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### Synthesis, NMR and Conformational Studies of Fucoïdan Fragments. V.[1] Linear 4,4',4''-Tri-O-Sulfated and Parent Non-sulfated (1→3)-Fucotrioside Fragments

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## Synthesis, NMR, and Conformational Studies of Fucoidan Fragments. V.<sup>[1]</sup> Linear 4,4',4''-Tri-*O*-Sulfated and Parent Non-sulfated (1→3)-Fucotrioside Fragments

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### ABSTRACT

Propyl 4,4',4''-tri-*O*-sulfated and non-sulfated (1→3)- $\alpha$ -L-fucotriosides which are related to fragments of natural fucoidans have been synthesized. Their spectral and conformational properties have been investigated by <sup>1</sup>H and <sup>13</sup>C NMR, NOE and molecular modeling. Molecular mechanics calculations of the tri-*O*-sulfated compound as a trianion did not give agreement with the experimental NOE values, while the model with the non-dissociated sulfo group on the non-reducing end worked successfully. (1→3)-Fucobioside fragments in both trisaccharides investigated were shown to have the same range of conformations as in previously described propyl (1→3)- $\alpha$ -L-fucobiosides, but with the increase of the relative

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population of the conformer with the spatial proximity of H-1' and H-4 in the case of non-sulfated fucotrioside.

**Key Words:** Fucoidan fragments; Synthesis; NMR; Conformational analysis; NOE.

## INTRODUCTION

Fucoidans, a group of highly sulfated polysaccharides of brown seaweeds, have received increasing interest as readily available biopolymers having many promising biological activities. Complete structural characterization of fucoidans can not generally be performed due to their irregularity and heterogeneity. In the framework of the investigation of fucoidan structure and assessment of pharmacophores in fucoidan chains, we perform the systematic synthesis of their supposed fragments for the study of their NMR and conformational characteristics and various types of biological activities (see the citations in Ref. [2]). Recently we described the synthesis and NMR studies of propyl 3-*O*- $\alpha$ -L-fucopyranosyl- $\alpha$ -L-fucopyranoside,<sup>[1,2]</sup> its 4-*O*-sulfate and 4,4'-di-*O*-sulfate.<sup>[1]</sup> In this communication we report the synthesis, NMR and conformational analyses of propyl glycosides of linear (1  $\rightarrow$  3)-linked  $\alpha$ -L-fucotrioside (**1**) and its 4,4',4''-tri-*O*-sulfate (**2**). The presence of 1  $\rightarrow$  3-linked  $\alpha$ -L-fucopyranose sequences was long ago suggested as the main structural feature of many fucoidans<sup>[3]</sup> and unambiguously confirmed by several investigators recently.<sup>[4-6]</sup>

## RESULTS AND DISCUSSION

**Synthesis of compounds 1 and 2.** The target linear fucotriosides **1** and **2** were prepared by use of the readily accessible partially protected disaccharide **3**<sup>[1]</sup> as glycosyl acceptor. Its regioselective and stereospecific fucosylation by 3,4-di-*O*-benzoyl-2-*O*-benzylfucosyl bromide<sup>[2]</sup> in the presence of Hg(CN)<sub>2</sub> and HgBr<sub>2</sub> gave the trisaccharide derivative **4** in 65% yield. The  $\alpha$ -anomeric configuration of the unit at the non-reducing end in **4** was confirmed by a characteristic value of  $J_{1,2} = 3.0$  Hz in the <sup>1</sup>H NMR spectrum. The location of the newly introduced fucosyl residue at O-3 followed from characteristic chemical shifts of C-3 in the <sup>13</sup>C NMR spectrum (Table 1).

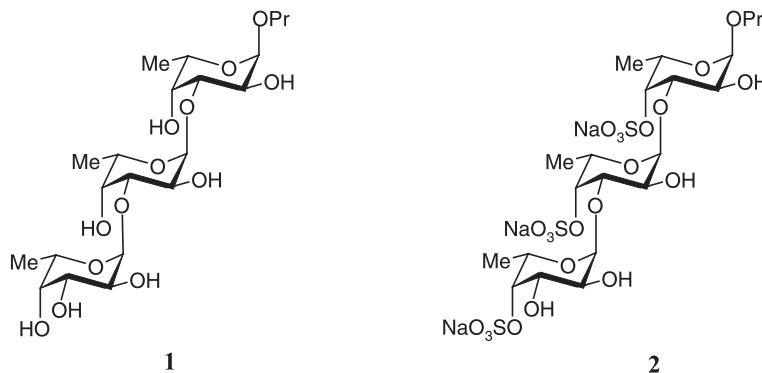


Table 1.  $^{13}\text{C}$  NMR chemical shifts<sup>a</sup> for compound 4.

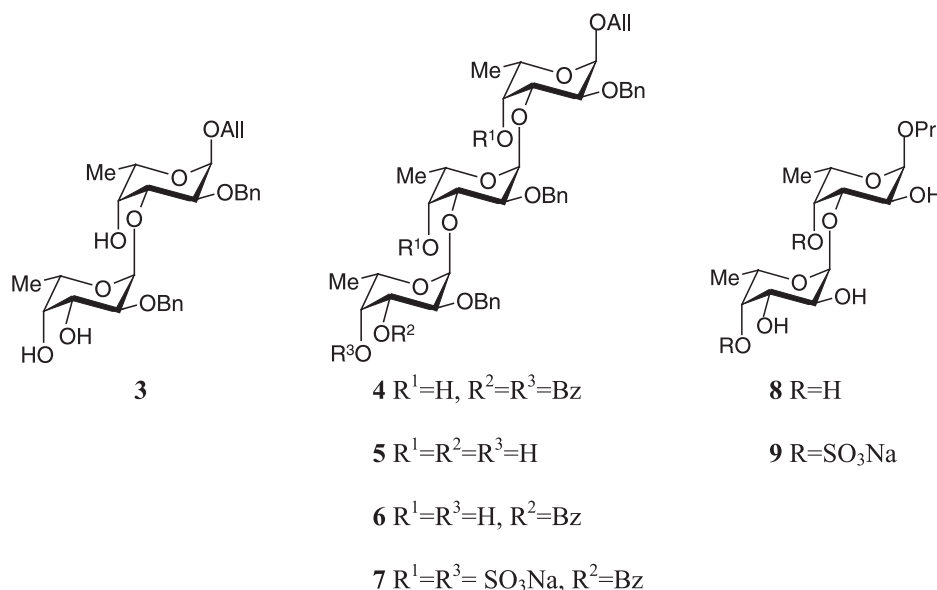
Residue	C-1	C-2	C-3	C-4	C-5	C-6
$\rightarrow 3)\alpha\text{-L-Fuc-OAll}$	95.8	74.5	74.8	68.2	64.9	16.2
$\rightarrow 3)\alpha\text{-L-Fuc-(1}\rightarrow$	94.0	75.1	75.5	68.2	65.7	16.0
$\alpha\text{-L-Fuc-(1}\rightarrow$	94.3	73.2	71.1	72.3	65.5	15.8

<sup>a</sup>In ppm, recorded at 30 °C in  $\text{CDCl}_3$ . Signals of allyl aglycon:  $\text{OCH}_2\text{CH} = \underline{\text{CH}_2}$   $\delta$  117.7;  $\text{OCH}_2\text{CH} = \underline{\text{CH}_2}$   $\delta$  134.0;  $\text{OCH}_2\text{CH} = \underline{\text{CH}_2}$   $\delta$  68.2.

*O*-Debenzoylation of 4 with methanolic sodium methoxide gave tetraol 5. Its regioselective benzylation *via* a stannylidene intermediate gave the 3''-*O*-benzoylated derivative 6 in 62% yield. A downfield chemical shift of the H-3'' signal to  $\delta = 5.54$  ppm (Table 2) evidenced the location of the benzoate group at C-3''.

Treatment of triol 6 with sulfur trioxide-pyridine complex in DMF gave sulfated derivative 7 in 80% yield. Characteristic downfield chemical shifts of the H-4 signals (Table 2) indicated the presence of sulfate groups in 7 at C-4, C-4' and C-4''.

Catalytic hydrogenolysis and subsequent saponification of products 4 and 7 gave the corresponding target trisaccharides 1 and 2 which were purified by gel filtration on a Sephadex G-10 column. The presence of *O*-sulfonato groups in 2 was confirmed by low-field chemical shifts of the respective H-4 and C-4 signals (Tables 3–5).



**NMR analysis of compounds 1 and 2.** Tables 3–5 show the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR chemical shifts of compounds 1 and 2. Assignments of the  $^1\text{H}$  NMR spectra (Tables 3, 4) of these compounds were made using a combination of  $^1\text{H}$ – $^1\text{H}$  COSY and




**Table 2.**  $^1\text{H}$  NMR chemical shifts<sup>a</sup> for compounds 4–7.

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6
<b>4</b>	→ 3)α-L-Fuc-OAll	4.91	3.85	4.10	3.74	3.99	1.33
	→ 3)-α-L-Fuc-(1 →	4.91	3.94	4.06	3.62	4.17	1.20
	α-L-Fuc-(1 →	4.99	4.15	5.76	5.52	4.35	1.01
<b>5</b>	→ 3)α-L-Fuc-OAll	4.79–4.89	3.73–3.80	3.95–4.16	3.49–3.65	3.90	1.28
	→ 3)-α-L-Fuc-(1 →					4.02	1.13
	α-L-Fuc-(1 →					4.02	1.13
<b>6</b>	→ 3)α-L-Fuc-OAll	4.82	3.70	3.94	3.65	4.15	1.10
	→ 3)-α-L-Fuc-(1 →	4.89	3.86	4.07	3.68	3.93	1.29
	α-L-Fuc-(1 →	4.89	4.11	5.54	3.97	4.31	1.08
<b>7</b>	→ 3)α-L-Fuc-OAll	4.83	3.90	4.25	4.81	n.d. <sup>b</sup>	0.98–
	→ 3)-α-L-Fuc-(1 →	5.69	4.07	4.50	4.81	n.d. <sup>b</sup>	1.32
	α-L-Fuc-(1 →	5.42	4.04	5.66	4.42	n.d. <sup>b</sup>	

<sup>a</sup>In ppm, compounds 4–7 were recorded in  $\text{CDCl}_3$ . Other signals:  $\text{OCH}_2\text{CH} = \text{CH}_2$   $\delta$  5.13–5.41;  $\text{OCH}_2\text{CH} = \text{CH}_2$   $\delta$  5.78–6.05 and 5.70–5.87;  $\text{OCH}_2\text{CH} = \text{CH}_2$   $\delta$  4.22–4.45 and 4.00–4.15;  $\text{PhCH}_2$   $\delta$  4.50–4.80;  $\text{C}_6\text{H}_5\text{CH}_2$   $\delta$  6.90–8.20;  $\text{C}_6\text{H}_5\text{CO}$   $\delta$  7.45–7.93;

<sup>b</sup>Not determined due to the overlap of the multiplets.

**Table 3.**  $^1\text{H}$  NMR chemical shifts<sup>a</sup> of oligosaccharides **1** and **2**.

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6
<b>1</b>	→3)- $\alpha$ -L-Fuc-OPr	4.92	3.93	3.96	4.03	4.08	1.23
	→3)- $\alpha$ -L-Fuc-(1 →	5.10	3.93	4.01	4.02	4.28	1.23
	$\alpha$ -L-Fuc-(1 →	5.06	3.81	3.97	3.83	4.32	1.23
<b>2</b>	$\alpha$ -L-Fuc-OPr	4.92	3.95	4.03	4.75	4.19	1.25
	→3)- $\alpha$ -L-Fuc-(1 →	5.12	3.87	4.05	4.77	4.43	1.25
	$\alpha$ -L-Fuc-(1 →	5.14	3.76	4.01	4.61	4.48	1.25

<sup>a</sup>In ppm, recorded at 40 °C in D<sub>2</sub>O with acetone as an internal standard. Signals of aglycon: OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>,  $\delta$  0.92; OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>,  $\delta$  1.62–1.64; OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>,  $\delta$  3.49–3.65 and 3.62–3.84.

2D TOCSY experiments. Assignments of the  $^{13}\text{C}$  NMR spectra (Table 5) were made using 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC and HMQC correlation spectroscopy.

The location of sulfates at C-4 in **2** was confirmed by the characteristic low-field position of the H-4 and C-4 signals in the spectra of **2**. The *O*-sulfation effects observed in  $^{13}\text{C}$  NMR spectra of compound **2** are shown in Table 6. These effects ( $\Delta\delta$ ) are the differences in the chemical shifts of the respective signals in spectra of the *O*-sulfated compound and its non-sulfated parent. The sulfation effect on the C-4 of the terminal residue at the non-reducing end is ca. 2 ppm smaller than those in the other two residues. At the same time, C-3 atoms in these two residues lack a  $\beta$ -effect (slight negative change of the chemical shift<sup>[7]</sup>) present in the terminal residue. Also substantial positive sulfation effects are observed on C-1 in the terminal and middle residues, while for the reducing end  $\Delta\delta^{13}\text{C}$  is 0. Similar tendencies were observed previously in the case of fucobiosides **8** and **9**.<sup>[1]</sup>

The similarity in the  $^{13}\text{C}$  spectra of compounds **1**, **2** and **8**, **9** suggests their conformational similarity. To confirm this we studied the trisaccharides using the approach suggested before<sup>[1]</sup> for (1 → 3)-fucobiosides **8** and **9** by means of molecular mechanics calculations and NOE experiments.

**Conformational analysis of compounds 1 and 2.** In a previous work<sup>[1]</sup> we accomplished the experimental conformational analysis of fucosides *via* a steady-state NOE technique. This method cannot be applied to compounds **1** and **2**, because the

**Table 4.** Coupling constants in  $^1\text{H}$  NMR spectra of oligosaccharides **1** and **2**.

Compound	Residue	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
<b>1</b>	→3)- $\alpha$ -L-Fuc-OPr	2.6	n.d. <sup>a</sup>	<2 Hz	<2 Hz	6.5
	→3)- $\alpha$ -L-Fuc-(1 →	3.8	n.d. <sup>a</sup>	<2 Hz	<2 Hz	6.5
	$\alpha$ -L-Fuc-(1 →	3.8	10.7	3.0	<2 Hz	6.5
<b>2</b>	→3)- $\alpha$ -L-Fuc-OPr	3.9	10.3	2.4	<2 Hz	6.4
	→3)- $\alpha$ -L-Fuc-(1 →	3.7	10.4	2.3	<2 Hz	6.5
	$\alpha$ -L-Fuc-(1 →	3.6	10.5	2.5	<2 Hz	6.5

<sup>a</sup>Not determined due to the overlap of the multiplets.



**Table 5.**  $^{13}\text{C}$  NMR chemical shifts<sup>a</sup> for oligosaccharides **1** and **2**.

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6
<b>1</b>	→3)α-L-Fuc-OPr	99.6	67.7	75.9	69.6	67.6	16.7
	→3)-α-L-Fuc-(1 →	96.5	67.8	76.4	69.9	68.0	16.7
	α-L-Fuc-(1 →	97.1	69.2	70.9	73.3	68.3	16.7
<b>2</b>	→3)α-L-Fuc-OPr	99.6	67.7	77.3	80.4	67.2	17.0
	→3)-α-L-Fuc-(1 →	99.5	68.3	76.8	80.4	67.7	17.0
	α-L-Fuc-(1 →	98.9	69.8	70.2	82.5	67.7	17.0

<sup>a</sup>In ppm, recorded at 40 °C in D<sub>2</sub>O with acetone as an internal standard. Signals of propyl aglycon: OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> δ 11.1; OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> δ 23.2–23.3; OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> δ 71.3.

resonance lines of the two protons of interest, H-1' and H-1'', are separated by less than 15 Hz, which makes impossible their selective pre-irradiation. In the current study the 2D-NOESY method followed by quantitative integration of cross-peaks was used. The resulting numbers correspond to transient NOE values. At the employed mixing time of 500 ms the build-up of NOE is in the linear range and NOE values can be estimated<sup>[8,9]</sup> as

$$f \propto \frac{1}{r^6}$$

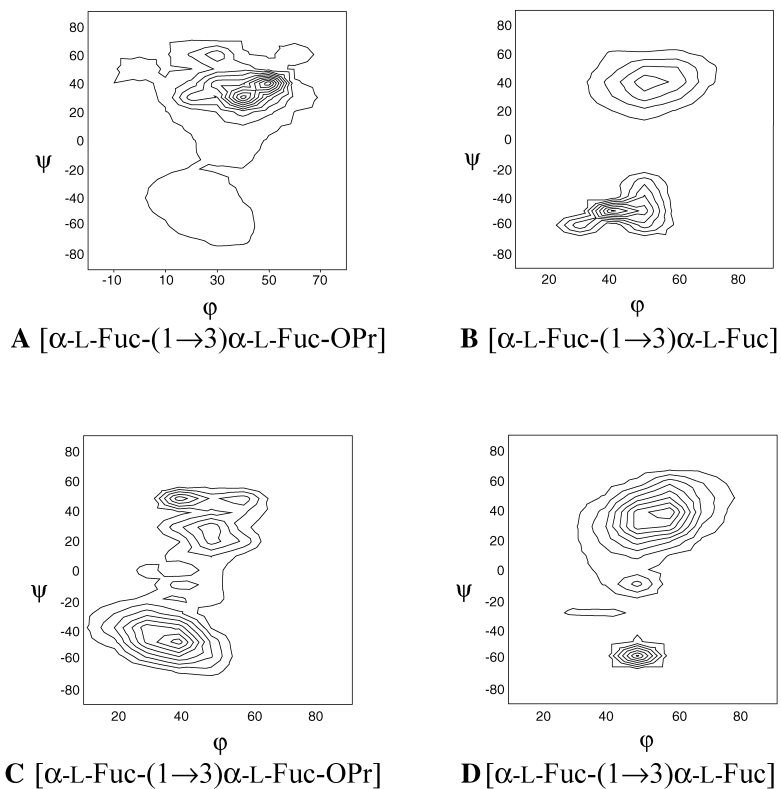
A theoretical conformational analysis was performed using the MM3 force field.<sup>[10]</sup> Conformational maps of the fucotriosides were built *via* a procedure similar to the one previously described.<sup>[11]</sup> Glycosidic linkages were treated independently: first, one linkage was restrained and the other allowed to rotate freely, then *vice-versa*. Thus, two conformational maps were produced for each fucotrioside. For minima found on these maps we analyzed the state of the glycosidic linkage that was not restrained during the construction of the current map. This yielded four conformations for each fucotrioside, which were checked further against identity: that is, if φ and ψ angle values for two conformations differed by less than 10° and their energies by less than 0.2 kcal/mol, we considered these conformations the same. Conformational maps of trisaccharides **1** and **2** are shown in Figure 1.

One of the approaches to the conformational analysis of higher oligosaccharides was to use pre-defined glycosidic linkages<sup>[11]</sup> in the same conformation as in a disaccharide fragment. Though we did not apply such constraints to the conformation of (1 → 3)-linkages in the studied compounds, our calculations showed that its conformational behavior in general resembles that of (1 → 3)-linkage in fucobiosides. For

**Table 6.** *O*-Sulfation effects (Δδ) in  $^{13}\text{C}$  NMR spectrum of *O*-sulfated derivative **2**.

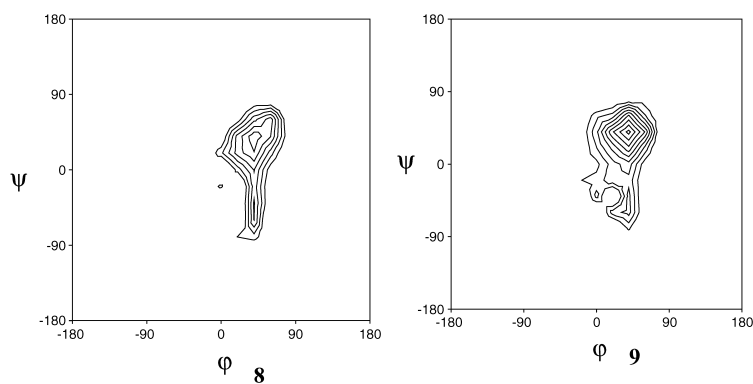
Residue	ΔδC-1	ΔδC-2	ΔδC-3	ΔδC-4	ΔδC-5	ΔδC-6
→3)α-L-Fuc-OPr	0.0	0.0	1.4	10.8	-0.4	0.3
→3)-α-L-Fuc-(1 →	3.0	0.5	0.4	10.5	-0.3	0.3
α-L-Fuc-(1 →	1.8	0.6	-0.7	8.8	-0.6	0.3





**Figure 1.** Conformational maps for the inter-unit linkages in trisaccharides **1** (A,B) and **2** (C,D).

each linkage there exist two main conformers. The first is characterized by the spatial proximity of protons H-1' and H-4 (positive  $\psi$ ), while in the second the proton H-1' is closer to H-3 (negative  $\psi$ ). We also encountered some noteworthy details that were not present in the disaccharides, and they are discussed below.



**Figure 2.** Conformational maps of disaccharides **8** and **9**.





**Table 7.** Experimental relative integral values of cross-peaks in 2D NOESY spectra of compounds **1** and **8** and computed relative transient NOE values (in parenthesis).

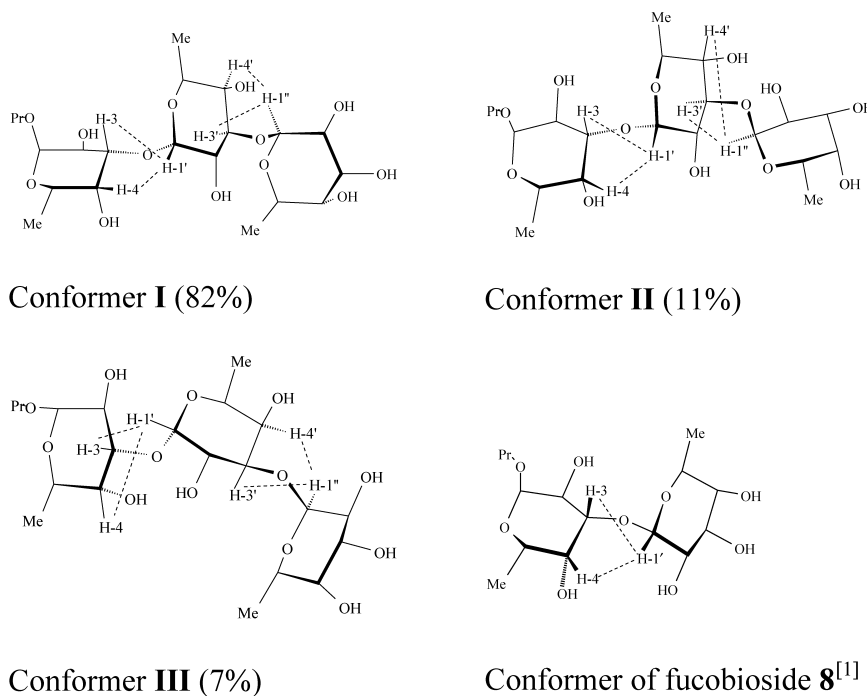
Linkage		H-2'/H-1'	H-3/H-1'	H-4/H-1'	
				NOE <sup>a</sup>	NOE <sup>b</sup>
<b>1</b>	$\alpha$ -L-Fuc-(1 $\rightarrow$ 3)- $\alpha$ -L-Fuc-OPr	214 <sup>c</sup> (120 + 100)		143 (155)	(250)
	$\alpha$ -L-Fuc-(1 $\rightarrow$ 3)- $\alpha$ -L-Fuc	119 (110)	100 (100)	143 (160)	(235)
<b>8</b>		125 (102)	100 (100)	109 (92)	(180)

<sup>a</sup>Theoretical NOEs calculated over all conformations lying within 10% of the global energy minimum.

<sup>b</sup>Theoretical NOEs calculated over 3 conformations shown on Figure 3.

<sup>c</sup>Measured for the sum of H-3/H-1' and H-2'/H-1' cross-peaks.

Compounds **1**, **8** and **9**. For non-sulfated trisaccharide **1** three different conformers were observed. Their schematic representations and proportion in the total ensemble are given in Figure 2. The dominant conformation is that with spatial proximity of H-1'/H-4 and H-1''/H-4' in both linkages. It is the same as in fucobioside **8** (Figure 2), but the value of its statistical weight is greater than 80%, while the weight of the conformer with such geometry of the inter-unit linkage in the fucobioside was about 60%. Rotation about either inter-unit linkage in order to shorten the H-1'—H-3 distance results in the increase of the energy and consequently in the decrease of the population. Con-



**Figure 3.** Conformers of the non-sulfated trisaccharide **1** with their statistical weights and schematic representation of one of the conformers of the related fucobioside **8**<sup>[1]</sup>.

formations in which both H-1 protons are close to H-3 are populated scarcely more than 1% and are not shown.

Values obtained from 2D-NOESY experiments for compounds **1** and **8** (Table 7) confirmed the tendency predicted from the calculations. Differences in ratio of NOE values of H-4 and H-3 protons between compounds **8** and **1** reveal that the average H-4/H'-1 distance in the fucotrioside is shorter than in fucobioside **8**. The same can be concluded from the conformational changes.

Comparison of the theoretical NOE values calculated from the weights shown above to the experimental data gave only qualitative agreement (Table 7): computed NOE values on H-4 were about twice greater than expected both for the disaccharide and trisaccharide. This observation means that the representation of a saccharide as an ensemble of only few conformers may be a rather coarse approximation.

It is worth noting that other researchers<sup>[12]</sup> also admitted that there are situations when NOEs computed directly from the map minima fail to reproduce experimental results. One way to avoid this is to perform molecular dynamics simulations, which give an average conformation but no information about local minima. To keep illustrativity provided by the conformational map, we employed the procedure described in the previous work,<sup>[1]</sup> taking into calculation of transient NOE all the conformations with energies lying within 10% of the global minimum. Thus computed values are shown in Table 7.

Compound **2**. The method described above for the parent trifucoside did not provide correspondence to the experimental 2D-NOESY data. Surprisingly, our calculations showed no significant changes in conformation of the glycosidic linkage upon introduction of sulfates. At the same time according to the experimental NOESY data the conformation of the trisaccharide should change in the same manner as for the difucoside (Tables 7 and 8), that is, the weight of the conformation with spatial proximity of H-1' and H-3 for both linkages should increase.

We supposed that such trisulfated molecule might not be fully dissociated in solution. As there are three sulfonato-groups and no presumptions could be made which of them is more likely to be non-dissociated, three computations were carried out, each time leaving one of the sulfo groups protonated. After that NOESY values over all conformers lying within 10% of the energy of the global minimum were calculated for each hypothetical compound. Resulting values (Tables 8, 9) showed good correlation with the experiment only when the proton was left on the sulfate group of the reducing

**Table 8.** Experimental relative integral values of cross-peaks in 2D NOESY spectra of compounds **2** and **9** and computed transient NOE values (in parenthesis).

Linkage	H-2'/H-1'	H-3/H-1'	H-4/H-1'		
			NOE <sup>a</sup>	NOE <sup>b</sup>	
<b>2</b>	$\alpha$ -L-Fuc-(1 $\rightarrow$ 3)- $\alpha$ -L-Fuc-OPr	80 (90)	100 (100)	57.5 (35)	(85)
	$\alpha$ -L-Fuc-(1 $\rightarrow$ 3)- $\alpha$ -L-Fuc	70 (95)	100 (100)	57.5 (40)	(95)
<b>9</b>		90 (82)	100 (100)	49 (42)	(70)

<sup>a</sup>Theoretical NOEs calculated over all conformations lying within 10% of the global energy minimum.

<sup>b</sup>Theoretical NOEs calculated over 3 conformations shown on Figure 4.



**Table 9.** Theoretical transient H4/H1' NOE values for different anions of compound **2**.

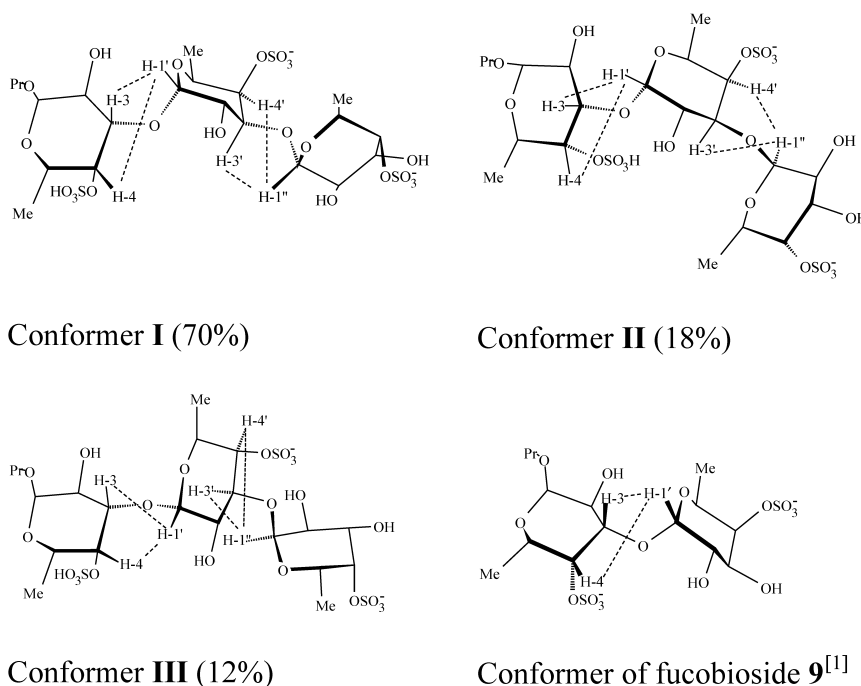
Fragment	H-4/H-1'		
	Trianion	dianion-1 <sup>a</sup>	dianion-2 <sup>b</sup>
$\alpha$ -L-Fuc-(1 $\rightarrow$ 3)- $\alpha$ -L-Fuc-OPr	148	155	145
$\alpha$ -L-Fuc-(1 $\rightarrow$ 3)- $\alpha$ -L-Fuc	140	150	140

<sup>a</sup>dianion with the sulfate in the non-reducing end.<sup>b</sup>dianion with the sulfate in the middle ring.

end. The resulting conformations and their statistical weights are presented in Figure 4. This compound also had three main conformations, but in the most stable one both H-1'—H-3 and H-1''—H-3' distances are short.

**Analysis of <sup>13</sup>C chemical shifts in spectra of fucotriosides **1** and **2**.** In the case of fucobiosides<sup>[1]</sup> it was possible to rationalize differences in sulfation effects (Table 6) in terms of the Grant-Cheney theory.<sup>[13]</sup> The same could be applied in part to the studied trisaccharides. Thus the lack of the  $\beta$ -effect on C-3 in the reducing and the middle ring is due to the decrease of the average distance between H-1' and H-3 upon 4-*O*-sulfation. A positive sulfation effect on C-1 of the middle and non-reducing ring appears due to the increase of the H-1'—H-4 distance.

In this study we also had an opportunity to compare trifucosides **1** and **2** to the corresponding disaccharide fragments **8** and **9**. In Table 10 the values of deviations

**Figure 4.** Conformers of tri-*O*-sulfated trisaccharide **2**, their statistical weights and schematic representation of related fucobioside **9** conformer.

**Table 10.** Deviations from additivity in compounds **1** and **2**, computed relative to the fucobiosides.

Compound	Residue	$\Delta\delta\text{C-1}$	$\Delta\delta\text{C-2}$	$\Delta\delta\text{C-3}$	$\Delta\delta\text{C-4}$	$\Delta\delta\text{C-5}$	$\Delta\delta\text{C-6}$
<b>1</b>	$\rightarrow 3)\alpha\text{-L-Fuc-OPr}$	0	-0.1	-0.1	0.1	0	0
	$\rightarrow 3)\alpha\text{-L-Fuc-(1} \rightarrow$	0	0	0.4	0.4	0.4	0
	$\alpha\text{-L-Fuc-(1} \rightarrow$	0.6	-0.2	0	0	0	0
<b>2</b>	$\rightarrow 3)\alpha\text{-L-Fuc-OPr}$	0.1	-0.1	0.7	0.4	0.1	0.0
	$\rightarrow 3)\alpha\text{-L-Fuc-(1} \rightarrow$	0.8	0.5	0.2	0.4	0.6	0.0
	$\alpha\text{-L-Fuc-(1} \rightarrow$	0.2	0.1	0.0	0.4	0.1	0.0

from additivity are presented. They are calculated as differences between chemical shifts of a trisaccharide and the corresponding disaccharide fragment (**8** or **9**).

For trisaccharide **2** both NOE data and the theoretical calculations show a similarity to disulfated fucobioside **9**. According to the Grant and Cheney concept<sup>[13]</sup>  $\Delta\delta\text{C}$  values should be 0. In fact, they are slightly positive for some atoms. More interestingly, the same tendency takes place in compound **1**, where  $\Delta\delta\text{C}$  are also positive or close to 0. This evidence is contradictory to that provided by NOE and calculations, that show significant decrease of the H-1'—H-4 distance. This observation shows that the Grant and Cheny theory may not correlate with experimental NOE data.

## CONCLUSIONS

Two linear fucotrioxide fucoidan fragments have been synthesized and investigated by  $^1\text{H}$ ,  $^{13}\text{C}$  and NOE NMR spectroscopy and molecular modeling. For the non-sulfated saccharide combined molecular mechanics modeling and 2D NOESY experiments have shown that the statistical weight of the dominant conformation with spatial proximity of H-1' and H-4 protons is increased if compared with that of the previously studied fucobioside. It was shown that molecular modeling results for the tri-O-sulfated trisaccharide do not correspond to the experimental NOESY data when the molecule is considered as a tri-anion, while theoretical regard of the sulfate group in the reducing end in the non-dissociated state restores the agreement with the experiment. It was found that NOE data and  $\Delta\delta^{13}\text{C}$  values predicted from the Grant-Cheney concept do not always correlate.

## EXPERIMENTAL

**General methods.** TLC was performed on silica gel 60 F<sub>254</sub> (Merck) with EtOAc–toluene (A, 1:1, B, 1:2, C, 1:5), CH<sub>2</sub>Cl<sub>2</sub>–MeOH (D, 4:1); spots were detected by charring with H<sub>3</sub>PO<sub>4</sub>. Column chromatography was performed on Silica Gel 0.063–0.2  $\mu\text{m}$  (Fluka) by gradient elution. Gel chromatography was performed on a Sephadex G-10 column (2  $\times$  20 cm) by elution with water at a flow rate of 1 mL/min, and a Sephadex LH-20 column (2  $\times$  40 cm) by elution with methanol at a flow rate of 1 mL/min. Optical rotations were determined with a Jasco DIP-360 digital polarimeter at 26–30°C. All solvents used for syntheses were purified according to conventional procedures.<sup>[14]</sup> NMR spectra for substituted compounds **4–7** were recorded on Bruker spectrometers WM-250 and AM-300 at 303 K.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for oligo-



saccharides **1** and **2** were recorded in D<sub>2</sub>O on a Bruker spectrometer DRX-500 with 0.05% acetone as reference (<sup>1</sup>H 2.225 ppm; <sup>13</sup>C 31.45 ppm). Gradient enhanced 2D gCOSY, gNOESY and gHSQC experiments as well as TOCSY experiments were used for resonance assignment. Experimental NOEs were measured using a 2D-NOESY technique with pulse field gradient (PFG) on a Bruker DRX-500 instrument in D<sub>2</sub>O (99.98% D, Merck) solutions at 303 K, mixing time 500 ms, relaxation delay 5 s. The following PFG parameters were used: homospile/gradient pulse of 1 ms, delay for homospile/gradient recovery of 1 ms. Computations were performed using TINKER software package with the implemented MM3 force field. The dielectric constant  $\epsilon$  was set to 81. All sulfate groups were treated as anions, unless stated otherwise. Parameters for their modelling were based on published work.<sup>[15]</sup> No solvent molecules were considered in the calculation. 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 force constant of 10 kcal/deg<sup>2</sup> before the optimization.

**Allyl 3,4-Di-O-benzoyl-2-O-benzyl- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl- $\alpha$ -L-fucopyranoside (4).** A mixture of triol **3** (111 mg, 0.21 mmol), Hg(CN)<sub>2</sub> (58 mg, 0.23 mmol), HgBr<sub>2</sub> (10 mg), and molecular sieves 4 Å (500 mg) in CH<sub>2</sub>Cl<sub>2</sub> (3.5 mL) was stirred for 1 h at 20 °C under Ar. Using a syringe, a solution of 3,4-di-O-benzoyl-2-O-benzyl- $\alpha$ -L-fucosyl bromide (prepared from 3,4-di-O-benzoyl-2-O-benzyl-L-fucoside,<sup>2</sup> 146 mg, 0.32 mmol) was added portionwise during 1 h. The mixture was stirred for 24 h, then filtered through Celite, diluted with CH<sub>2</sub>Cl<sub>2</sub>, washed with satd aq KBr and NaHCO<sub>3</sub> solutions and concentrated. Adsorption chromatography on silica of the residue followed by gel chromatography on a Sephadex LH-20 column gave the trisaccharide **4** (133 mg, 65%): [ $\alpha$ ]<sub>D</sub> – 253° (*c* 2, MeOH); *R*<sub>F</sub> 0.57 (solvent B). The <sup>1</sup>H and <sup>13</sup>C NMR data for **4** are presented in Tables 1 and 2.

Anal. Calcd for C<sub>56</sub>H<sub>62</sub>O<sub>15</sub> (975.11): C, 68.98%; H, 6.41%. Found: C, 68.91%; H, 6.55%.

**Allyl 2-O-Benzyl- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl- $\alpha$ -L-fucopyranoside (5).** To a solution of **4** (97 mg, 0.10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) a solution of 1.2 M MeONa in MeOH (0.1 mL) was added. The mixture was kept for 1 h at rt and then neutralized with KU-2 (H<sup>+</sup>) resin, filtered, and concentrated to dryness. Column chromatography of the residue gave compound **5** (68 mg, 90%): [ $\alpha$ ]<sub>D</sub> – 115° (*c* 0.67, MeOH); *R*<sub>F</sub> 0.17 (solvent A). The <sup>1</sup>H NMR data for **5** are presented in Table 1.

Anal. Calcd for C<sub>42</sub>H<sub>54</sub>O<sub>13</sub> (766.89): C, 65.78%; H, 7.10%. Found: C, 65.63%; H, 6.99%.

**Allyl 3-O-Benzoyl-2-O-benzyl- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl- $\alpha$ -L-fucopyranoside (6).** A mixture of compound **5** (20 mg, 0.026 mmol), Bu<sub>2</sub>SnO (7 mg, 0.03 mmol) and toluene (1.7 mL) was refluxed until complete dissolution and then concentrated to a volume of 0.7 mL. BzCl (4  $\mu$ L, 0.034 mmol) was added, the solution was kept at rt for 1.5 h and concentrated *in vacuo*. Column chromatography of the residue gave amorphous **6** (14 mg, 62%): [ $\alpha$ ]<sub>D</sub> – 159° (*c* 1, EtOAc); *R*<sub>F</sub> 0.79 (solvent C). The <sup>1</sup>H NMR data for **6** are presented in Table 1.

Anal. Calcd for C<sub>49</sub>H<sub>58</sub>O<sub>14</sub> (871.00): C, 67.57%; H, 6.71%. Found: C, 67.45%; H, 6.69%.

**Trisodium salt of Allyl 3-*O*-Benzoyl-2-*O*-benzyl-4-*O*-sulfonato- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-*O*-benzyl-4-*O*-sulfonato- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-2-*O*-benzyl-4-*O*-sulfonato- $\alpha$ -L-fucopyranoside (7).** A solution of **6** (25 mg, 0.03 mmol) in DMF (0.5 mL) was treated with SO<sub>3</sub>-Py complex (57 mg, 0.36 mmol) for 1 h at rt, then quenched with NaHCO<sub>3</sub> (30 mg) and stirred for 1 h. The solid was filtered off and washed with MeOH (10 mL). The filtrate was treated with KU-2 (Na<sup>+</sup>) cation-exchange resin for 20 min, the resin was filtered off, and the filtrate was concentrated. Column chromatography of the residue gave amorphous **7** (28 mg, 80%):  $[\alpha]_D - 143^\circ$  (*c* 1, MeOH); *R*<sub>F</sub> 0.27 (solvent D). The <sup>1</sup>H NMR data for **7** are presented in Table 1.

Anal. Calcd for C<sub>49</sub>H<sub>55</sub>Na<sub>3</sub>O<sub>23</sub>S<sub>3</sub>(1177.13): C, 50.00%; H, 4.71%. Found: C, 50.13%; H, 4.87%.

**Propyl  $\alpha$ -L-Fucopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-fucopyranoside (1).** A solution of **4** (43 mg, 0.047 mmol) in MeOH (6 mL) was subjected to catalytic hydrogenolysis with 10% Pd-C at 20°C and atm. pressure for 2 h. The mixture was filtered through Celite, and the solvent was evaporated *in vacuo*. The residue was dissolved in water (1 mL), 0.1 M NaOH (0.5 mL) was added. The mixture was kept for 1 h at rt and then was subjected to gel chromatography on a Sephadex G-10 column in water to give amorphous **1** (18 mg, 75%):  $[\alpha]_D - 248^\circ$  (*c* 0.33, H<sub>2</sub>O). The <sup>1</sup>H and <sup>13</sup>C NMR data for **1** are presented in Tables 3–5.

Anal. Calcd for C<sub>21</sub>H<sub>38</sub>O<sub>13</sub> (498.53): C, 50.60%; H, 7.68%. Found: C, 50.72%; H, 7.88%.

**Trisodium salt of Propyl 4-*O*-sulfonato- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-4-*O*-sulfonato- $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 3)-4-*O*-sulfonato- $\alpha$ -L-fucopyranoside (2).** Debenzylation and debenzoylation of **7** (28 mg, 0.024 mmol) via the same procedure as described for **1** gave amorphous **2** (15 mg, 78%):  $[\alpha]_D - 150^\circ$  (*c* 0.5, H<sub>2</sub>O). The <sup>1</sup>H and <sup>13</sup>C NMR data for **2** are presented in Tables 3–5.

Anal. Calcd for C<sub>21</sub>H<sub>35</sub>Na<sub>3</sub>O<sub>22</sub>S<sub>3</sub> (804.66): C, 31.35%; H, 4.38%. Found: C, 31.23%; H, 4.48%.

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