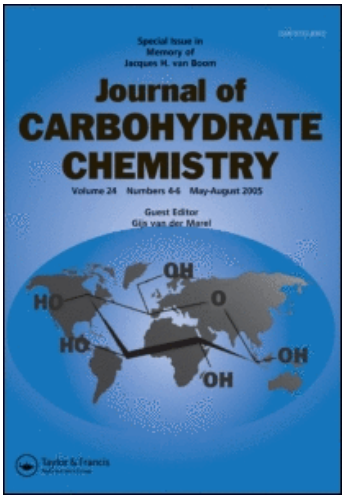

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SYNTHESIS, NMR AND CONFORMATIONAL STUDIES OF FUCOIDAN FRAGMENTS 1:[1] DESULFATED 2,3- AND 3,4-BRANCHED TRISACCHARIDE FRAGMENTS AND CONSTITUTING DISACCHARIDES

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ABSTRACT

Two fucotriosides with vicinal disubstitution α-L-Fuc-(1→2)[α-L-Fuc-(1→3)]α-L-Fuc-OPr (**1**) and α-L-Fuc-(1→3)[α-L-Fuc-(1→4)]α-L-Fuc-OPr (**2**), which are related to fragments of natural polysaccharides fucoidans, have been synthesized together with constituent disaccharides **3-5**. Spectral and conformational properties of tri- and disaccharides have been investigated by ^{1}H, ^{13}C and NOE NMR spectroscopy.

INTRODUCTION

Fucoidans are a group of highly sulfated polysaccharides consisting essentially of α-L-fucopyranose residues. During the last decade these biopolymers received increasing interest due to their various biological activities. Among them are blood-anticoagulant[2-8] (for review see ref. 9), antithrombotic,[7,10] antiangiogenic,[11] antiproliferative,[12] antitumor,[13-

[15] anti-inflammatory action,[16-19] and inhibition of cell-cell binding interactions mediated by P- and L-selectins,[18,20-22] but not E-selectin.[23] In addition, fucoidans prevent infection with envelope viruses, including HIV, herpes and cytomegalovirus.[24-27] Fucoidans also can block sperm-egg binding.[28-30]

Fucoidans were isolated from two natural sources, brown seaweeds and echinoderms (they are present in the jelly coat of sea urchin eggs and in the body wall of sea cucumbers). The structure of fucoidans obtained from several echinoderms was recently elucidated.[31-34] These polysaccharides were shown to be (1→3)- and (1→4)-linked linear polysulfated fucans with repeating tetrasaccharide blocks differing in sulfation pattern, which seems to be species-specific.

Structural features of algal fucoidans, however, are less known, though these compounds are involved in the majority of reports on biological studies. The structure for fucoidan from *Fucus vesiculosus*, proposed by Percival over 50 years ago,[35,36] contained 4-*O*-sulfated (1→2)-linked α-L-fucopyranosyl units. Recent reinvestigation[37] of this polysaccharide led to the conclusion that it is built up mostly of 4-*O*-sulfated (1→3)-linked α-L-fucopyranosyl units with occasional 2-*O*-sulfation and some branches at position 2 of the backbone. Similar structural details in different proportions were found in fucoidans isolated from *Ecklonia kurome*,[2,3] *Laminaria saccharina*,[38] *Chorda filum*,[39] *Cladosiphon okamuranus* [40] and several other brown seaweeds.[9,41] However, structural investigations of fucoidan from *Ascophyllum nodosum*[6] showed a high proportion of α-(1→4)-linkages. The presence of some branches at position 4 of the backbone cannot be excluded according to the data obtained.[3,37,39]

Determination of fucoidan structure and particularly the localization of inter-residue linkages, sulfate groups and other carbohydrate substituents is strongly hindered by irregularity and heterogeneity of its chains, as well as by the presence of minor monosaccharides (e.g., galactose, xylose, mannose, uronic acids).[35,40,41] As a result, a complete structural characterization of fucoidans could not generally be performed. Nevertheless, combined application of traditional destructive methods of analysis (total acid hydrolysis for determination of monosaccharide composition and sulfate content, linkage analysis by methylation, etc.) together with NMR spectroscopy may be quite informative.

With the objective of more profound elucidation of fucoidan structures and the development of a computer-assisted method for their analysis, we started systematic synthesis and NMR studies of hypothetical fragments of fucoidans, in order to reveal "structural reporter groups",[42] which indicate the presence of specific types of fragments. This project also comprises investigation of pharmacophore fucoidan fragments which determine their anticoagulant, antiviral and other types of activities. To date only the following fucoidan fragments have been synthesised: (1→2)-linked fucooligosaccharides which comprise up to 6 fucose moieties[43-45] and 3-,[45] 3′-,[46] 4-[47] and 4′-*O*-sulfated[46] derivatives of (1→2)-linked fucobioside.

In this communication we report synthesis and NMR studies of non-sulfated 2,3- and 3,4-branched propyl α-L-fucotriosides **1** and **2**, and the consistent fucobiosides **3-5**:

α-L-Fuc-(1→2)\
α-L-Fuc-OPr
α-L-Fuc-(1→3)/
1

α-L-Fuc-(1→3)\
α-L-Fuc-OPr
α-L-Fuc-(1→4)/
2

α-L-Fuc-(1→2)-α-L-Fuc-OPr
3

α-L-Fuc-(1→3)-α-L-Fuc-OPr
4

α-L-Fuc-(1→4)-α-L-Fuc-OPr
5

RESULTS AND DISCUSSION

Synthesis of oligosaccharides 1-5. As our target compounds **1-5** comprise only fucose units, the readily accessible allyl α-L-fucopyranoside **6**[48] was chosen as a common precursor. The allyl aglycon was selected due to the possibility of (a) its reduction with formation of propyl glycosides which are useful haptens in bioassays and models for NMR studies, (b) removal with formation of 1-OH derivatives for assembling larger oligosaccharides, and (c) transformation into a spacer-arm[49] which is necessary for design of neoglycoconjugates.

To prepare the oligosaccharides **1-5** the following series of selectively protected allyl fucoside derivatives were used as initial blocks: 3,4-acetonide **7**,[50] 4-acetate **9**, 2-*O*- and 3-*O*-benzyl ethers **10** and **11** and 2,3-di-*O*-benzyl ether **12**. Their synthesis is based on

functionalization of the 3-equatorial,4-axial-diol fragment according to well developed procedures. Particularly, 4-acetate **9** was obtained in an overall yield of 90 % by treatment of fucoside **6** with trimethyl orthoacetate and subsequent regioselective cleavage of orthoester **8** by acid hydrolysis. Position of acetate at C-4 in compound **9** was confirmed by downfield location of the H-4 signal in the ^{1}H NMR spectrum (δ 5.20, Table 1).

Diol **10** was readily assessed by benzylation and de-isopropylidenation of acetonide **7**. The presence of OH groups at C-3 and C-4 was confirmed by specific coupling of H-3 and H-4 with protons of the corresponding hydroxyls in the ^{1}H NMR spectrum. Mono- and di-benzylated compounds **11** and **12** were synthesized by benzylation of fucoside **6** with benzyl bromide *via* a dibutylstannylidene intermediate. To elucidate the location of a benzyl group in diol **11**, it was treated with trichloroacetyl isocyanate to give carbamate **13**. Downfield chemical shifts (δ 3.87→5.12 and 3.76→5.47, Table 1) of H-2 and H-4 signals evidenced the presence of OH groups in diol **11** at C-2 and C-4. Similarly, downfield location of the H-4 signal (δ 5.40) in the ^{1}H NMR spectrum of carbamate **14** showed the presence of a free OH group at C-4 in 2,3-di-*O*-benzylated fucoside **12**.

To choose appropriate fucosyl donors for the syntheses of trisaccharides **1** and **2**, model 2-, 3-, and 4-*O*-glycosylations of alcohols **7** and **10-12** with fucosyl bromides **22-24** (Table 2) were performed in CH_2Cl_2 in the presence of $Hg(CN)_2$ and $HgBr_2$. Donors **22-24** were prepared by deallylation of precursors **15-17** in the presence of $PdCl_2$ in methanol (yields of hemiacetals **18-20** 83-96%) and subsequent bromination under treatment with Ph_3P and CBr_4 in refluxing CH_2Cl_2. The final step was almost quantitative and gave CH_2Cl_2 solutions of fucosyl bromides **22-24** which were used without any treatment or purification. Alternative two-step deallylation *via* isomerization into the corresponding 2-propenyl derivative (e.g., **21**) and subsequent hydrolysis was less effective (see Experimental for preparation of **18**).

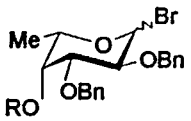
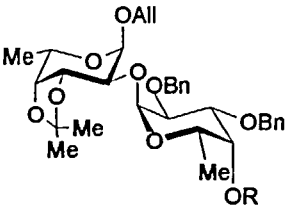
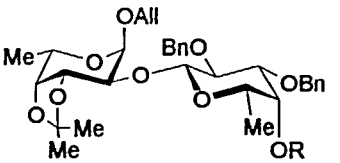
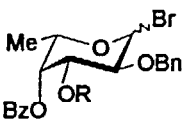
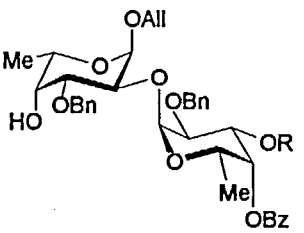
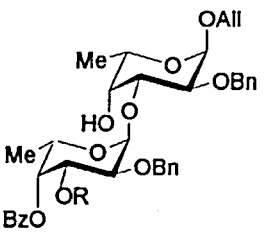
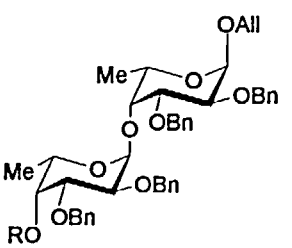
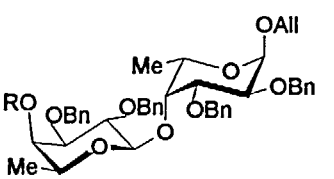
The results of glycosylations of alcohols **7** and **10-12** with fucosyl bromides **22-24** are summarized in Table 2. These data clearly show higher efficiency and better α-stereoselectivity of glycosylation with 4-*O*- and 3,4-di-*O*-benzoyl-fucosyl bromides **23** and **24** as compared with the results for 2,3,4-tri-*O*-benzylated bromide **22**. The presence of an acyl group at O-4 in fucosyl bromide is known[1,51,52] to favor the formation of the α-linkage. Another reason to use a benzoate group at O-3 and O-4 is connected with the

Table 1. ^{1}H NMR shifts[a] for compounds **9-21, 26-32, 34-36.**

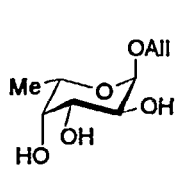
Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6
9		4.95	3.79	3.97	5.20	4.01	1.16
10		4.86	3.72	4.03	3.78	3.96	1.28
11		4.84	3.87	3.61	3.76	3.86	1.23
12		4.78	3.48	3.82		3.90	1.21
13		5.18	5.12	4.06	5.47		1.25
14		4.86	3.81	4.06	5.40	4.13	1.22
15		4.93	4.18	4.08	3.72	3.96	1.18
16		4.95	3.96	4.16	5.66		1.20
17		5.06	4.17	5.79	5.69	4.37	1.22
18α		5.31	4.07	3.91	3.68	4.11	1.20
18β		5.28	4.07	3.91	3.68	4.07	1.12
19α		5.34	3.92	4.07	5.66	4.39	1.21
19β		4.87	3.68		5.60	3.82	1.29
20α		5.46	4.13	5.76	5.66	4.58	1.22
20β			3.87	5.41	5.61	3.99	1.30
21[b]		5.03	4.12	4.12	3.72	3.92	1.26
26	N[c]	5.05	3.95	4.27	5.70	4.50	1.19
	R[c]	4.97	3.87	4.38	4.09	4.19	1.38
27	N	5.06	3.96	4.14	5.47	4.35	1.01
	R	5.10	4.03	4.17	3.91	4.03	1.34
28	N	5.16	4.16	5.88	5.52	4.50	0.93
	R	5.10	4.09	4.16	3.93	4.01	1.34
29	N	4.88	3.92	4.05	5.53	4.26	1.05
	R	4.91	3.85	4.06	4.62	3.96	1.34
30	N	5.01	4.15	5.78	5.76	4.48	1.02
	R	4.94	3.93	4.12	3.78	3.97	1.32
31	N	4.94	3.94	4.10	3.89		0.91
	R	4.78			3.79		1.27
32	N	4.95	3.95	4.08	5.59	4.54	0.89
	R	5.00	3.98	3.92	3.85	3.91	1.35
34	N	4.77	3.76	3.66	5.54	3.73	1.23
	R	5.10	3.91	4.44	4.10	4.23	1.38
35	N	4.87	3.80				1.15
	R	4.83	3.47		3.94		1.26
36	N	5.30	3.74	3.63	5.51		1.21
	R	4.89	4.00	3.94	3.90	3.90	1.32

a. In ppm, recorded in $CDCl_3$. Other signals: $OCH_2C\underline{H}=CH_2$ δ 5.13-5.41; $OCH_2CH=C\underline{H}_2$ δ 5.78-6.05 and 5.70-5.87; $OC\underline{H}_2CH=CH_2$ δ 4.22-4.45 and 4.00-4.15; $PhC\underline{H}_2$ δ4.50-4.80; $C_6\underline{H}_5CH_2$ δ 6,90-8,20; $C_6\underline{H}_5CO$ δ 7.45-7.93; $C\underline{H}_3CO$ δ 2,1-2,3. b. $OCH=CHC\underline{H}_3$ δ 1.69; $OCH=C\underline{H}CH_3$ δ 4.64; $OC\underline{H}=CHCH_3$ δ 6.10; c. N - "non-reducing" unit; R - "reducing" unit.

Table 2. Glycosylation of fucosyl acceptors **7** and **10-12** with donors **22-24**.

Entry	Fucosyl Donor	Fucosyl acceptor	α-Disaccharide product	β-Disaccharide product
				
1	**22** R=Bn	**7**	**25*** R=Bn	**33*** R=Bn
2	**23** R=Bz	**7**	**26** R=Bz (74%)	**34** R=Bz (21 %)
				
3	**23** R=Bn	**11**	**27** R=Bn (75 %)	not observed
4	**24** R=Bz	**11**	**28** R=Bz (83 %)	not observed
				
5	**23**	**10**	**29** R=Bn (71 %)	not observed
6	**24**	**10**	**30** R=Bz (81 %)	not observed
				
7	**22**	**12**	**31** R=Bn (43 %)	**35** R=Bn (21 %)
8	**23**	**12**	**32** R=Bz (38 %)	**36** R=Bz (17 %)

* The mixture (1:1) of disaccharides **26** and **28** in total yield of 65% was obtained.



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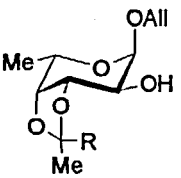
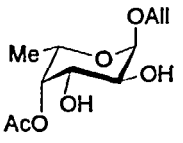


7 R=Me
8 R=OMe



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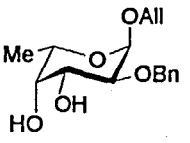

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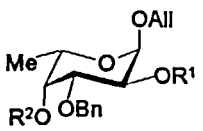
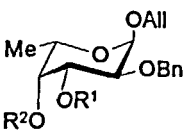
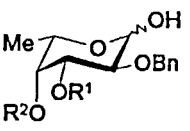


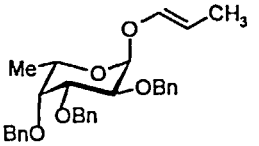
11 R^1=H, R^2=H
12 R^1=Bn, R^2=H
13 R^1= R^2=NHCOCCl$_3$
14 R^1=Bn, R^2=NHCOCCl$_3$



15 R^1=R^2=Bn
16 R^1=Bn, R^2=Bz
17 R^1=R^2=Bz



18 R^1=R^2=Bn
19 R^1=Bn, R^2=Bz
20 R^1=R^2=Bz



21

possibility of subsequent sulfation of these positions with the view to the preparation of sulfated fucoidan fragments.

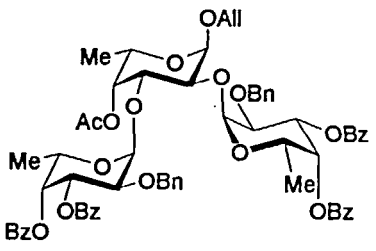
Fucosyl bromide **23** which gave good results in glycosylation of monohydroxyl glycosyl acceptors **7** and **12**, was used in 1.5 eq amounts for mono-fucosylation of diols **10** and **11**. In both cases the reaction proceeded regio- and stereoselectively and afforded α-(1→2)-linked disaccharide **27** and α-(1→3)-linked disaccharide **29**, respectively, which were obtained as sole products in yields of 75 and 71%. Surprisingly, no β-isomers of compounds **27** and **29** nor trisaccharide products were detected. Similar results were obtained for glycosylation of diols **10** and **11** with 3,4-di-*O*-benzoylated fucosyl bromide **24** (1.5 eq), which gave respective α-disaccharides **30** (81%) and **28** (83%).

The structure of the disaccharides was assessed by NMR spectroscopy. The α-anomeric configuration of the unit at the non-reducing end in disaccharides **25-30** and **32** was confirmed by a characteristic value of $J_{1,2}$ = 3.0-3.7 Hz in the ^{1}H NMR spectra. Similarly, the values of $J_{1,2}$ = 7.3-7.7 Hz for the unit at the non-reducing end in the ^{1}H NMR spectra of disaccharides **33**, **34**, and **36** evidenced their β-configuration. The anomeric configuration of fucosyl residues at the non-reducing end in isomeric disaccharides **31** and **35** was confirmed by the characteristic position of C-1 signals (δ 99.7 for **31** and δ 102.9 for **35**) in their ^{13}C NMR spectra.

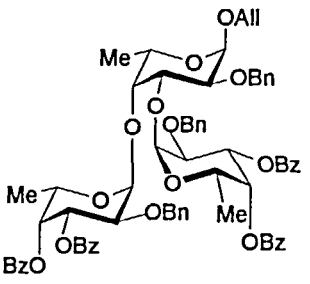
The glycosylation sites in disaccharides **27-30** were unambiguously located by NOE experiments. Pre-irradiation of H-1 of the glycosylating moiety of **27-30** caused an NOE-signal of H-2 of the glycosylated moiety, which evidenced the presence of an (1→2)-interresidual linkage in disaccharides **27** and **28** and of a (1→3)-linkage in disaccharides **29** and **30**.

Protected compounds **27**, **29**, and **31** were deblocked by conventional methods to give target disaccharides **3**, **4** and **5**.

In order to prepare trisaccharides **1** and **2** bis-fucosylation of appropriate 2,3- and 3,4-diols **9** and **10** by 3,4-di-*O*-benzoylated fucosyl bromide **24** (3 eq) was performed under the same conditions to give substituted trisaccharides **37** and **38** in yields of ~70% and 76%, respectively. The α-configuration of the terminal fucose units in **37** and **38** was confirmed by the characteristic values of respective $J_{1,2}$ = 3.3-3.7 Hz in the ^{1}H NMR spectra of trisaccharides **1** and **2**. These were prepared by hydrogenolysis and saponification of the substituted derivatives **37** and **38** (see below).



37



38

NMR analysis of oligosaccharides 1-5. Tables 3 and 4 show the ^{1}H NMR and ^{13}C NMR chemical shifts for compounds **1-5** and propyl α-L-fucopyranoside (**39**) prepared by hydrogenation of **6**. Assignments of the ^{1}H NMR spectra (Table 3) of these compounds were made using a combination of ^{1}H-^{1}H COSY and 2D TOCSY experiments. Assignments of the ^{13}C NMR spectra (Table 4) were made using 2D ^{1}H-^{13}C HMQC correlation spectroscopy.

Using the data from Table 4 the values of glycosylation effects (Δδ) in ^{13}C NMR spectra of disaccharides and deviations from additivity (ΔΔδ) in ^{13}C NMR spectra of trisaccharides were calculated which are the increments for the calculation of hypothetical ^{13}C NMR spectra of oligo- and polysaccharides.[53,54] The glycosylation effects[55] (Δδ) in the

Table 3. ^{1}H NMR shifts[a] for oligosaccharides **1-5** and monosaccharides **39** and **40**.

Compound	Residue	H-1	H-2	H-3	H-4	H-5	H-6
1	α-L-Fuc-(1→2)	5.13	3.78	3.72	3.79	4.15	1.24
	α-L-Fuc-(1→3)	5.08	3.83	3.74	3.79	4.15	1.24
	α-L-Fuc-OPr	5.17	4.04	4.06	4.09	4.08	1.24
2	α-L-Fuc-(1→3)	5.10	3.76	3.93	3.81	4.31	1.21
	α-L-Fuc-(1→4)	5.02	3.82	3.98	3.81	4.28	1.25
	α-L-Fuc-OPr	4.94	4.00	3.93	4.05	4.17	1.35
3	α-L-Fuc-(1→2)	5.04	3.76	3.95	3.81	4.25	1.21
	α-L-Fuc-OPr	5.10	3.85	3.96	3.84	4.09	1.22
4	α-L-Fuc-(1→3)	5.07	3.81	3.95	3.82	4.28	1.21
	α-L-Fuc-OPr	4.91	3.91	3.91	4.02	4.08	1.27
5	α-L-Fuc-(1→4)	4.96	3.80	3.93	3.83	4.51	1.18
	α-L-Fuc-OPr	4.93	3.83	3.95	3.84	4.13	1.31
39	α-L-Fuc-OPr	4.88	3.78	3.86	3.80	4.10	1.22
40	α-L-Fuc-OH	5.19	3.76	3.85	3.80	4.19	1.20

a. Recorded at 60-70 °C in D_2O with acetone as an internal standard. b. Signals of propyl 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 4. ^{13}C NMR shifts[a] for oligosaccharides **1-5** and monosaccharides **39** and **40**.

Compound	Residue	C-1	C-2	C-3	C-4	C-5	C-6
1	α-L-Fuc-(1→2)	96.6	68.95	71.1	72.9	68.3	16.5
	α-L-Fuc-(1→3)	95.4	69.1	71.0	72.9	68.4	16.5
	α-L-Fuc-OPr	96.2	70.6	73.1	68.6	67.2	16.5
2	α-L-Fuc-(1→3)	97.2	69.6	71.3	73.2	68.3	16.5
	α-L-Fuc-(1→4)	102.1	70.0	70.3	73.1	68.6	17.0
	α-L-Fuc-OPr	99.4	68.1	76.1	80.2	68.9	17.1
3	α-L-Fuc-(1→2)	97.1	69.2	70.6	73.0	68.2	16.4
	α-L-Fuc-OPr	96.6	73.3	69.2	73.1	67.7	16.5
4	α-L-Fuc-(1→3)	96.4	69.2	70.7	73.1	68.1	16.5
	α-L-Fuc-OPr	99.45	67.6	75.8	69.3	67.5	16.5
5	α-L-Fuc-(1→4)	101.9	69.9	70.7	73.2	68.2	16.6
	α-L-Fuc-OPr	99.6	69.4	70.4	81.4	68.3	16.5
39	α-L-Fuc-OPr	99.5	69.2	70.9	73.1	67.7	16.5
40	α-L-Fuc-OH	93.4	69.3	70.5	73.05	67.5	16.7

a. Recorded at 60-70 °C in D_2O with acetone as an internal standard. b. Signals of propyl aglycon: $OCH_2CH_2C\underline{H}_3$ δ 11.1; $OCH_2C\underline{H}_2CH_3$ δ 23.2-23.3; $OC\underline{H}_2CH_2CH_3$ δ 71.3.

Table 5. Glycosylation effects (ppm) in ^{13}C NMR spectra of disaccharides **3-5**

Compound	Residue	ΔδC-1	ΔδC-2	ΔδC-3	ΔδC-4	ΔδC-5	ΔδC-6
3	α-L-Fuc-OPr	-2.9	4.1	-1.7	0.0	0.0	0.0
	α-L-Fuc-(1→2)	3.7	-0.1	0.1	-0.05	0.7	-0.3
4	α-L-Fuc-OPr	-0.05	-1.6	4.9	-3.8	-0.2	0.0
	α-L-Fuc-(1→3)	3.0	-0.1	0.2	0.05	0.6	-0.2
5	α-L-Fuc-OPr	0.1	0.2	-0.5	8.3	0.6	0.0
	α-L-Fuc-(1→4)	8.5	0.6	0.2	0.15	0.7	-0.1

Table 6. Deviations from additivity (ppm) in ^{13}C NMR spectra of trisaccharides **1** and **2**

Compound	ΔΔδC-1	ΔΔδC-2	ΔΔδC-3	ΔΔδC-4	ΔΔδC-5	ΔΔδC-6	ΔΔδC-1′	ΔΔδC-1″
1	-0.35	-1.1	-1.0	-0.7	-0.3	0.0	-0.5[a]	-1.0[b]
2	-0.15	0.3	0.8	2.6	0.8	0.6	0.8[b]	0.2[c]

a. (1→2)-Linked residue. b. (1→3)-Linked residue. c. (1→4)-Linked residue.

^{13}C NMR spectra of disaccharides, which are the differences in chemical shifts of the respective carbon atoms in the monosaccharide fragments of disaccharide and in non-substituted propyl fucoside **39** and α-L-fucopyranose (**40**) (^{13}C NMR data for **40** are also included in Table 4), are summarized in Table 5.

The deviations from additivity values (ΔΔδ), which were observed in the case of branched trisaccharides **1** and **2**, are presented in Table 6. These values represent the deviation between experimental and calculated chemical shift values for respective carbons C1-C6 of the bis-glycosylated unit and for C1′ and C1″ (anomeric carbons of the 2- and 3-substituents in case of trisaccharide **1** and the 3- and 4-substituents in case of trisaccharide **2**).

The ΔΔ values for carbon atoms in the propyl fucoside fragment, for example for C*i*, of trisaccharide **1** were calculated using Eqn (1) and for C1′ and C1″ of glycosylating residues - using Eqns (2) and (3), respectively. In Eqn (1)-(3), δCi_{TS}, $\delta Ci_{(1\rightarrow2)}$, $\delta Ci_{(1\rightarrow3)}$ and $\delta Ci_{\alpha\text{-L-Fuc-OPr}}$ are the chemical shifts of C*i* in ^{13}C NMR spectra of the examined trisaccharide, (1→2)- and (1→3)-linked disaccharides **3** and **4** and propyl fucoside **39**, respectively.

Calculation of ΔΔ values in the spectrum of trisaccharide **2** was performed by Eqns (4)-(6), which are similar to Eqns. (1)-(3). The data from the Table 6 show that the spectrum of trisaccharide **1** is characterized by small and medium[56] ΔΔ values, while the spectrum of trisaccharide **2** displays the large ΔΔ value of 2.6 ppm for the C-4 atom of the bis-fucosylated moiety.

$$\Delta\Delta\delta Ci = \delta Ci_{TS} - \delta Ci_{(1\rightarrow2)} - \delta Ci_{(1\rightarrow3)} + \delta Ci_{\alpha\text{-L-Fuc-OPr}} \quad (1)$$

$$\Delta\Delta\delta C1' = \delta C1'_{TS} - \delta C1'_{(1\rightarrow2)} \quad (2)$$

$$\Delta\Delta\delta C1'' = \delta C1''_{TS} - \delta C1''_{(1\rightarrow3)} \quad (3)$$

$$\Delta\Delta\delta Ci = \delta Ci_{TS} - \delta Ci_{(1\rightarrow3)} - \delta Ci_{(1\rightarrow4)} + \delta Ci_{\alpha\text{-L-Fuc-OPr}} \quad (4)$$

$$\Delta\Delta\delta C1' = \delta C1'_{TS} - \delta C1'_{(1\rightarrow3)} \quad (5)$$

$$\Delta\Delta\delta C1'' = \delta C1''_{TS} - \delta C1''_{(1\rightarrow4)} \quad (6)$$

It is known (see refs. 56-58 and papers cited herein) that NMR chemical shifts, particularly ^{13}C NMR shifts, and conformational properties of oligosaccharides are closely connected. The origin of the observed ΔΔ values in ^{13}C NMR spectra of trisaccharides **1** and **2** can be revealed by comparison of the NOE values for trisaccharides **1** and **2** and constituting disaccharides **3-5** in the terms of Grant and Cheney equation (7)[59] which describes the influence of the proton-proton distance (*r*) on the chemical shifts of the carbons bearing these protons (Fig. 1). Previously we used the same approach to explain ΔΔ values in ^{13}C NMR spectra of blood Le^d related trisaccharides.[58]

$$\Delta\delta^{13}C = 1680 \cdot \exp(-2.671r) \cdot \cos\Theta \quad (7)$$

According to equation (7)[59] the increase of the distance between two protons causes the upfield shifts of the resonance of corresponding carbon atoms of the same sign and order as a function of the $cos\Theta$ term, where Θ is the angle between proton-carbon and proton-proton vectors (Fig. 1). In case of the pairs of protons at anomeric (A) and trans-glycosidic (T) carbons the angles Θ_{AT} and Θ_{TA} are usually <90° ($cos\Theta$ is positive), but they can be also >90° ($cos\Theta$ is negative) when one of the protons in the pair is located at the anomeric carbon of the glycosylating residue and the second one is an equatorial (E) proton at the carbon atom (of glycosylated residue) which is neighboring to the substituted one [e.g., H1 in (1→2)-linked fucobiosyl fragments and H4 in (1→3)-linked fucobiosyl

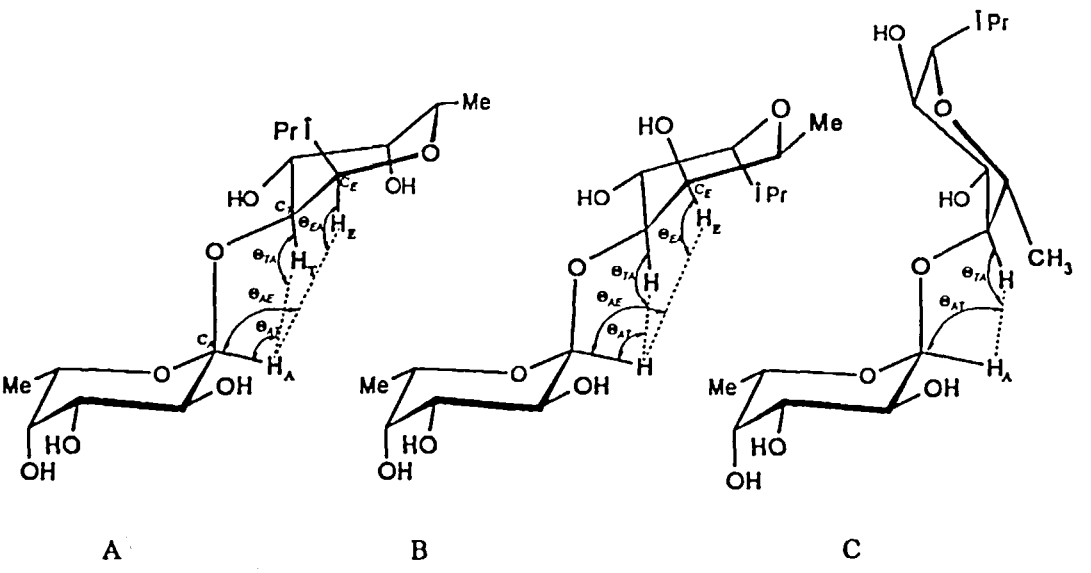


Figure 1. Proton-proton spatial contacts (dotted lines) which determine the values of NOEs and glycosylation effects for the carbon atoms around inter-monosaccharide bridges in (1→2)-, (1→3)-, and (1→4)-disaccharide fragments (A)-(C) of trisaccharides **1** and **2** and corresponding disaccharides **3**, **4** and **5**.

fragments, Figs. 1A,B]. Thus the decrease of the distance *r* between H_A and H_T protons should result in down-field chemical shifts of the corresponding carbons, but the decrease of the distance *r* between H_A and H_E protons should cause high-field chemical shift of the corresponding carbon atoms. The sign of the glycosylation effect ($\Delta\delta$) for the corresponding carbons depends also on the value angles Θ_{AT} between vectors H_A–C_A and H_A–H_T, Θ_{TA} between vectors H_T–C_T and H_T–H_A, Θ_{AE} between vectors H_A–C_A and H_A–H_E, Θ_{EA} between vectors H_E–C_E and H_E–H_A.

Comparison of NOEs values (Table 7) which were observed after pre-irradiation of H-1′ in the α-(1→2)- and α-(1→3)-linked disaccharides **3** and **4** and of H-1′ and H-1″ in the trisaccharide **1** showed that the average distances H-1′–H-2 and H-1″–H-3 in trisaccharide **1** are shorter but H1′-H1 is larger when compared to respective distances in disaccharides **3** and **4**. These differences are supposed to cause negative deviations from additivity for the shifts of C-1, C-1′, and C-2 that is in agreement with the observed ΔΔ

Table 7. Experimental inter- and intraunit NOEs values for oligosaccharides **1-5**.

Compound	Proton saturated	NOEs				
1	H-1'	H-1	H-2	H-2'		
		109	55	100		
	H-1"	H-3,4	H-3	H-4	H-2"	H-5"
		178	-	-	100	21
2	H-1'	H-3,3'	H-4	H-2'		
		140.5	52	100		
	H-1"	H-4	H-6	H-2"		
		106	26	100		
3	H-1'	H-1	H-2	H-3	H-5	H-3'
		64	67	100	5	12.5
4	H-1'	H-3	H-4	H-2'	H-5'	
5	H-1'	H-4	H-6	H-2'	H-5'	
		132	14.5	100	10	

values (Table 6). Similar correlation between negative ΔΔ values for shifts of C-3 and C-4 of trisaccharide **1** and differences of NOEs on H-3 and H-4 after pre-irradiation of H-1′ in disaccharide **4** and H-1″ in trisaccharide **1** may be tracked down in the same manner.

The observed positive ΔΔ values for the shifts of C-1′, C-3 and C-4 of the trisaccharide **2** may be also predicted and explained from the results of comparative analyses. NOEs values which were observed after pre-irradiation of H-1′ in the α-(1→3)- and α-(1→4)-linked disaccharides **4** and **5** and of H-1′ and H-1″ in the trisaccharide **2** revealed for trisaccharide a shorter average distance H-1′–H-3 but larger H-1″–H-4 one (see NOE data for oligosaccharides **2** and **4** in the Table 7).

CONCLUSION

The oligosaccharide syntheses performed have shown once again high α-stereoselectivity of fucosylation with 4-*O*- and 3,4-di-*O*-benzylated fucosyl bromides as compared to 4-*O*-benzylated ones. Analyses of NOEs values for oligosaccharides **1-5** confirmed conformational origin of deviations from additivity in ^{13}C NMR spectra of

vicinally branched trisaccharides **1** and **2**. The ^{13}C NMR data obtained for oligosaccharides **1-5** were used in structural assessment of fucoidan from the brown seaweed *Chorda filum*.[39]

EXPERIMENTAL

General methods. TLC was performed on Silica Gel 60 F_{254} (Merck) with EtOAc-petroleum ether (A, 1:1; B, 1:2; C, 1:3; D, 1:5) or EtOAc-toluene (E, 1:3; F, 1:2), and with detection by charring with H_3PO_4. Medium pressure liquid chromatography was performed on Silica Gel L 40-100 μm (Fluka) by gradient elution with benzene-EtOAc (solvent G) or hexane-EtOAc (solvent H). Optical rotations for substituted compounds were determined with a Jasco DIP-360 digital polarimeter at 26-30 °C. All solvents used for syntheses were purified according to conventional procedures. Glycosylation reactions were carried out under argon with freshly distilled solvents.

NMR spectra for substituted compounds **9-21** and **25-38** were recorded in $CDCl_3$ on Bruker spectrometers WM-250 and AMX-300 at 303 K. One and two-dimensional spectra were acquired using standard Bruker softwares for ASPECT-2000 and ASPECT-3000, respectively. ^1H and ^{13}C NMR spectra for non-substituted oligosaccharides **1-5** and monosaccharides **39** and **40** were recorded in D_2O on Bruker spectrometer DRX-500 (500.13 MHz) with acetone as the reference (^1H 2.225 ppm; ^{13}C 31.45 ppm). Gradient enhanced 2D gCOSY, gNOESY and gHSQC experiments as well as TOCSY experiments were used for resonance assignment. The mixing time used for the NOESY experiment was 400 ms. 1D transient NOE experiments were performed at 500.13 MHz and 307 K according to the described protocol.[60] The following parameters were used: relaxation delay: 6s; number of scans: 512 - 1024; number of dummy scans: 64; sweep width: 5000 Hz; time domain size: 16 K. The gaussian-shaped pulse was used for selective resonance inversion of 90-160 ms length according to desired selectivity. The mixing time was fixed at 900 ms. Atmosphere Pressure Chemical Ionization Mass Spectrometry (APCI-MS) was performed on a LCQ Finnigan MAT mass spectrometer.

Allyl 4-*O*-acetyl-α-L-fucopyranoside (9). A solution of allyl fucoside **6**[48] (100 mg, 0.49 mmol), triethyl orthoacetate (0.33 mL, 1.8 mmol) and a catalytic amount of TsOH in MeCN (0.3 mL) was kept at rt for 15 min, 80% aqueous AcOH (0.64 mL) was

added and the mixture was kept for 15 min at rt. Then Et_3N (0.1 mL) was added and the solution was concentrated and co-evaporated with toluene (2x5 mL). Column chromatography (solvent G) of the residue gave diol **9** (121 mg, 90%), $[\alpha]_D$ -196° (*c* 1, EtOAc), R_F 0.36 (A). APCI-MS: *m/z* 279.1 $[M+CH_3OH+H]^+$, 189.1 $[M-OAll]^+$. The ^1H NMR data for **9** are presented in Table 1.

Anal. Calcd for $C_{11}H_{18}O_6$: C 53.66; H 7.32. Found: C 53.67; H 7.33.

Allyl 2-*O*-benzyl-α-L-fucopyranoside (10). A solution of acetonide **7**[50] (264 mg, 1.08 mmol) in DMF (5.5 mL) was added at 0 °C under stirring to a 60% oil suspension of NaH (80 mg, 2 mmol). The mixture was stirred at 0 °C for 30 min, and BnBr (0.45 mL, 2.7 mmol) was added portionwise under stirring which was continued for 2 h at rt. The mixture was diluted with $CHCl_3$ (40 mL) and washed with water (2x500 mL). The organic layer was separated and concentrated, the residue was dissolved in the mixture of CH_2Cl_2 (2 mL) and 90% aqueous CF_3COOH (5 mL), kept for 30 min, then concentrated and co-evaporated with toluene (5x5 mL). Column chromatography of the residue (solvent H) gave diol **10** (263 mg, 83 %), $[\alpha]_D$ -117° (*c* 2, $CHCl_3$), R_F 0.29 (B). APCI-MS: *m/z* 311.2 $[M+H_2O-H]^+$. The ^1H NMR data for **10** are presented in Table 1.

Anal. Calcd for $C_{16}H_{22}O_5$: C 65.31; H 7.48. Found: C 65.36; H 7.39.

Allyl 3-*O*-benzyl-α-L-fucopyranoside (11) and allyl 2,3-di-*O*-benzyl-α-L-fucopyranoside (12). A mixture of allyl fucoside **6** (580 mg, 2.8 mmol), Bu_2SnO (731 mg, 2.9 mmol) and toluene (15 mL) was refluxed until complete dissolution and then concentrated to the volume of ~3 mL. Bu_4NBr (1.0 g, 3 mmol) and BnBr (3 mL, 6.2 mmol) were added. The solution was refluxed for 3 h and concentrated *in vacuo*. Column chromatography of the residue (solvent H) gave amorphous **11** (251 mg, 30%) and **12** (747 mg, 69%).

Data of **11**: $[\alpha]_D$ -114° (*c* 2, $CHCl_3$), R_F 0.14 (F). APCI-MS: *m/z* 311.2 $[M+H_2O-H]^+$. The ^1H NMR data for **11** are presented in Table 1.

Anal. Calcd for $C_{16}H_{22}O_5$: C 65.31; H 7.48. Found: C 65.36; H 7.39.

Data of **12**: $[\alpha]_D$ -50° (*c* 2, $CHCl_3$), R_F 0.66 (F). APCI-MS for **12**: *m/z* 402.2 $[M+H_2O]^+$. The ^1H NMR data for **12** are presented in Table 1.

Anal. Calcd for $C_{23}H_{30}O_5$: C 71.48; H 7.82. Found: C 71.33; H 7.93.

Allyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranoside (15). Benzylation of allyl fucoside **6** (1.015 g, 4.98 mmol) with BnBr (2 mL, 12.5 mmol) in the presence of NaH (1.08 g, 27

mmol, 60% suspension in oil) in DMF (22 mL), as described for **10**, yielded amorphous **15** (2.0 g, 85%), $[\alpha]_D$ -36° (*c* 2, $CHCl_3$), R_F 0.38 (solvent D). APCI: calcd for $C_{30}H_{36}O_6$ $[M+H_2O]^+$ 492.2, found 492.0; calcd for $C_{27}H_{29}O_4$ $[M-OAll]^+$ 417.2, found 416.8. The ^1H NMR data for **15** are presented in Table 1.

Anal. Calcd for $C_{30}H_{36}O_5$: C 75.60; H 7.61. Found: C 75.71; H 7.69.

Allyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranoside (17). Benzoylation of **10** (347 mg, 1.18 mmol) with BzCl (3.30 mL, 2.84 mmol) and Py (8 mL), as described for **19**, yielded **17** (491 mg, 95%), $[\alpha]_D$ -214° (*c* 1, EtOAc), R_F 0.51 (solvent C). APCI-MS: *m/z*: 520.2 $[M+H_2O]^+$. The ^1H NMR data for **17** are presented in Table 1.

Anal. Calcd for $C_{30}H_{30}O_7$: C 71.71; H 5.98. Found: C 71.53; H 6.01.

2,3,4-Tri-*O*-benzyl-α- and -β-L-fucopyranose (18). *Method 1.*- A solution of **15** (1.0 g, 2.1 mmol) in MeOH (35 mL) was stirred for 2 h at rt with $PdCl_2$ (150 mg, 0.84 mmol). Then triethylamine (0.5 mL) was added, the mixture was filtered through a Celite pad, and the filtrate was concentrated. Column chromatography of the residue (solvent H) gave **18** (900 mg, 95%), R_F 0.23 (solvent C). APCI-MS: *m/z* 452.2 $[M+H_2O]^+$, 417.2 $[M-OH]^+$. The ^1H NMR data for **18** are presented in Table 1.

Method 2.- A mixture of **15** (1.27 g, 2.67 mmol), potassium *tert*-butoxide (588 mg, 5.34 mmol), and DMSO (26 mL) was stirred at 100 °C for 4 h, then cooled to rt, diluted with CH_2Cl_2 (100 mL), washed with water and concentrated to dryness. Flash column chromatography of the residue by elution with 8:1 petroleum ether-AcOEt mixture containing 0.5% of Et_3N gave **21** (1.02 g, 81%), $[\alpha]_D$ -28° (c 2, $CHCl_3$), R_F 0.36 (solvent D), the ^1H NMR data are presented in Table 1. A mixture of **21** (748 mg, 1.58 mmol), iodine (553 mg, 2.4 mmol), $NaHCO_3$ (2.3 g), THF (23 mL), and water (4.6 mL) was stirred for 15 min at room temperature, diluted with $CHCl_3$ (300 mL), washed with water (100 mL) and concentrated. Column chromatography of the residue gave **18** (520 mg, 76%).

Anal. Calcd for $C_{27}H_{30}O_5$: C 74.63; H 6.96. Found: C 74.51; H 7.04.

4-*O*-Benzoyl-2,3-di-*O*-benzyl-α- and -β-L-fucopyranose (19). To a solution of **12** (1.085 g, 2.82 mmol) in Py (10 mL) BzCl (393 mL, 3.4 mmol) was added at 0 °C under stirring. The solution was kept at rt for 6 h, concentrated, diluted with CH_2Cl_2 (100 mL) and washed with water (2x200 mL). The organic layer was separated and concentrated.

Column chromatography of the residue (solvent H) gave **16** (1.11 g, 81%), $[\alpha]_D$ -79° (*c* 2, $CHCl_3$), R_F 0.47 (solvent D). Deallylation of **16** (1.1 g, 2.25 mmol), as described for **18** (method 1), yielded **19** [840 mg, 83%; R_F 0.25 (solvent C)]. The ^1H NMR data for **16** and **19** are presented in Table 1.

Anal. Calcd for $C_{27}H_{28}O_6$: C 72.30; H 6.29. Found: C 72.45; H 6.33.

3,4-Di-*O*-benzoyl-2-*O*-benzyl-α- and -β-L-fucopyranose (20). Deallylation of **17** (530 mg, 1.05 mmol), as described for **18** (method 1), yielded **20** (465 mg, 96%), R_F 0.25 (solvent C). APCI-MS: *m/z* 480.2 $[M+H_2O]^+$, 445.2 $[M-OH]^+$. The ^1H NMR data for **20** are presented in Table 1.

Anal. Calcd for $C_{27}H_{26}O_7$: C 70.12; H 5.67. Found: C 70.15; H 5.70.

Preparation of fucosyl bromides (22) - (24). A solution of 1-OH compound **18**, **19** or **20** (1 mmol), PPh_3 (1.3 mmol) and CBr_4 (1.3 mmol) in 10 mL of CH_2Cl_2 was refluxed for 3 h, cooled to room temperature, and the solution was used in glycosylation reactions.

Glycosylation by fucosyl bromides (22) - (24) (typical procedure). A mixture of glycosyl acceptor (1 mmol), $Hg(CN)_2$ (1.5 mmol), $HgBr_2$ (catalytic amount), and molecular sieves 4A (1.4 g) in CH_2Cl_2 (10 mL) was stirred for 45 min at 20° under Ar. Using a syringe, a solution of fucosyl bromide (1.5 mmol) in CH_2Cl_2 (see above for preparation) was added portionwise during 1 h. The mixture was stirred for 10 h, and CH_2Cl_2 (50 mL) and satd aq KBr (50 mL) were added. The mixture was stirred for 10 min and filtered through Celite. The organic layer was separated, washed with satd aq KBr and $NaHCO_3$, filtered through cotton wool, and concentrated. Column chromatography of the residue gave fucosylation products.

Allyl 2-*O*-(2,3,4-tri-*O*-benzyl-α- (25) and -β-L-fucopyranosyl)-3,4-*O*-isopropylidene-α-L-fucopyranoside (33). Glycosylation of acetonide **7** (56 mg, 0.17 mmol) by fucosyl bromide **22**, prepared from **18** (108.5 mg, 0.25 mmol), gave a ~1:1 mixture (72 mg, 65%) of amorphous **25** and **33**. APCI-MS: *m/z* 678.3 $[M+H_2O]^+$.

Data of **25**: R_F 0.54 (E), ^{13}C NMR data (δ_C $CDCl_3$): 94.77 (C1), 95.16 (C1'), 16.25 and 16.48 (C6, C6').

Data of **33**: R_F 0.51 (E), ^{13}C NMR data (δ_C $CDCl_3$): 97.78 (C1), 103.48 (C1'), 16.30 and 16.85 (C6, C6').

Anal. Calcd for $C_{39}H_{48}O_9$: C 70.89; H 7.32. Found: C 70.98; H 7.40.

Allyl 2-*O*-(4-*O*-benzoyl-2,3-di-*O*-benzyl-α- (26) and -β-L-fucopyranosyl)-3,4-*O*-isopropylidene-α-L-fucopyranoside (34). Glycosylation of acetonide 7 (22 mg, 0.1 mmol) by fucosyl bromide **23**, prepared from **19** (67 mg, 0.15 mmol), gave amorphous **26** (46 mg, 74%) and **34** (14 mg, 21%).

Data of **26**: $[\alpha]_D$ -109° (*c* 1, $CHCl_3$), R_F 0.49 (solvent C). APCI-MS: *m/z* 692.3 $[M+H_2O]^+$, 617.3 $[M\text{-}OAll]^+$. The ^1H NMR data for **26** are presented in Table 1.

Anal. Calcd for $C_{39}H_{46}O_{10}$: C 69.42; H 6.87. Found: C 69.55; H 6.93.

Data of **34**: $[\alpha]_D$ -133° (*c* 1, $CHCl_3$), R_F 0.42 (solvent C). APCI-MS: *m/z* 692.3 $[M+H_2O]^+$, 617.3 $[M\text{-}OAll]^+$. The ^1H NMR data for **34** are presented in Table 1.

Anal. Calcd for $C_{39}H_{46}O_{10}$: C 69.42; H 6.87. Found: C 69.63; H 6.70.

Allyl 2-*O*-(4-*O*-benzoyl-2,3-di-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-α-L-fucopyranoside (27). Glycosylation of diol **11** (167 mg, 0.57 mmol) by fucosyl bromide **23**, prepared from **19** (383 mg, 0.85 mmol), gave amorphous **27** (294 mg, 71%), $[\alpha]_D$ -109° (*c* 0.3, EtOAc), R_F 0.33 (solvent B). APCI: calcd for $C_{43}H_{50}O_{11}$ $[M+H_2O]^+$ 742.3, found 742.1. The ^1H NMR data for **27** are presented in Table 1.

Anal. Calcd for $C_{43}H_{48}O_{10}$: C 71.25; H 6.67. Found: C 71.34; H 6.69.

Allyl 2-*O*-(3,4-di-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-α-L-fucopyranoside (28). Glycosylation of diol **11** (106 mg, 0.36 mmol) by fucosyl bromide **24**, prepared from **20** (250.3 mg, 0.54 mmol), gave amorphous **28** (216 mg, 81%), $[\alpha]_D$ -242° (*c* 2, $CHCl_3$), R_F 0.46 (solvent B). APCI: calcd for $C_{43}H_{48}O_{12}$ $[M+H_2O]^+$ 756.3, found 755.9. The ^1H NMR data for **28** are presented in Table 1.

Anal. Calcd for $C_{43}H_{46}O_{11}$: C 69.90; H 6.28. Found: C 70.05; H 6.33.

Allyl 3-*O*-(4-*O*-benzoyl-2,3-di-*O*-benzyl-α-L-fucopyranosyl)-2-*O*-benzyl-α-L-fucopyranoside (29). Glycosylation of diol **10** (30 mg, 0.10 mmol) by fucosyl bromide **23**, prepared from **19** (68 mg, 0.15 mmol), gave amorphous **29** (57 mg, 75%), $[\alpha]_D$ -113° (*c* 2, $CHCl_3$), R_F 0.51 (EtOAc-toluene, 1:10). APCI-MS: *m/z* 742.3 $[M+H_2O]^+$. The ^1H NMR data for **29** are presented in Table 1.

Anal. Calcd for $C_{43}H_{46}O_{11}$: C 69.90; H 6.28. Found: C 70.05; H 6.33.

Allyl 3-*O*-(3,4-di-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl)-2-*O*-benzyl-α-L-fucopyranoside (30). Glycosylation of diol **10** (100 mg, 0.34 mmol) by fucosyl bromide **24**, prepared from **20** (235 mg, 0.51 mmol), gave amorphous **30** (208 mg, 83%), $[\alpha]_D$ -

226° (*c* 1, $CHCl_3$), R_F 0.64 (solvent A). APCI-MS: *m/z* 756.3 $[M+H_2O]^+$. The ^1H NMR data for **30** are presented in Table 1.

Anal. Calcd for $C_{43}H_{46}O_{11}$: C 69.90; H 6.28. Found: C 70.03; H 6.30.

Allyl 4-*O*-(2,3,4-tri-*O*-benzyl-α- (31) and -β-L-fucopyranosyl)-2,3-di-*O*-benzyl-α-L-fucopyranoside (35). Glycosylation of **12** (51 mg, 0.132 mmol) with bromide **22**, prepared from **18** (87 mg, 0.2 mmol), gave amorphous **31** (49 mg, 43%) and **35** (23 mg, 21%).

Data of **31**: $[\alpha]_D$ -125° (*c* 1, $CHCl_3$), R_F 0.37 (solvent D). APCI-MS for **31**: *m/z* 831.4 $[M+CH_3OH-H]^+$. The ^{1}HNMR data for **31** are presented in Table 1.

Anal. Calcd for $C_{50}H_{56}O_9$: C 74.98; H 7.05. Found: C 75.09; H 7.12.

Data of **35**: $[\alpha]_D$ -98° (*c* 0.6, EtOAc), R_F 0.45 (solvent D). APCI-MS for **35**: *m/z* 818.4 $[M+H_2O]^+$. The ^{1}HNMR data for **35** are presented in Table 1.

Anal. Calcd for $C_{50}H_{56}O_9$: C 74.98; H 7.05. Found: C 75.22; H 7.25.

Allyl 4-*O*-(4-*O*-benzoyl-2,3-di-*O*-benzyl-α- (32) and -β-L-fucopyranosyl)-2,3-di-*O*-benzyl-α-L-fucopyranoside (36). Glycosylation of **12** (38 mg, 0.1 mmol) with **23**, prepared from **19** (67 mg, 0.15 mmol), gave amorphous **32** (31 mg, 38 %) and **36** (14 mg, 17%). The ^1H NMR data for **32** and **36** are presented in Table 1.

Data of **32**: $[\alpha]_D$ -90° (*c* 1, $CHCl_3$), R_F 0.55 (solvent D). APCI-MS for **32**: *m/z* 832.4 $[M+H_2O]^+$. The ^1H NMR data for **32** are presented in Table 1.

Anal. Calcd for $C_{50}H_{54}O_{10}$: C 73.69; H 6.68. Found: C 73.55; H 6.63.

Data of **36**: $[\alpha]_D$ -128° (*c* 0.7, EtOAc), R_F 0.53 (solvent D). APCI-MS for **36**: *m/z* 831.4 $[M+H_2O-H]^+$. The ^1H NMR data for **36** are presented in Table 1.

Anal. Calcd for $C_{50}H_{54}O_{10}$: C 73.69; H 6.68. Found: C 73.55; H 6.63.

Allyl 2-*O*-benzyl-3,4-di-*O*-(3,4-di-*O*-benzoyl-2-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranoside (38). Glycosylation of diol **10** (27.2 mg, 0.09 mmol) by fucosyl bromide **24**, prepared from **20** (124.8 mg, 0.27 mmol), gave amorphous **38** (83 mg, 76%), $[\alpha]_D$ -207° (*c* 0.5, $CHCl_3$), R_F 0.5 (solvent A). APCI-MS: *m/z* 1199.5 $[M+H_2O-H]^+$.

Anal. Calcd for $C_{70}H_{70}O_{17}$: C 71.05; H 5.96. Found: C 71.27; H 6.00.

Propyl 2,3-di-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside (1). Glycosylation of diol **9** (11 mg, 0.045 mmol) by fucosyl bromide **24**, prepared from **20** (95.5 mg, 0.21 mmol), gave amorphous **37** (36 mg, ~70%), R_F 0.36 (solvent B). According to ^1H- (Table

1) and ^{13}C NMR spectra compound **37** included 5-10% of an unknown impurity having chromatographic properties equal to **37**. A solution of crude **37** (46 mg, 0.04 mmol) in 1 mL of 0.1M MeONa in MeOH was kept for 15 min at rt and then was neutralized with KU-2 (H^+) resin, filtered, and concentrated to dryness. The crude residue was subjected to catalytic hydrogenolysis in EtOH-EtOAc (1:2, 12 mL) with 10% Pd-C at 40 °C and atm pressure for 20 h. The mixture was filtered and the solvent was evaporated *in vacuo*. The residue was subjected to gel filtration on fracto-gel TSK HW-40(S) (25 x 400 mm, V_o 50 mL), in water, to give amorphous **1** (15 mg, 80%), $[\alpha]_D$ -157° (*c* 0.3, H_2O). APCI-MS: *m/z* 439.2 $[M\text{-}OPr]^+$. The ^1H and ^{13}C NMR data for **1** are presented in Tables 2 and 3.

Anal. Calcd for $C_{21}H_{38}O_{13}$ C 50.60%; H 7.68%. Found: C 50.63%; H 7.70%.

Propyl 3,4-di-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside (2). Debenzylation and saponification of **38** (34.6 mg, 0.03 mmol) followed by gel filtration, as described for **1**, yielded amorphous **2** (10 mg, 75%), $[\alpha]_D$ -290° (*c* 0.2, H_2O). APCI-MS: *m/z* 439.2 $[M\text{-}OPr]^+$. The ^1H and ^{13}C NMR data for **2** are presented in Tables 2 and 3.

Anal. Calcd for $C_{21}H_{38}O_{13}$ C 50.60%; H 7.68%. Found: C 50.62%; H 7.73%.

Propyl 2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside (3). Debenzylation and debenzoylation of **27** (94 mg, 0.13 mmol), as described for **1**, yielded amorphous **3** (37 mg, 81%), $[\alpha]_D$ -205° (*c* 1, H_2O). APCI-MS: *m/z* 293.2 $[M\text{-}OPr]^+$. The ^1H and ^{13}C NMR data for **3** are presented in Tables 2 and 3.

Anal. Calcd for $C_{15}H_{28}O_9$ C 51.13%; H 8.01%. Found: C 51.08%; H 7.95%.

Propyl 3-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside (4). Debenzylation of **29** (56 mg, 0.077 mmol), as described for **1**, yielded amorphous **4** (21 mg, 78%), $[\alpha]_D$ -224° (c 1, H_2O). APCI-MS: *m/z* 293.2, $[M\text{-}OPr]^+$. The ^1H and ^{13}C NMR data for **4** are presented in Tables 2 and 3.

Anal. Calcd for $C_{15}H_{28}O_9$ C 51.13%; H 8.01%. Found: C 51.08%; H 7.95%.

Propyl 4-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside (5). Debenzylation of **31** (70 mg, 0.08 mmol), as described for **1**, yielded amorphous **5** (26 mg, 78%), $[\alpha]_D$ -187° (*c* 0.4, H_2O). APCI-MS: *m/z* 293.2 $[M\text{-}OPr]^+$. The ^1H and ^{13}C NMR data for **5** are presented in Tables 2 and 3.

Anal. Calcd for $C_{15}H_{28}O_9$ C 51.13%; H 8.01%. Found: C 51.08%; H 7.94%.

Propyl α-L-fucopyranoside (39). Hydrogenation of **6** (60 mg, 0.3 mmol), as

described for **1**, yielded amorphous **39** (58 mg, 95%), $[\alpha]_D$ -142° (*c* 0.8, H_2O). APCI-MS: *m/z* 147.3 $[M\text{-}OPr]^+$. ^1H and ^{13}C NMR data for **39** are presented in Tables 2 and 3.

Anal. Calcd for $C_9H_{18}O_5$ C 52.41%; H 8.80%. Found: C 52.44%; H 8.92%.

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