Enzyme-assisted synthesis of a bivalent high-affinity dodecasaccharide inhibitor of mouse gamete adhesion
The length of the chains carrying distal α1,3-bonded galactose residues is critical

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Received 10 April 1995; revised version received 7 May 1995

Abstract Proposing to study the molecular mechanisms of mouse gamete adhesion with the aid of high affinity adhesion inhibitors of saccharide nature, we report here the enzymatic synthesis of a bivalent oligosaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (*4*), consisting of two long arms that link together two distal α1,3-galactose residues. Binding data reported elsewhere (E. Litscher et al., Biochemistry, 1995, 34, 4662–4669) show that *4* is a high affinity inhibitor of mouse gamete adhesion in vitro (IC_{50} = 9 μM), while a related octasaccharide Galα1-3Galβ1-4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc, consisting of two short arms is of very low inhibitory activity. The data highlight the importance of the two α-galactose residues of *4*, and the length of the sugar chains joining them.

Key words: Enzymatic synthesis; Mouse sperm receptor saccharide; NMR; MALDI-MS; Poly-(*N*-acetyllactosamino)glycan

1. Introduction

Adhesion of mouse gametes is mediated by large *O*-linked saccharides of the zona pellucida glycoprotein ZP3 [1–3]. These saccharides are believed to bind one or several kinds of saccharide binding proteins on the sperm surface [4,5]. Bleil and Wassarman [2] showed that distal α-linked galactose residues are an essential structural feature of the adhesion saccharides of mouse eggs. Miller et al. [3] in turn, have provided evidence of an important role of distal β-linked GlcNAc residues.

The adhesion-mediating saccharides of mouse eggs are difficult to obtain in quantity, and remain poorly defined structurally. In the lack of precise chemical information on the interacting molecules, a good approach to study the molecular mechanisms of gamete adhesion is to use high affinity adhesion inhibitors of saccharide nature [6]. By measuring the inhibitory potency of a number of different oligosaccharides of known structure and conformation, important conclusions may eventually be reached regarding the nature of the adhesion saccharides of the eggs as well as the adhesion proteins of the sperm. Such oligosaccharides can now be obtained in a high degree of purity by chemical [7] and enzyme-assisted [8] synthetic methods. Here, we describe enzyme-assisted synthesis and characterization by ^{1}H-NMR-spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of the 12-meric bi-antennary oligosaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**4**); its analog carrying glucose at the reducing end (**8**) was also synthesized for comparison. As reported elsewhere [6], glycan **4** revealed considerable sperm receptor activity, while the decasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**3**), lacking the distal α-linked galactose residues, was only marginally active at the concentrations studied. Also the monovalent pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc as well as the bivalent octasaccharide Galα1-3Galβ1-4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc were almost inactive at the concentrations studied. The data imply that the important structural features in the 'sperm receptor saccharides' of mouse eggs probably include the presence of several binding determinants as well as sufficiently long carbohydrate chains as spacers between them.

2. Materials and methods

2.1. Oligosaccharide primers

The hexasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**1**) was constructed essentially as described earlier [9]. The primer octasaccharide GlcNAcβ1-3Galβ1-4GlcNAcβ1-3(GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**2**) was synthesized as described [10]. Radiolabeled isotopomers of **1** and **2** were obtained by using radiolabeled sugar nucleotides instead of unlabeled

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Abbreviations: Gal, D-galactose; GlcNAc, *N*-acetyl-D-glucosamine; IC_{50}, inhibitory concentration that prevents 50% of sperm binding; LacNAc, *N*-acetyllactosamine; ManNAc, *N*-acetyl-D-mannosamine; MH, maltoheptaose; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; NMR, nuclear magnetic resonance.

Footnote: The positions of given monosaccharide residues in the oligosaccharides are specified by superscripts indicating the glycosidic linkages that lead to the reducing end [35]. The residues at the reducing end disaccharide bear no superscripts. The residues of glycan **7**, for example, are specified as follows: ^{3,3}Galβ1-4^{3,3}GlcNAcβ1-3^{3}Galβ1-4^{3}GlcNAcβ1-3(^{3,6}Galβ1-4^{3,6}GlcNAcβ1-3^{6}Galβ1-4^{6}GlcNAcβ1-6)Galβ1-4Glc.

SSDI 0014-5793(95)00535-8

ones at selected stages of the enzymatic syntheses. The primer hexasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1–6)Galβ1-4Glc (**5**) from human milk was from BioCarb, Lund, Sweden. The radiolabeled isotopomer of **5**, [^{3}H]Galβ1-4GlcNAcβ1-3([^{3}H]Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc was prepared by cleaving the unlabeled **5** with jack bean β-galactosidase (EC 3.2.1.23; Sigma, St Louis, MO, USA), and re-β1,4-galactosylating the cleavage product enzymatically with UDP-[^{3}H]Gal [11].

2.2. Enzymatic methods

Bovine milk β1,4-galactosyltransferase (EC 2.4.1.22; Sigma) and bovine thymus α1,3-galactosyltransferase (EC 2.4.1.87) reactions were carried out essentially as described previously [12, 13]. Human serum β1,3-GlcNAc transferase (EC 2.4.1.149) reactions were performed by incubating the radiolabeled acceptor saccharide with human serum and UDP-GlcNAc as described by Seppo et al. [11]. Digestions with β-galactosidase from *Diplococcus pneumoniae* (EC 3.2.1.23; Boehringer, Mannheim, Germany) were carried out as described [14].

2.3. Chromatographic methods

Gel filtration was performed in a column of Superdex 75 HR 10/30 (Pharmacia, Uppsala, Sweden), with water, at a flow rate of 1 ml/min. The eluant was monitored at 205 nm, and oligosaccharides were quantified against external *N*-acetylglucosamine. Paper chromatography was carried out using the mixture of n-butanol/acetic acid/water (10:3:7, by volume) as solvent. The mobility of oligosaccharides is given in relation to maltoheptaose (Sigma). The oligosaccharides were desalted by filtration in water through AG-1 (AcO^-) and AG-50W (H^+) (Bio-Rad, Richmond, CA, USA).

2.4. NMR-spectroscopy

^{1}H-NMR spectroscopy of the deuterium exchanged oligosaccharides was performed on ^2H_2O (99.996% ^{2}H; CIL, Andover, MA, USA) at 500 MHz in a Varian Unity 500 spectrometer. 1D spectra were recorded at 23°C or 25°C using a modification of the WEFT sequence as described [15] for water suppression. Chemical shifts were measured by reference to internal acetone (δ = 2.225 ppm) with an accuracy of 0.002 ppm.

2.5. MALDI-MS

Matrix-assisted laser desorption/ionization mass spectrometry was performed in the positive ion mode with irradiation from a nitrogen laser (337 nm) and 2,5-dihydroxybenzoic acid as the matrix with the Vestec VT-2000 linear time-of-flight instrument operated at 30 kV accelerating voltage. External calibration was used; this method has an accuracy of ±0.1% (±2 u at *m/z* 2000).

2.6. Distance estimations

To estimate the maximum spatial separation of O-3:s in the distal galactoses, models of glycans **1** and **3** of Table 1 were built using the Insight II program (Biosym Technologies Inc., San Diego, CA, USA). Dihedral angles $\varphi = -66°$, $\psi = 120°$ for Galβ1-4GlcNAc and $\varphi = -69°$, $\psi = -131°$ for GlcNAcβ1-3Gal, representing the minima in the extended conformation of poly-*N*-acetyllactosamine chain were used [16]. The torsion angles of the single GlcNAcβ1-6Gal linkage in both molecules were rotated to achieve the maximum separation between the O-3:s of the distal galactose residues. The structures were then relaxed with AMBER force field.

3. Results

*3.1. Synthesis of the decasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1–3(Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**3**)*

The primer octasaccharide GlcNAcβ1-3Galβ1-4GlcNAcβ1-3(GlcNAcβ1–3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**2**) (See Fig. 1A and Table 2 for the 1D ^{1}H-NMR data) was synthesized essentially as described previously [10]. It was further incubated with β1,4-galactosyltransferase and UDP-Gal, yielding the decasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1–3(Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**3**) that was purified by gel filtration, and in some experiments by paper chromatography (R_{MH} = 0.69). The reaction appeared to be complete in exhaustive experiments using a four-fold molar excess of the donor, no monogalactosylated products were detected. The acceptor-based yields of isolated **3** were 58–85%. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) revealed that two hexose residues were transferred to the acceptor; a major peak, assigned to $(M + Na)^+$ (calculated *m/z* = 1,867.7), was observed at *m/z* = 1,868.5, and a minor peak, assigned to $(M + K)^+$ (calculated *m/z* = 1,883.7), was seen at *m/z* = 1,884.6. No significant impurities in the size range of 1,700–2,020 *m/z* were visible in the MALDI-MS (data not shown). 1D ^{1}H-NMR-spectroscopy at 500 MHz (Fig. 1B, Table 2) confirmed that two β1,4-linked galactose residues had become transferred to the acceptor. When compared to the spectrum of glycan **2** (Fig. 1A, Table 2), a new 7.8 Hz doublet of two equivalents appears at 4.479 ppm. The doublet is assigned to H-1's of newly added terminal ^{3,3}Gal and ^{3,6}Gal (see footnote on title page) as in [17–19]. The 8.3 Hz doublet at 4.702 ppm from H-1 of ^{3,3}GlcNAc and ^{3,6}GlcNAc is shifted downfield by 0.021 ppm, specifying the galactosylation sites at both branches of glycan **2**.

*3.2. Synthesis of the dodecasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1–4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1–3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc (**4**)*

Incubation of radiolabeled **3** (4.4 nmol) with UDP-Gal and α1,3-galactosyltransferase of bovine thymus and subsequent paper chromatography revealed the formation of the dodecamer **4** (2.3 nmol) with two α-linked galactoses (R_{MH} = 0.47), together with products containing only one α-bonded galactose (0.9 nmol) (R_{MH} = 0.57). Another incubation, performed with 73 nmol of **3**, yielded 67 nmol of pure **4**. MALDI-MS revealed that two hexose residues had become transferred to the acceptor (Fig. 2); a major peak, assigned to $(M + Na)^+$ (calculated *m/z* = 2,192.0), was observed at *m/z* = 2,192.1, and a minor peak, assigned to $(M + K)^+$ (calculated *m/z* = 2,208.0), was seen at *m/z* = 2,208.1. No significant impurities in the size range of 1,900–2,420 *m/z* were visible in the MALDI-MS. The 1D ^{1}H NMR-spectrum of **4** is shown in Fig. 1C. The transfer of two galactoses in α1,3-linkage is indicated by the appearance of signals of $^{3,3+3,6}$Galα H-1 at 5.145 ppm and H-5 at 4.200 ppm, and H-4 of the penultimate $^{3,3+3,6}$Galβ at 4.183 ppm, amounting two equivalents each. Also, the H-1 signals of subterminal ^{3,3}Galβ and ^{3,6}Galβ are shifted downfield 0.072 ppm, completing the typical pattern of signals characteristic to terminal Galα1-3Galβ1-4GlcNAc-R structure in *N*-acetyllactosamine [20], oligo-*N*-acetyllactosaminoglycans [8], *O*-glycans [21] and *N*-glycans [22]. No signals arising from partially α-galactosylated molecules at the expected position of unsubstituted ^{3,3}Galβ H-1 or ^{3,6}Galβ H-1 at 4.479 ppm can be detected on the spectrum. Some signals of minor unidentified impurities were detected at 4.367 and 4.329 ppm; in addition a small amount of signals from H-1 of ManNAc at the reducing end were observed at 5.133 and 5.031 ppm. The same impurities were present in glycan **3**.

*3.3. Synthesis of the dodecasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1–4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1–3Galβ1-4GlcNAcβ1-6)Galβ1-4Glc (**8**)*

The hexasaccharide [^{3}H]Galβ1-4GlcNAcβ1-3([^{3}H]Galβ1-4GlcNAcβ1-6)Galβ1-4Glc (**5**) (93 nmol) was converted into

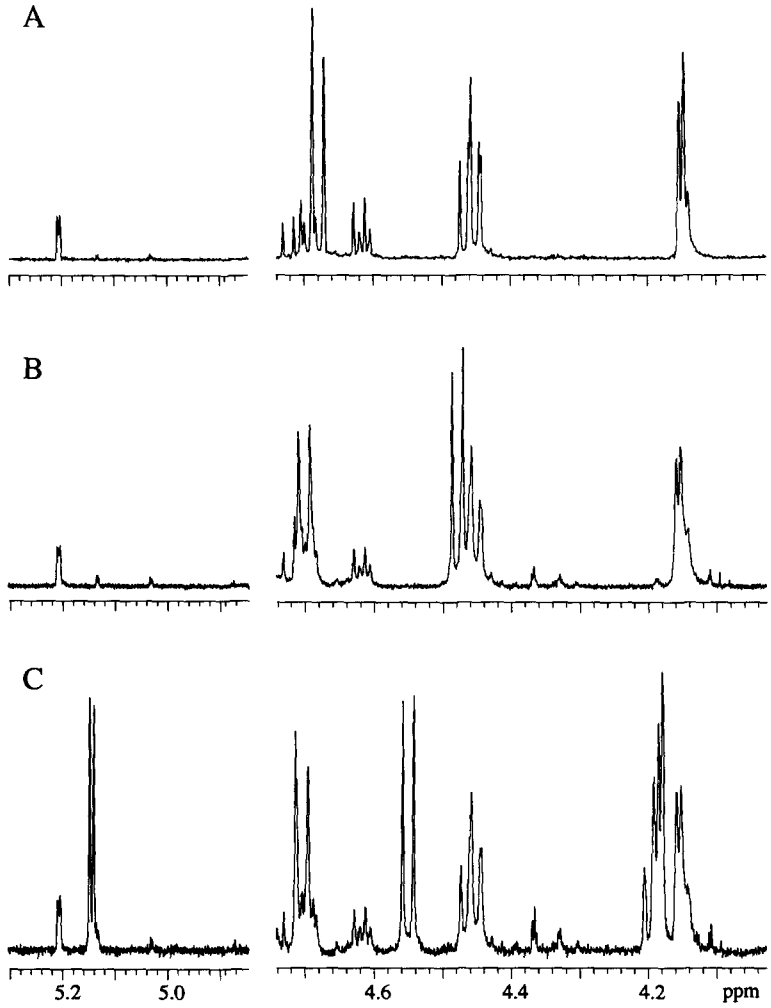


Fig. 1. Partial 1D ^{1}H-NMR spectra. **A**, Octasaccharide **2**; **B**, Decasaccharide **3**; **C**, Dodecasaccharide **4**.

GlcNAcβ1-3[^{3}H]Galβ1-4GlcNAcβ1–3(GlcNAcβ1-3[^{3}H]Galβ1-4GlcNAcβ1–6)Galβ1-4Glc (**6**) (57 nmol) (R_{MH} = 0.96) by means of UDP-GlcNAc and β1,3-GlcNAc transferase present in human serum. The NMR-spectrum of **6** (see Table 2) revealed that two terminal β1,3-linked *N*-acetylglucosamine units were transferred to the acceptor: A new 8.3 Hz doublet of two proton equivalents appearing at 4.681 ppm was assigned to H-1's of ^{3,3}GlcNAc and ^{3,6}GlcNAc, as in glycan **2**. 3-Substitution of the penultimate galactoses of **6** is supported by the appearance of an unresolved H-4 signal of ^{3}Gal and ^{6}Gal at 4.152 ppm, and by the upfield shift (-0.016 ppm) of the H-1 signals of ^{3}Gal and ^{6}Gal of **6**. A similar shift resulted from the conversion of saccharide **1** to **2**, as shown in Table 2.

Incubation of ^{3}H-labelled **6** (54 nmol) with UDP-Gal and β1,4-galactosyltransferase gave the decasaccharide Galβ1-4GlcNAcβ1-3[^{3}H]Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-3[^{3}H]Galβ1-4GlcNAcβ1-6)Galβ1-4Glc (**7**) (41nmol) that appeared almost pure in paper chromatography (R_{MH} = 0.54). The ^{1}H NMR-spectrum of **7** revealed the transfer of two terminal β1,4-linked galactoses (see Table 2). MALDI-MS confirmed the structure of **7** as $Gal_5GlcNAc_4Glc$: The $(M + Na)^+$ signal revealed *m/z* 1,826.7 (calculated 1,826.6), while the $(M + K)^+$ peak was of *m/z* 1,843.3 (calculated 1,842.6).

Incubation of **7** (10.2 nmol) with UDP-Gal and α1,3-galactosyltransferase gave pure Galα1-3Galβ1-4GlcNAcβ1-3[^{3}H]Galβ1-4GlcNAcβ1-3(Galα1-3Galβ1-4GlcNAcβ1-3[^{3}H]Galβ1-4GlcNAcβ1–6)Galβ1-4Glc (**8**) (7.3 nmol) (R_{MH} = 0.36). The ^{1}H NMR-spectrum confirmed the presence of two α1,3-linked Gal units in the same way as in glycan **4** (see Table 2). In MALDI mass spectrum, the $(M + Na)^+$ signal of **8** revealed *m/z* 2,151.3 (calculated 2,150.9), while the $(M + K)^+$ peak was of *m/z* 2,167.7 (calculated 2,166.9).

3.4. *Inhibition of mouse gamete adhesion by the dodecasaccharide 4 and related saccharides*

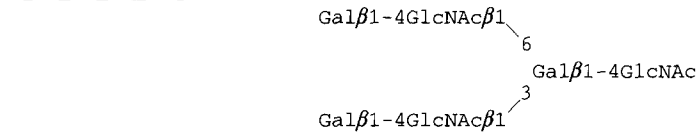
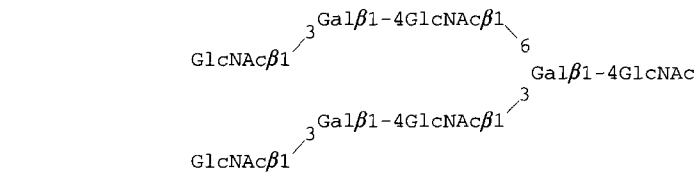
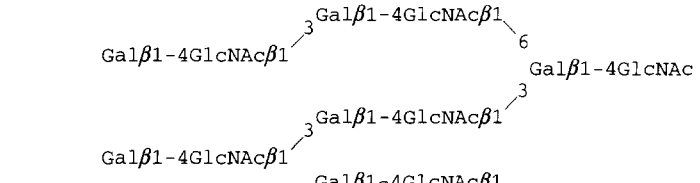
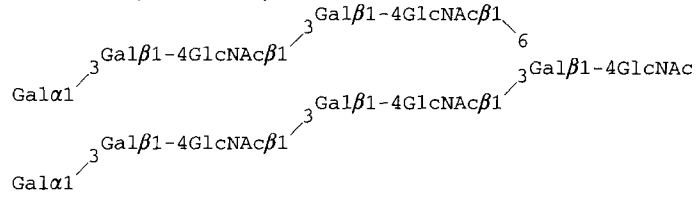
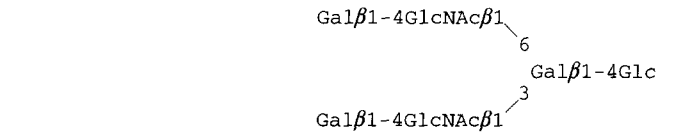
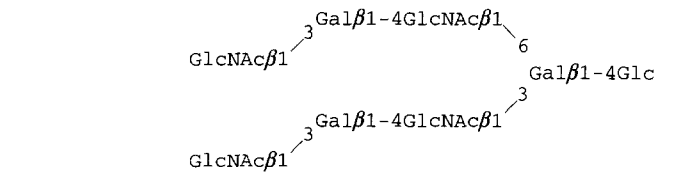
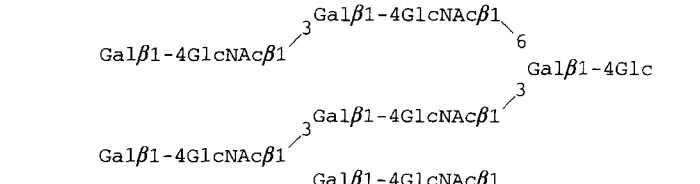
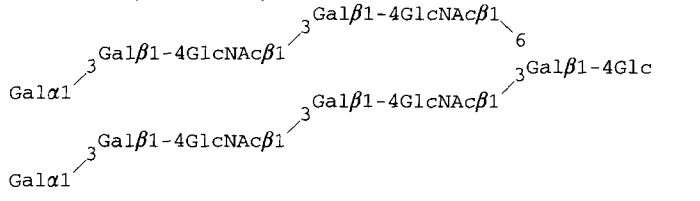
Fewer sperm were bound to mouse eggs in vitro in the presence of the dodecasaccharide **4** than in parallel control experiments performed without added saccharide [6]. Data of this and some similar experiments performed with related saccharides are collected in Table 3. The dodecasaccharide **4**, at 10 μM, reduced the number of egg-bound sperm by as much as 55 ± 23%. In contrast, the decasaccharide **3**, lacking the distal α-galactose residues, inhibited gamete binding only marginally at 10 μM, the highest concentration tested. The monovalent pentasaccharide Galα1-3Galβ1-4GlcNAcβ1-3Galβ1–4GlcNAc, too, was nearly inactive at this concentration.

Most important, the bivalent octasaccharide Galα1-3Galβ1-4GlcNAcβ1-3(Galα1–3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc, which differs from **4** only in the much smaller length of its two arms, was inactive as an adhesion inhibitor. Taken together, the data of Table 3 show that besides the presence of two α-galactose residues, also the presentation of the binding epitopes at the distal ends of two *long* arms is critically important for a high affinity inhibitor of mouse gamete adhesion.

4. Discussion

Mouse egg oligosaccharides are known to mediate the adhesion of sperm to egg [1–3], but they are difficult to obtain in sufficient quantities for analysis, and remain poorly defined structurally. To identify the adhesion saccharides, and to obtain adhesion inhibitors, we have performed inhibition experiments of gamete adhesion by using enzymatically synthesized

Table 1
Structures and numbering of the oligosaccharides discussed in this study

No.	Oligosaccharide
1	
2	
3	
4	
5	
6	
7	
8	

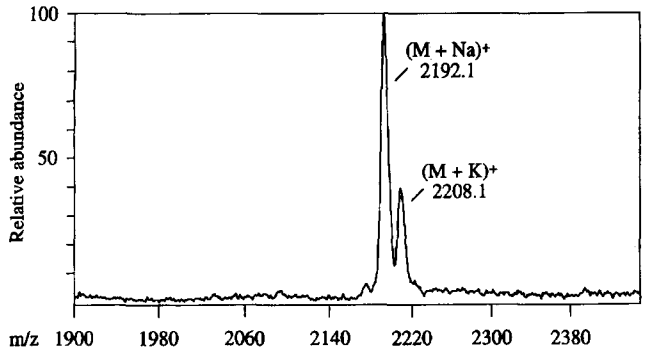


Fig. 2. MALDI mass spectrum of dodecasaccharide **4**.

oligosaccharides of well defined structures. Recently we described enzymatic de novo synthesis of a 18-meric poly-*N*-acetyllactosaminoglycan that contains four distal α1,3-bonded galactose residues [8], and possesses considerable 'sperm receptor activity' (IC_{50} = 2.7 μM) [6]. As the monovalent Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc revealed insignificant activity, the data highlighted the central role of multivalency as a structural element of 'sperm receptor saccharides'. The present syntheses were carried out in order to study the effect of the length of the saccharide chain spacers joining the important α-linked galactose residues in the active 'sperm receptor saccharides'.

The binding assay data reported elsewhere [6] are collected to Table 3, showing that glycan **4** possessed significant 'sperm receptor activity', reducing the number of egg-bound sperm to half of their control value at 9 μM. The high 'sperm receptor activity' of glycan **4** of the present experiments is contrasted with the apparent inactivity of the octasaccharide Galα1-3Galβ1-4GlcNAcβ1-3(Galα1–3Galβ1-4GlcNAcβ1-6)Galβ1-4GlcNAc synthesized by Seppo et al. [8]. Glycan **4** has much longer α-galactose bearing arms than the octasaccharide; the maximal spatial separation between the terminal α-galactoses is estimated to be about 4 nm in glycan **4**, but only about 2 nm in the octasaccharide. The length of the spacer chains joining two binding determinants of saccharide ligands is known to be decisive also in other cases. For example, the synthetic ligands of chicken hepatic lectin [23] and serum mannose binding protein [24] show optimal binding affinities when the maximal spatial separations between the terminal monosaccharides are 3.3 nm and 2.8–3.0 nm, respectively. The binding affinities of bivalent sialosides to whole influenza virus, too, are quite sensitive to changes in the spacer length [25].

In contrast to glycan **4**, glycan **3** revealed only marginal 'sperm receptor activity' (Table 3). These observations resemble those of Bleil and Wassarman [2], who showed that removal or modification of distal α-linked galactose residues of the natural sperm receptor saccharides of mouse eggs renders them inactive as sperm receptors.

The high efficiency of glycan **4** suggests that it binds to two sites, most likely to the protein sp56 [4] on sperm surface. The notion of multiple binding of oligosaccharide ligands to isolated lectin molecules and also to lectins on cell or virus surfaces has been put forward experimentally by several groups [23–30]. Multiple binding of the ligands and ensuing crosslinking of the receptors on cell surfaces may have also signalling functions: While small glycopeptides of mouse ZP3 [31] and the synthetic 'sperm receptor active' saccharides [6] fail to induce the acrosome reaction, the induction is achieved with the glycopeptides that are crosslinked in situ by anti-ZP3-IgG [32].

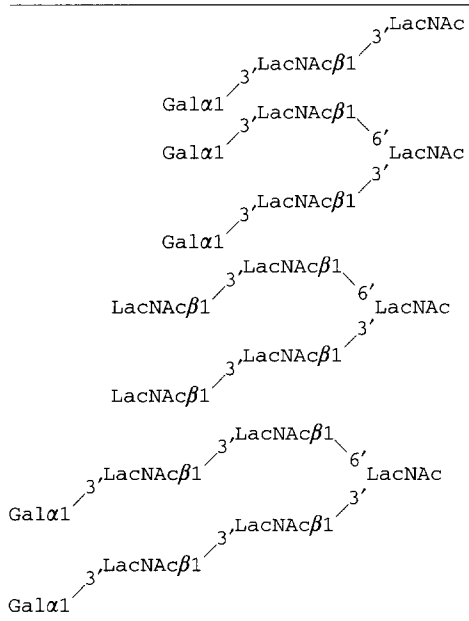
The saccharides **6**–**8** of Table 1, bearing glucose instead of *N*-acetylglucosamine at the reducing end, were synthesized for structural comparison with the analogous glycans **2**–**4**. Remarkably, the reporter group resonances of the distal trisaccharide sequences were completely identical in the two series of saccharides, and also in the 1→3 and the 1→6 bonded arms; the similarity extended even to the H-4 resonances of ^{3}Gal and ^{6}Gal (see Table 2). Some differences were observed between the two series of saccharides in the reporter group resonances of Gal, ^{6}GlcNAc, as well as in ^{6}Gal H-1s. These resonance differencies suggest that ^{6}GlcNAc and/or ^{6}Gal of **1**–**4** may interact with the reducing end GlcNAc; indirect observations on similar interactions have been described also previously [33,34].

Table 2
^{1}H-NMR chemical shifts[a] of structural reporter group signals of synthetic saccharides

		Structure							
Residue[b]	proton	**1**	**2**	**3**	**4**	**5**[c]	**6**	**7**	**8**
Glc(Nac)	H-1	5.208/4.725	5.207/4.724	5.207/4.724	5.207/4.724	5.220/4.664	5.218/4.665	5.218/4.664	5.217/4.664
Gal	H-1	4.457	4.455	4.455	4.455	4.431	4.426	4.426	4.426
	H-4	4.151	4.145	4.146	4.145	4.145	4.140	4.140	4.140
^{3}GlcNAc	H-1	4.700/4.695	4.698/4.693	4.698/4.693	4.697/4.693	4.706	4.697	4.697	4.697
^{6}GlcNAc	H-1	4.624/4.618	4.622/4.614	4.622/4.614	4.622/4.613	4.645/4.639	4.638/4.632	4.638/4.631	4.639/4,631
^{3}Gal	H-1	4.480	4.467	4.467	4.467	4.483	4.467	4.467	4.467
	H-4	3.924	4.152	4.156	4.156	3.929	4.152	4.156	4.156
^{6}Gal	H-1	4.464/4.467	4.452	4.451	4.452	4.475	4.458	4.458	4.458
	H-4	3.924	4.152	4.156	4.156	3.929	4.152	4.156	4.156
$^{3,3+3,6}$GlcNAc	H-1	–	4.681	4.702	4.705	–	4.681	4.702	4.706
$^{3,3+3,6}$Gal	H-1	–	–	4.479	4.551	–	–	4.479	4.551
	H-4	–	–	N.D.	4.183	–	–	N.D.	4.183
$^{3,3+3,6}$Galα	H-1	–	–	–	5.145	–	–	–	5.145
	H-5	–	–	–	4.200	–	–	–	4.199

[a]Chemical shifts are given in ppm scale by reference to internal acetone signal set to 2.225 ppm. If two values for a resonance are given, they refer to α/β anomers of the molecule in question.
[b]For naming of monosaccaride residues see Footnote 1.
[c]Data from [36].
N.D. Not determined.

Table 3
Sperm receptor activities of oligosaccharides presented here[a]

Oligosaccharide	Inhibition of sperm binding (±S.D.) at 10 μM
	12%[b]
	3% (±2%)
	8% (±6%)
	55% (±23%)

[a]Data from [6].
[b]The average of two individual experiments, which gave 10% and 14% inhibition.

Acknowledgements: The work was supported in part by grants from the University of Helsinki, from the Finnish Academy of Science, from Jenny and Antti Wihuri Foundation, from Leo and Regina Wainstein Foundation and from the Technology Development Centre. Mass spectrometry experiments were carried out by C.E.C. at the MIT Mass Spectrometry Resource which is supported by Grant No. RR00317 (to K. Biemann) from the NIH Center for Research Resources.

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