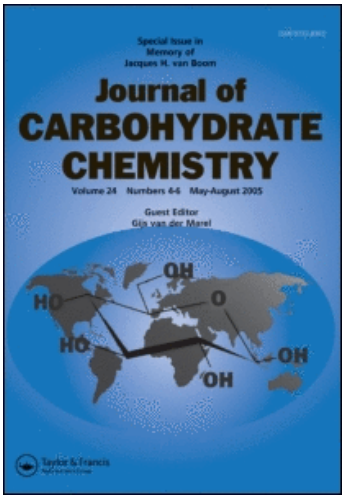

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NMR Investigation of the Influence of Sulfate Groups at C-2 and C-4 on the Conformational Behavior of Fucoidan Fragments with Homo-(1→3)-Linked Backbone#

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NMR Investigation of the Influence of Sulfate Groups at C-2 and C-4 on the Conformational Behavior of Fucoidan Fragments with Homo-(1→3)-Linked Backbone[#]

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The conformational behavior of 2-*O*- and 4-*O*-sulfated derivatives of linear (1 → 3)-linked di-, tri-, and tetrafucosides and 2,3-branched tetrafucoside was studied by means of theoretical molecular modeling and experimental determination of trans-glycosidic vicinal coupling constants $^{3}J_{C,H}$. It was shown that *O*-sulfation of (1 → 3)-linked oligofucosides restricts their conformational flexibility and changes the conformational equilibrium if compared with the parent nonsulfated oligosaccharides. In the case of 2-*O*-sulfated oligofucosides, the conformations of *O*-glycoside linkages depend on its location within the oligosaccharide chain and the chain length as well as on the presence of a 2,3-branch, whereas the conformation of the (1 → 3)-linkage in the presence of a 4-*O*-sulfate group only depends on the presence of a 2,3-branch.

Keywords Fucoidan fragments, Sulfate, NMR, Transglycosidic coupling constants, NOE, Conformational analysis

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[#]Synthesis, NMR and Conformational Studies of Fucoidan Fragments. Part 9. For Part 8, see [1].
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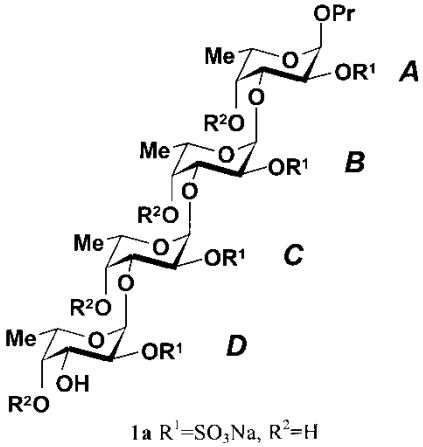
INTRODUCTION

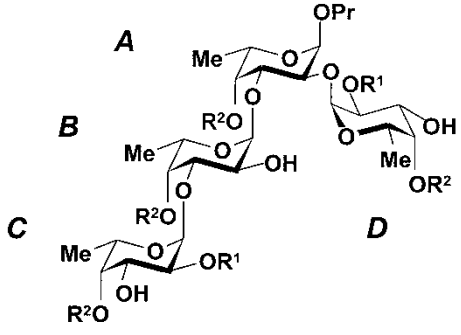
This paper continues the series of previous communications dedicated to the synthesis, NMR, and conformational studies of synthetic fucoidan fragments[1–5] in order to determine structural factors influencing spatial organization of fucoidans and their biological activity. Previously we reported the results of conformational analysis of linear homo-(1 → 3)-linked propyl tetrafucoside **1c**, its 2,3-branched isomer **2c**, and their di- and trifucoside fragments **3c** and **4c**.[2] In this paper we report the conformational analysis of 2-*O*-sulfated and 4-*O*-sulfated derivatives **1a–4a** and **1b–4b** of these molecules (Scheme 1).



1a R^1=SO_3Na, R^2=H

1b R^1=H, R^2= SO_3Na

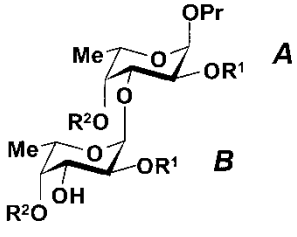
1c R^1=R^2=H



2a R^1=SO_3Na, R^2=H

2b R^1=H, R^2= SO_3Na

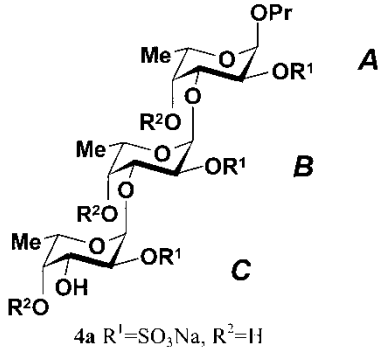
2c R^1=R^2=H



3a R^1=SO_3Na, R^2=H

3b R^1=H, R^2= SO_3Na

3c R^1=R^2=H



4a R^1=SO_3Na, R^2=H

4b R^1=H, R^2= SO_3Na

4c R^1=R^2=H

Scheme 1: Investigated oligosaccharides.

These oligofucosides are related to the fragments of natural fucoidans comprising $(1 \rightarrow 3)$-linked α-L-fucopyranose residues. Fucoidans with this type of chain were isolated from seaweeds *Laminaria saccharina*,[6] *Chorda filum*,[7] and *Cladosiphon okamuranus*.[8]

During the study of nonsulfated tetrafucosides **1c** and **2c**, it was found that NOE NMR-spectroscopy is inapplicable in this case[2] because of the overlap of the signals in 2D NOESY and ROESY spectra. To overcome this restriction, experimental determination of transglycosidic vicinal coupling constants $^{3}J_{C,H}$ was successfully employed for the conformational study of nonsulfated oligofucosides **1c** and **2c**.

NOESY and ROESY spectra of sulfated tetrafucosides **1a,b** and **2a,b** and trifucosides **4a,b** are also uninformative. Therefore, experimental conformational analysis was performed again with the use of the long-range coupling constants $^{3}J_{C,H}$.

RESULTS AND DISCUSSION

^{1}H and ^{13}C NMR studies of compounds 1a,b–4a,b. Tables 1 and 2 represent the ^{1}H NMR and ^{13}C NMR chemical shifts for 2-*O*-sulfated (**1a**–**4a**), 4-*O*-sulfated (**1b**–**4b**), and parent nonsulfated (**1c**–**4c**) oligofucosides. Assignments of chemical shifts in the ^{1}H NMR spectra (Table 1) were made using a combination of 2D ^{1}H–^{1}H COSY and TOCSY experiments. ^{13}C NMR spectra (Table 2) were assigned using 2D ^{1}H–^{13}C HSQC and J-HMBC experiments.

The location of sulfates at C-2 in **1a**–**4a** and at C-4 in **1b**–**4b** was confirmed by the characteristic low-field position of the signals, either of H-2 and C-2 or of H-4 and C-4 in their ^{1}H and ^{13}C NMR spectra in comparison with the corresponding shifts for nonsulfated parent structures **1c**–**4c**. The *O*-sulfation effects ($\Delta\delta$C) observed in ^{13}C NMR spectra of the series of compounds **1a**–**4a** and **1b**–**4b** are summarized in Table 3. The $\Delta\delta$C values show the differences in chemical shifts of the respective signals in the spectra of sulfated derivatives and parent nonsulfated compounds **1c**–**4c**.[2]

In the case of 2-*O*-sulfated compounds **1a**–**4a** a significant positive sulfation effect of about 6.7 ppm was observed for C-2 signals of all sulfated residues. In addition, some negative sulfation effects (up to -2.1 ppm) were also observed for C-1 and C-3 signals. They are due to the so-called β-effect, that is, a slight up-field shift of the β-carbon atom resonance upon the introduction of a substituent.[9]

In the case of 4-*O*-sulfated compounds **1b**–**4b**, the positive sulfation effect for C-4 signals was about 11 ppm for all residues except the terminal ones on the nonreducing ends, where it was $\sim$2 ppm smaller. A negative β-effect was observed for the C-5 signals of most of the residues and for the C-3 signals of the residues at the nonreducing ends. Contrary to the case of 2-*O*-sulfated oligosaccharides, the 4-*O*-sulfation effect on the signals of C-1 and C-3 atoms

Table 1: ^{1}H NMR chemical shifts[a] of oligosaccharides **1a**–**4c**.

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6
1a	**A**	5.20	4.53	4.02	4.08	4.10	1.25
	B	5.33	4.54	4.10	4.08	4.43	1.25
	C	5.33	4.54	4.10	4.08	4.43	1.25
	D	5.33	4.44	4.08	3.88	4.45	1.23
2a	**A**	5.14	4.02	4.12	4.07	4.08	1.25
	B	5.15	3.95	3.84	4.01	4.16	1.27
	C	5.28	4.44	4.06	3.87	4.36	1.20
	D	5.31	4.41	3.88	3.84	4.18	1.25
3a	**A**	5.19	4.52	4.01	4.07	4.11	1.25
	B	5.32	4.43	4.09	3.89	4.45	1.22
4a	**A**	5.23	4.55	4.04	4.10	4.10	1.25
	B	5.34	4.57	4.12	4.10	4.42	1.25
	C	5.36	4.46	4.10	3.90	4.44	1.22
1b	**A**	4.93	3.95	4.03	4.77	4.19	1.27
	B	5.15	3.88	4.05	4.77	4.43	1.25
	C	5.11	3.84	4.06	4.79	4.47	1.27
	D	5.12	3.75	4.01	4.63	4.47	1.27
2b	**A**	5.22	4.12	4.28	4.86	4.18	1.29
	B	5.29	3.94	3.98	4.77	4.37	1.31
	C	5.09	3.78	4.01	4.61	4.52	1.24
	D	5.14	3.86	3.84	4.61	4.21	1.31
3b[(4)]	**A**	4.89	3.89	4.00	4.73	4.15	1.25
	B	5.10	3.75	3.97	4.57	4.41	1.23
4b[(5)]	**A**	4.92	3.95	4.03	4.75	4.19	1.25
	B	5.12	3.87	4.05	4.77	4.43	1.25
	C	5.14	3.76	4.01	4.61	4.48	1.25
1c[(2)]	**A**	4.92	3.93	3.95	4.04	4.08	1.23
	B	5.11	3.95	4.02	4.05	4.29	1.23
	C	5.10	3.93	3.99	4.03	4.34	1.21
	D	5.07	3.81	3.96	3.83	4.32	1.21
2c[(2)]	**A**	5.19	4.05	4.11	4.10	4.09	1.26
	B	5.17	3.96	3.83	4.03	4.17	1.27
	C	5.00	3.80	3.93	3.82	4.35	1.19
	D	5.09	3.79	3.73	3.81	4.12	1.28
3c[(4)]	**A**	4.91	3.91	3.91	4.02	4.08	1.27
	B	5.07	3.81	3.95	3.82	4.28	1.21
4c[(5)]	**A**	4.92	3.93	3.96	4.03	4.08	1.23
	B	5.10	3.93	4.01	4.02	4.28	1.23
	C	5.06	3.81	3.97	3.83	4.32	1.23

[a]In ppm, recorded at 298–307 K in D_2O with acetone as an internal standard (2.225 ppm). Signals of aglycon: $OCH_2CH_2C\underline{H}_3$ δ 0.92–0.93, $OCH_2C\underline{H}_2CH_3$ δ 1.62–1.64, $OC\underline{H}_2CH_2CH_3$ δ 3.49–3.65 and 3.62–3.84.

Table 2: ^{13}C NMR chemical shifts[a] of oligosaccharides **1a–4c**.

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6
1a	**A**	97.6	74.3	75.2	70.4[d]	67.3	16.5
	B	96.2[b]	74.3	75.0[c]	70.3[d]	67.8	16.5
	C	96.1[b]	74.3	74.5[c]	70.2[d]	67.8	16.5
	D	95.6	76.4	68.6	73.4	68.1	16.5
2a	**A**	96.8	71.8	73.0	68.6	67.1	16.5
	B	95.2	67.3	76.7	69.5	68.1	16.5
	C	95.3	76.4	68.6	73.1	67.8	16.5
	D	96.0	76.1	68.6	73.0	68.4	16.8
3a	**A**	97.5	74.3	74.4	69.9	67.4	16.4
	B	95.5	76.5	68.5	73.3	68.1	16.4
4a	**A**	97.5	74.3	75.0	70.2[b]	67.3	16.4
	B	96.0	74.3	74.3	70.1[b]	67.8	16.4
	C	95.5	76.4	68.6	73.3	68.1	16.4
1b	**A**	99.7	67.9	77.2	80.6[b]	67.3	16.9
	B	99.5	68.4	77.5	80.3[b]	67.9	16.9
	C	99.7	68.4	76.8	80.6	67.7[c]	16.9
	D	98.8	69.9	70.3	82.1	67.9[c]	16.9
2b	**A**	96.3	71.2	72.3	78.7	67.3	16.9
	B	96.5	68.0	76.4	79.9	67.8	17.1[b]
	C	98.7	69.7	70.2	81.9	67.5	17.1
	D	96.6	69.2	70.0	81.3	68.0	17.3[b]
3b[(4)]	**A**	99.5	67.8	76.6	80.0	67.1	17.0
	B	98.7	69.7	70.2	81.9	67.6	17.0
4b[(5)]	**A**	99.6	67.7	77.3	80.4	67.2	17.0
	B	99.5	68.3	76.8	80.4	67.7	17.0
	C	98.9	69.8	70.2	82.5	67.7	17.0
1c[(2)]	**A**	99.4	67.5[b]	75.6	69.2[c]	67.4	16.6
	B	96.2	67.6[b]	76.0	69.7[c]	67.8	16.5
	C	96.9	67.7[b]	76.1	69.7[c]	67.8	16.6
	D	96.8	69.3	70.7	73.1	68.2	16.6
2c[(2)]	**A**	96.1	70.1	73.2	68.8	67.2	16.6[c]
	B	95.6	67.4	76.2	69.6	68.1	16.6[c]
	C	97.1	69.3[b]	70.8	72.9	68.0	16.7[c]
	D	96.2	69.1[b]	71.1	72.9	68.4	16.8[c]
3c[(4)]	**A**	99.5	67.6	75.8	69.3	67.5	16.5
	B	96.4	69.2	70.7	73.1	68.1	16.5
4c[(5)]	**A**	99.6	67.7	75.9	69.6	67.6	16.7
	B	96.5	67.8	76.4	69.9	68.0	16.7
	C	97.1	69.2	70.9	73.3	68.3	16.7

[a]In ppm, recorded at 298–307 K in D_2O with acetone as an internal standard (31.45 ppm). Signals of propyl aglycon: $OCH_2CH_2C\underline{H}_3$ δ 11.0–11.1, $OCH_2C\underline{H}_2CH_3$ δ 23.2–23.3, $OC\underline{H}_2CH_2CH_3$ δ 71.3.
[b]The assignment may be reversed.
[c]The assignment may be reversed.
[d]The assignment may be reversed.

Table 3: *O*-Sulfation effects ($\Delta\delta$) in ^{13}C NMR spectra of 2-*O*-sulfated **1a–4a** and 4-*O*-sulfated **1b–4b** oligofucosides.

Compound	Residue	ΔδC-1	ΔδC-2	ΔδC-3	ΔδC-4	ΔδC-5	ΔδC-6
1a	**A**	−1.8	6.8	−0.4	1.2	−0.1	−0.1
	B	0	6.7	−1.0	0.6	0	0
	C	−0.8	6.6	−1.6	0.5	0	−0.1
	D	−1.2	7.1	−2.1	0.3	−0.1	−0.1
2a	**A**	0.7	1.7	−0.2	−0.2	−0.1	−0.1
	B	−0.4	−0.1	0.5	−0.1	0	−0.1
	C	−1.8	7.1	−2.2	0.2	−0.2	−0.2
	D	−0.2	7.0	−2.5	0.1	0	0
3a	**A**	−2.0	6.7	−1.4	0.6	−0.1	−0.1
	B	−0.9	7.3	−2.2	0.2	0	−0.1
4a	**A**	−2.1	6.6	−0.9	0.6	−0.3	−0.3
	B	−0.5	6.5	−2.1	0.2	−0.2	−0.3
	C	−1.6	7.2	−2.3	0	−0.2	−0.3
1b	**A**	0.3	0.4	1.6	11.4	−0.1	0.3
	B	3.3	0.8	1.5	10.6	0.1	0.4
	C	2.8	0.7	0.7	10.9	−0.1	0.3
	D	2.0	0.6	−0.4	9.0	−0.3	0.3
2b	**A**	0.2	1.1	−0.9	9.9	0.1	0.3
	B	0.9	0.6	0.2	10.3	−0.3	0.5
	C	1.6	0.4	−0.6	9.0	−0.5	0.4
	D	0.4	0.1	−1.1	8.4	−0.4	0.5
3b[4]	**A**	0	0.2	0.8	10.7	−0.4	0.5
	B	2.3	0.5	−0.5	8.8	−0.5	0.5
4b[5]	**A**	0	0	1.4	10.8	−0.4	0.3
	B	3.0	0.5	0.4	10.5	−0.3	0.3
	C	1.8	0.6	−0.7	9.2	−0.6	0.3

involved in inter-unit linkages had positive values. The positive sulfation effect on the signals of C-1 and C-3 atoms was observed previously during the study of a series of 4-*O*-sulfated (1 → 3)-linked fucobiosides.[4] In that work it was shown that 4-*O*-sulfation of glycosylated fucopyranose residues changed the conformation of (1 → 3)-linkage in fucobiosides. Obviously, the same conformational changes occur in tri- and tetrafucosides upon 4-*O*-sulfation. These conformational changes are also the cause of the increase of the sulfation effect from 9 to 11 ppm on atoms C-4 next to the glycosidic linkages Scheme.

Determination of $^3J_{C,H}$ constants for compounds 1a,b–4a,b. The values of constants $^3J_{C,H}$ depend on the values of the corresponding dihedral angles φ and ψ in the inter-unit linkages. This dependence is defined by the Karplus Equation (1)[10] as illustrated in Figure 1. The values of J_φ and J_ψ constants for sulfated oligofucosides **1a,b–4a,b** were obtained by 2D J-HMBC[11,12] and

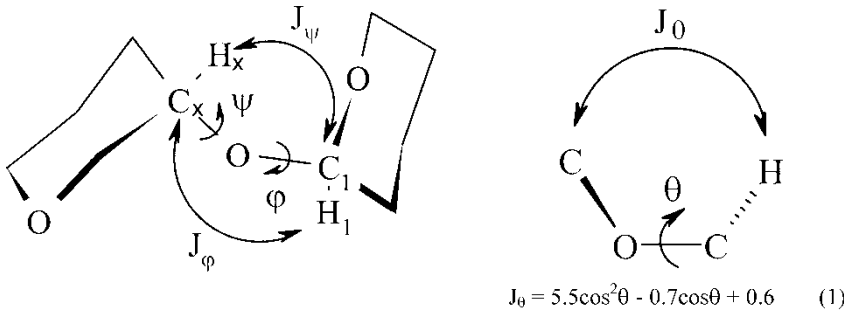


Figure 1: The coupling constants J_φ and J_ψ are determined by the values of the corresponding dihedral angles φ and ψ and are defined by Karplus Equation (1).[10]

J-resolved[13,14] NMR experiments and are summarized in Table 5. The detailed description of the NMR technique for the measurement of transglycosidic coupling constants in oligofucosides was given in the previous work of the series[2] and in the experimental part of this article.

Most of the values were measured by 2D J-HMBC experiment except for some of J_φ constants (marked with footnote **a** in Table 5), whose cross-peaks overlapped with the neighboring ones, making impossible their accurate measurement. In these particular cases 2D J-resolved method[13,14] was employed in addition to J-HMBC.

To estimate the experimental error of $J_{C,H}$ constants measurement we performed repetitive determination of $J_{H1,C5}$ constant values both within 2D J-resolved and J-HMBC experiments. The dihedral angle around (C-1)–O bond in the fragments (H-1)–(C-1)–O–(C-5) of fucopyranose rings in all compounds is close to 180°. According to the Karplus Equation (1), this corresponds to characteristic large values of constant $J_{H1,C5}$ of about 6.5 Hz. Their cross-peaks were well resolved both in J-HMBC and J-resolved spectra, showing that the experimental error is not exceeding 0.5 Hz in both methods similarly to the case of nonsulfated oligofucosides.[2] On the other hand, calculation of constants according to Equation (1) can only be made with the accuracy of 1 Hz.[10]

The molecular modeling of compounds 1a,b–4a,b. Conformational maps of the studied saccharides were constructed using MM3 force field and the grid search method as described previously.[2–5] The starting structures were produced by geometry optimization with MM3. In each point of a conformational map the same starting geometry was used, and the dihedral angles were restrained with a force constant of 10 kcal/deg^2 before the optimization. The step value used during scanning of both φ and ψ directions was 10°. As the calculated values of coupling constants dramatically depend on the values of angles φ and ψ, this procedure was only used for the rough estimate of the regions containing principal energy minima. These regions of conformational space ($\varphi = 0° \div 70°$ and $\psi = -80° \div 80°$) were more thoroughly rescanned

using a step value of 4°, and the resulting conformations were used for the calculation of coupling constants. The constants J_φ and J_ψ were determined for each point (φ, ψ) with the energy lying within 10% of the global minimum of conformational map according to Equation (1) and subsequently averaged by the procedure analogous to averaging of NOE as described in our previous paper.[4]

The electrostatic constant was fixed at 81. No solvent molecules were explicitly included in the calculations. Inclusion of water molecules in case of sulfated compounds would most likely have resulted in the necessity to include explicitly also counter ions. The calculation times would have increased, while the accuracy of calculations on such complicated systems is still questionable.

It was observed previously[5] during the investigation of trisulfated trifucoside **4b** that calculated NOE values did not correlate with the experimental ones when all sulfates were regarded as ions in calculations, while better results were obtained when one of these groups was left undissociated. For disulfated difucosides good correlation was obtained when both groups were modeled as dissociated. In this study we also investigated the influence of the number of ionic groups on the coincidence between calculated and experimental coupling constants. It was found that in the case of tri- and tetrafucosides, better coincidence was achieved when the sulfate group on the reducing end was left undissociated. It may be explained by the fact that in some oligosulfated molecules, dissociation of several sulfate groups may impede the dissociation of others the way it takes place in polymers. On the other hand, disulfated molecules behave normally; that is, both sulfate groups are dissociated. In this work difucosides **3a** and **3b** were modeled as fully dissociated.

The (1 → 3)-α-fucoside linkage was previously found to exist predominantly in two conformations,[4] shown in Figure 2. In 4-*O*-sulfated tetrafucoside **1b** and the (1 → 3)-linked backbone of 4-*O*-sulfated branched tetrafucoside **2b**, we found the same tendency as was published earlier[4,5] for di- and trifucosides **3b** and **4b**: the relative weight of conformer **II** (Fig. 2) significantly increased as the result of 4-*O*-sulfation. The only exception was the 2,3-branch in tetrafucoside **2b**, whose conformational behavior was similar to that of branched 4-*O*-sulfated trifucosides[3]; that is, the weight of conformer **I** was much greater for the (1 → 3)-linkage at the 2,3-branch.

For 2-*O*-sulfated structures we did not observe any significant conformational changes upon the introduction of sulfates. In the case of disaccharide **3a** the statistical weight of conformer of type **I** was comparable to that of the parent nonsulfated difucoside.[2] For tri- and tetrafucosides **4a** and **1a** and for (1 → 3)-linked chain of tetrafucoside **2a**, the statistical weight of conformer **I** was increased in comparison with difucoside **3a**. The same situation was observed with the nonsulfated compounds; therefore, this increase is most likely to be attributed to the chain elongation rather than to the introduction of sulfates.

Conformational maps of (1 → 3)-linkages in 2-*O*-sulfated and 4-*O*-sulfated structures in comparison with disaccharides are shown in Figure 3. Table 4

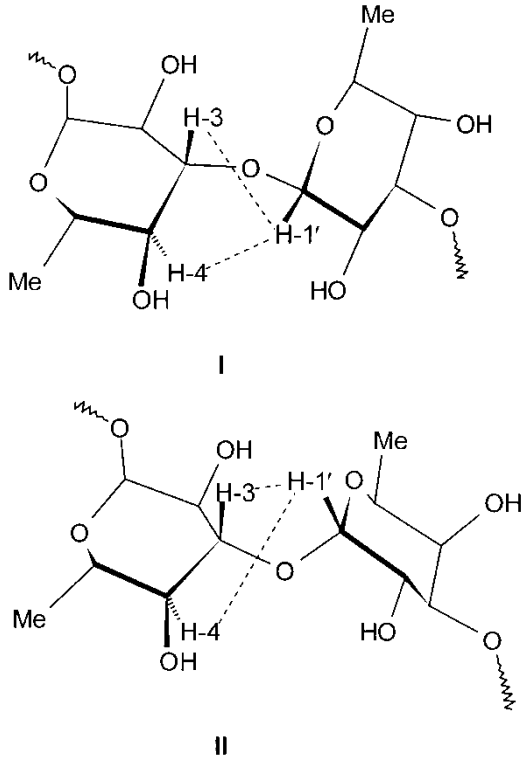


Figure 2: Dominating conformations **I** and **II** around the inter-unit linkage in α-(1 → 3)-fucobiosides.[4,5]

shows statistical weights of conformers **I** and **II** for (1 → 3)-linkages calculated from energy maps.

Analysis of $^3J_{C,H}$ constant values for compounds 1a,b–4a,b

2-*O*-Sulfated oligofucosides 1a–4a. Experimental values of J_φ constants are almost the same for all (1 → 3)-linkages in difucoside **3a** and linear trifucoside **4a** and are about 4.1 Hz. In the case of linear tetrafucoside **1a,** the experimental value of J_φ constant for the disaccharide unit between rings A and B is similar and equals 4.3 Hz. On the other hand, the values of J_φ constants for two other disaccharide units are bigger and are about 5.2 Hz.

The experimental values of J_ψ constants appear to be dependent on the length of the (1 → 3)-linked chain and the position of the corresponding linkage in the chain. Thus, the values of J_ψ constants in tetrafucoside **1a** and trifucoside **4a** tend to increase for inter-unit linkages in the order from nonreducing toward reducing ends. The absolute values of corresponding

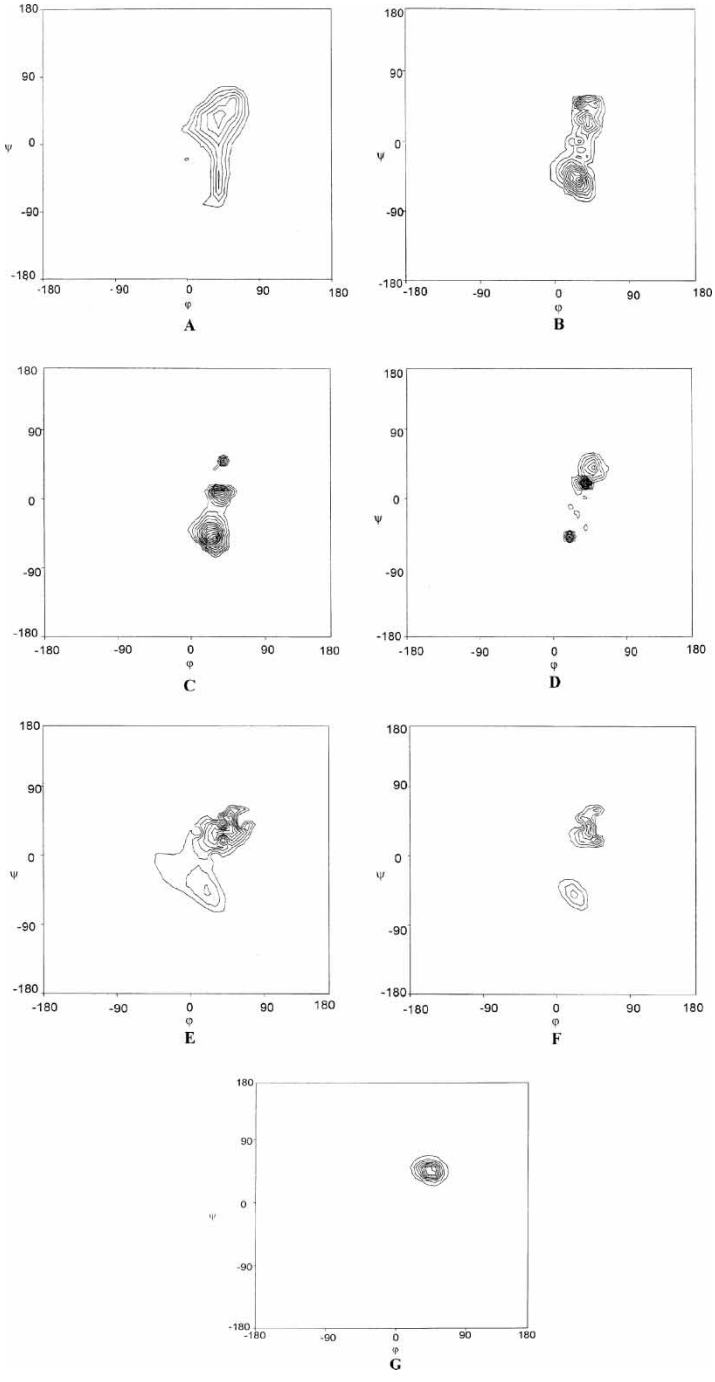


Figure 3: Conformational maps of (1 → 3)-linkages in 4-*O*-sulfated di- (**A**), tri- (**B**), and linear tetrasaccharides (**C**), 2-*O*-sulfated di- (**D**), tri- (**E**), and linear tetrafucosides (**F**), and (1 → 3)-linkage at the branch in tetrafucoside **2a** (**G**).

Table 4: Calculated statistical weights of conformers **I** and **II** of α-(1 → 3)-fucoside linkages for compounds **1a–4b**

Compound	Linkage	I (%)	II (%)
1a	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	74	26
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	72	28
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	72	28
2a	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	80	20
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	75	25
3a	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	55	45
4a	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	72	28
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	69	31
1b	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	21	79
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	23	77
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	23	77
2b	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	80	20
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	40	60
3b	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	39	61
4b	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	20	80
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	19	81

angles ψ (Table 5, Fig. 4) decrease in the same order. At the same time the values of J_ψ constants for the disaccharide units (B-A) on the reducing ends in the di-, tri-, and tetrafucosides all lie within 0.1 Hz range from 4.8 Hz. The values of J_ψ constants for the disaccharide units on the nonreducing ends in the tri- and tetrafucosides are all close to 1.9 Hz. The difference between the values of J_ψ constants for the disaccharide units on reducing and nonreducing ends suggests that the conformation of fucoside linkages in these molecules depends on the size of the corresponding aglycon and glycon moieties.

Previously,[2] we reported that in the case of nonsulfated linear (1 → 3)-linked oligofucosides, the absolute values of angles ψ increased in the order from nonreducing toward reducing ends (Table 5, Fig. 4). Thus, 2-*O*-sulfation of linear (1 → 3)-linked oligofucosides causes changes in conformation of glycoside linkages and reverses the direction of the increase of ψ values along (1 → 3)-linked oligofucoside chain.

The comparison of the experimental and calculated values of J_φ and J_ψ for the studied saccharides **1a**–**3a** showed good coincidence for most disaccharide units in oligofucosides displaying deviations less than 1 Hz with the exception of several cases of terminal disaccharide units. It is also noteworthy that the molecular mechanics calculations did not show differences in conformations of fucoside linkages within one molecule in the case of linear tri- and tetrafucosides **4a** and **1a**. In our opinion, this discrepancy between the experimental and theoretical

Table 5: Experimental and calculated (in parentheses) values of J_φ and J_ψ and corresponding absolute values of angles φ and ψ (calculated from the experimental values according to equation (1)) of compounds **1a–4c**

Compound	Linkage	J_φ, Hz	J_ψ, Hz	φ	ψ
1a	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	4.2[a] (3.9)	4.8 (4.3)	29°	20°
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	5.2[a] (4.5)	2.8 (3.0)	11°	46°
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	5.1[a] (4.5)	1.8 (3.0)	14°	58°
2a	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	4.4[a] (3.5)	2.8 (3.0)	26°	46°
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	3.7 (3.4)	4.2 (3.7)	35°	29°
	α-L-Fuc-(1 → 2)-α-L-Fuc-OPr	2.3 (2.7)	3.7 (3.3)	51°	35°
3a	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	4.1 (3.8)	4.7 (3.4)	30°	22°
4a	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	4.1[a] (3.8)	4.9 (3.1)	30°	18°
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	4.0 (3.6)	2.0 (2.9)	32°	55°
1b	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	4.1[a] (3.8)	5.2 (4.0)	30°	11°
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	4.5[a] (4.3)	5.4 (5.0)	25°	0°
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	4.1[a] (4.3)	4.9 (4.7)	30°	18°
2b	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	2.7 (3.1)	3.9 (3.7)	47°	33°
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	3.7 (3.7)	4.0 (3.5)	35°	32°
	α-L-Fuc-(1 → 2)-α-L-Fuc-OPr	2.5[a] (3.0)	2.6 (3.1)	49°	48°
3b	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	4.0 (3.8)	5.1 (4.0)	32°	14°
4b	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.9[a] (4.0)	4.8 (4.9)	33°	20°
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	4.4[a] (4.0)	5.2 (5.0)	26°	11°
1c[(2)]	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.4[a] (3.6)	1.7 (3.4)	39°	59°
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	3.9[a] (3.6)	2.7 (3.1)	33°	47°
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	3.8 (3.3)	4.3 (3.1)	34°	28°
2c[(2)]	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.1[a] (3.0)	3.1 (2.8)	42°	42°
	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	3.2[a] (3.2)	4.0 (3.4)	41°	32°
	α-L-Fuc-(1 → 2)-α-L-Fuc-OPr	2.2[a] (3.2)	2.1 (2.8)	53°	54°
3c[(2)]	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.5 (3.6)	2.6 (3.4)	38°	48°
4c[(2)]	→ 3)-α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.6[a] (3.8)	2.2 (3.5)	36°	53°
	α-L-Fuc-(1 → 3)-α-L-Fuc-(1 →	3.7 (3.3)	4.3 (3.1)	35°	28°

[a]The value of constant was measured by J-resolved experiment.

data results from the fact that the molecular mechanics calculations reproduce the conformational properties of the terminal disaccharide units in oligofucosides less accurate because they are less rigid than the internal ones.

In the case of 2,3-branched tetrafucoside **2a**, the introduction of two sulfate groups in the terminal residues C and D changed the values of J_φ constants for (1 → 3)-linkages in a greater extent than those of J_ψ. On the contrary, for the (1 → 2)-linkage of tetrafucoside **2a** the J_ψ constant was changed more significantly than J_φ. The differences beetwen the experimental and calculated values of constants for tetrafucoside **2a** were less than 1 Hz for all glycosidic linkages.

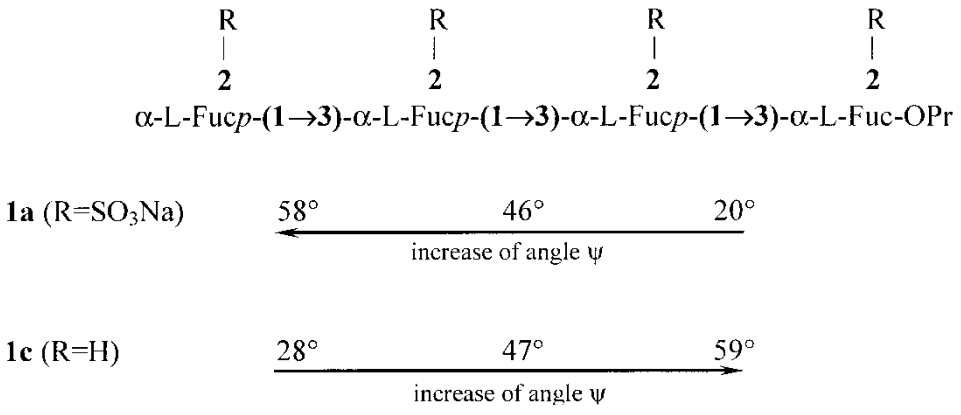


Figure 4: The opposite dependences of the absolute value of angle ψ on the position of the glycosidic linkage within the oligosaccharide homo-(1 → 3)-linked chain in 2-*O*-sulfated and nonsulfated tetrafucosides **1a** and **1c**.

4-*O*-Sulfated oligofucosides 1b–4b. Contrary to the tendencies observed for 2-*O*-sulfated oligofucosides, the experimental constants J_φ and J_ψ changed very slightly for the same linkages within 4-*O*-sulfated linear (1 → 3)-linked difucoside **3b,** trifucoside **4b**, and tetrafucoside **1b**. Their averaged values are 4.1 and 5.2 Hz for J_φ and J_ψ, respectively. The differences between experimental and calculated values of constants J_φ and J_ψ for these molecules did not exceed 1 Hz, except the case of J_ψ constants for the disaccharide units B-A in difucoside **3b** and tetrafucoside **1b** (Table 5). In the latter case the deviation of the calculated values from the experimental ones was slightly larger (Table 5).

Thus, according to the theoretical molecular modeling and experimental determination of transglycosidic vicinal coupling constants $^3J_{C,H}$, the introduction of 4-O-sulfate groups into linear (1 → 3)-linked oligofucosides makes their difucoside units more rigid. All of these units exist predominantly in conformation with angles $\varphi > 0^\circ$ and $\psi < 0^\circ$ (conformer **II**, Fig. 2). This fact is opposite to the case of nonsulfated[2] and 2-*O*-sulfated (1 → 3)-linked oligofucosides, as described above.

Comparison of the corresponding experimental values of J_φ and J_ψ constants for trifucoside **4b** and tetrafucoside **2b** showed that the introduction of a 4-*O*-sulfated (1 → 2)-linked fucosyl unit into a 4-*O*-sulfated (1 → 3)-linked trifucoside chain reduced the values of both constants by about 1 Hz. Theoretical calculations suggested the reduction of the weight of conformation **II** for (1 → 3)-linkages in tetrasaccharide **2b**. At the same time, calculated and experimental constants J_φ and J_ψ for this compound were in good agreement, supporting the idea of the theoretically predicted conformational change in (1 → 3)-linkages upon the introduction of a 2,3-branch.

CONCLUSION

The conformational behavior of 2-*O*-sulfated and 4-*O*-sulfated derivatives of $(1 \rightarrow 3)$-linked oligofucosides, which are related to fragments of natural fucoidans, were studied by theoretical molecular modeling and experimental determination of transglycosidic vicinal coupling constants $^{3}J_{C,H}$. It was shown that the conformations of difucoside units in the 2-*O*-sulfated $(1 \rightarrow 3)$-linked oligofucosides similarly to the case of parent nonsulfated compounds[2] depended on their location within the oligosaccharide backbone and on the presence or absence of a 2,3-branch point. At the same time the 2-*O*-sulfation of $(1 \rightarrow 3)$-linked oligofucosides caused changes in the conformation of glycoside linkages and reversed the direction of the increase of ψ values along $(1 \rightarrow 3)$-linked oligofucoside chain. The conformations of the difucoside units in the 4-*O*-sulfated $(1 \rightarrow 3)$-linked oligofucosides did not depend on their location within the oligosaccharide backbone, but did depend on the presence or absence of a 2,3-branch point. The 4-*O*-sulfation of $(1 \rightarrow 3)$-linked oligofucosides might complicate the rotation at glycoside linkages in difucoside units and make them rigid. The comparison of experimental and calculated values of transglycoside constants $^{3}J_{C,H}$ for sulfated oligofucosides showed better coincidence than in the case of nonsulfated precursors.[2] This could be due to a better prediction of the molecular mechanics calculations of the behavior of less conformationally flexible sulfated structures.

EXPERIMENTAL

The preparation of oligofucosides **1a,b**,[1] **3b**,[4] and **4b**[5] was described previously. Preparation of compounds **2a,b**, **3a**, and **4a** will be described elsewhere. The NMR spectra of oligofucosides **1a,b**–**4a,b** (10–15 mg) were recorded in D_2O (99.98% D, Merck, 0.5 mL) solutions on a Bruker spectrometer DRX-500 with 0.05% acetone as reference (^{1}H 2.225 ppm, ^{13}C 31.45 ppm). The temperature was chosen so that the signal of residual protons of HOD in ^{1}H NMR spectra did not overlap with the signals of protons of oligofucosides. Thus, the spectra for 2-*O*-sulfated (**1a**–**4a**) and 4-*O*-sulfated (**1b**–**4b**) oligofucosides were recorded at 298 K and 307 K, respectively. A microtube (Shigemi, Inc.) was sometimes used for sensitivity enhancement. The resonance assignment in ^{1}H and ^{13}C NMR spectra was performed by gradient-enhanced 2D gCOSY, gHSQC, and gJ-HMBC experiments as well as TOCSY experiments.

Experimental $^{3}J_{C,H}$ constants were measured using J-HMBC and J-resolved techniques. The 2D J-HMBC experiment was performed in the constant-time version.[11] The spectral widths were about 1000 ($\pm$50) Hz for ^{1}H and 4700 Hz for ^{13}C and did not include resonances of methyl groups. The data were collected in the echo/antiecho mode. For echo selection the two sinusoidal field gradients in a ratio of $5 : -3$ were applied, and for antiecho selection

the ratio was $-3:5$. The length of gradients was 1 ms, and the recovery time was 100 μs. The spectra were acquired with 60 to 80 t_1 increments and 500 to 700 scans per increment. Five hundred and twelve points were collected during the acquisition time t_2. The HMBC preparation delay Δ for the reliable measurement of a coupling constants was chosen to be at least 60% of the inversion values of the smallest coupling of interest ($\Delta = 0.6/J^{min}_{C,H}$).[12] Smaller values of Δ led to the overestimation of J because of the antiphase character of the peaks. $\Delta = 300$ ms was used corresponding to $J^{min}_{C,H} = 2.0$ Hz. The upscaling coefficient **k** was 40 to 60. The relaxation delay was 1 s. Thus, the resulting acquisition time was 10 to 15 h. The third order low-pass J-filter[12] was made on suppression of one bond constants ($^1J_{C,H}$) in the range from 125 Hz to 180 Hz. Sinusoidal field gradient sequence with the ratio $+7:-4:-2:-1$ was applied during low-pass J-filter. The forward linear prediction to 1024 points was used in F_1 corresponding to 5 to 6 Hz resolution, and zero-filling to 1024 points was used in F_2. The processing was performed with $\pi/2$ shifted sine square function in both dimensions.

J-resolved experiments were performed in direct ^{13}C-detecting mode with the PENDANTE preparation sequence for the enhancement of the sensitivity of carbon atoms. The spectral widths were 4800 Hz for ^{13}C dimensional and 14 Hz for J dimensional. The spectra were acquired with 44 t_1 increments and 500 to 740 scans per increment. Two thousand and forty-eight points were collected during the acquisition time t_2, giving a spectral resolution of about 2.3 Hz in ^{13}C dimension. The relaxation time between each individual scans was 1 s. The resulting acquisition time was 12 to 15 h. A Gauss-shaped pulse was used; its duration τ was 20 to 50 ms corresponding to the required selectivity of 50 to 20 Hz. Zero-filling to 128 points was used for the J dimension prior to Fourier transformation, giving a spectral resolution of about 0.3 Hz. The 2D spectra were processed with $\pi/2$ shifted sine square function in ^{13}C dimension and in the J dimension.

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