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Focus Article, *Mendeleev Commun.*, 2007, **17**, 57–62
Mendeleev Communications

Modeling of polysaccharides with oligosaccharides: how large should the model be?

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DOI: 10.1016/j.mencom.2007.03.001

Polysaccharides represent an important class of biopolymers. Advance in their investigation depends considerably on the adequate modeling of their structures by using appropriate oligosaccharides. Herein, we discuss the role of the length of oligosaccharide models used in predicting polysaccharide properties, in particular, their conformational and spectral characteristics.

The usual approach for predicting conformational, spectral and other characteristics of polysaccharides is based on the analysis of data for corresponding simple oligosaccharide fragments.[1–4] Such a practice is uncertain because there is no rule for the selection of an adequate length for oligosaccharide models. Clarification of this question is generally complicated by the low availability of large oligosaccharides of distinct structure. In our investigation of synthesis, spectral (NMR) and conformational properties of fucoidan fragments,[5–10] a unique set of oligosaccharide models was obtained that gave us a rare possibility to investigate the influence of model size on the predicted conformational and spectral fucoidan characteristics.

Fucoidans are a unique class of sulfated polysaccharides of brown seaweeds and echinoderms, consisting mainly of L-fucopyranose, which are characterised by different types of physiological activities including anticoagulant, antiviral, anti-inflammatory *etc.*[11,12] Recent investigations have shown the existence of two types of backbones in algal fucoidans. The first one is comprised of (1→3)-linkages between α-L-fucopyranose units, while the second one contains alternating (1→3)- and (1→4)-linkages (Figure 1). For our study, we used data for selectively 2-O-sulfated and non-sulfated oligosaccharides **1a**–**10b**[5–8]

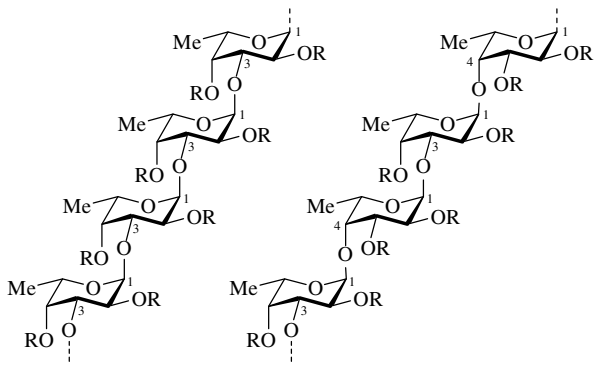

Figure 1 Two types of backbones in natural fucoidan chains, which may carry carbohydrate (R = Fuc, GlcA) and non-carbohydrate substituents (R = SO_3^-, Ac).

(Figure 2), which represent the fragments of fucoidan chains of corresponding types.

α-L-Fucopyranose rings in oligofucosides are conformationally rigid enough to be considered as $^1C_4$ in all cases.[5–7] Thus, the spatial structure of oligo- and polysaccharides is

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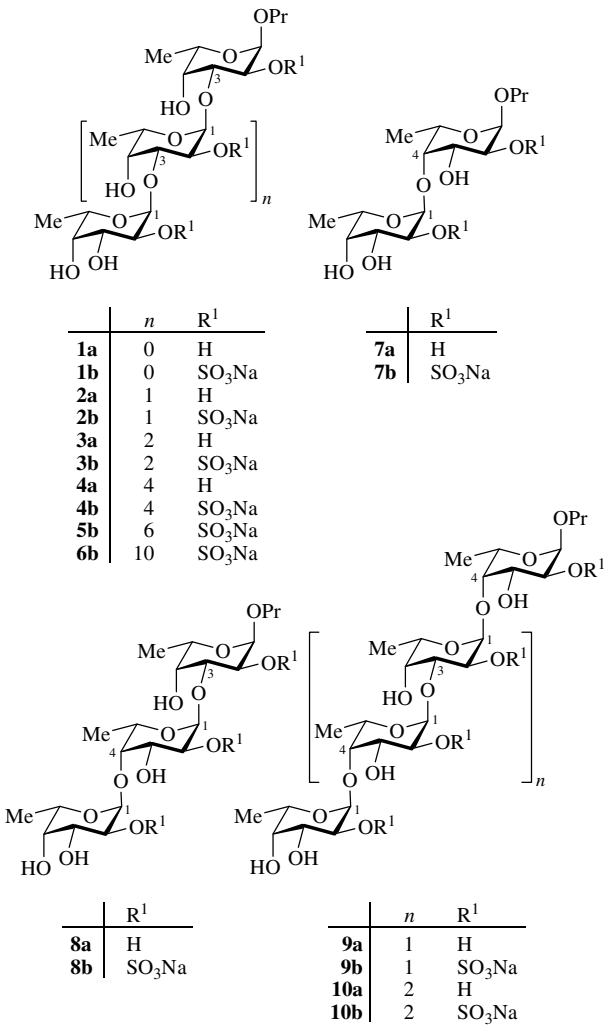
Nadezhda E. Ustyuzhanina, Alexey G. Gerbst and Alexey A. Grachev are the scientific researchers of the Laboratory of Glycoconjugate Chemistry (IOC RAS). In 2004 they were awarded the Gold Medal of Russian Academy of Science for young researchers.

Table 1 Experimental and calculated (in parentheses) values of J_φ and J_ψ for difucoside units of compounds **1a**,**b**–**4a**,**b** and **7a**,**b**–**10a**,**b**.[a]

Unit		J_φ/Hz	J_ψ/Hz		J_φ/Hz	J_ψ/Hz
α-L-Fuc-(1→3)-α-L-Fuc-OPr	**1a**	***3.5 (3.6)***	***2.6 (3.4)***	**1b**	4.1 (3.8)	4.7 (3.4)
→3)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**2a**	3.6 (3.8)	2.2 (3.5)	**2b**	4.1 (3.8)	4.9 (3.1)
α-L-Fuc-(1→3)-α-L-Fuc-(1→		3.7 (3.3)	4.3 (3.1)		***4.0 (3.6)***	***2.0 (2.9)***
→3)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**3a**	3.4 (3.6)	1.7 (3.4)	**3b**	***4.2 (3.9)***	***4.8 (4.3)***
→3)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		***3.9 (3.6)***	***2.7 (3.1)***		***5.2 (4.5)***	***2.8 (3.0)***
α-L-Fuc-(1→3)-α-L-Fuc-(1→		3.8 (3.3)	4.3 (3.1)		5.1 (4.5)	1.8 (3.0)
→3)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**4a**	5.0[c] (3.5)	2.0[c] (3.4)	**4b**[a]		
→3)-α-L-Fuc-(1→3)-α-L-Fuc-(1→[b]		***4.5***[c] ***(3.5)***	***3.6***[c] ***(3.3)***			
α-L-Fuc-(1→3)-α-L-Fuc-(1→		4.0[c] (3.5)	2.1[c] (3.4)			
α-L-Fuc-(1→4)-α-L-Fuc-OPr	**7a**	3.9 (3.7)	5.9 (4.6)	**7b**	***4.5 (3.9)***	***5.3 (4.7)***
→4)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**8a**	3.1 (3.5)	2.3 (3.7)	**8b**	***4.1 (3.9)***	***4.5 (4.2)***
α-L-Fuc-(1→4)-α-L-Fuc-(1→		3.8 (3.5)	5.9 (4.0)		***4.4 (3.8)***	***5.4 (4.8)***
→3)-α-L-Fuc-(1→4)-α-L-Fuc-OPr	**9a**	3.9 (3.8)	5.6 (4.5)	**9b**	4.9 (3.9)	5.4 (3.9)
→4)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		***3.9 (3.1)***	***3.8 (3.3)***		***3.2 (3.5)***	***4.9 (4.2)***
α-L-Fuc-(1→4)-α-L-Fuc-(1→		3.9 (3.8)	5.6 (4.4)		***4.6 (3.8)***	***5.4 (4.5)***
→3)-α-L-Fuc-(1→4)-α-L-Fuc-OPr	**10a**	—[a] (3.9)	6.0 (4.5)	**10b**	4.7 (4.3)	5.5 (4.2)
→4)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		—[a] (3.5)	***3.8 (3.4)***		***3.4 (3.5)***	***4.7 (3.8)***
→3)-α-L-Fuc-(1→4)-α-L-Fuc-(1→		—[a] (3.8)	6.0 (4.0)		***4.5 (4.3)***	***5.5 (4.5)***
→4)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		—[a] (3.6)	***3.8 (3.5)***		***3.3 (3.6)***	***4.7 (3.8)***
α-L-Fuc-(1→4)-α-L-Fuc-(1→		—[a] (4.0)	6.0 (4.2)		4.4 (4.0)	5.5 (4.2)

[a]The experimental values of $^3J_{C,H}$ constants were not determined for oligofucosides **4b**–**6b** and **10a** due to the overlap of the signals in the corresponding regions of J-HMBC and J-resolved spectrum. [b]All the internal difucoside units of the oligosaccharide. [c]The experimental error for this $^3J_{C,H}$ value was > 0.5 Hz due to the overlap of the signals in J-HMBC and J-resolved spectra.
The values in bold italics correspond to the disaccharide units for which the differences between the experimental and calculated values of J_φ and J_ψ constants do not exceed 1 Hz.



	n	R^1
1a	0	H
1b	0	SO_3Na
2a	1	H
2b	1	SO_3Na
3a	2	H
3b	2	SO_3Na
4a	4	H
4b	4	SO_3Na
5b	6	SO_3Na
6b	10	SO_3Na

	R^1
7a	H
7b	SO_3Na

	R^1
8a	H
8b	SO_3Na

	n	R^1
9a	1	H
9b	1	SO_3Na
10a	2	H
10b	2	SO_3Na

Figure 2 Model oligofucosides with homo-(1→3)-linked chains (**2**–**6**), alternating (1→3)- and (1→4)-linkages (**8**–**10**) and corresponding disaccharides **1** and **7**.

dependent mainly on the dihedral angles φ and ψ around the bridges between the monosaccharide units (Figure 3). The dihedral angles φ and ψ could be calculated from corresponding experimental NMR transglycosidic ^{13}C–^1H coupling constants J_φ and J_ψ according to the Karplus equation (1)[13] illustrated in Figure 4.

In our previous studies,[9,10] the coupling constants J_φ and J_ψ (Table 1) in oligosaccharides **1**–**10** were determined using 2D J-HMBC[14,15] and 2D J-resolved[16,17] experiments. An example of the measurement of J_ψ constants for compound **2a** is shown in Figure 5. These methods permit the measurements of coupling constants with a low experimental error of < 0.5 Hz,[9,10] which is acceptable for a comparison with the results of calculations according to equation (1) with an accuracy of 1 Hz.[13]

It was found that the experimental $^3J_{C,H}$ constants for (1→3)-linked difucoside units in the studied molecules critically depend on the length of the oligofucoside chain, the position of the unit within the chain and the presence of O-sulfate groups in fucose residues. On the contrary, the constants for (1→4)-linked difucoside units were significantly changed only upon sulfation (Table 1).

For each of the studied oligofucosides, the molecular mechanics calculations were carried out using MM3 force field (TINKER program suit). The conformational maps obtained for each disaccharide unit of the oligofucosides showed the dependence of energy of a molecule on the φ and ψ angles and thus characterised the broadness of conformational distribution and

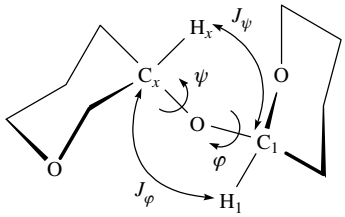


Figure 3 Dihedral angles φ and ψ describing the conformation of the linkage between monosaccharide units and influencing the corresponding NMR coupling constants J_φ and J_ψ.

Table 2 Calculated statistical weights of conformers **A**–**D** of difucoside units in oligosaccharides **1a**,**b**–**4a**[a] and **7a**,**b**–**10a**,**b**.

Disaccharide unit		A	B	C	D		A	B	C	D
α-L-Fuc-(1→3)-α-L-Fuc-OPr	**1a**	60%	40%			**1b**	55%	45%		
→3)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**2a**	80%	20%			**2b**	72%	28%		
α-L-Fuc-(1 3)-α-L-Fuc-(1→		80%	20%				69%	31%		
→3)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**3a**	100%	0%			**3b**	74%	26%		
→3)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		100%	0%				72%	28%		
α-L-Fuc-(1→3)-α-L-Fuc-(1→		100%	0%				72%	28%		
→3)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**4a**	100%	0%							
→3)-α-L-Fuc-(1→3)-α-L-Fuc-(1→[b]		100%	0%							
α-L-Fuc-(1→3)-α-L-Fuc-(1→		100%	0%							
α-L-Fuc-(1→4)-α-L-Fuc-OPr	**7a**			70%	30%	**7b**			30%	70%
→4)-α-L-Fuc-(1→3)-α-L-Fuc-OPr	**8a**	100%	0%			**8b**	100%	0%		
α-L-Fuc-(1→4)-α-L-Fuc-(1→				40%	60%				20%	80%
→3)-α-L-Fuc-(1→4)-α-L-Fuc-OPr	**9a**			40%	60%	**9b**			25%	75%
→4)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		100%	0%				100%	0%		
α-L-Fuc-(1→4)-α-L-Fuc-(1→				40%	60%				25%	75%
→3)-α-L-Fuc-(1→4)-α-L-Fuc-OPr	**10a**			93%	7%	**10b**			50%	50%
→4)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		100%	0%				100%	0%		
→3)-α-L-Fuc-(1→4)-α-L-Fuc-(1→				50%	50%				50%	50%
→4)-α-L-Fuc-(1→3)-α-L-Fuc-(1→		100%	0%				100%	0%		
α-L-Fuc-(1→4)-α-L-Fuc-(1→				50%	50%				35%	65%

[a]The molecular mechanics calculations for oligofucosides **4b**–**6b** were not performed. [b]All the internal difucoside units of the oligosaccharide.

φ and ψ angles for dominant conformers, as illustrated in Figure 6.

According to molecular mechanics calculations, the α-(1→3)-difucoside unit may adopt two dominating conformations **A** and **B**[2,3] (Figure 7) corresponding to the minima on conformational maps. In conformer **A** ($\varphi = 40°$, $\psi = 40°$), the proton H-1 of the glycosylating unit is equidistant from H-3 and H-4 protons of the glycosylated unit (Figure 7), while in conformer **B** ($\varphi = 30°$, $\psi = -40°$) the proton H-1 of the glycosylating unit is in spatial proximity with the H-3 proton of the glycosylated unit. Table 2 shows statistical weights of conformers for difucoside units calculated from the energy maps. It was found that, for α-(1→3)-difucoside units in the studied oligosaccharides, conformer **A** is dominant (Table 2). At the same time, the statistical weight of conformer **A** increases with the elongation of an oligofucoside chain.[5–7,9,10] For example, the internal α-(1→3)-difucoside units in nonsulfated tetra- and hexafucosides **3a** and **4a** exist only in conformation **A**. A similar trend was observed for 2-O-sulfated oligosaccharides **1b**–**3b**. Thus, the introduction of sulfate groups into oligofucosides changes the ratio of **A** and **B** conformers from 55:45 for difucoside **1b** to 72:28 for tetrafucoside **3b**.

Molecular mechanics calculations revealed the existence of two dominant conformers **C** and **D** (Figure 6) for (1→4)-linked difucoside units. Conformer **C** ($\varphi = 35°$, $\psi = 20°$) is characterised by an equidistant location of the proton H-1 of the glycosylating unit in relation to both H-4 and the methyl group of the glycosylated unit (Figure 6), whereas in conformer **D** ($\varphi = 20°$, $\psi = -35°$) the spatial proximity is only between the protons H-1 and H-4. Both conformers have significant statistical weights for most α-(1→4)-linked difucoside units in the studied oligofucosides (Table 2). However, the weight of conformer **D** slightly increased upon the introduction of sulfate groups in the oligosaccharides (Table 2).

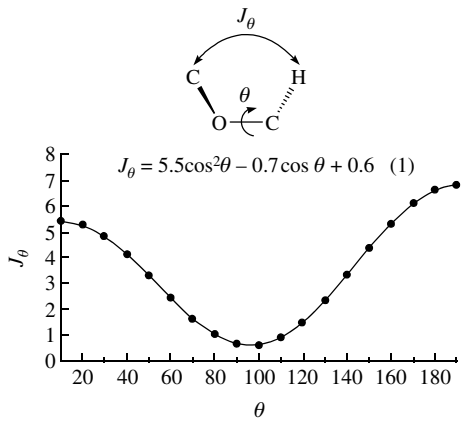


Figure 4 Karplus function[13] for the determination of the constants $^3J_{C,H}$ in the ^{13}C–O–C–^1H fragment of carbohydrates.

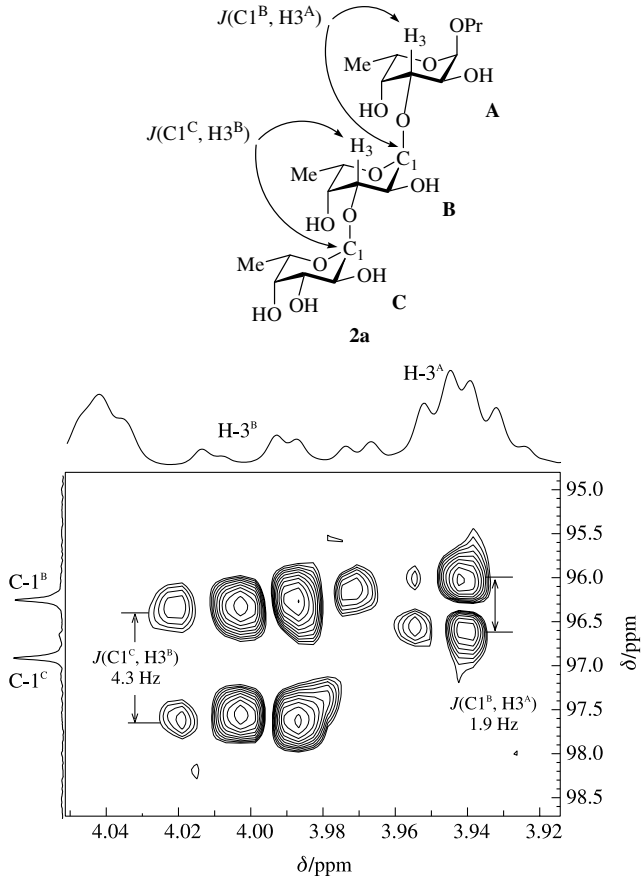


Figure 5 Fragment of the J-HMBC spectrum of trifucoside **2a** reflecting the interactions of the protons H-3 and the carbons C-1. There are two correlations on the spectrum corresponding to two inter-unit linkages of the molecule. These correlations are split into doublets along the vertical ^{13}C axis. The values of the split are proportional to the J_ψ constants.

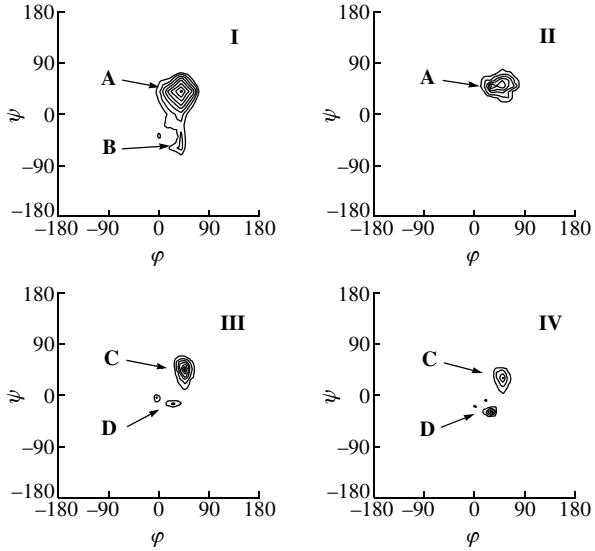


Figure 6 Regions on the conformational maps corresponding to main conformers for (1→3)-linkages in difucoside **1a** (**I**) and tetrafucoside **3a** (**II**) and (1→4)-linkages in difucoside **7a** (**III**) and tetrafucoside **9a** (**IV**). The step value used during scanning of both φ and ψ directions was 4°. For all experimental details, see refs. 5–10. **A**, **B**, **C** and **D** are the main conformers, which are shown in Figure 7.

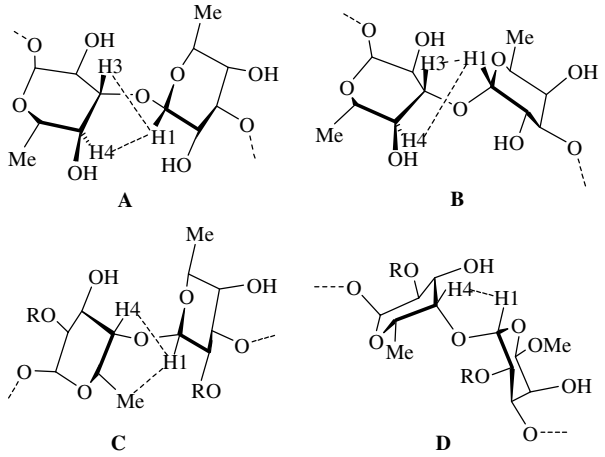


Figure 7 Conformers found by molecular mechanics calculations: **A** (φ = 40°, ψ = 40°) and **B** (φ = 30°, ψ = –40°) for α-(1→3)-difucoside units,[5,6] as well as **C** (φ = 35°, ψ = 20°) and **D** (φ = 20°, ψ = –35°) for α-(1→4)-difucoside units.

Based on data from molecular mechanics calculations, the theoretical values of transglycosidic constants J_φ and J_ψ (Table 1) were computed. For each conformer with energy lying within 10% of the global minimum of the conformational map, the constants J_φ and J_ψ were determined according to the Karplus equation (1) and subsequently averaged according to the Boltzmann distribution of conformers.[9,10]

The comparison of the experimental and calculated values of J_φ and J_ψ constants for (1→3)-linked difucoside units shows good coincidence for the internal units of all tetra- and hexa-

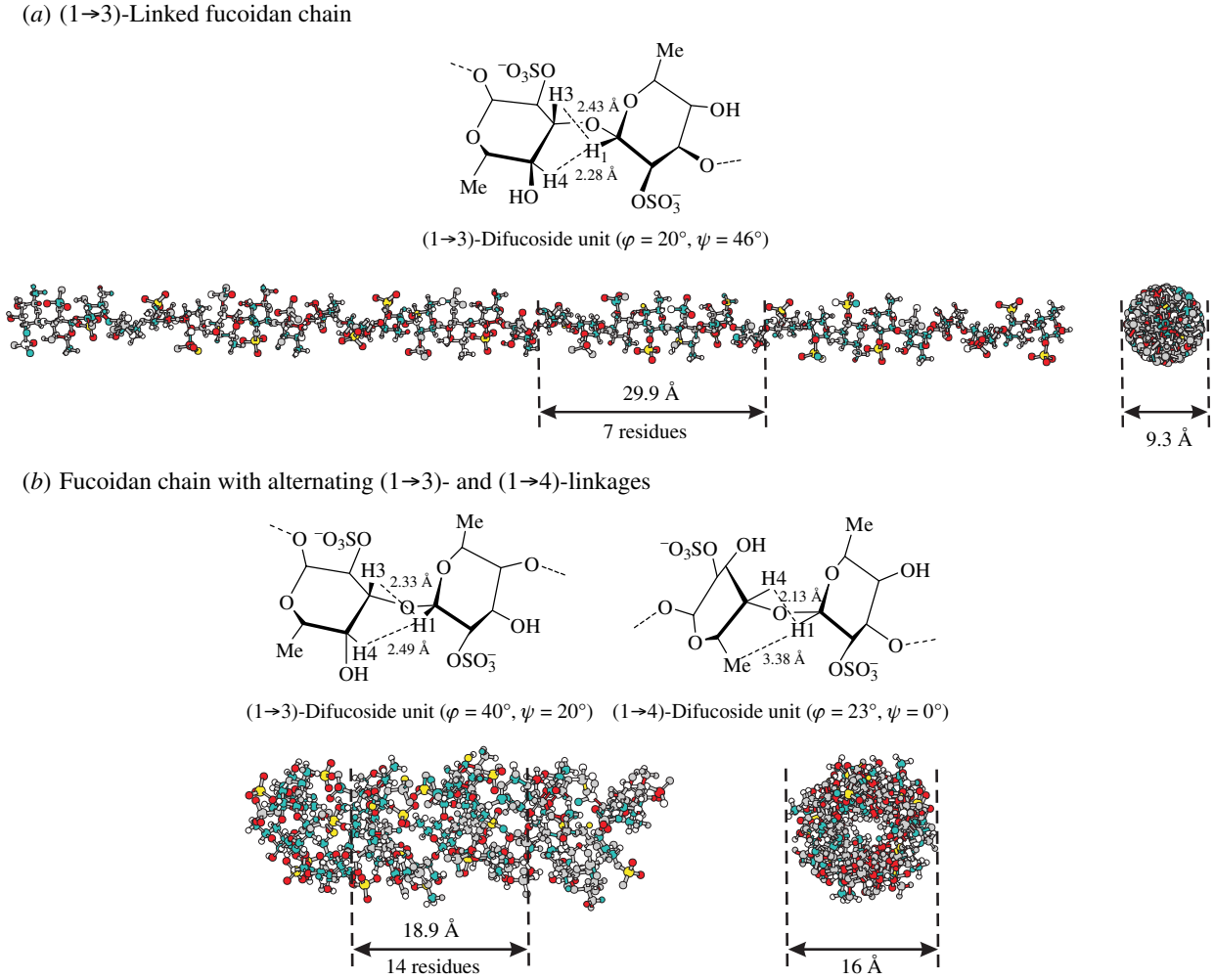


Figure 8 Conformational shapes and parameters of helices (helix translation periods and diameters) of 2-O-sulfated fucoidan chains comprising (*a*) α-(1→3)- or (*b*) alternating α-(1→3)- and α-(1→4)-linkages. These chains were constructed by joining together shown internal disaccharide units (obtained on the basis of conformational data of tetrafucosides **3b** and **9b**) with subsequent geometry optimization to remove van der Waals clashes.

fucosides and for some terminal units of all studied molecules (deviation is less than 1 Hz, Table 1). Note that, contrary to experimental J_φ and J_ψ constants, the calculated values do not depend greatly on the location of a disaccharide unit within the chains of α-(1→3)-linked tri-, tetra- and hexafucosides (Table 1). In our opinion, this discrepancy between the experimental and theoretical data results from the fact that the molecular mechanics calculations reproduce less accurately the properties of more flexible terminal disaccharide units than of more rigid internal ones.[9,10] It is noteworthy that, in the case of sulfated oligofucosides, the coincidence was observed for a larger number of (1→3)-linked difucoside units than in the case of non-sulfated structures because sulfated oligofucosides are more rigid.

In the case of (1→4)-linked difucoside units, the good coincidence between the experimental and calculated values of J_φ and J_ψ constants was obtained only for some units of conformationaly more rigid sulfated oligofucosides. At the same time, for (1→4)-linked difucoside units, the differences between experimental and calculated values for all J_φ still do not exceed 1 Hz, but for all J_ψ constants the calculated values were underestimated (Table 1). In our opinion, this results from the fact that the molecular mechanics calculations overestimate the degree of flexibility around the bond described by the angle ψ. According to calculations, the angle ψ varied from –40 to +40° (maps **III** and **IV** in Figure 6). However, the experimental values of J_ψ varied from 5.4 to 6.0 Hz (Table 1), which correspond to the maximum of the Karplus function reachable at the angle $\psi = 0°$ (Figure 4). The presence of a large amount of conformers in solution with the angles ψ exceeding 0° in absolute values causes a decrease of the experimental value of J_ψ. One could conclude that the (1→4)-linked difucoside units are characterised by the domination of conformers with $\psi = 0°$ and φ varying from 25 to 35°. We suppose that conformers with the angle $\psi = 0°$ are stabilised by solvation.

In summary, the results of the conformational analysis of oligofucosides **1**–**10** point out that the conformations of (1→3)-linked difucoside units in these compounds critically depend on the length of the oligofucoside chain, the position of the unit within the chain and the presence of sulfate groups, while the conformations of (1→4)-linked units depend mainly on the presence of sulfate groups. These results also demonstrate that the internal difucoside units of tetra- and hexafucosides are less flexible than the terminal ones. In addition, the conformational similarity of internal disaccharide units in corresponding tetra- and hexasaccharides was observed, which indicates that their internal disaccharide units are equivalent to corresponding ones in polysaccharides.

To illustrate this conclusion, we carried out the modeling of long fragments of 2-O-sulfated fucoidan chains consisting of 32 fucose units comprising only α-(1→3)-linkages or alternating α-(1→3)- and α-(1→4)-linkages based on conformational data for the internal units of tetrafucoside models **3b** and **9b** (Figure 8).

The conformational shapes of the chains have regular helical structures (Figure 8) when built up from the data for tetrasaccharide models. On the contrary, the use of more flexible disaccharide and trisaccharide models (not shown in Figure 8) leads to the lack of regularity, and the generation of disperse non-helical structures clearly showing again the critical role of the size of oligosaccharides used in polysaccharide modeling.

In addition to the above conformational studies, we also investigated the role of the length of oligosaccharide models in the analysis of ^{13}C NMR characteristics of polysaccharides. The ^{13}C NMR chemical shifts of carbon atoms near the glycosidic linkage depend on its conformation.[1–4] Therefore, if the conformations of disaccharide fragments from an oligofucoside resemble those from a fucoidan, the chemical shifts of carbon atoms in these disaccharide units must be similar.

According to the well-known method of additive schemes,[1,3] the chemical shift values of carbon atom C_i in a monosaccharide unit in a poly- or oligosaccharide chain (for example, in the fucoidan fragment shown in Figure 9) could be calculated by the following equation (2):

$$\delta C_i = \delta C_i^0 + A(k, i) + B(k', i), \quad (2)$$

where δC_i^0 is the chemical shift of atom C_i in parent fucose unit; $A(k, i)$ and $B(k', i)$ represent the spectral 'glycosylation effects' due to the formation of glycoside linkage k and k' (Figure 9) and whose values could be simply calculated from the spectra of corresponding oligosaccharide models.[1,3]

$$\overset{k}{\underline{\rightarrow 3)\text{-Fuc-}\alpha\text{-}(1\rightarrow 3)\text{-Fuc-}\alpha\text{-}(1}}\overset{k'}{\underline{\rightarrow 3)\text{-Fuc-}\alpha\text{-}(1\rightarrow}}$$

Figure 9 Fragment of a fucoidan chain and indexes k and k' of linkages used in the discussion.

We have calculated ^{13}C chemical shifts for 2-O-sulfated α-(1→3)-linked fucoidan using di-, tri-, tetra-, hexa-, octa- and dodecasaccharide models and compared the results with experimental spectral data for corresponding natural fucan sulfate from the sea urchin *Strongylocentrotus franciscanus*[18] (Table 3).

To compare the calculated and experimental chemical shifts, the root mean square deviations are calculated according to the equation

$$S = \sqrt{\frac{1}{n}\left[\sum_{i=1}^{n}(\delta C_i^{\text{exp}} - \delta C_i^{\text{calc}})^2\right]}, \quad (3)$$

where n is the number of carbon atoms in the repeating unit of a fucoidan chain. The S values also allow the estimation of the suitability of an oligosaccharide model.

Analysis of S values showed their decrease with increasing chain length of the model. Thus, the S values for tetra-, hexa-, octa- and dodecasaccharide models were close to each other and lay within the range of the admissible error of polysaccharide chemical shift determination (Table 3). However, the S values obtained with the use of di- and trisaccharide models exceeded this limit and thus should not be used to model the fucoidan chain.

Summarising the above discussion, the selection of a suitable oligosaccharide model is critical for polysaccharide investigations. In any case, the use of data for internal disaccharide units of larger oligosaccharides, preferentially of tetrasaccharides or longer ones, is necessary for the accurate prediction of polysaccharide properties.

Table 3 ^{13}C NMR chemical shifts in the spectrum[18] of 2-O-sulfated α-(1→3)-linked fucan sulfate from the sea urchin *S. franciscanus*, the differences between experimental and calculated chemical shifts ($\Delta\delta^{13}C^a$) and corresponding root-mean-square deviations from additivity[1] (S) obtained in different oligofucoside models.

Compound	C-1	C-2	C-3	C-4	C-5	C-6	S^b
Experimental chemical shifts	96.0	74.7	75.1	70.4	67.8	16.5	—
	$\Delta\delta^{13}$C/ppm						
difucoside model **1b**	0.5	0.4	0.7	0.5	0.4	0.1	0.5
trifucoside model **2b** (**A**)^c	0	0.4	0.1	0.2	0.5	0.1	0.3
trifucoside model **2b** (**B**)^c	0.5	0.4	0.8	0.3	0	0.1	0.4
tetrafucoside model **3b**^{d}	–0.2	0.4	0.1	0.1	0	0	0.2
hexafucoside model **4b**^{d}	–0.1	0.4	0.1	0	0	–0.1	0.2
octafucoside model **5b**^{d}	–0.2	0.5	0	–0.1	0.1	0.1	0.2
dodecafucoside model **6b**^{d}	–0.3	0.4	–0.1	–0.1	0.1	0	0.2

$^a\Delta\delta^{13}C_i$ is the difference between chemical shifts for corresponding i atoms in the experimental fucoidan spectrum and the shifts being calculated according to formula (2). ^{b}S-value of 0.2 ppm corresponds to admissible error in determination of chemical shifts values.[1] ^{c}Calculation from the data for difucoside unit on reducing (**A**) and non-reducing (**B**) ends of the molecule.^{d} Data for internal difucoside unit were used in calculation.

References

1 G. M. Lipkind, A. S. Shashkov, Y. A. Knirel, E. V. Vinogradov and N. K. Kochetkov, *Carbohydr. Res.*, 1988, **175**, 59.
2 F. V. Toukach and A. S. Shashkov, *Carbohydr. Res.*, 2001, **335**, 101.
3 P.-E. Jansson, L. Kenne and G. Widmalm, *Carbohydr. Res.*, 1989, **188**, 169.
4 P.-E. Jansson, R. Stenutz and G. Widmalm, *Carbohydr. Res*., 2006, **341**, 1003.
5 A. G. Gerbst, N. E. Ustuzhanina, A. A. Grachev, E. A. Khatuntseva, D. E. Tsvetkov, A. S. Shashkov, A. I. Usov and N. E. Nifantiev, *J. Carbohydr. Chem.*, 2002, **21**, 313.
6 A. G. Gerbst, N. E. Ustuzhanina, A. A. Grachev, N. S. Zlotina, E. A. Khatuntseva, D. E. Tsvetkov, A. S. Shashkov, A. I. Usov, M. E. Preobrazenskaya, N. A. Ushakova and N. E. Nifantiev, *J. Carbohydr. Chem.*, 2003, **22**, 109.
7 A. G. Gerbst, A. A. Grachev, N. E. Ustuzhanina, E. A. Khatuntseva, D. E. Tsvetkov, A. I. Usov, A. S. Shashkov, M. E. Preobrazenskaya, N. A. Ushakova and N. E. Nifantiev, *Zh. Bioorg. Khim.*, 2004, **30**, 156 (*Russ. J. Bioorg. Chem.*, 2004, **30**, 137).
8 N. Ustuzhanina, V. Krylov, A. Grachev, A. Gerbst and N. Nifantiev, *Synthesis*, 2006, 4017.
9 A. A. Grachev, A. G. Gerbst, N. E. Ustyuzhanina, E. A. Khatuntseva, A. S. Shashkov, A. I. Usov and N. E. Nifantiev, *J. Carbohydr. Chem.*, 2005, **24**, 85.
10 A. A. Grachev, A. G. Gerbst, N. E. Ustyuzhanina, A. S. Shashkov, A. I. Usov and N. E. Nifantiev, *J. Carbohydr. Chem.*, 2006, **25**, 315.
11 O. Berteau and B. Mulloy, *Glycobiology*, 2003, **13** (6), 29R–40R.
12 A. Cumashi, N. A. Ushakova, A. E. Berman, G. E. Morozevich, M. E. Preobrazhenskaya, M. I. Bilan, A. I. Usov, N. E. Ustuzhanina, A. A. Grachev, C. J. Sanderson, M. Kelly, R. Miller, A. Morrison, S. Iacobelli and N. E. Nifantiev, *Glycobiology*, 2007, in press.
13 B. Mulloy, T. A. Frenkiel and D. B. Davis, *Carbohydr. Res*., 1988, **184**, 39.
14 C. H. Gotfredsen, A. Meissner, J. Ø. Duus and O. W. Sørensen, *Magn. Reson. Chem*., 2000, **38**, 692.
15 A. Meissner and O. W. Sørensen, *Magn. Reson. Chem.*, 2001, **39**, 49.
16 A. Bax and R. Freeman, *J. Am. Chem. Soc.*, 1982, **104**, 1099.
17 M. Liu, R. D. Farant, J. M. Gillam, J. K. Nicholson and J. C. Lindon, *J. Magn. Reson., Ser. B*, 1995, **109**, 275.
18 A.-C. Vilela-Silva, A.-P. Alves, A.-P. Valente, V. D. Vacquier and P. A. S. Mourao, *Glycobiology*, 1999, **9**, 927.

Received: 13th November 2006; Com. 06/2821