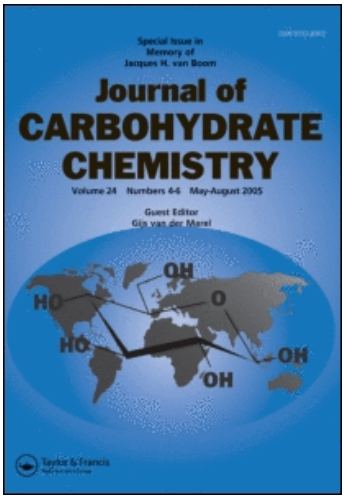

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Synthesis, NMR, and Conformational Studies of Fucoidan Fragments. VII.1 Influence of Length and 2,3-Branching on the Conformational Behavior of Linear (1→3)-Linked Oligofucoside Chains

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Synthesis, NMR, and Conformational Studies of Fucoidan Fragments. VII.[1] Influence of Length and 2,3-Branching on the Conformational Behavior of Linear (1 → 3)-Linked Oligofucoside Chains

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The conformational behavior of linear (1 → 3)-linked propyl di-, tri-, tetrafucosides and 2,3-branched tetrafucosides with linear (1 → 3)-linked trisaccharide backbone related to fragments of natural fucoidans were studied by theoretical molecular modeling and experimental determination of transglycosidic vicinal coupling constants $^{3}J_{C,H}$. The application of NOE NMR-spectroscopy, which is traditionally used in conformational analysis of oligosaccharides, was accompanied by experimental difficulties in the case of tetrafucosides, due to the overlap of cross-peaks and their trend to be close to zero. It was shown that conformations of difucoside units in the studied compounds

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depend on their position within the oligosaccharide backbone, on the chain length, and on the presence or absence of 2,3-branch point. The comparison of experimental and calculated values of transglycosidic constants $^3J_{C,H}$ showed good coincidence for the middle disaccharide units of tetrafucosides, indicating that these units are more rigid than terminal ones.

Keywords Fucoidan Fragments, NMR, Transglycosidic coupling constants, NOE, Conformational Analysis

INTRODUCTION

Fucoidans are a group of highly sulfated polysaccharides of brown seaweeds and echinoderms. They are characterized by different types of physiologic activities, including anticoagulant, antiviral, anti-inflammatory, and others (for a review see reference 2). These properties could be explained by the ability of fucoidans to mimic the carbohydrate ligands of cell receptors. Complete structural characterization of fucoidans could not generally be performed due to their irregularity and heterogeneity. To understand the mechanism of mimicry and to assess pharmacophores in fucoidan chains, we performed NMR and theoretical conformational analyses of their synthetic fragments. In this paper we report the conformational analysis of linear $(1 \rightarrow 3)$-linked propyl tetrafucoside **1**, its 2,3-branched isomer **2**, and their di- and trifucoside fragments **3** and **4**. The conformational behavior of di- and trifucoside **3**[4] and **4**[5] by using transient NOE values from 2D NOESY spectra and data of molecular mechanics calculations was studied previously.[2–5] The use of the same procedure for conformational analysis of tetrafucosides **1** and **2** was problematic because of the trend of transient NOE values to be close to zero and to overlap. Here we describe the use of long-range coupling constants $^3J_{C,H}$ for the conformational analysis of compounds **1** and **2** and their fragments **3**[4] and **4**.[5] The synthesis of tetrafucosides **1** and **2** will be described elsewhere.

RESULTS AND DISCUSSION

^{1}H-, ^{13}C-, and NOE NMR Studies of Compounds 1–4.

Tables 1 and 2 represent the ^{1}H-NMR and ^{13}C-NMR chemical shifts for tetrafucosides **1** and **2** and earlier synthesized di- **3**[4] and trifucoside **4**.[5] Assignments of chemical shifts in the ^{1}H-NMR spectra (Table 1) of tetrafucosides **1** and **2** were made using a combination of 2D ^{1}H–^{1}H COSY and TOCSY

experiments, while in the ^{13}C NMR spectra (Table 2) by using 2D $^{1}H-^{13}C$ HSQC and J-HMBC ones.

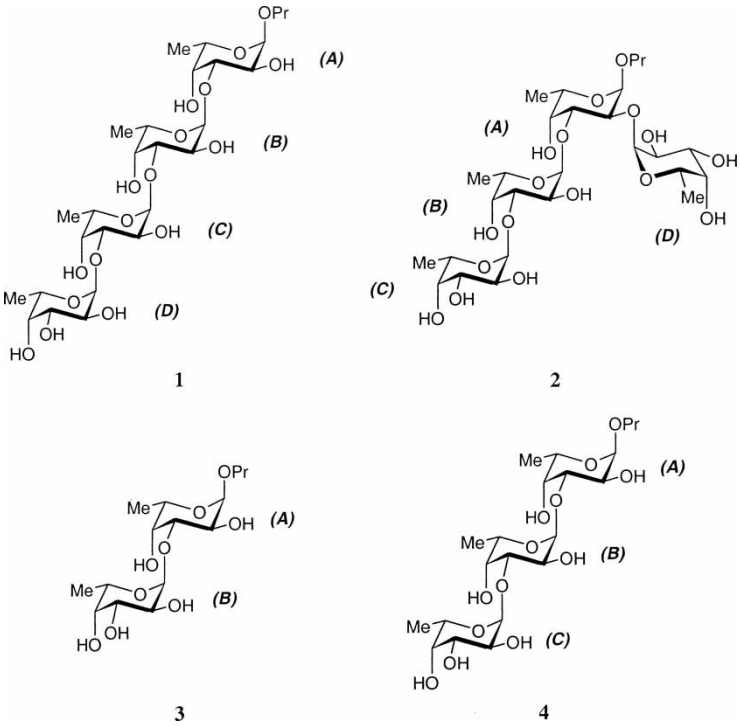

Table 1: ^{1}H-NMR chemical shifts[a] of oligosaccharides **1–4**.

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6
1	α-L-Fuc-OPr	4.92	3.93	3.95	4.04	4.08	1.23
	→ 3)-α-L-Fuc-(1 →	5.11	3.95	4.02	4.05	4.29	1.23
	→ 3)-α-L-Fuc-(1 →	5.10	3.93	3.99	4.03	4.34	1.21
	α-L-Fuc-(1 →	5.07	3.81	3.96	3.83	4.32	1.21
2	α-L-Fuc-OPr	5.19	4.05	4.11	4.10	4.09	1.26
	→ 3)-α-L-Fuc-(1 →	5.17	3.96	3.83	4.03	4.17	1.27
	α-L-Fuc-(1 →	5.00	3.80	3.93	3.82	4.35	1.19
	α-L-Fuc-(1 → 2	5.09	3.79	3.73	3.81	4.12	1.28
3[4]	→ 3)-α-L-Fuc-OPr	4.91	3.91	3.91	4.02	4.08	1.27
	α-L-Fuc-(1 →	5.07	3.81	3.95	3.82	4.28	1.21
4[5]	→ 3)-α-L-Fuc-OPr	4.92	3.93	3.96	4.03	4.08	1.23
	→ 3)-α-L-Fuc-(1 →	5.10	3.93	4.01	4.02	4.28	1.23
	α-L-Fuc-(1 →	5.06	3.81	3.97	3.83	4.32	1.23

[a]In ppm, recorded at 30°C in D_2O with acetone as an internal standard. Signals of the aglycon: $OCH_2CH_2C\underline{H}_3$ δ 0.92; $OCH_2C\underline{H}_2CH_3$ 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 **1–4**.

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6
1	α-L-Fuc-OPr	99.4	67.5[b]	75.6	69.2[b]	67.4	16.6
	→ 3)-α-L-Fuc-(1 →	96.2	67.6[b]	76.0	69.7[b]	67.8	16.5
	→ 3)-α-L-Fuc-(1 →	96.9	67.7[b]	76.1	69.7[b]	67.8	16.6
	α-L-Fuc-(1 →	96.8	69.3	70.7	73.1	68.2	16.6
2	α-L-Fuc-OPr	96.1	70.1	73.2	68.8	67.2	16.6[b]
	→ 3)-α-L-Fuc-(1 →	95.6	67.4	76.2	69.6	68.1	16.6[b]
	α-L-Fuc-(1 →	97.1	69.3[b]	70.8	72.9	68.0	16.7[b]
	α-L-Fuc-(1 → 2	96.2	69.1[b]	71.1	72.9	68.4	16.8[b]
3[4]	→ 3)-α-L-Fuc-OPr	99.5	67.6	75.8	69.3	67.5	16.5
	α-L-Fuc-(1 →	96.4	69.2	70.7	73.1	68.1	16.5
4[5]	→ 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

[a]In ppm, recorded at 30°C in D_2O with acetone as an internal standard. Signals of the propyl aglycon: $OCH_2CH_2CH_3$ δ11.1; $OCH_2CH_2CH_3$ δ 23.2–23.3; $OCH_2CH_2CH_3$ δ 71.3.
[b]The assignments may be reversed.

In the previous studies[1,5] we used the 2D transient NOESY technique with a mixing time of 500 ms for the experimental conformational analysis. In the case of tetrafucosides **1** and **2** the 2D NOESY spectra were obtained in the same format but the measured NOE values were practically inapplicable for conformational analysis. Particularly, the spectrum of tetrafucoside **1** had zero values of NOEs while in the case of di- and trifucosides[1,5] they were measurable (Table 3). Such a difference could be explained by the increase of correlation time τ_c for compound **1** that decreases the contribution of double quantum transients W_2 in the cross-relaxation rates of protons $\sigma = W_2 - W_0$[6] for the used magnetic field $\omega_0 = 500$ MHz, and reduce the values of NOEs to zero.

The 2D NOESY spectrum of branched tetrafucoside **2**, unlike that of its linear isomer **1**, contained inter- and intraunit proton correlations with positive NOEs (Table 3), but many of them overlapped and could not be integrated separately (Table 3).

Another method for the measurement of NOE values is the group of rotating frame experiments such as 2D ROESY, but its use does not eliminate the overlap of the signals for tetrafucosides **1** and **2**. This circumstance necessitated the use of another method for the experimental conformational analysis of oligosaccharide fucoidan fragments. Here we investigated the approach based on the analysis of the values of vicinal coupling constants $^3J_{C,H}$.

The value of three-bond coupling constant $^3J_{C,H}$ for the chemical fragment C–O–C–H in carbohydrates is determined by the values of the corresponding dihedral angle θ and is defined by Karplus Equation (1)[7] being illustrated in Fig. 1.

Table 3: Experimental relative integral values of cross-peaks in 2D NOESY spectra of compounds **1**–**4** and calculated relative transient NOE[a] values (in parenthesis).

	Units and linkage type (X[b] *and* Y[b])	NOE values				
		H-2^X/H-1^X	**H-3^Y/H-1^X**	**H-4^Y/H-1^X**	**H-2^Y/H-1^X**	**H-1^Y/H-1^X**
1	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	100 (120)	–[c] (100)	–[c] (180)		
	α-L-Fuc-(1 → 3)-α-L-Fuc	434[d]	–[c] (100)	–[c] (180)		
	α-L-Fuc-(1 → 3)-α-L-Fuc	(120 + 120)	–[c] (100)	–[c] (180)		
2	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	71 (115)	212[e] (100 + 170)			
	α-L-Fuc-(1 → 3)-α-L-Fuc	209[f] (130 + 100)		100 (150)		
	α-L-Fuc-(1 → 2)-α-L-Fuc-OPr	145 (130)			95 (70)	–[c] (100)
3[(4)]	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	125 (102)	100 (100)	109 (92)		
4[(5)]	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	214[f] (120 + 100)		143 (155)		
	α-L-Fuc-(1 → 3)-α-L-Fuc	119 (110)	100 (100)	143 (160)		

[a]Theoretical NOEs calculated over all conformations lying within 10% from the global energy minimum.
[b]Indexes X and Y correspond to the protons of glycosylating and glycosylated fucosyl units, respectively.
[c]The corresponding cross-peak was not observed in the 2D NOESY spectra.
[d]Measured for the sum of cross-peaks H-2^B/H-1^A + H-2^C/H-1^B.
[e]Measured for the sum of cross-peaks H-3^Y/H-1^X and H-4^Y/H-1^X.
[f]Measured for the sum of cross-peaks H-2^X/H-1^X and H-3^Y/H-1^X.

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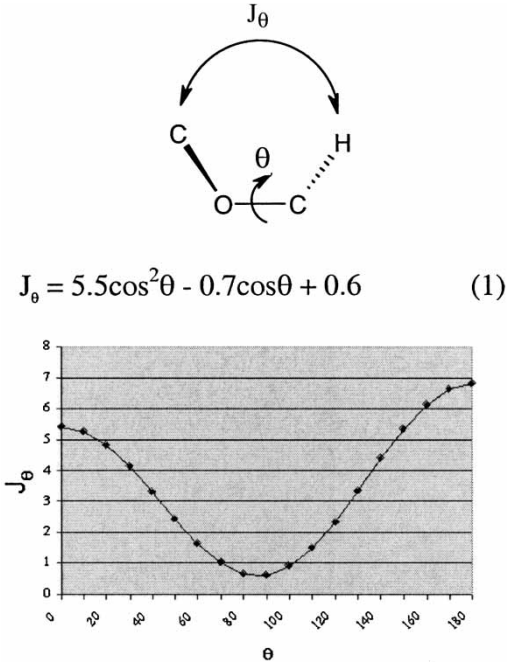


Figure 1: The equation and graph of the Karplus function.

The conformation of the disaccharide unit is determined by the values of dihedral angles φ and ψ at the interunit linkage (Fig. 2). Thus, each conformation of the disaccharide unit can be characterized by two corresponding constants, J_φ and J_Ψ (Fig. 2). Their analysis permits the study the conformational state of a interunit linkage.

Several different NMR experiments have been suggested for the measurement of long-range coupling constants $J_{C,H}$: E.COSY-type experiments like X-filtered NOESY[8,9] and X-filtered TOCSY,[10] selective 2D J-resolved,[11,12] quantitative HMBC,[13] and 2D J-HMBC[14,15] experiments. The 2D J-HMBC

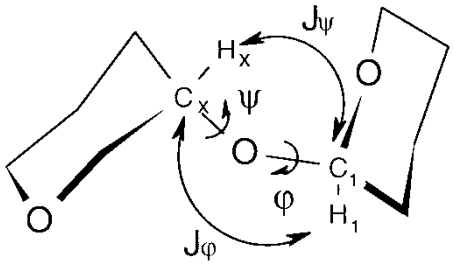


Figure 2: The dihedral angles φ and Ψ and corresponding couplin constants J_φ and J_Ψ at the interunit linkage.

protocol by Sørensen and co-workers[16,17] in the constant-time version with low-pass J-filter was employed first as one of the most powerful techniques for the detection of proton-carbon long-range couplings. In this case the 2D J-HMBC spectrum is in the same format as simple HMBC but the cross-peaks, which correspond to proton-carbon long-range interactions, are split into doublets with the appropriate heteronuclear constants $J_{C,H}$ along ^{13}C-axis (F1-dimension). The values of splits observed in the spectrum are the upscaled corresponding coupling constants $J_{C,H}$. The coefficient of proportionality **k** is chosen so as to increase the values of splits and facilitate the determination of constants, as well as to avoid the overlapping of the neighboring cross-peaks. Thus, obtained 2D J-HMBC spectra of oligofucosides **1–4** have higher resolution than the corresponding 2D NOESY spectra, due to the larger range of chemical shifts in ^{13}C-NMR spectra in comparison with the range of ^{1}H-NMR spectra that mapped the F1-dimension of corresponding 2D spectra.

The values of J_{ψ} constants were successfully determined for all interunit linkages of oligofucosides **1–4** using the J-HMBC experiments. On the contrary, the registration of several J_{φ} constants for (1 → 3)-linkages was complicated due to the overlap of the cross-peaks corresponding to intra- and interunit interactions. Particularly, the overlap of the cross-peaks of H-1^{B}/C-3^{A} and H-1^{B}/C-3^{B} in the case of trifucoside **4** (Fig. 3) made impossible the precise measurement of J_{φ} between sugar units A and B. In the case of the (1 → 2)-linkage in compound **2**, the J_{φ} constant was unresolved from the noise. We used the 2D J-resolved method[14,15] in addition to J-HMBC one to overcome this problem.

The signals of protons H-1 of the studied α-oligofucosides were always well separated from the signals of other ring protons in ^{1}H-NMR spectra, and the 2D J-resolved spectroscopy could be used for the measurement of missing constants J_{φ}. Two different approaches[15] are used for 2D J-resolved experiments: direct heteronucleus-detected and inversion proton-detected. We used direct carbon-detected acquisition, because the resolution of ^{13}C-NMR spectra of oligofucosides was higher.

The applicability of 2D J-resolved spectroscopy may be illustrated with the example of tetrafucoside **1**. In its ^{1}H-NMR spectrum, H-1^{B} and H-1^{C} signals partly overlapped (Fig. 4), making impossible their selective irradiation. Both protons were excited in the 2D J-resolved experiment, and thus the cross-peaks of carbon atoms, which coupled with protons H-1^{B} and/or H-1^{C}, were split into multiplets in the 2D J-resolved spectrum. The cross-peak of carbon C-3^{A} of the reducing unit in the 2D J-resolved spectrum was split into a doublet with interunit constant $J_{\varphi} = 3.4$ Hz along the F1-dimension, while the cross-peak of carbon signal C-3^{B} was split into well-resolved doublet of doublets with J-constants of 3.9 and 5.3 Hz, because both preirradiated protons had coupling constants with carbon C-3^{B}. To determine which of these constants

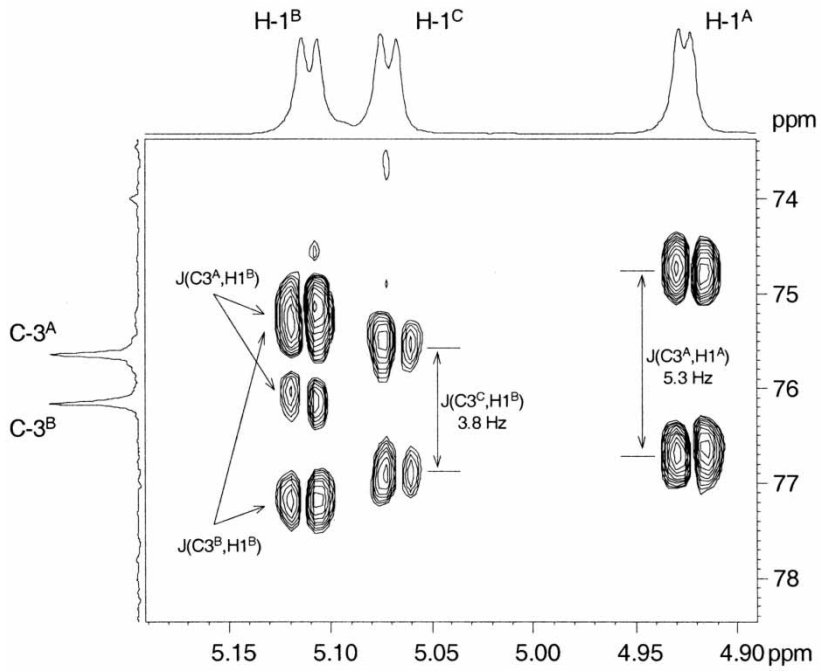


Figure 3: The fragment of J-HMBC spectrum of trifucoside **4** reflecting the interactions of protons H-1 and carbons C-3.

was the interunit one (J_{φ}), we used the fact that intraunit constants changed only slightly within the fucopyranose rings. The analysis of 2D J-HMBC spectra of compounds **1–4** showed that for intraunit cross-peaks H-1/C-3, which were not overlapped with interunit cross-peaks H-1/C-3 (for example, cross-peak H-1^A/C-3^A in 2D J-HMBC spectrum of trifucoside **4**; Fig. 3), the corresponding intraunit constants $J_{H1,C3}$ were about 5.2–5.4 Hz. This lead to the conclusion that the value J = 3.9 Hz in the case of carbon C-3^B in the 2D J-resolved spectrum of tetrafucoside **1** belonged to the interunit constant J_{φ} (Table 4).

The dihedral angle around the (C-1)–O bond in fragments (H-1)–(C-1)–O–(C-5) of fucopyranose rings in all compounds is close to 180° and gives characteristic large values of constant $J_{H1,C5}$ of 6–7 Hz in accordance to Karplus Equation (1). These constants are well resolved in J-HMBC and J-resolved spectra and could therefore be used for the estimation of the experimental deviation of the measured constants. It was found to be less than 0.5 Hz.

The Molecular Modeling of Compounds 1 and 2

Conformational maps of the studied saccharides were constructed using the MM3 force field and the grid search method as described previously.[1,4,5]

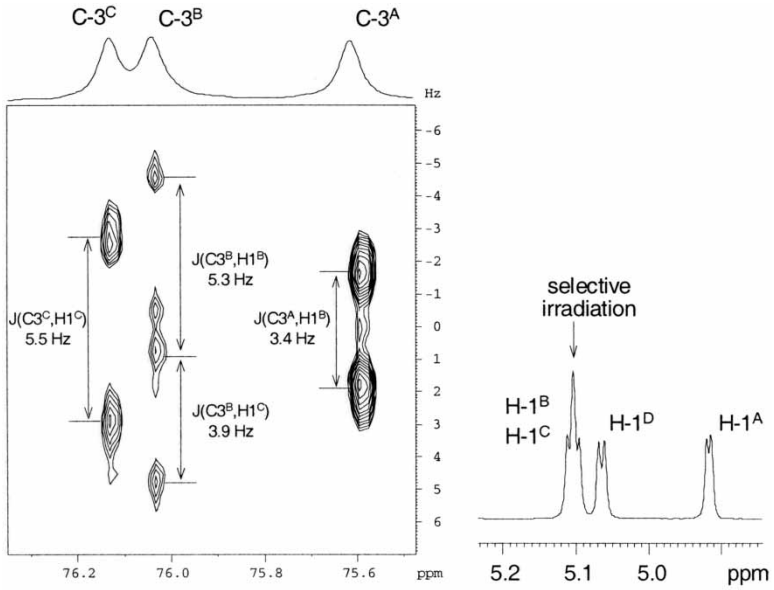


Figure 4: The fragment of 2D J-resolved spectra of tetrafucoside **1** (left) containing the correlations of glycosylated C-3 carbons, which was obtained on irradiation of NMR frequency corresponding to resonances of H-1^B and H-1^C proton signals (right).

The step value used during scanning of both φ and Ψ directions was 10°, and theoretical NOEs were calculated based on the resulting points. As could be seen from Table 4, the differences in $^3J_{C,H}$ constants for different compounds often corresponded to the differences in angles smaller than 10°. Therefore, the regions of the conformational maps containing principal minima of the studied compounds ($\varphi = 0^\circ \div 70^\circ$ and $\Psi = -80^\circ \div 80^\circ$) were rescanned with the step of 4°, and theoretical values of $^3J_{C,H}$ were computed using these points. 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 a procedure analogous to averaging of NOE as described in the recent work.[4] The calculations for the (1 → 2)-linkage in tetrafucoside **2** were carried out using the MM3 force field. Its applicability for the investigation of this linkage type in the presence of vicinal branching was shown in recent work.[1]

We reported previously[4] that an α-(1 → 3)-fucoside fragment may adopt two dominating conformations, namely **I** and **II** (Fig. 5), in which proton H-1 of the glycosylating unit is in spatial proximity with protons H-4 and H-3 of the glycosylated unit. It was also shown that the statistical weight of conformation **A** increased from di- to trisaccharide.[4,5]

The conformational maps for interunit linkages for the tetrafucoside **1** are presented in Fig. 6 (a–c). Each of these maps contains a single minimum

Table 4: Experimental and calculated (in parenthesis) values of J_φ and J_ψ and corresponding averaged values of angles φ and ψ (calculated from the experimental values of constants) of compounds **1–4**.

	Linkage	J_φ, Hz	J_ψ, Hz	φ	ψ
1	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.4[a] (3.6)	1.7 (3.4)	39°	59°
	α-L-Fuc-(1 → 3)-α-L-Fuc	3.9[a] (3.6)	2.7[b] (3.1)	33°	47°
	α-L-Fuc-(1 → 3)-α-L-Fuc	3.8 (3.3)	4.3 (3.1)	34°	28°
2	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.1[a] (3.0)	3.1 (2.8)	42°	42°
	α-L-Fuc-(1 → 3)-α-L-Fuc	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°
3	α-L-Fuc-(1 → 3)-α-L-Fuc-OPr	3.5 (3.6)	2.6 (3.4)	38°	48°
4	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	3.7 (3.3)	4.3 (3.1)	35°	28°

[a]The values of constants were measured by J-resolved experiment.
[b]Error may be greater than 0.5 Hz due to the overlap of the neighboring peaks.

centered on the point (40°; 40°). Thus, according to the calculations, each linkage in tetrafucoside **1** exists mainly in conformation **I**. The statistical weight of conformers where at least one linkage adopts conformation **II** is negligible in tetrafucoside **1**.

The conformational maps for the interunit linkages of 2,3-branched tetrafucoside **2** are presented in Fig. 6 (d–f). The maps for (1 → 3)-linkages in this compound (Fig. 6 d, e) are similar to those of tetrasaccharide **1** and contain only one minimum corresponding to conformation **I**. The conformational map for (1 → 2)-linkage of tetrafucoside **2** (Fig. 6) contains two minima, with the

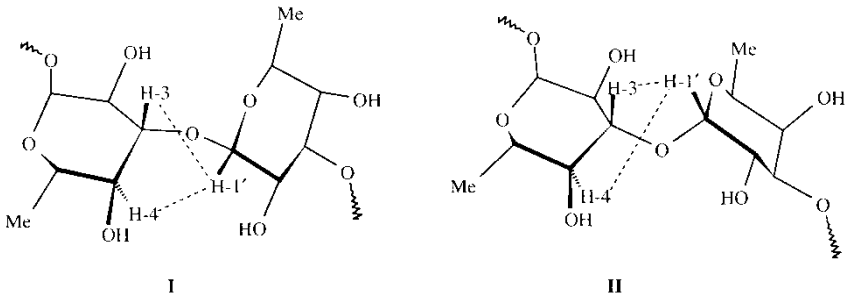


Figure 5: Conformations **I** and **II** around the interunit linkage in α-(1 → 3)-fucobiosides.[4,5]

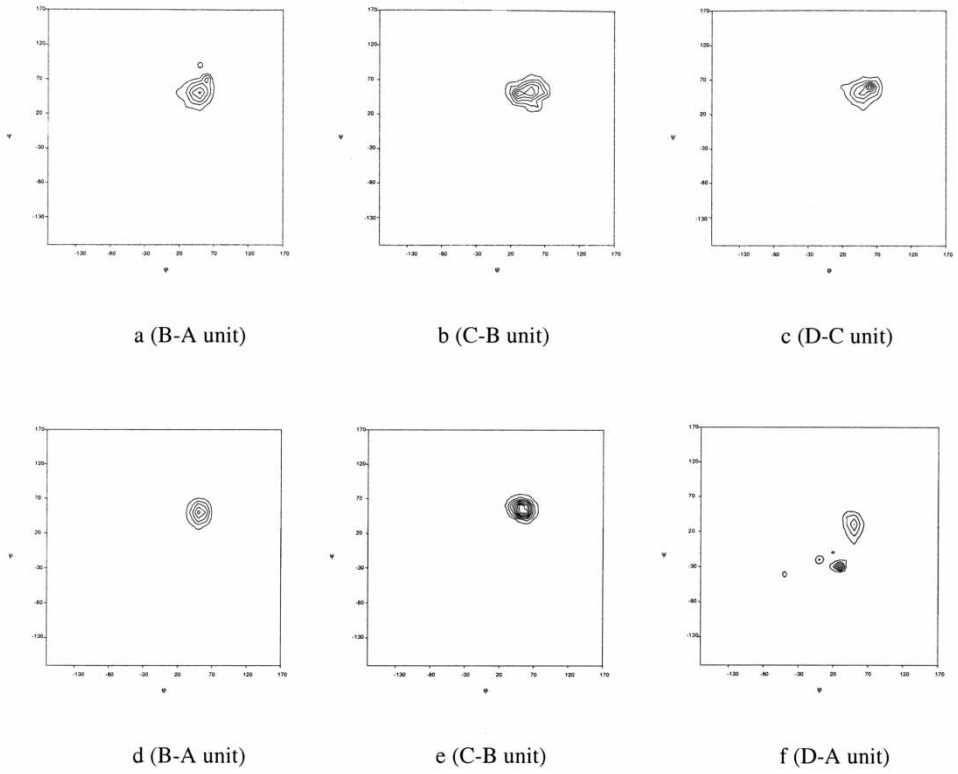
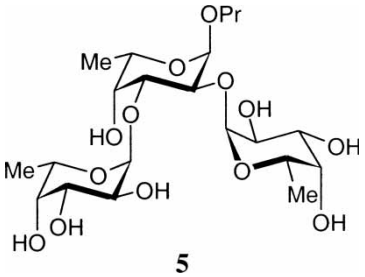


Figure 6: Conformation maps for the α-(1 $\rightarrow$ 3)-linkages in tetrafucosides **1** (a, b, c) and **2** (d, e) and for the α-(1 $\rightarrow$ 2)-linkage in tetrafucoside **2** (f).

principal one centered around the point (30°; −40°) that is similar to the conformational behavior of (1 $\rightarrow$ 2)-linkage in 2,3-branched propyl trifucoside **5**.[1]



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Analysis of NOE and $^3J_{C,H}$ Values for Compounds 1–4

The values of transient NOEs for difucoside **3** and trifucoside **4** were calculated in previous work[4,5] and are shown in Table 3. The calculations for tetrafucosides **1** and **2** were performed in the same manner, taking into account the points in conformational maps with energies lying within 10% of the global minima.

According to the calculation results, the NOE values for tetrafucoside **1** (Table 3) on protons H-4 are 1.8 times greater than those on protons H-3 for all three linkages, while in the case of difucoside **3** and trifucoside **4**, the calculated ratios were 0.9 and 1.6, respectively (Table 3). The comparison of the calculated NOE values for linear oligofucosides **1**, **3**, and **4** (Table 3) showed that the elongation of $(1 \rightarrow 3)$-linked chain from di- to tetrafucoside is accompanied by the increase of NOE values on proton H-4 with respect to proton H-3 of the glycosylated unit. This trend correlates well with the increase of the statistical weight for the conformer with positive angle Ψ (conformer **A**). The same tendency was observed for the $(1 \rightarrow 3)$-chain of branched tetrafucoside **2**.

In contrast to theoretical NOE values, the calculated constants J_{φ} and J_{Ψ} changed very slightly for the same linkages in the row of linear $(1 \rightarrow 3)$-linked difucoside **3**, trifucoside **4**, and tetrafucoside **1**. Their averaged values are 3.5 and 3.3 Hz for J_{φ} and J_{Ψ} accordingly. The differences in behavior of NOE and J values could be explained by the even character of Karplus function (1); that is, the conformers with the same absolute values of dihedral angles but with different signs have the same values of constants J. Conformers **I** $(40^{\circ}; 40^{\circ})$ and **II** $(40^{\circ}; -40^{\circ})$ (Fig. 5) in linear oligofucosides differ only in the sign of angle Ψ, and hence, they the have same values of J_{Ψ}. Thus, difucoside **3**, trifucoside **4**, and tetrafucoside **1** have very close calculated values for constants J_{φ} and J_{Ψ}. However, the values of J_{Ψ} for the disaccharide unit between rings A and B (Table 4) are slightly bigger. This can be explained by the influence of the propyl aglycon moiety (it was taken into account in the calculations) on the conformation of the corresponding interunit linkages.

Experimental values of J_{φ} constants are almost the same for all $(1 \rightarrow 3)$-linkages in linear difucoside **3**, trifucoside **4**, and tetrafucoside **1**. The differences between experimental and calculated values in this case were equal or less than 0.5 Hz. In contrast to constants J_{φ}, the J_{Ψ} ones appear to be dependent on the length of the $(1 \rightarrow 3)$-linkage chain and position of this linkage in the chain. Thus, for tetrafucoside **1** and trifucoside **4**, the values of constants J_{Ψ} decrease for interunit linkages in the order from nonreducing to reducing ends.

The observed differences between experimental and calculated values J_{Ψ} show that they are overestimated in the calculation for interunit linkages on the reducing ends of linear $(1 \rightarrow 3)$-linked oligofucosides **1**, **3**, and **4**. At the same time, they are underestimated for interunit linkages on the nonreducing ends of compounds **1** and **4**. The best coincidence of theoretical and experimental $^{3}J_{C,H}$ was obtained for the middle difucoside unit (C-B) in tetrafucoside **1**.

The introduction of the $(1 \rightarrow 2)$-linked fucosyl unit in the $(1 \rightarrow 3)$-linked trifucoside chain of tetrafucoside **2** changed the values of both constants J_{φ} and J_{Ψ} for $(1 \rightarrow 3)$-linkages, so that the differences between experimental and calculated ones became smaller. They are less than 0.3 Hz in the case of the $(1 \rightarrow 3)$-linkage on the reducing end and less than experimental deviation (0.5 Hz).

The values of calculated constants J_φ and J_Ψ for $(1 \rightarrow 2)$-linkage are close to the calculated values for the $(1 \rightarrow 3)$-linkage in 2,3-branched fragment of tetrafucoside **2**. The difference is less than 0.3 Hz (Table 4). On the other hand, the experimental constants J_φ and J_Ψ for $(1 \rightarrow 2)$-linkage are 2.2 and 2.1 accordingly, and they are 1 Hz less than the corresponding values for $(1 \rightarrow 3)$-linkage, which can be explained by the different influence of the 1-propyloxy and 4-hydroxy vicinal substituent groups on the conformational behavior of $(1 \rightarrow 2)$- and $(1 \rightarrow 3)$-linkages, accordingly. Thus, the best coincidence of calculated and experimental values was obtained for the middle difucoside unit (B-A) of tetrafucoside **2**, as in the case of tetrafucoside **1**.

CONCLUSION

The possibility of the application of vicinal coupling constants $^3J_{C,H}$ to the conformational analysis of $(1 \rightarrow 3)$-linked oligofucosides was investigated. Both the experimental and the calculated values of J_φ change very slightly for the different linkages in the studied oligosaccharides. The obtained experimental data revealed the dependence of the values of J_Ψ on the length of the $(1 \rightarrow 3)$-linked chain and the position of the transglycosidic linkage in the chain. Good coincidence of experimental and calculated values of J_Ψ constants was obtained for the middle disaccharide units of tetrafucosides **1** and **2**, which suggests that these units are more rigid, and thus, molecular mechanics calculations predict better their behavior. To verify this expectation, the study of larger oligofucosides is started.

EXPERIMENTAL

The NMR spectra for oligofucosides **1–4** (10–15 mg) were recorded in D_2O (99.98% D, Merck; 0.5 mL) solutions at 303 K on a Bruker spectrometer DRX-500 with 0.05% acetone as reference (^{1}H 2.225 ppm; ^{13}C 31.45 ppm). Microtube (Shigemi, Inc.) was used for sensitivity enhancement in the case of tetrafucosides **1** and **2**. The resonance assignment in ^{1}H- and ^{13}C-NMR spectra was performed by gradient enhanced 2D gCOSY, gNOESY, gHSQC, and gJ-HMBC experiments as well as TOCSY experiments.

Experimental NOEs were measured using a field gradient–enhanced 2D gNOESY technique in D_2O solutions at 303 K, mixing time 500 ms, relaxation delay 5 s. A sinusoidal field gradient of 1 ms length and a recovery time of 1 ms were used. The processing was performed with $\pi/2$ shifted sine-square function in both dimensions.

Experimental $^3J_{C,H}$ constants were measured using J-HMBC and J-resolved techniques. The 2D J-HMBC experiment was performed in the constant-time version.[16] The spectral widths were about 1000 ($\pm$50) Hz for the ^{1}H region and 4700 Hz for the ^{13}C region 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 60–80 t_1 increments and 500–700 scans per increment. Collected during the acquisition time t_2 were 512 points. The HMBC preparation delay Δ for the reliable measurement of a coupling constant should be taken at least 60% of inversion values of smallest coupling of interest ($\Delta = 0.6/J^{min}_{C,H}$).[17] Smaller values of Δ lead to the overestimation of J because of the antiphase character of the peaks. $\Delta = 375$ ms was used that corresponds to $J^{min}_{C,H} = 1.6$ Hz. The upscaling coefficient **k** was 40–60. The relaxation delay was 1 s. Thus, resulting acquisition time was 10–15 hr. The third order low-pass J-filter[17] was made on suppresion 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 that corresponds to resolution 5–6 Hz, 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 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 500–740 scans per increment. Collected during the acquisition time t_2 were 2048 points, 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 12–15 hr. A Gauss shaped pulse was used; its duration τ was 20–30 ms corresponding to the required selectivity of 50–30 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 with no window function in the J dimension.

Computations were performed using TINKER software package with the implemented MM3 force field. The dielectric constant ε was set to 81. 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 a force constant of 10 kcal/deg^2 before the optimization.

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