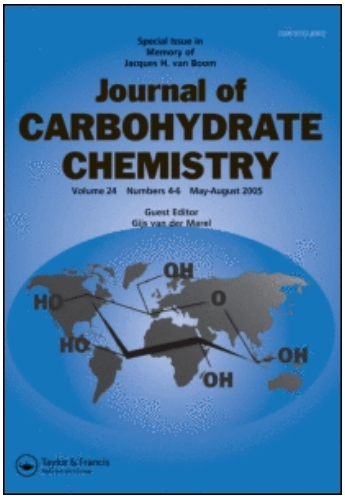

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Structural Elucidation of the Component Trisaccharide Obtained from the Acrosome Reaction-Inducing Substance of the Starfish *Asterias Amurensis* by Chemical Synthesis

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COMMUNICATION

STRUCTURAL ELUCIDATION OF THE COMPONENT TRISACCHARIDE OBTAINED FROM THE ACROSOME REACTION-INDUCING SUBSTANCE OF THE STARFISH *ASTERIAS AMURENSIS* BY CHEMICAL SYNTHESIS

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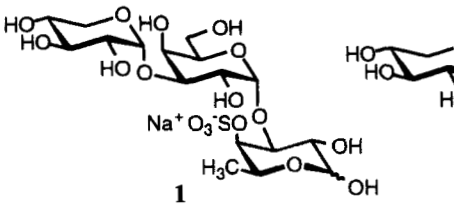
Okinaga et al.[1] have recently reported a novel trisaccharide obtained from the acrosome reaction-inducing substance (ARIS) of the starfish *Asterias amurensis*. The ARIS is essential for triggering the acrosome reaction in homologous spermatozoa, and the biological activity is due to the sugar moiety. The trisaccharide, composed of xylose (Xyl), galactose (Gal), and fucose (Fuc), and proposed to have a sequence of Xyl1→3Gal1→3 or 4[4 or 3-(SO_3^-)]Fuc, was deduced to be one of the major structural units constructing the side chain of the high molecular carbohydrate portion of the ARIS. The sequence differs from similar oligosaccharides, found in hemicellulose[2] and composed of D-xylose, D-galactose, and L-fucose. The ARIS contains a unique saccharide chain having sulfated L-fucose as an internal residue. This unique structure prompted us to synthesize the trisaccharide as well as to reveal the anomeric configuration of Xyl and Gal moieties and the sulfated position of Fuc residue.

The sequence was proposed based on the negative FAB MS fragment ions indicating the elimination of Xyl, and partial fragmentation of Xyl as well as Fuc(SO_3^-) residues.[1] Further, the sulfated position was deduced by a fragment ion of *m/z* 137 stemmed from the C-4 to C-6 portion of Fuc(SO_3^-) in the MS/MS spectrum using linked scanning[3] with a constant B/E at *m/z* 537, which is $[M-H]^-$ for the trisaccharide.[4] Due to deficiency in the amount of the pure natural trisaccharide, the ^1H NMR signals (D_2O) could scarcely be assigned. However, the signals in the anomeric proton region indicated the presence of more than two α-anomers including the reducing end.[5] Among the possible 8 isomers originated from two interglycosidic linkages and one regioisomeric linkage, the most plausible isomeric pair due to xylosyl linkage, *i.e.*, D-Xylα1→3-D-Galα1→3[4-(SO_3^-)]-L-Fuc **1**, D-Xylβ1→3-D-Galα1→3[4-(SO_3^-)]-L-Fuc **2**, and one of its regioisomers, D-Xylβ1→3-D-Galα1→4[3-(SO_3^-)]-L-Fucα-OMe **3**, were selected as our synthetic targets.



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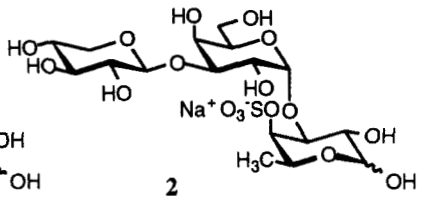

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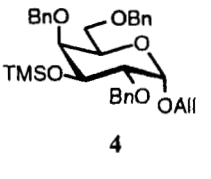

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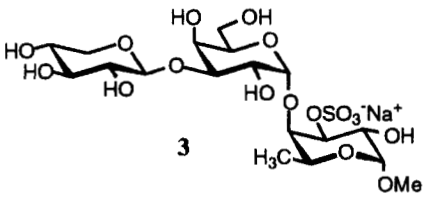

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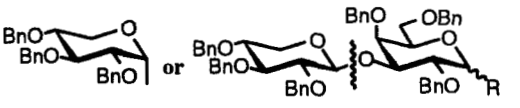
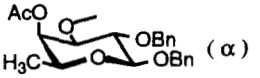
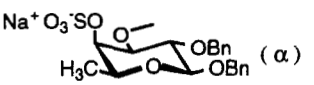
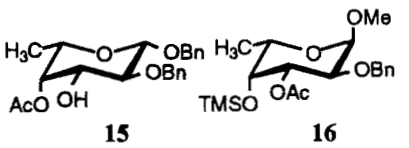
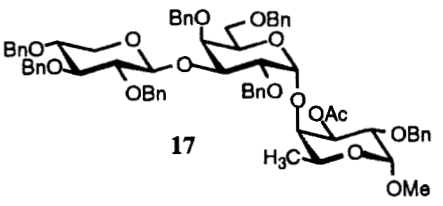


αXyl	βXyl	R
5	**10**	**OAll** (α)
6	**11**	**OH** (α, β)
7	**12**	**F** (α, β)
8	**13**	 (α)
9	**14**	 (α)



15 **16**



17

The two former trisaccharides, **1** and **2**, were synthesized by coupling of the disaccharide glycosyl donors **7** and **12**, respectively, with L-fucoside acceptor **15.** The 3-*O*-sulfated regioisomer **3** was synthesized using methyl α-fucoside acceptor **16.**

D-Galactoside acceptor **4** was prepared in 35% total yield from allyl α-D-galactopyranoside by temporary protection of the 3-hydroxyl group with *p*-methoxybenzyl residue followed by stannylene acetalation, benzylation, de-*O*-*p*-methoxybenzylation and finally trimethylsilylation. Condensation of 2,3,4-tri-*O*-benzyl-D-xylopyranosyl fluoride (α : β = 1 : 5) and **4** in the presence of trimethylsilyl triflate[6] in dichloromethane gave a mixture of disaccharides, which were easily separated by silica gel column chromatography to give α-linked **5** [^{13}C NMR ($CDCl_3$): C-1' 96.37 ppm; ^{1}H NMR ($CDCl_3$): H-1' 5.03 ppm, $J_{1',2'}$ = 3.3 Hz] and β-linked disaccharides **10** [C-1' 104.17 ppm; H-1' 4.83 ppm, $J_{1',2'}$ = 7.6 Hz] in 52% and 46% yields, respectively. Each disaccharide was easily converted into the corresponding glycosyl fluoride **7** and **12** by fluorination of the de-*O*-allylated derivative **6** and **11** using diethylaminosulfur trifluoride in 68% and 75% yield in two steps, respectively.

Glycosylation of L-fucoside acceptor **15** with the glycosyl fluoride **7** (α : β = 1 : 1) in the presence[7] of $SnCl_2$-$AgClO_4$ gave only α-(1→3)-linked trisaccharide **8** (H-1', 5.48 ppm, $J_{1',2'}$ = 3.3 Hz in $CDCl_3$) in 34% yield. Similarly, the coupling between **15** and **12** (α : β = 1 : 1) gave also only α-linked product **13** (H-1', 5.48 ppm, $J_{1',2'}$ = 3.3 Hz in $CDCl_3$) in 47% yield. De-*O*-acetylation of thus obtained trisaccharides **8** and **13**, followed by sulfation with ten molar equivalents of sulfur trioxide-pyridine complex in *N*,*N*-dimethylformamide, gave, after ion exchange, sodium 4-sulfates **9** and **14**, whose benzyl groups were directly hydrogenolyzed to provide the desired trisaccharides **1** and **2** in 80% and 79% yield, respectively.

The regioisomer of **2** was synthesized as the corresponding methyl α-glycoside **3** in a similar conversion sequence starting from the coupling of the disaccharide donor **12** and a fucoside acceptor **16** followed by de-*O*-acetylation, sulfation, and de-*O*-benzylation.

In examination of the ^{1}H NMR spectra of the sulfated trisaccharides **1** - **3** in D_2O only that of **2** coincides with that of the trisaccharide obtained from natural source. Although some signals of the natural trisaccharide, especially originated from the minor

Table Compared ^{1}H NMR shift[a] for natural and synthesized trisaccharides

Compound		H-1	H-4	H-6	H-1'	H-4'	H-1''	H-3''
Natural	α	5.21	-[b]	1.29	5.30	4.16	4.61	3.47
	β	4.62	4.64	1.32	5.32			
Synthesized **2**	α	5.22	4.72	1.27	5.32	4.17	4.63	3.475
	β	4.62	4.65	1.30	5.35			

a. Recorded at 270 MHz in D_2O at 25 °C with acetone as an indirect internal standard, where the signal resonates at δ 2.225. b. Not clearly observed.

anomer of the reducing end, are not obvious, now their characteristic signals could be assigned with the help of the fully assigned spectrum[8] of the synthesized trisaccharide **2** and the identity was confirmed by the good agreement of the significant signals as shown in Table. It is noteworthy that the ^{1}H NMR spectrum[9] of the regioisomeric trisaccharide **3** displays characteristic signals for H-1' (d, 5.57 ppm, $J_{1',2'}$ = 2.6 Hz) and H-4 (d, 4.37 ppm, $J_{4,3}$ = 2.6 Hz), which are quite different from those for H-1' (d, 5.28 ppm, $J_{1',2'}$ = 4.0 Hz) and H-4 (d, 4.69 ppm, $J_{4,3}$ = 2.3 Hz) of the corresponding methyl glycoside[10] of **2**.

These results indicate that the trisaccharide **2** must be one of the major oligosaccharide structures in the the ARIS and thus the interglycosidic anomeric linkages of xylose and galactose are now elucidated to be β and α, respectively. Further, the sulfate group was confirmed to be attached to the 4-position of fucose residue.

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8. All signals of the synthesized trisaccharide **2** were fully assigned as shown below by means of double quantum-filtered COSY and HOHAHA. Recorded at 500 MHz in D_2O at 25 °C. The chemical shifts are calculated assuming that HOD signal resonates at δ 4.806. ^{1}H NMR δ 5.328 (d, $J_{1',2'}$ = 4.0 Hz, H-1'β), 5.301 (d, $J_{1',2'}$ = 4.0 Hz, H-1'α), 5.207 (d, $J_{1,2}$ = 3.9 Hz, H-1α), 4.705 (s, H-4α), 4.631 (d, $J_{3,4}$ = 3.3 Hz, H-4β), 4.613 (d, $J_{1,2}$ = 8.0 Hz, H-1β), 4.607 (d, $J_{1'',2''}$ = 7.8 Hz, H-1''), 4.335 (q, H-5α), 4.300 (t, H-5'α), 4.288 (t, H-5'β), 4.151 (d, $J_{3',4'}$ = 3.3 Hz, H-4'α), 4.146 (d, $J_{3',4'}$ = 3.1 Hz, H-4'β), 4.134 (dd, $J_{2',3'}$ = 9 Hz, H-3'β), 4.128 (m, H-3'α), 4.111 (dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.1 Hz, H-3α), 4.012 (dd, H-2α), 4.092 (dd, $J_{2',3'}$ = 9.8 Hz, H-2'α), 4.091 (dd, H-2'β), 3.929 (q, H-5β), 3.927 (dd, H-5''eq), 3.925 (dd, $J_{2',3'}$ = 10.0 Hz, H-3β), 3.737(dd, J_{gem} = 12.0 Hz, H-6'aβ), 3.732 (dd, J_{gem} = 12.0 Hz, H-6'aα), 3.707, 3.703 (each of m, H-6'bα and β), 3.689 (dd, H-2β), 3.608 (ddd, $J_{3'',4''}$ = 9.1, $J_{4'',5''eq}$ = 5.5, $J_{4'',5''ax}$ = 10.5 Hz, H-4''), 3.460 (t, H-3''), 3.336 (dd, $J_{2'',3''}$ = 9.4 Hz, H-2''), 3.329 (dd, H-5''ax), 1.248 (d, J = 6.5 Hz, H-6α), 1.287 (d, J = 6.6 Hz, H-6β).
9. These spectra were recorded in D_2O at 25 °C with DSS as an indirect internal standard.
10. The methyl α-glycoside of trisaccharide **2** was synthesized **6** by the same route starting from the coupling of the donor **12** with the corresponding methyl α-glycoside of **15**.