide–pyridine complex in dry pyridine per hydroxyl group.

Table 1
Synthesis of peracetylated alkyl laminara-tetraoside and -pentaoside with stannic chloride catalyst

Sample	Peracetylated laminara-saccharide		Alkanol		$SnCl_4$ (mol%)[a]	Yield (%)	$[\alpha]_D^{20}$ (°)[b]
	mg	(mmol)	Carbon number	(Equiv)[a]			
Tetraoside							
L4C4	200	(0.16)	4	(2.0)	200	45	−33.6
L4C6	200	(0.16)	6	(2.0)	200	56	−37.6
L4C8	200	(0.16)	8	(2.0)	200	55	−33.1
L4C10	200	(0.16)	10	(2.0)	200	45	−35.3
L4C12	200	(0.16)	12	(2.0)	200	54	−35.3
Pentaoside							
L5C4	300	(0.19)	4	(2.0)	200	40	−56.7
L5C6	250	(0.16)	6	(2.0)	200	63	−57.0
L5C8	300	(0.19)	8	(2.0)	200	70	−58.7
L5C10	190	(0.12)	10	(2.0)	200	56	−55.4
L5C12	500	(0.32)	12	(2.0)	100	53	−52.9

[a] Equivalent to acetylated oligosaccharide.
[b] Measured in $CHCl_3$ (*c* 1.0).

To examine the effects of degree of sulfation on anti-HIV activity, dodecyl laminara-pentaosides and -hexaosides having different degrees of sulfation were prepared, and the results are shown in Table 2. For all pentaosides and hexaosides, the degree of sulfation was controlled by changing the ratio of SO_3–pyridine complex in the reagents, giving sulfated oligosaccharides having 0.7–3.0 sulfate groups per glucose residue.

NMR analysis of the sulfate group positions revealed that the 6-hydroxyl groups were the most reactive, followed by the 2- and the 3-hydroxyl groups at the nonreducing end. The 4-hydroxyl groups were sulfated only at the final stage of sulfation, probably because of steric hindrance.

Next, the peracetylated laminara-pentaoside was allowed to react with various alcohols using Lewis acid catalysts in dichloromethane or 1,2-dichloroethane. The results of these glycosidations are summarized in Table 3. Peracetylated laminara-pentaosides glycosidated by various aliphatic alcohols were obtained in 50–68% yields, except for the derivatives with cholesterol and L-menthol, whose hydroxyl groups were of low reactivity. Also, highly hydrophilic or hydrophobic alcohols, such as those containing oligo(ethyleneoxy) or fluoroalkyl moieties, were formed in only low yields. For the fluoro compounds, the use of perfluoro-*m*-xylene as solvent gave almost the same yield as with dichloromethane. After deacetylation, the oligosaccharide glycosides were sulfated with 2.2 equiv of sulfur trioxide–pyridine complex per hydroxyl group to afford highly sulfated compounds.

Effects of number of glucose residues on anti-HIV activity.—Since it has been found that oligosaccharides higher than pentaosides give highly anti-HIV active compounds [20–22], we tried to synthesize active tetraoside derivatives by changing the alkyl chain from *n*-butyl to *n*-dodecyl. Table 4 shows the anti-HIV activity of the sulfated alkyl laminara-tetraoside in comparison with that of the pentaoside homologs.

The sulfated butyl tetraoside L4C4S exhibited low anti-HIV activity (EC_{50} of 43 μg/mL). The activity increased with increasing alkyl chain length, reaching an EC_{50} of 5.8 μg/mL for the dodecyl tetraoside L4C12S. The EC_{50} of 43 μg/mL for the butyl tetraoside was about one-tenth of that for the butyl pentaoside (3.4 μg/mL). The activity (5.8 μg/mL) of the dodecyl tetraoside was only one-thirtieth of that of the highly active dodecyl pentaoside (0.18 μg/mL). It is therefore assumed that, in a series of sulfated alkyl oligosaccharides, the sulfated pentasaccharide had stronger interactions with the HIV protein gp120 than the sulfated tetrasaccharide. For

Table 2
Sulfation of alkyl laminara-oligosaccharide glycosides with different equivalents of sulfur trioxide–pyridine complex

Dodecyl laminara-oligosaccharide glycoside (mg)	Sulfur trioxide–pyridine complex (mg (equiv))	Sulfated dodecyl laminara-pentaoside						
		Yield (mg (%))	DS[a]	Elem anal. (Found)				
				%C	%H	%N	%S	%Na
Pentaosides								
100	76 (0.3)	60 (44)	0.7	35.7	5.9	0	7.9	4.0
100	125 (0.5)	110 (66)	1.3	28.9	4.9	0	12.3	7.2
100	125 (0.5)	88 (51)	1.4	28.0	4.9	0	12.4	6.9
100	178 (0.7)	73 (53)	1.5	26.9	4.7	0	13.0	6.9
100	254 (1.0)	195 (94)	2.1	23.7	3.6	0	15.6	9.7
100	254 (1.0)	200 (90)	2.4	21.8	3.4	0	16.4	11.1
200	1260 (2.5)	440 (90)	2.8	16.8	3.5	0	16.9	13.7
Hexaosides								
90	95 (0.4)	70 (60)	0.7	33.3	5.3	0	8.3	6.1
50	75 (0.6)	52 (66)	1.1	28.7	4.7	0	10.8	7.9
50	130 (1.0)	90 (87)	2.0	21.6	3.4	0	15.5	12.0
20	166 (2.2)	31 (70)	2.5	13.7	2.9	0	14.2	12.9
90	600 (2.5)	180 (85)	2.6	17.0	3.2	0	17.0	14.1
90	600 (2.5)	210 (94)	2.8	16.1	3.4	0	17.0	13.8

[a] Degree of sulfation (DS) designates the number of sulfate groups per glucose residue.

the natural sulfated polysaccharide heparin and synthetic dextrans, their interacting portions are composed of pentasaccharide sequences [12,23,24]. In addition, it is assumed that the interacting portion of a sulfated polysaccharide to the HIV protein gp120 consists of oligosaccharide segments [25].

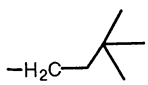
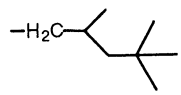
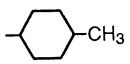
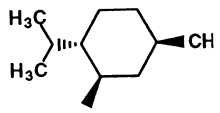
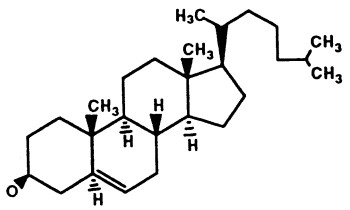
Molecular weights and molecular-weight distributions of the sulfated alkyl oligosaccharides could not be measured by gel-permeation chromatography, because these sulfated alkyl oligosaccharide glycosides behave as surfactants, and may exist in the form of molecular aggregates. The apparent molecular weights of sulfated dodecyl laminara-tetraoside, -pentaoside, and -hexaside, determined in water, were about 30–70 times higher than the calculated molecular weights. However, these compounds showed sharp NMR signals characteristic of compounds of medium molecular weight. Therefore, it is reasonable to assume that for highly sulfated samples, in which no scission of sugar bonds occurred during sulfation and almost all hydroxyl groups were sulfated, there was no significant distribution of molecular weights.

Effects of the degree of sulfation.—Table 5 shows that the anti-HIV activity of sulfated dodecyl laminara-pentaosides and -hexaosides depends to a large extent on the DS. Sulfated dodecyl pentaosides of DS below 0.7 exhibited almost no activity. With DS levels of 1.3–1.5, the activity was low, in the EC_{50} range of 220–860 μg/mL. On the other hand, as the DS increased above 2, the activity increased to a great extent and reached a maximum value of 0.18 μg/mL at DS 2.8. The sulfated dodecyl hexaosides showed a similar tendency. The highest activity was attained at DS 2.5 or above, possibly because they contained a larger number of glucose residues, namely six.

The finding that a sulfated dodecyl pentaoside containing altogether about 15 sulfate groups exhibits high anti-HIV activity might indicate that putative positively charged virus protein interacts strongly with the sulfated oligosaccharide having a similar number of negatively charged sulfate groups, although the protein does not necessarily interact with all negative charges.

Effects of hydrophobicity and branching structure.—Since it was found that the alkyl chain-length (and thus hydrophobicity)

Table 3
Synthesis of peracetylated laminara-pentaoside glycosides using a Lewis acid catalyst

Sample	Laminara-pentaose peracetate (mg)	Alcohol		Catalyst		Yield (%)
		Alkyl portion	Equiv[a]	Type	Equiv[a]	
Alkyloligoethyleneoxy						
L5C2OE6	250	$-(C_2H_4O)_2C_6H_{13}$	2.0	$SnCl_4$	2.0	22
L5C3O2	500	$-(C_2H_4O)_3C_2H_5$	3.5	$BF_3{\cdot}Et_2O$	3.9	36
L5C4O1	500	$-(C_2H_4O)_4CH_3$	2.0	$BF_3{\cdot}Et_2O$	5.0	29
L5C12OH	250	$-C_{12}H_{24}OH$	2.0	$SnCl_4$	2.0	32
Fluoroalkyl						
L5C6F8	300	n-$C_6H_{12}C_8F_{17}$	2.0	$SnCl_4$	2.0	33
Branched and cyclic alkyls						
L5C33DM4	250		2.0	$SnCl_4$	2.0	54
L5C244TM5	250		2.0	$SnCl_4$	2.0	55
L5C4M^{C}H (methylcyclohexyl)	250		2.0	$SnCl_4$	2.0	68
L5Men (menthyl)	308		2.0	$SnCl_4$	2.0	37
L5Chol (cholesteryl)	308		2.0	$SnCl_4$	2.0	43

[a] Equivalent to peracetylated oligosaccharide.

markedly affects the anti-HIV activity, hydrophilic groups (oligoethylenoxy groups) at the reducing end were evaluated. The anti-HIV activity of these compounds is shown, along with results for branched and cyclic alkyl-substituted compounds, in Table 6.

Compounds L5O3C2S and L5O4C1S, which contained tri(ethylenoxy) and tetra(ethylenoxy) aglycons, respectively, exhibited very low activities: $EC_{50} = 110$ and 88 μg/mL. However, with the hexyloxyethylenoxy group, having the shorter ethylenoxy group, the activity (1.8 μg/mL) was a little higher than for the foregoing two compounds. A dodecyl derivative having a terminal sodium sulfate group showed low activity ($EC_{50} = 18$ μg/mL).

On the other hand introduction of the $-C_6H_{12}C_8F_{17}$ group, having a fairly long perfluoroalkyl portion, led to high activity. In fluoro-containing alkyl derivatives, the fluoroalkyl group contributed to the activity with hydrophobicity almost equivalent to that of the alkyl group [26]. The 2-methyl-4,4-dimethylpentyl derivative exhibited high activity, although a 3,3-dimethylbutyl derivative was slightly less active. This comparison of the activity of branched alkyl derivatives with that

Table 4
Anti-HIV activity of sulfated alkyl laminara-tetraosides and -pentaosides[a]

Sample designation	Carbon number of alkyl chain	Anti-HIV activity[b] EC_{50} (μg/mL)	Cytotoxide effect[c] CC_{50} (μg/mL)	SI[d] (CC_{50}/EC_{50})
Tetraosides				
L4C4S	4	43	>1000	>23
L4C6S	6	36	>1000	>28
L4C8S	8	25	>1000	>40
L4C10S	10	9.0	>1000	>110
L4C12S	12	5.8	>1000	>170
Pentaosides				
L5C4S	4	3.4	>1000	>290
L5C6S	6	1.1	>1000	>910
L5C8S	8	1.1	>1000	>910
L5C10S	10	1.1	>1000	>910
L5C12S	12	0.18	>1000	>5600
Curdlan sulfate[e]		0.18	>1000	>5600

[a] All compounds have high DS values (>2.9).
[b] Drug concentration effective for 50% inhibition of virus infection in a 5-day HIV-infected MT-4 cell culture.
[c] Drug concentration for 50% cytotoxicity in a 5-day MT-4 cell culture.
[d] Selectivity index.
[e] Curdlan sulfate with molecular weight of 79×10^3 used for measurement of anti-HIV activity as reference.

of the linear alkyl derivatives showed that the total number of carbon atoms in the alkyl group also plays an important contributory role toward high anti-HIV activity.

Although a compound containing the (small) methylcyclohexyl group showed only low activity, compounds having the bulky L-menthyl and cholesteryl groups displayed high activities.

It was concluded that the sulfated alkyl oligosaccharide glycoside having the highest anti-HIV activity coupled with the lowest cytotoxicity is the fully sulfated dodecyl laminara-pentaoside, and that when a branched alkyl group is used, a little shorter alkyl group is tolerated.

3. Experimental

General.—NMR spectra were measured on Jeol LA-400 and LA-500 spectrometers in $CDCl_3$ or D_2O using Me_4Si and sodium 4,4-dimethyl-4-silapentanoate as the internal standards, respectively. Optical rotations were measured for solutions in H_2O using a Perkin–Elmer 241 polarimeter working with a 1-mL cell. For column chromatography, silica gel (E. Merck's Kieselgel 60, 70–230 mesh ASTM) was used. Sulfur trioxide–pyridine complex (Tokyo Kasei Kogyo Co., Ltd.) was used without further purification. GPC analysis were carried out on a Tosco Series 8000 liquid chromatograph system equipped with packed column of TSKGel G2500PWXL, G3000PWXL, and G4000PWXL in phosphate buffer solution or water.

Acetylation of laminara-oligosaccharides.—To boiling Ac_2O (20 mL) in a three-necked flask, 200 mg of KOAc was added. Then, 200 mg of laminara-pentaose was gradually added under vigorous stirring. The solution was kept for 1 h at 140 °C, and then cooled to room temperature. After conventional isolation, 360 mg (98%) of peracetylated laminara-pentaose was obtained. Other laminara-oligosaccharides were acetylated similarly. All peracetates were purified by column chromatography on silica gel.

Glycosidation of peracetylated laminara-oligosaccharide with n-alkanols.—Percetylated laminara-pentaose (500 mg) and 111 mg of dodecyl alcohol were added to 30 mL of dry 1,2-dichloroethane at 45 °C, followed by the addition of 83 mg of $SnCl_4$. The mixture was stirred for 5 h. After standard processing

work-up, 0.30 g of dodecyl laminara-pentaoside peracetate was obtained in 53% yield. Other glycosides were synthesized similarly.

Deacetylation of alkyl oligosaccharide glycoside peracetates.—A total of 100 mg of alkyl oligosaccharide glycoside peracetate was stirred in MeOH containing 0.3 equiv of NaOMe per acetyl group at room temperature for 5 h, followed by neutralization with H^+ type ion-exchange resin (Daia Ion SK-1B) to pH 6.0–6.5. Evaporation of solvent gave the colorless alkyl oligosaccharide glycoside in quantitative yield.

Sulfation of dodecyl laminara-pentaoside.—A solution containing 100 mg of dodecyl laminara-pentaoside in dry pyridine (5 mL) was heated to 85 °C, and then 340 mg (2.2 equiv to hydroxyl groups) of sulfur trioxide–pyridine complex was added, followed by stirring for 90 min. After cooling, the solution was brought to pH 7.5–8.0 with saturated $Ba(OH)_2$ solution. The precipitated $BaSO_4$ was separated by centrifugation, and the supernatant was passed through a Na^+ type ion-exchange resin column. The crude product was purified by dissolving it in a very small amount of water followed by precipitation from acetone. The aqueous solution of the compound was brought to pH 6.9–7.2 by 0.2 M HCl.

Finally, the product was freeze-dried from water to give 190 mg of off-white powdery sulfated dodecyl laminara-pentaoside; ^{13}C NMR δ 102.7, 102.13, 101.92, 101.92, 101.13 (C-1), 80.00, 79.77, 79.69, 79.69, 79.26 (C-2), 81.73, 81.56, 81.25, 80.31, 79.43 (C-3), 76.93, 76.85, 76.80, 76.05, 75.75 (C-4), 78.56, 78.15, 78.97, 77.41, 76.96 (C-5), 71.25, 70.81, 70.76, 70.58, 70.58 (C-6).

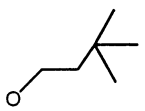
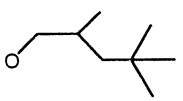
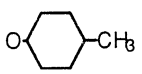
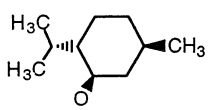
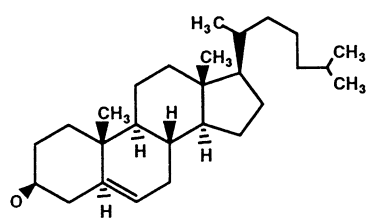
Anti-HIV assay.—The anti-HIV activity of a series of sulfated alkyl oligosaccharide glycosides against HIV infection was determined by protection from HIV-induced cytopathic effects (CPE) [11]. HIV-$1_{HTLV\text{-}IIIB}$ was prepared from the culture supernatant of MOLT-4/HTLV-IIIB cells which were persistently infected with HTLV-IIIB. 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-diphenyl-tetrazolium bromide (MTT) to a blue-colored formazan product according to Pauwels and co-workers [27]. The absorbances were determined in a microcomputer-controlled photometer (Titertek Multiskan, Lab-

Table 5
Anti-HIV activity of sulfated dodecyl laminara-oligosaccharides with different degrees of sulfation

Number of glucose units	DS[a]	Anti-HIV activity[b] EC_{50} (μg/mL)	Cytotoxic effect[c] CC_{50} (μg/mL)
5	0.7	>1000	480
5	1.3	590	>1000
5	1.4	860	>1000
5	1.5	220	>1000
5	2.1	30	>1000
5	2.4	5.0	>1000
5	2.8	0.18	>1000
6	0.7	>1000	890
6	1.1	400	>1000
6	2.0	4.2	>1000
6	2.5	0.18	>1000
6	2.6	0.18	>1000
6	2.8	0.14	>1000
Curdlan sulfate[d]	1.5	0.18	>1000

[a] Degree of sulfation.
[b] Concentration of the drug inhibiting 50% virus infection.
[c] Drug concentration for 50% cytotoxicity.
[d] Curdlan sulfate with molecular weight of 79×10^3 used for measurement of anti-HIV activity as reference.

Table 6
Anti-HIV activity of sulfated alkyl laminara-pentaosides

Sample	Alkyl portion	Carbon number	Anti-HIV activity[a] EC_{50} (μg/mL)	Cytotoxic effect[b] CC_{50}/EC_{50} (μg/mL)	SI[c] (CC_{50}/EC_{50})	DS
Alkyloligoethyleneoxy						
L5C2OE6S	$-(C_2H_4O)_2C_6H_{13}$	$C_{10}O_2$	1.8	>1000	>560	
L5C3O2S	$-(C_2H_4O)_3C_2H_5$	C_8O_3	110	>1000	>9	3.2
L5C4O1S	$-(C_2H_4O)_4CH_3$	C_9O_4	88	>1000	>11	3.0
L5C12OS	$-C_{12}H_{24}OSO_3Na$	C_{12}	18	>1000	>56	3.4
Fluoroalkyl						
L5C6F8S	n-$C_6H_{12}C_8F_{17}$	C_8	0.53	945	1780	3.0
Branched and cyclic alkyls						
L5C33DM4S		C_6	0.78	>1000	>1280	
L5C244TM5S		C_8	0.42	>1000	>2380	
L5C4M^{C}HS (methyl-cyclohexyl)		C_7	0.97	>1000	>1000	3.1
L5MenS (menthyl)		C_{10}	0.60	>1000	>1670	3.2
L5CholS (cholesteryl)		C_{27}	0.24	720	2980	3.2
Curdlan sulfate[d]			0.18	>1000	>5600	1.4

[a] Concentration of the drug inhibiting 50% virus infection.
[b] Drug concentration for 50% cytotoxicity.
[c] Selectivity index.
[d] Curdlan sulfate with molecular weight of 79×10^3 used for measurement of anti-HIV activity as reference.

system Oy, Helsinki, Finland). The activity is represented as EC_{50}, which denotes 50% inhibition of HIV, and cytotoxicity is represented as CC_{50}.

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