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Chemo-Enzymatic Synthesis of Asymmetrical Multi-Antennary *N*-glycans to Dissect Glycan-Mediated Interactions between Human Sperm and Oocytes[†]

Zoeisha S. Chinoy[a], Frédéric Friscourt[a], Chantelle J. Capicciotti[a], Philip Chiu[d], Geert-Jan Boons*[a,b,c]

[a]Complex Carbohydrate Research Center; [b]Department of Chemistry, University of Georgia, 315 Riverbend Road, Athens, GA 30602 (USA); and [c]Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, and Bijvoet Center for Biomolecular Research, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht (Netherlands), [d]Department of Obstetrics and Gynaecology, Faculty of Medicine Building, The University of Hong Kong, Hong Kong (CHINA)

E-mail: gjboons@ccrc.uga.edu or g.j.p.h.boons@uu.nl

[†]Electronic supplementary information (ESI) available. Procedures for chemical and enzymatic synthesis of compounds, copies of NMR spectra, experimental methods for bio-assays.

Abstract. Complex *N*-glycans of glycoproteins of the zona pellucida (ZP) of human oocytes have been implicated in the binding of spermatozoa. The termini of these unusual bi-, tri-, and tetra-antennary *N*-glycans consist of the tetrasaccharide sialyl-Lewis^x (SLe^x), which was previously identified as the minimal epitope for sperm binding. We describe here the chemo-enzymatic synthesis of highly complex tri-antennary *N*-glycans derived from ZP carrying SLe^x moieties at the C-2 and C-2' arm and a sialyl-Lewis^x-Lewis^x (SLe^x-Le^x) residue at the C-6 antenna and two closely related analogs. The compounds were examined for their ability to inhibit the interaction of human sperm to ZP. It was found that the SLe^x-Le^x moiety is critical for inhibitory activity, whereas the other SLe^x moieties exerted minimal effect. Further studies with SLe^x-Le^x and SLe^x showed that the extended structure is the more potent inhibitor. In addition, trivalent SLe^x-Le^x and SLe^x were prepared which showed greater inhibitory activity compared to their monovalent counterparts. Our studies show that although SLe^x can inhibit the binding of spermatozoa, presenting this epitope in the context of a complex *N*-glycan results in

a loss of inhibitory potential, and in this context only SLe^x-Le^x can make productive interactions. It is not the multivalent display of SLe^x on a multi-antennary glycan but the presentation of multiple SLe^x-Le^x on the various glycosylation sites of ZP that accounts for high avidity binding.

1. Introduction

The first committed step of animal fertilization is the binding between an oocyte and sperm. A specialized extracellular matrix surrounding the oocyte, called the zona pellucida (ZP) in mammals, is crucial for this process by mediating species-restricted recognition between gametes.[1,2] Human ZP is composed of 4 glycoproteins termed as ZP1, ZP2, ZP3 and ZP4 which are organized into long cross-linked fibrils. Both ZP2 and ZP3 have been implicated as binding partners for sperm to induce spermatozoa exocytosis.[3, 4]

A number of studies have indicated that glycans of the ZP glycoproteins are critical for binding of mammalian sperm.[5,6,7,8]. These ZP glycans may take part in direct interactions with the sperm ZP receptors or may provide the proper tertiary structure that increases the availability of the ZP glycoproteins to their binding proteins on spermatozoa.[4] Recently, Dell and coworkers determined the structures of human ZP glycans by employing ultrasensitive mass spectrometry.[9] This in-depth analysis showed the presence of bi-, tri-, and tetra-antennary *N*-glycans, many of which terminate in SLe^x epitopes. Heterogeneity of the bi-, tri-, and tetra-antennary *N*-glycans was limited to differences in the length of the antenna and the level of fucosylation. In healthy humans, where SLe^x plays a key role in leukocyte trafficking, fewer than 1% of *N*-glycans carry this structure and none were found with more than one SLe^x containing antenna.[10] On the other hand, SLe^x epitopes are highly expressed by many human cancer cells. [11] The presence of SLe^x-Le^x epitopes is another unusual feature of a number of *N*-glycans of ZP, and previously this structure was only found on tumor cells.[9]

It was shown that SLe^x alone or conjugated to bovine serum albumin (BSA) for multivalent presentation can partially inhibit the binding of human sperm to isolated human ZP in a hemizona assay.[9] Furthermore, the trisaccharide Lewis^x (Le^x) had no effect on the binding of sperm indicating that the sialic acid moiety of SLe^x is critical for recognition. The latter finding was supported by the observation that an antibody against SLe^x can inhibit the binding of sperm to ZP by ~60%, whereas this was not the case for an anti-Le^x antibody.

We expected that the *N*-glycans of oocytes, which are modified by several SLe^x moieties, can bind with high avidity to sperm cells through multivalent interactions.[12,13] In this regard, the binding of multiple ligands expressed on a single entity to multiple receptors on a complementary entity can result in a substantial increase in functional affinity and is common in glycan-mediated biological recognition events. The glycoprotein ZP3 has three identified *N*-glycosylated sites (Asn125, Asn147 and Asn272),[14] and thus, it is possible that the binding avidity between sperm and the ZP is further increased by the presentation of three *N*-glycans each carrying several SLe^x moieties. Since glycan structures are highly heterogeneous when isolated from natural sources, the identification of the biological relevant epitope is very challenging. Synthetic chemistry can address this challenge by providing well-defined glyco-structures for interrogation of glycan-receptors interactions.[15,16]

We report here the chemo-enzymatic preparation of three synthetically challenging tri-antennary *N*-glycans (**1**, **2** and **3**). Compound **1** is a representative structure of a tri-antennary *N*-glycan derived from human ZP carrying a SLe^x moiety at the C-2 and C-2' arm and a SLe^x-Le^x residue at the C-6 antenna (Fig. 1). Compounds **2** and **3** are positional isomers of each other and are derived from **1** where one of the SLe^x moieties is replaced by Le^x. The compounds were evaluated as inhibitors of the binding of human sperm to ZP, and surprisingly it was found that the SLe^x-Le^x moiety at the C-6 antenna contributes significantly to the inhibitory potential of the *N*-glycans, whereas the other SLe^x epitopes exerted a minimal effect. Further studies with SLe^x-Le^x (**4**) and SLe^x (**5**) showed that the extended structure is the more potent inhibitor. In addition, trivalent SLe^x-Le^x and SLe^x (**7**-**8**) were prepared and these compounds exhibited greater inhibitory potency compared to their monovalent counterparts, indicating that the interaction of multiple glycan epitopes on the surface of oocytes with multiple receptors on sperm cells results in high avidity of binding.

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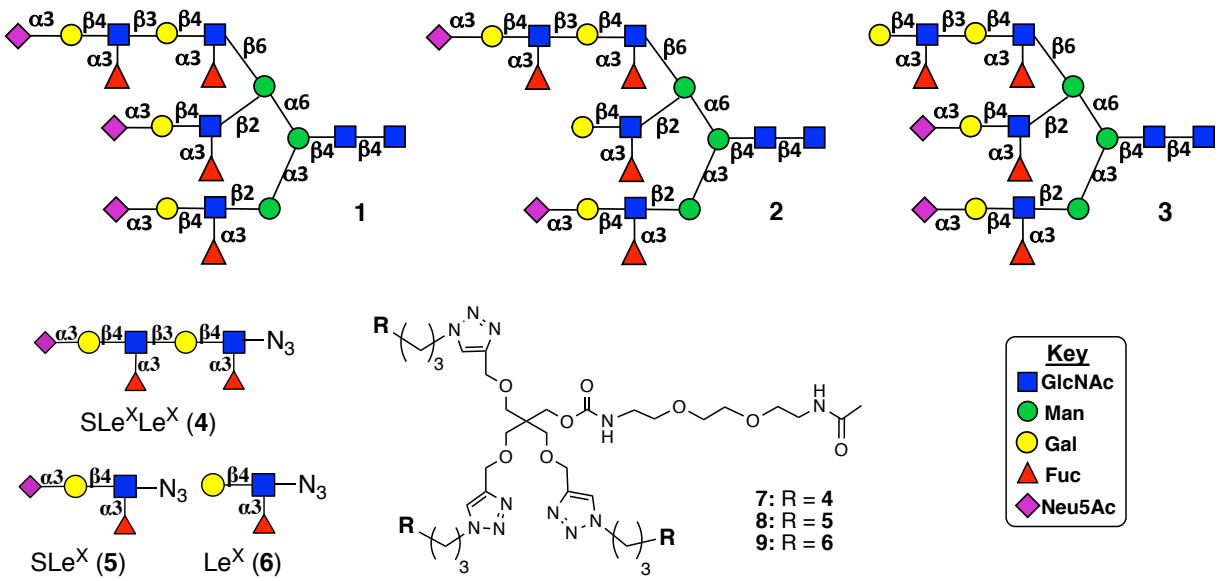

Figure 1. Structures of *N*-glycan derivative **1** found on human ZP and analogs thereof (**2**, **3**), SLe^x and Le^x containing oligosaccharides **4**-**6** and glycodendrimers **7**-**9**.

2. Results and Discussion

2.1 Chemoenzymatic synthesis. The synthesis of *N*-glycans such as **1** represents a formidable challenge due to their asymmetrical architecture. Although *N*-glycans are usually asymmetrically branched, synthetic efforts have almost exclusively focused on the preparation of simpler symmetrical structures. [17,18,19,20,21,22,23] This stems from the difficulties of controlling diversification at the various branching sites, especially when several different complex terminal structures need to be appended. Recently, we reported a flexible approach for the preparation of asymmetrical multi-antennary glycans. It employs protected pentasaccharide **10**[24] (Scheme 1) that is modified at positions where branching points in *N*-linked glycans can occur[25] with the orthogonal protecting groups levulinoyl (Lev), fluorenylmethyloxycarbonate (Fmoc), allyloxycarbonate (Alloc), and 2-naphthylmethyl (Nap). These protecting groups can sequentially be removed and chemically glycosylated using benzylated or acetylated *N*-acetylglucosamine (GlcNAc) and *N*-acetyllactosamine (LacNAc) donors (*e.g.* **11**, **12** and **13**)[24]. Removal of all protecting groups, except the acetyl esters, gives precursor glycans that at each antenna can selectively be extended by a panel of glycosyltransferases to give highly complex asymmetric *N*-glycans. We employed this methodology to chemically synthesize decasaccharide **19**, which was then extended by a panel of glycosyltransferases to provide the

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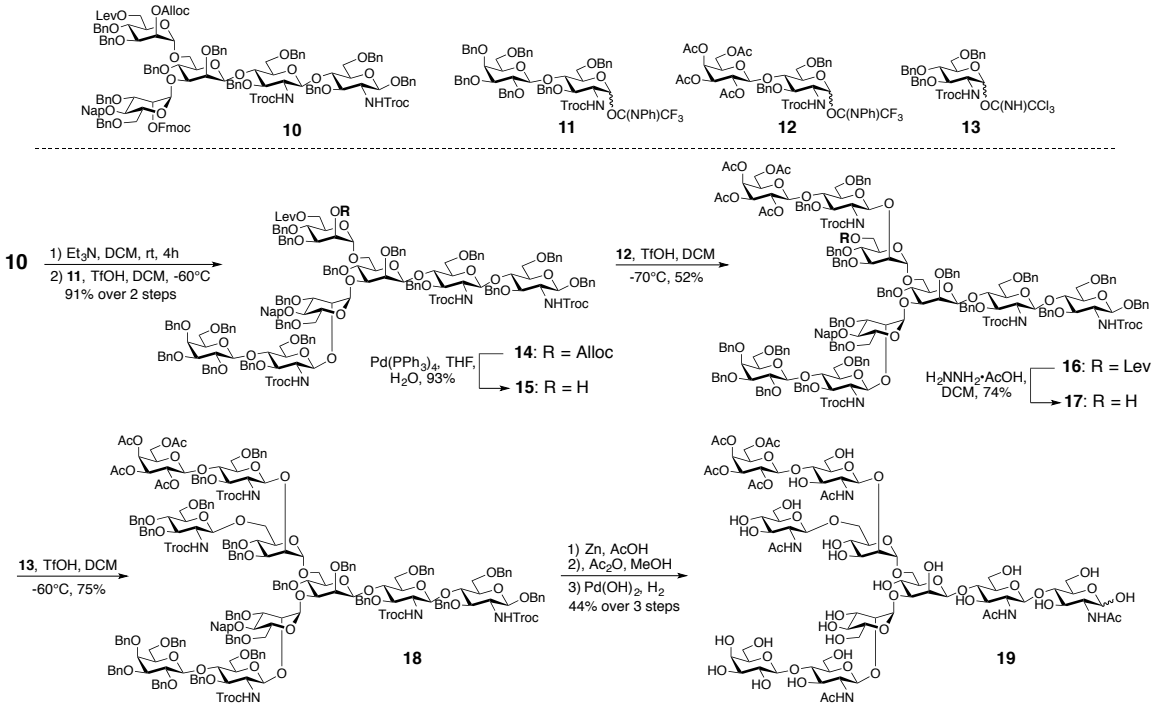
target glycans **1**-**3**. Thus, the Fmoc group of **10** was selectively removed by the hindered base triethylamine to give an alcohol that was glycosylated with **11** using triflic acid as the promoter to provide heptamer **14** in 91% yield over 2 steps (Scheme 1). Next, the Alloc protecting group of **14** was selectively removed with $Pd(PPh_3)_4$ and the resulting acceptor **15** was glycosylated with **12** to provide nonasaccharide **16** in 52% yield as only the β-anomer due to neighboring group participation of the Troc functionality at C-2 of the glycosyl donor. The Lev ester of **16** was selectively cleaved with hydrazine acetate to give **17**, which was then coupled with glycosyl donor **13** to afford decasaccharide **18** in an excellent yield of 75%. Partial deprotection of the latter compound was accomplished by a three-step procedure to give target compound **19** entailing the removal of Troc protecting groups with zinc in acetic acid, acetylation of the resulting free amines with acetic anhydride in methanol, and finally catalytic hydrogenolysis of the benzyl and Nap ethers.



Scheme 1. Chemical synthesis of precursor decasaccharide **19** for enzymatic extension.

For the preparation of glycan **1**, the acetyl esters of **19** were cleaved by treatment with ammonium hydroxide and the two LacNAc moieties of the resulting compound **20** were sialylated enzymatically using α-2,3-sialyltransferase from *Pasteurella multocida* (α2,3SiaT) in

the presence of cytidine 5'-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac), and alkaline phosphatase from calf intestine (CIAP) to give **21** (Scheme 2). The dodecasaccharide was then fucosylated with α-1,3-fucosyltransferase (α1,3FucT) from *Helicobacter pylori*, guanosine 5'-diphospho-L-fucose (GDP-Fuc) and CIAP resulting in the formation of the bis-fucosylated derivative **22**. Next, the GlcNAc moiety of the C-6 antenna was converted into LacNAc by employing a β-1,4-galactosyltransferase from bovine milk (GalT-1), uridine 5'-diphosphogalactose (UDP-Gal), and CIAP to give **23**. Treatment of the latter compound with β-1,3-*N*-acetylglucosaminyltransferase from *Helicobacter pylori* (β1,3GlcNAcT), UDP-GlcNAc, and CIAP resulted in the selective addition of a β(1,3)-linked GlcNAc moiety to the LacNAc moiety of the β1-6 branch to provide glycan **24**. This branch was further extended by GalT-1 to give **25**, which was sialylated and fucosylated to give the target glycan **1**. Interestingly, when α2,3SiaT from *Pasteurella multocida* was employed for the sialylation of **25**, no product formation was observed. However, switching from the bacterial enzyme to the mammalian sialyltransferase (ST3Gal-IV) resolved the issue. Intermediate **25** could also be fucosylated with α1,3FucT to give glycan **3**, which has a Le^x-Le^x epitope at the C-6 arm, and SLe^x moieties at the C-2 and C-2' antennae. After each enzymatic transformation, the product was purified by size exclusion column chromatography using Sephadex G-25 gel filtration and the resulting compounds were fully characterized by NMR spectroscopy (800 or 900 MHz) and mass spectrometry of the permethylated derivatives. If any starting material was detected by mass spectrometry, the compound was resubjected to the enzyme and sugar nucleotide until a homogeneous product was obtained.

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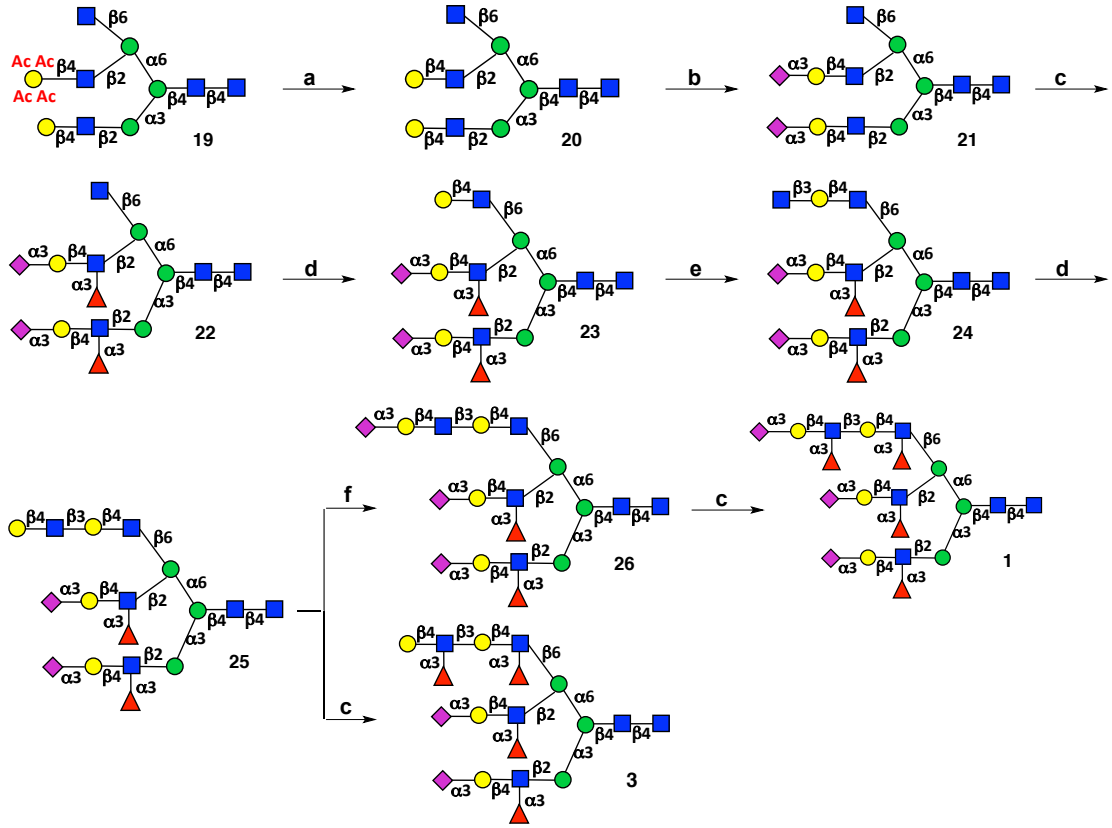

Scheme 2. Chemoenzymatic synthesis of human ZP *N*-glycan derivatives **1** and **3**. Reagents and conditions: **a)** NH_4OH, H_2O; **b)** α2,3SiaT, CMP-Neu5Ac, CIAP, sodium cacodylate buffer (pH 7.3), 37 °C; **c)** α1,3FucT, GDP-Fuc, CIAP, $MnCl_2$, Tris buffer (pH 7.5), 37 °C; **d)** β1,4GalT, UDP-Gal, CIAP, $MnCl_2$, Tris buffer (pH 7.5), 37 °C; **e)** β1,3GlcNAcT, UDP-GlcNAc, CIAP, $MgCl_2$, HEPES buffer (pH 7.3), 37 °C; **f)** ST3Gal-IV, CMP-Neu5Ac, CIAP, sodium cacodylate buffer (pH 7.3), 37 °C.

Glycan **2**, which has unique appendages at the C-2, C-2' and C-6 arm, was prepared starting from precursor compound **19**. In this case, the C-2 arm of **19** was selectively sialylated with α2,3SiaT from *Pasteurella multocida,* which was followed by base treatment and fucosylation to give SLe^x and Le^x moieties at C-2 and C-2', respectively. The C-6 antenna was then extended into an SLe^x-Le^x residue as described for compound **1** (for details see Scheme S1)

Recent advances in glycomics have highlighted the importance of multivalent presentation of glycan epitopes in order to significantly increase the avidity and specificity of protein-carbohydrate interactions.[26] Synthetic multivalent scaffolds are powerful tools for displaying multiple copies of monomeric glyco-epitopes in a controlled spatial arrangement.[27] Therefore, we first synthesized monomeric azido-linkered Le^x, SLe^x and SLe^x-Le^x using a chemo-

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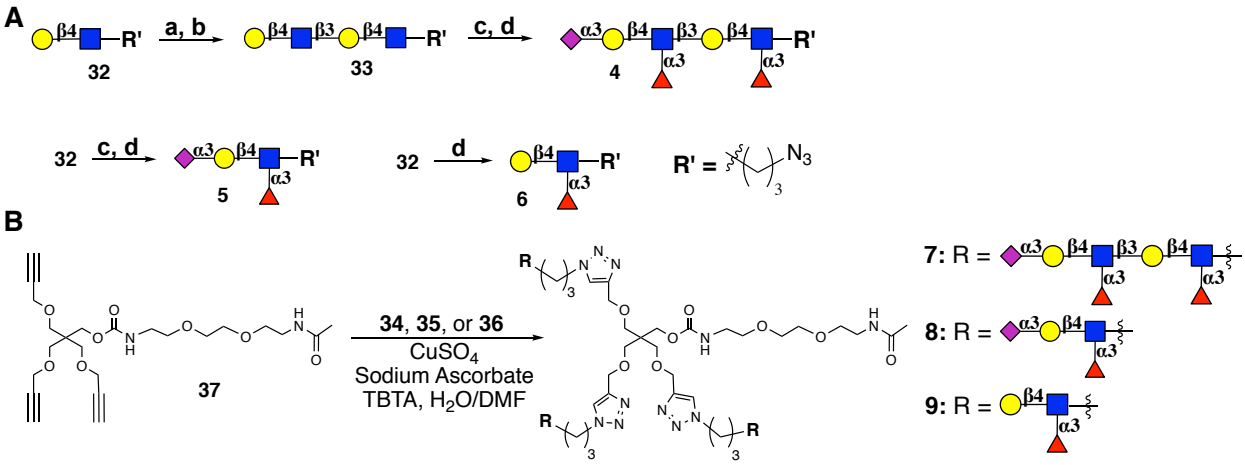
enzymatic approach and attached these to pentaerythritol-based scaffold modified with alkynes for trivalent presentation. Briefly, the azido-SLe^x-Le^x **4** was synthesized by subjecting chemically synthesized azido-LacNAc (**32**) (for synthesis see SI) to mammalian β-1,3-*N*-acetylglucosaminyltransferase (B3GnT2), UDP-GlcNAc and CIAP, followed by treatment with GalT-1, UDP-Gal and CIAP to produce the azido-di-LacNAc **33**. Sialylation of **33** followed by bis-fucosylation using ST3Gal-IV and mammalian α-1,3-fucosyltransferase (FUT5) respectively, yielded target azido-SLe^x-Le^x **6**. Similarly, sialylation and fucosylation of **32** provided azido-SLe^x **5**, while fucosylation of **32** gave azido-Le^x **6** (Scheme 3A). Finally, azido-SLe^x-Le^x **4**, -SLe^x **5** and -Le^x **6** were conjugated to the pentaerythritol-based scaffold displaying three alkyne functionalities (**37**) (for details see Scheme S3) using Cu-catalyzed alkyne-azide cycloaddition (CuAAC), followed by purification using Sephadex G-25 gel filtration, to provide the trivalent glycoconjugates **7**, **8** and **9**, respectively (Scheme 3B).



Scheme 3. Preparation of glycodendrimers **7**-**9**. **A)** Chemoenzymatic synthesis of linkered SLe^X-Le^X (**4**), SLe^X (**5**) and Le^X (**6**). Reagents and conditions: **a)** B3GnT2, UDP-GlcNAc, CIAP, $MnCl_2$, HEPES buffer (pH 7.3), 37 °C; **b)** GalT-1, UDP-Gal, CIAP, $MnCl_2$, Tris buffer (pH 7.5), 37 °C; **c)** ST3Gal-IV, CMP-Neu5Ac, CIAP, sodium cacodylate buffer (pH 7.3), 37 °C; **d)** FUT5, GDP-Fuc, CIAP, $MnCl_2$, Tris buffer (pH 7.5), 37 °C. **B)** Chemical synthesis of glycodendrimers **7**-**9** using CuAAC.

2.2 Biological evaluation. The hemizona assay was employed to determine the effect of the synthetic glycans on sperm-ZP binding. In this assay, non-living human eggs are bisected to generate two equivalent hemispheres of ZP (hemizona). The influence of a compound of interest on capacitated sperm binding can then be evaluated by an internally controlled

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comparison because of matched hemizona surfaces. Due to the limited availability of human eggs, each compound was examined at one concentration (200 μM, 10 replicates) at which differences in potency should be detectable. As anticipated, the number of sperm bound to the hemizona was significantly decreased after treatment with SLe^x whereas this was not the case for Le^x (Figure **2**). Interestingly, treatment with SLe^x-Le^x led to a greater reduction in sperm binding compared to SLe^x. The complex tri-antennary glycans **1** and **2**, which contain an extended SLe^x-Le^x moiety at the C-6 antenna and additional SLe^x residues at the other arm(s), gave a similar reduction in sperm-hemizona binding compared to SLe^x-Le^x. Importantly, glycan **3**, which has SLe^x moieties at the C-2 and C-2' arm and a Le^x-Le^x residue at the C-6 antenna was much less potent compared to monomeric SLe^x, highlighting the importance of glyco-epitopes topology for stronger binding.

This surprising observation led us to investigate the effect of multivalent presentation of key monomeric epitopes such as Le^x, SLe^x and SLe^x-Le^x, on egg-sperm binding. Inhibition experiments with trivalent derivatives **7-9** showed a similar trend in inhibitory activity as their monomeric structures, with trivalent SLe^x-Le^x (**7**) being the most potent compound whereas no activity was observed for the Le^x-containing derivative **9**. Interestingly, trivalent SLe^x (**8**) and SLe^x-Le^x (**7**) exhibited greater inhibitory potency than their monovalent counter parts SLe^x and SLe^x-Le^x, respectively, confirming that not only the glycan-structure is highly important for proper egg-sperm binding but also that the multi-presentation of the epitopes is a key factor for enhancing the interaction between the glycan and its receptor.

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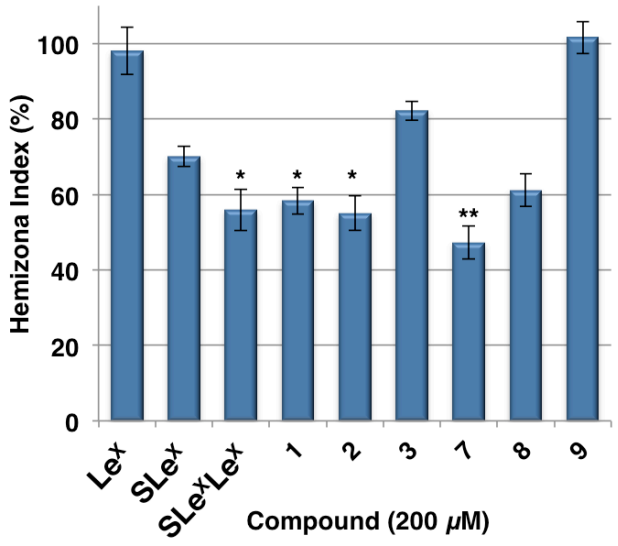

Figure 2. Hemizona binding index (HZI) of capacitated spermatozoa incubated in the presence of Le^X, SLe^X, SLe^XLe^X (**4**-**6**), glycans **1**-**3**, or glycodendrimers **7**-**9** compared with medium alone (control). All compounds were assessed for HZI at 200 μM. The dendrimer concentration is based on molar concentrations of SLe^x. Data represents mean ± SEM (n = 10). $^{\star}P<0.05$, $^{\star\star}P<0.01$ compared with SLe^X.

3. Conclusions

A previous study indicated that SLe^x-containing *N*-glycans of human oocytes are involved in human sperm-oocyte binding.[9] The data presented here demonstrates that SLe^x-Le^x is actually the key epitope that facilitates this interaction. Although the tetrasaccharide SLe^x can inhibit oocyte-sperm binding in the hemizona assay, it was found that presenting this epitope in the context of a complex *N*-glycan results in a loss of inhibitory potential. During the past decade, it has become clear that glycan binding proteins can recognize with high selectivity relatively small oligosaccharide motifs that are often found at termini of complex glycans.[28] For example, Galectins recognize terminal LacNAc moieties, Siglecs bind 2,3- or 2,6-sialylated LacNAc, and Selectins complex SLe^x structures. A more complex picture of protein-glycan recognition is emerging in which the topology of a complex glycan can modulate terminal glycan recognition.[21,29,30,31,32,33,34] It has, however, been difficult to examine, in a systematic way, such features of glycan-protein binding. This study shows that contemporary chemo-enzymatic synthesis can provide highly complex asymmetrical *N*-glycans such as **1**, as well as closely related analogs that can probe in a systematic manner which features of complex glycans are involved in the binding to proteins. We have also found that the presentation of the SLe^x-Le^x

epitope on a trivalent scaffold results in a greater inhibitory potential compared to the monovalent counterparts. These results are in agreement with the previous observation that attachment of multiple copies of SLe^x to BSA provides a conjugate that exhibits inhibitory potential at lower concentrations. While the protein receptor is currently unknown,[35] it is likely that sperm cells express multiple receptors that can bind to SLe^x-Le^x containing glycans. Furthermore, ZP3, which is abundantly expressed by oocytes, has three sites of *N*-glycosylation that can carry glycans such as **1**. Thus, it is likely that the interaction of multiple glycan epitopes on the surface of oocytes with multiple receptors on sperm cell, results in high avidity of binding. This study indicates that it is not the presentation of multiple SLe^x epitopes within a single *N*-linked glycan, but the presentation of multiple *N*-glycans carrying a SLe^x-Le^x epitope that contributes to high avidity binding.

Acknowledgements

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References

[1] K. J. Mengerink, V. D. Vacquier, *Glycobiology* **2001**, *11*, 37r-43r.

[2] P. M. Wassarman, L. Jovine, E. S. Litscher, *Nat. Cell Biol*. **2001**, *3*, E59-64.

[3] M. A. Avella, B. A. Baibakov, M. Jimenez-Movilla, A. B. Sadusky, J. Dean, *Sci. Transl. Med.* **2016**, *8*, 336ra360-336ra360.

[4] P. C. Chiu, K. K. Lam, R. C. Wong, W. S. Yeung, *Semin. Cell. Dev. Biol.* **2014**, *30*, 86-95.

[5] T. T. F. Huang, E. Ohzu, R. Yanagimachi, *Gamete Res.* **1982**, *5*, 355-361.

[6] M. S. Patankar, S. Oehninger, T. Barnett, R. L. Williams, G. F. Clark, *J. Biol. Chem.* **1993**, *268*, 21770-21776.

[7] H. Lucas, S. Bercegeay, J. Le Pendu, M. Jean, S. Mirallie, P. Barriere, *Hum. Reprod.* **1994**, *9*, 1532-1538.

[8] M. Jimenez-Movilla, M. Aviles, M. J. Gomez-Torres, P. J. Fernandez-Colom, M. T. Castells, J. de Juan, A. Romeu, J. Ballesta, *Hum. Reprod.* **2004**, *19*, 1842-1855.

[9] P.-C. Pang, P. C. N. Chiu, C.-L. Lee, L.-Y. Chang, M. Panico, H. R. Morris, S. M. Haslam, K.-H. Khoo, G. F. Clark, W. S. B. Yeung, A. Dell, *Science* **2011**, *333*, 1761-1764.

[10] P. Babu, S. J. North, J. Jang-Lee, S. Chalabi, K. Mackerness, S. R. Stowell, R. D. Cummings, S. Rankin, A. Dell, S. M. Haslam, *Glycoconj. J.* **2009**, *26*, 975-986.

[11] J. L. Magnani, *Glycobiology* **1991**, *1*, 318-320.

[12] J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555-578.

[13] M. Cohen, *Biomolecules* **2015**, *5*, 2056-2072.

[14] M. Zhao, E. S. Boja, T. Hoodbhoy, J. Nawrocki, J. B. Kaufman, N. Kresge, R. Ghirlando, J. Shiloach, L. Pannell, R. L. Levine, H. M. Fales, J. Dean, *Biochemistry* **2004**, *43*, 12090-12104.

[15] L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Angew. Chem. Int. Ed.* **2006**, *45*, 2348-2368.

[16] T. J. Boltje, T. Buskas, G.-J. Boons, *Nat. Chem.* **2009**, *1*, 611-622.

[17] C. Unverzagt, *Angew. Chem. Int. Ed.* **1996**, *35*, 2350-2353.

[18] S. Hanashima, S. Manabe, Y. Ito, *Angew. Chem. Int. Ed.* **2005**, *44*, 4218-4224.

[19] S. Jonke, K.-g. Liu, R. R. Schmidt, *Chem.-Eur. J.* **2006**, *12*, 1274-1290.

[20] B. Sun, B. Srinivasan, X. Huang, *Chem.-Eur. J.* **2008**, *14*, 7072-7081.

[21] C. Unverzagt, G. Gundel, S. Eller, R. Schuberth, J. Seifert, H. Weiss, M. Niemietz, M. Pischl, C. Raps, *Chem.-Eur. J.* **2009**, *15*, 12292-12302.

[22] S. Serna, J. Etxebarria, N. Ruiz, M. Martin-Lomas, N. C. Reichardt, *Chem.-Eur. J.* **2010**, *16*, 13163-13175.

[23] M. A. Walczak, S. J. Danishefsky, *J. Am. Chem. Soc.* **2012**, *134*, 16430-16433.

[24] Z. Wang, Z. S. Chinoy, S. G. Ambre, W. J. Peng, R. McBride, R. P. de Vries, J. Glushka, J. C. Paulson, G. J. Boons, *Science* **2013**, *341*, 379-383.

[25] K. W. Moremen, M. Tiemeyer, A. V. Nairn, *Nat. Rev. Mol. Cell. Biol.* **2012**, *13*, 448-462.

[26] J. C. Paulson, O. Blixt, B. E. Collins, *Nat. Chem. Biol.* **2006**, *2*, 238-248.

[27] A. Bernardi, J. Jimenez-Barbero, A. Casnati, C. De Castro, T. Darbre, F. Fieschi, J. Finne, H. Funken, K. E. Jaeger, M. Lahmann, T. K. Lindhorst, M. Marradi, P. Messner, A. Molinaro, P. V. Murphy, C. Nativi, S. Oscarson, S. Penades, F. Peri, R. J. Pieters, O. Renaudet, J. L. Reymond, B. Richichi, J. Rojo, F. Sansone, C. Schaffer, W. B. Turnbull, T. Velasco-

Torrijos, S. Vidal, S. Vincent, T. Wennekes, H. Zuilhof, A. Imberty, *Chem. Soc. Rev.* **2013**, *42*, 4709-4727.

[28] C. D. Rillahan, J. C. Paulson, *Annu. Rev. Biochem.* **2011**, *80*, 797-823.

[29] H. J. Gabius, S. Andre, J. Jimenez-Barbero, A. Romero, D. Solis, *Trends. Biochem. Sci.* **2011**, *36*, 298-313.

[30] T. L. Lowary, *Curr. Opin. Chem. Biol.* **2013**, *17*, 990-996.

[31] J. Hirabayashi, T. Hashidate, Y. Arata, N. Nishi, T. Nakamura, M. Hirashima, T. Urashima, T. Oka, M. Futai, W. E. Muller, F. Yagi, K. Kasai, *Biochim. Biophys. Acta.* **2002**, *1572*, 232-254.

[32] A. Antonopoulos, N. Demotte, V. Stroobant, S. M. Haslam, P. van der Bruggen, A. Dell, *J. Biol. Chem.* **2012**, *287*, 11240-11251.

[33] N. Srinivasan, S. M. Bane, S. D. Ahire, A. D. Ingle, R. D. Kalraiya, *Glycoconj. J.* **2009**, *26*, 445-456.

[34] C. M. Nycholat, R. McBride, D. C. Ekiert, R. Xu, J. Rangarajan, W. Peng, N. Razi, M. Gilbert, W. Wakarchuk, I. A. Wilson, J. C. Paulson, *Angew. Chem. Int. Ed.* **2012**, *51*, 4860-4863.