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RESEARCH ARTICLE Very Important Paper

A De Novo Chemoenzymatic Assembly Strategy Enables the Efficient Preparation of a Large Core-Xylosylated N-Glycan Library for Probing Antibodies Against *Schistosoma japonicum*

Fangyu Wei[1,2,3] | Xiao Tian[2,4] | Dingyi Mao[5] | Yang Dai[5] | Jiabin Zhang[2,3,4] | Liuqing Wen[1,2,3]

[1]State Key Laboratory of Chemical Biology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China | [2]Department of Carbohydrate-Based Drug Research Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China | [3]University of Chinese Academy of Sciences, Beijing, China | [4]Zhongshan Institute for Drug Discovery, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Zhongshan, Guangdong Province, China | [5]National Health Commission Key Laboratory of Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Provincial Medical Key Laboratory, Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu Province, China

Correspondence: Yang Dai (daiyang@jipd.com) | Jiabin Zhang (jiabinzhang@simm.ac.cn) | Liuqing Wen (lwen@simm.ac.cn)

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ABSTRACT

Core-xylosylated N-glycans represent a ubiquitous class of N-glycans widely distributed across nature. Recently, core-xylosylated N-glycans have attracted considerable attention in the medical field, as they could elicit strong immune responses. Notably, antibodies targeting core-xylosylated N-glycans could kill *Schistosoma* in vitro, and thus process potentials for protecting host from *Schistosoma* infections. However, the structural complexity has posed significant challenges to access structure-defined core-xylosylated N-glycans. Here, a powerful synthetic platform enabling the efficient preparation of core-xylosylated N-glycans is described. The strategy is based on de novo biosynthetic pathways to efficiently generate a sufficient quantity of N-glycan core precursors, coupled with the use of a robust β1,2-Xylosyltransferase to install core xylose in a stereoselective and regioselective manner. This approach further leverages diversity-oriented synthesis to systematically construct a comprehensive core-xylosylated N-glycan library, encompassing a broad range of structurally diverse N-glycans through enzymatic extension strategy. With the glycan array made from this glycan library, we investigated the binding activities of the antibodies against *Schistosoma japonicum*, which has caused widespread infections in Asia. In addition to the synthetic breakthroughs, this work also provides an insightful understanding of the structure–function relationships of core-xylosylated N-glycans, which will advance the rational design of carbohydrate-based vaccine against *Schistosoma* infections.

1 | Introduction

N-Glycan is the most abundant class of oligosaccharides that occurs in living cells [1]. They are structurally conserved during evolution and consist of a core pentasaccharide region substituted with numerous sugars in a non-stoichiometric manner [2]. Core-xylosylated N-glycans, the attachment of xylose to the central mannose through β1,2-linkage, are a ubiquitous class of

Fangyu Wei and Xiao Tian contributed equally to this work.

N-glycans in nature [3]. They are not found in humans, but are abundantly expressed in plants, and parasitic helminths of the genus *Schistosoma* [4]. Core-xylosylated N-glycans have garnered significant attention in the medical field due to their ability to elicit potent immune responses [5–8]. Notably, antibodies targeting schistosome glycans have been shown to kill *schistosomula* in vitro and thus process potential for protecting the host from *Schistosoma* infections [9–12]. Infection with *Schistosoma* parasites results in schistosomiasis, a disease that remains highly prevalent in many low- and middle-income countries and for which no effective vaccine is currently available. The World Health Organization (WHO) has recognized schistosomiasis as one of the twenty neglected tropical diseases [13, 14]. It is estimated that schistosomiasis affects more than 200 million people worldwide and causes up to seventy million disability-adjusted-life years [15, 16]. Therefore, Science magazine has identified schistosomiasis as one of the ten diseases for which the development of an effective vaccine is most urgently needed [17].

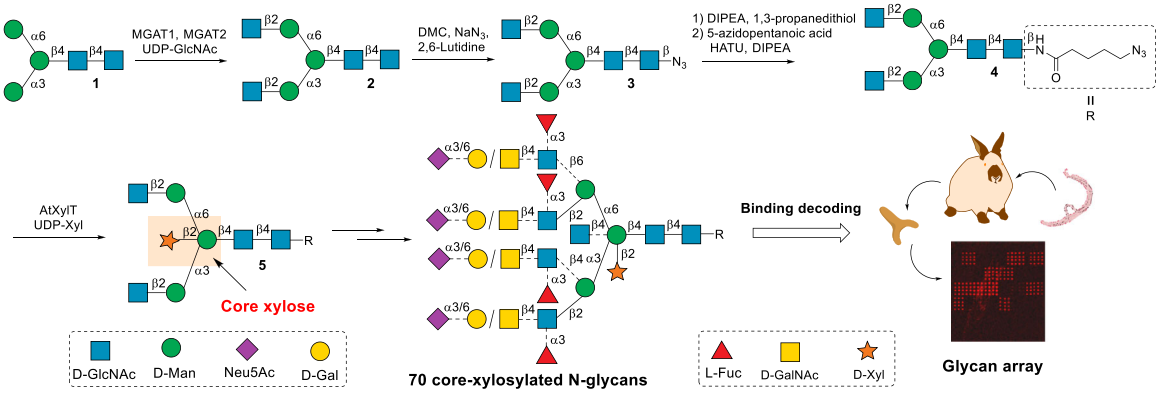
Considering the importance of N-glycans in many research fields [18–27], the synthesis of N-glycans has long been a major focus in the field of carbohydrate chemistry [28]. Numerous methodologies have been established to access structurally well-defined N-glycans by many leading research groups, for example, Wong and co-workers [29–31], Boons and co-workers [32, 33], Danishefsky and co-workers [34, 35], Unverzagt and co-workers [36, 37], Fukase and co-workers [38], Ito and co-workers [39, 40], Wang and co-workers [41, 42], and Wen and co-workers [43, 44]. However, the incorporation of core xylose modification introduces additional challenges to the already demanding synthesis of N-glycans. Chemical installation of core xylose needs extensive protecting group manipulations and requires a high level of technical expertise from researchers [45–47]. The numerous reaction steps typically lead to low overall yields, thereby limiting the application of chemical synthesis to prepare enough quantity precursors, which is the prerequisite to construct comprehensive glycan library that encompass a broad diversity of structural variants. Therefore, although a growing body of studies has highlighted the potential applications of core-xylosylated N-glycans in both fundamental research and medicinal chemistry, their biological roles and structure–function relationships remain poorly understood, largely due to the limited availability of structurally well-defined core-xylosylated N-glycans.

In this work, a chemoenzymatic synthetic strategy based on de novo biosynthetic pathway of N-glycans for preparation of core-xylosylated N-glycans is described (Scheme 1). By the strategy, 70 structure-defined core-xylosylated N-glycans that cover diverse typical N-glycan structures can be easily obtained from common starting materials in high yields. The obtained N-glycan library was subsequently fabricated into a microarray to evaluate the binding activities of selected lectins and antibodies from the *Schistosoma*-immunized host.

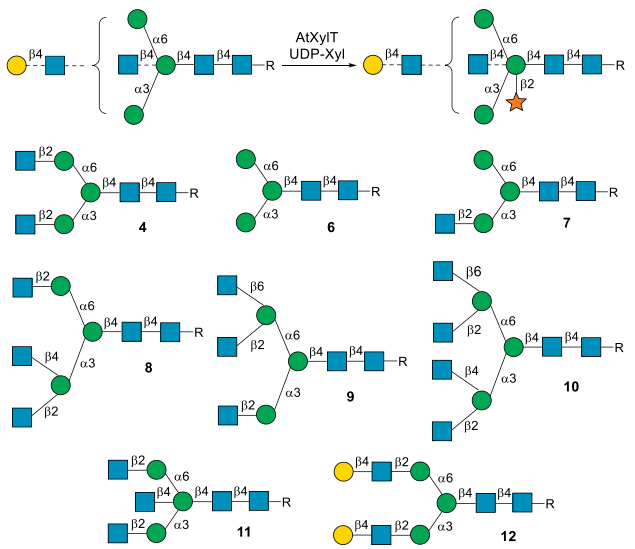
2 | Results and Discussion

Core-xylosylated N-glycans are broadly distributed across diverse species in nature, but are absent in humans. The core-xylosylated N-glycans discovered in *Schistosoma* contain bi-, tri-, or tetra-antennary branches [48]. However, study has only focused primarily on the bi-antennary core-xylosylated N-glycans [45], as the difficulty in obtaining tri- and tetra-antennary structures. Alternatively, glycan extraction from *Schistosoma* glycoproteins, in combination with mass spectrometry–based characterization, has also been employed to produce core-xylosylated N-glycans for investigation of their structure–function relationships [49]. Nevertheless, due to technical limitations, extraction-based approaches typically yield heterogeneous glycan mixtures, which complicates the precise delineation of structure–function relationships of antigenic glycans from Schistosoma. Here, we set out to prepare a core-xylosylated N-glycan library that covers diverse typical N-glycan structures. The library contains bi-, tri-, and tetra-antennary backbones, which are decorated with LacNAc or LacdiNAc-related epitopes. These epitopes are commonly found in *Schistosoma* and humans. To construct a large sugar library that contains diverse structures, readily available core N-glycan pentasaccharides and heptasaccharides are critical. As discussed above, highly demanding synthetic manipulations and low synthetic yields have restricted the application of chemical synthetic strategies for producing N-glycan core pentasaccharides or heptasaccharides on a large scale. Alternatively, the degradation of SGP, which can be extracted from egg yolk, provides a more efficient strategy to produce core precursors than chemical synthesis [50–52]. Nevertheless, SGP occurs in egg yolk at low abundance (~0.3%) [53], and its extraction process consumes a large amount of organic solvent and is both time-consuming and labor-intensive. Recently, we developed an efficient chemoenzymatic strategy, inspired by the de novo biosynthetic pathway of N-glycans, to assemble N-glycans from common starting materials [43]. By a few facile enzymatic and chemical steps [43], we were able to quickly prepare 3.9 g of N-glycan core pentasaccharide **1** for constructing a core-xylosylated N-glycan library in this work. With **1** in hand, 5.02 g of **2** was further prepared using human *N*-acetylglucosaminyltransferases 1 and 2 (MGAT1 and MGAT2) in a one-pot reaction (89% yield, Scheme 1). Then, a 5-azidopentanoic acid ($penN_3$) linker was installed in the reducing end of **2** to give compound **4** (3.51 g) [43]. The azido linker can be selectively reduced to an amine group later, enabling the covalent immobilization of the obtained compounds on an NHS-activated glass surface for the fabrication of a glycan array.

In addition to the availability of N-glycan core precursors, an efficient β1,2-xylosyltransferase is also essential for the successful synthesis of core-xylosylated N-glycans. We chose a β1,2-Xylosyltransferase from *Arabidopsis thaliana* (AtXylT) [54], for further test as it was reported that AtXylT prepared in insect cells could accept several bi-antennary and high mannose N-glycan structures as substrates [55]. In this work, we successfully expressed AtXylT using a mammalian cell protein expression system. The designed recombinant protein harbors a GFP tag to facilitate the protein solubility, and more than 100 mg protein can be obtained from 1L medium using the HEK293 expression system (Figure S1). More importantly, the prepared AtXylT exhibited remarkably high enzymatic activity (>1800 U mg^{-1}), enabling its efficient application in large-scale glycan synthesis. To test the possibility of the use of AtXylT to synthesize the designed structures, we first synthesized bi-, tri-, and tetra-antennary N-glycans **6** to **12** from compound **4** using the branching enzymes involved in biosynthesis of N-glycans as reported previously (Figure S2) [44]. In addition to MGAT1 and MGAT2, other



SCHEME 1 | De novo chemoenzymatic assembly of complex core-xylosylated N-glycans for probing antibodies from *Schistosoma*-immunized host.



Substrate	4	6	7	8	9	10	11	12
RA (%)[a]	100[b]	ND	77	ND	10	ND	ND	ND

FIGURE 1 | Substrate specificity study of AtXylT. [a] Relative activity, [b]The activity towards **4** was defined as 100%.

branching enzymes include N-acetylglucosaminyltransferases **3**, **4**, and **5** (termed as MGAT3, MGAT4, and MGAT5) [56]. MGAT3 is responsible for the addition of bisecting GlcNAc, which is generally regarded as structurally non-extendable. Substrate specificity study shows AtXylT processes high activity towards **4** and **7**, while no activity towards **6** was observed, indicating the activity of AtXylT needs β1,2-linked N-acetyl glucosamine (GlcNAc) residue at the nonreducing terminus of the α1,3-branched mannose. The existence of terminal β1,4 galactose (**12**) strongly inhibits the activity of AtXylT, according well with the previous study [55]. Meanwhile, the existence of β1,4-linked or β1,6-linked GlcNAc residue also strongly inhibits the activity of AtXylT (compounds **8**, **9**, and **10**), indicating AtXylT cannot be used to synthesize tri- and tetra-antennary core-xylosylated N-glycans directly (Figure 1).

We first synthesized bi-antennary core-xylosylated N-glycan precursor **5** from **4** using AtXylT. 2.66 g of **4** was incubated with AtXylT using UDP-xylose as donor. The reaction can proceed cleanly after overnight. Once finished, 2.50 g of **5** can be obtained easily after purification using a size-exclusion column in a yield of 86%. The structure was confirmed by NMR analysis (Figure 2) [57]. Comparing with the ^{1}H NMR spectra of compound **4**, a new signal appeared at 4.44 ppm, with the H1 of Man-1 downfield from 4.78 ppm to 4.88 ppm in compound **5**. HSQC and HMBC analysis of **5** showed that H1 of Xyl-1 is coupled with C2 of Man-1, and the $^1J_{H1,\,C1}$ of Xyl-1 is 164.05 Hz, indicating that Xyl-1 is linked to Man-1 through β1,2-linkage (Figure 2) [57]. As **6** could not be accept by AtXylT, **13** was prepared from **7** by using AtXylT (Figure 3A). Then, the removal of the GlcNAc residue of compound **13** by GlcNAcH afforded compound **14** (Figure 3A).

As discussed above, AtXylT cannot recognize tri- and tetra-antennary core-xylosylated N-glycan precursors such as **8**, **9**, or **10**. Therefore, we tried to develop other strategies to prepare multi-branched core-xylosylated N-glycans (Figure 3). We first evaluated the substrate specificities of MGAT3, MGAT4, and MGAT5 using substrate **5** to determine whether these enzymes can accept core-xylosylated N-glycans as substrates. Activity study shows that both MGAT3 and MGAT5 could efficiently recognize compound **5** (Figure S3). MGAT4 processes a low activity towards compound **5** in comparison to **4** (11%), indicating the existence of core xylose inhibits the activity of MGAT4. However, increasing the enzyme concentration and extending the reaction time could enhance the conversion efficiency, rendering the process synthetically valuable. In a large-scale synthetic reaction, **5** was incubated with MGAT5 using UDP-GlcNAc as glycosylation donor. Once no starting material was observed by TLC, the reaction was stopped and purified using size-exclusion column. 395 mg of compound **16,** which contains tri-antennary branches, can be easily obtained in 90% yield. Similarly, another tri-antennary structure **15** was synthesized from **5** using MGAT4. As discussed above, the presence of core xylose reduces the activity of MGAT4. Thus, more enzymes were added in the reaction system to achieve a higher yield. Once the reaction stopped moving forward, the reaction was quenched, and the product was purified. Finally, 439 mg of **15** was obtained in 47% yield regarding to **5**. Interestingly, **15** can also be accepted efficiently by MGAT5 to afford the tetra-antennary N-glycan **17** (Figure 3). In another synthetic route, **5** was incubated with MGAT3 using UDP-GlcNAc to afford **18** (Figure 3). **18** cannot be accepted by either MGAT4 or MGAT5. Nevertheless, in the substrate specificity studies, we found **15**, **16**, and **17** can all be

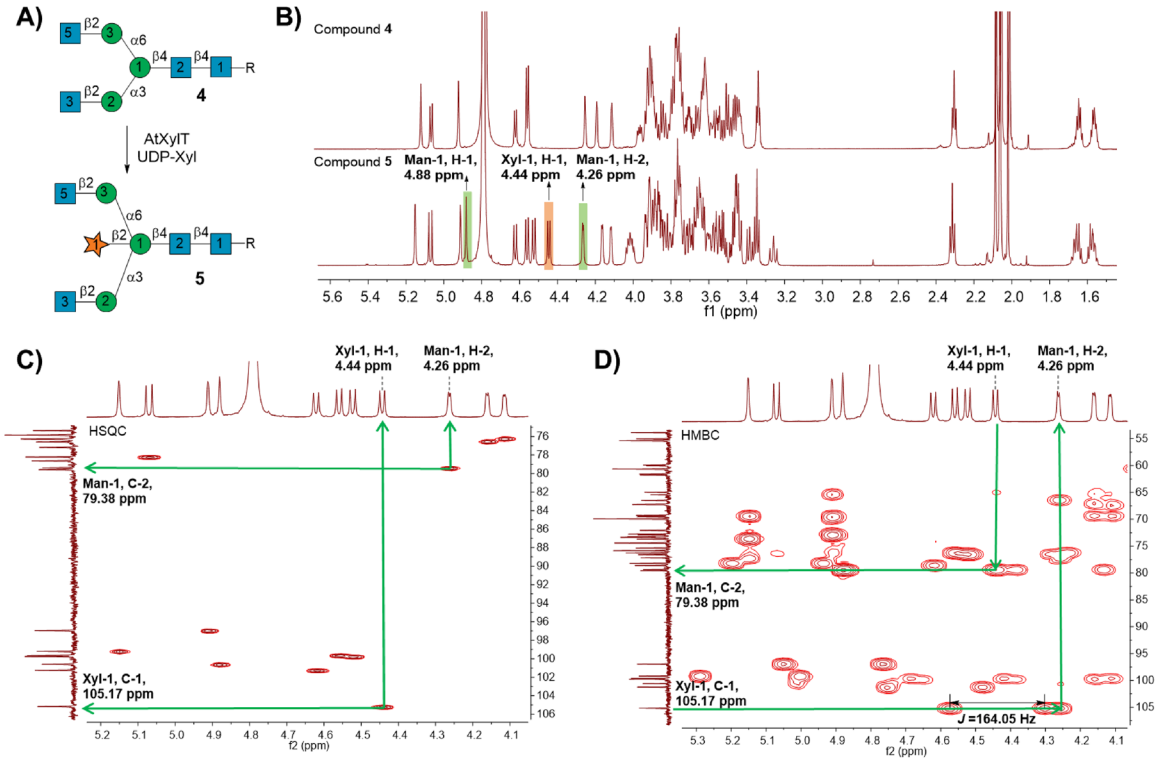


FIGURE 2 | NMR analysis of compound **5**. (A) AtXylT catalyzes the addition of β1,2-core Xyl residue to **4**. (B) ^{1}H NMR spectra of compounds **4** and **5** (C) HSQC spectra of **5**. (D) HMBC spectra of **5**.

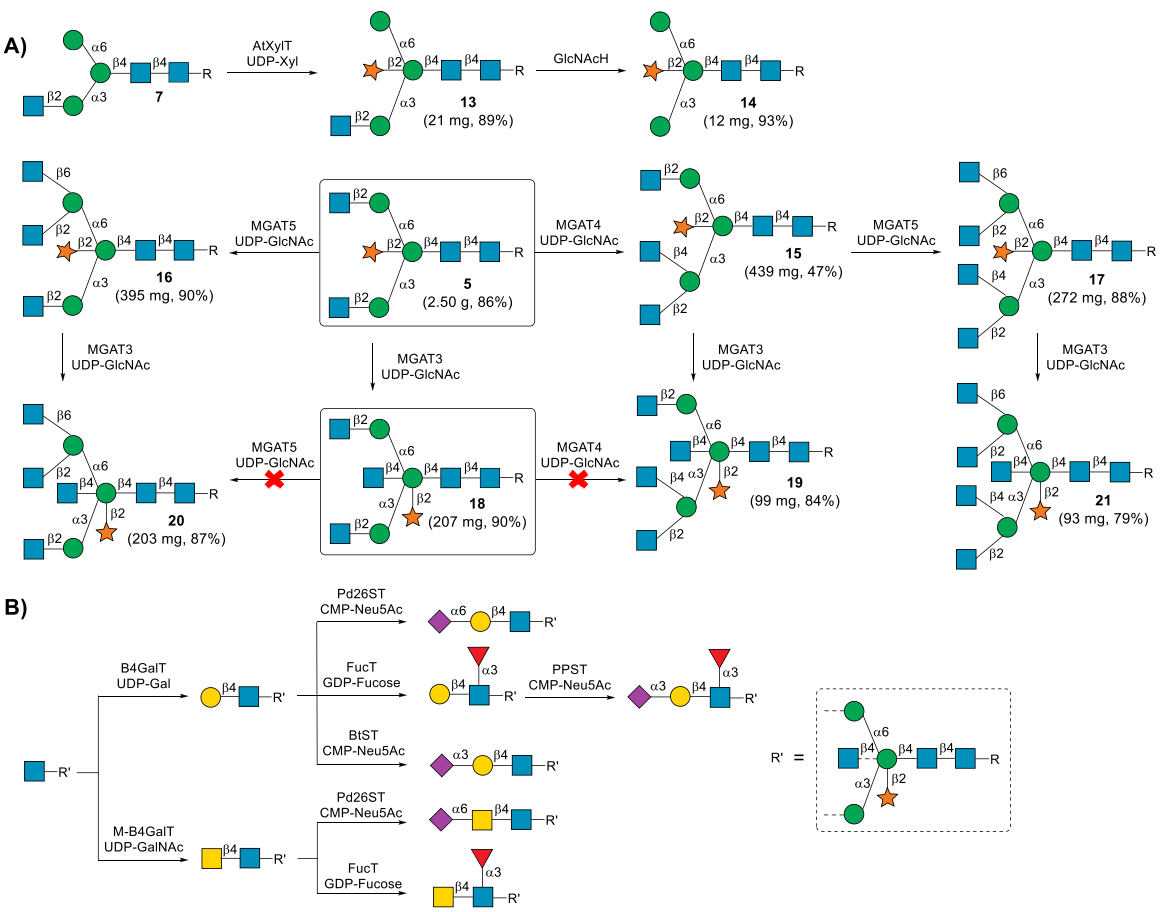


FIGURE 3 | (A) Enzymatic synthesis of core-xylosylated *N*-glycan precursors. (B) Enzymatic construction of glycan epitopes for diversifying sugar library.

FIGURE 4 | Diversity-oriented synthesis of 70 structures of core-xylosylated N-glycans in this work.

well accepted by MGAT3 to afford compounds **19**, **20**, and **21**, which contain both core xylose and bisecting GlcNAc structure. Large-scale synthetic reactions allow the access of 99 mg of **19** (84% yield), 203 mg of **20** (87% yield), and 93 mg of **21** (79% yield) for further enzymatic extension (Figure 3).

With these core-xylosylated N-glycan precursors in hand, we set out to construct a comprehensive N-glycan library (Figure 4). The library contains bi-, tri-, and tetra-antennary backbones which are decorated with LacNAc or LacdiNAc-related epitopes. Studies also indicated that these epitopes, including LacNAc, LacdiNAc, or their fucosylated forms, can be recognized by antibodies against *Schistosoma* [45, 49]. To prepare the compounds processing LacNAc epitope, core-xylosylated N-glycan precursor **5** was incubated with human β1,4-galactosyltransferase 1 (B4GalT) using UDP-Gal as glycosylation donor (Figure S4). The reaction can proceed cleanly in a short time. Once finished, the reaction was stopped and **22** can be obtained easily after purification by using size-exclusion column (137 mg, 90%). The following sialylation of **22** by a bacterial α2,6-sialyltransferase from

Photobacterium damselae (Pd26ST) affords compound **54** (Figure S4). After purification by using ion-exchange column, the product can be obtained in high yield (26 mg, 82%). Similarly, the sialylation by an α2,3-sialyltransferase from *Bibersteinia trehalosi* (BtST) gave compound **62** in high yield (9.9 mg, 86%). To synthesize the compound containing fucosylated LacNAc epitope, also known as Lewis x epitope (Galβ1,4(Fucα1,3)GlcNAc), **22** was incubated with an α1,3-fucosyltransferase from *Helicobacter pylori* (FucT) using GDP-L-Fucose as donor (Figure S4). Compound **30** can also be obtained easily after purification by using size-exclusion column in 88% yield (51 mg). **30** was further sialylated by an α2,3-sialyltransferase from *Photobacterium phosphoreum* (PPST) to afford compound **70** containing sialyl Lewis x epitope (18 mg, 47%). It is worth mentioning that PPST could catalyze the formation of sialyl Lewis x epitope without producing detectable α2,6-Neu5Ac by-product [43]. In contrast to LacNAc, LacdiNAc represents a relatively rare glycan epitope, and its biological significance has remained largely underappreciated since its initial discovery. The LacdiNAc epitope can be constructed using β1,4-N-Acetylgalactosaminyltransferase from *Caenorhabditis elegans* [58, 59] and mutant β1,4-galactosyltransferase from bovine (GalT Y289L) [60, 61]. In this study, we selected the mutant bovine β1,4-galactosyltransferase (GalT Y289L, M-B4GalT) for the synthesis of the LacdiNAc epitope as it can be readily produced in the HEK293 expression system with high yield and consistently strong enzymatic activity. Precursor **5** was incubated with M-B4GalT using UDP-GalNAc as glycosylation donor (Figure S4). The reaction can proceed cleanly in a short time. Once finished, compound **38** was obtained easily by purification using size-exclusion column in high yield (118 mg, 94%). In addition, compound **81** that contains α2,6-sialylated LacdiNAc epitope was synthesized from **38** using Pd26ST in high yield (Figure S4). Meanwhile, although we tested many α2,3-sialyltransferases, including human and bacterial sources, no enzyme that recognizes compound **38** was found. Therefore, the structures containing α2,3-sialylted LacdiNAc epitope were not synthesized successfully in this work. FucT is widely used for the synthesis of Lewis x epitope, while it also accepts N-glycan LacdiNAc epitope efficiently. Therefore, compound **46** that contains α1,3-fucosylated LacdiNAc can be easily synthesized by incubation of **38** with FucT and GDP-L-Fuc as glycosylation donor (Figure S4). After purification using size exclusion column, **46** was obtained in high yield (32 mg, 93%). As either human or bacterial α2,6-sialyltransferases fail to recognize fucosylated LacdiNAc epitope, the extension of **46** with α2,6-linked Neu5Ac is not successful. Following the similar extension strategy as discussed above, another 24 structures were prepared from precursors **15**, **16**, and **17** successfully without technical difficulty (Figures S5–S7). It is worth mentioning that the preparation of tri- and tetra-antennary core-xylosylated N-glycans processing sialyl Lewis x epitope suffers from low yields due to the low activity of PPST towards fucosylated LacNAc epitope. The attempt to find a more efficient enzyme to improve the yield is unsuccessful.

We next prepared the structures starting from precursors **18**, **19**, **20**, and **21** (Figures S8–S11). These structures are a unique class of N-glycans that process both core xylose and bisecting GlcNAc modifications to the central mannose. **26** to **29** processing LacNAc epitope, **34** to **37** processing fucosylated LacNAc epitope, **42** to **45** processing LacdiNAc, and **66** to **69** processing α2,3-linked Neu5Ac epitope were prepared successfully without any technical difficulty. **50** to **53** processing fucosylated LacdiNAc epitope, and **58** to **61** processing α2,6-linked Neu5Ac can all be prepared successfully. However, the compounds containing the MGAT4 branch suffer (**51**, **53**, **59**, and **61**) from low yields for both epitopes. This is mainly because of the incomplete reaction that requires intensive purification manipulation. Similarly, **74** to **77** processing sialyl Lewis x epitope is also prepared in relatively low yields. In addition to the core-xylosylated N-glycans shown in Figure 4, we also prepared 12 N-glycans processing no core xylose as controls (Figure S12).

With the large library comprising 70 core-xylosylated and 12 conventional N-glycans in hand, the azido linker was subsequently reduced to an amino group. The reductive glycans containing amino group were immobilized onto NHS-activated glass slides to construct a microarray comprising 82 well-defined glycan structures. To validate the quality of the constructed microarray, we performed binding assays using well-characterized lectins: including *Aleuria aurantia* Lectin (AAL, binding L-fucose residue) [62], *Erythrina cristagalli* Lectin (ECL, binding to Galβ1,4GlcNAc) [63], *Maackia amurensis* Lectin I (MAL I, binding to Neu5Acα2,3Galβ1,4GlcNAc) [64, 65], and *Sambucus nigra* Lectin (SNA, binding to Neu5Acα2,6Galβ1,4GlcNAc) [66, 67]. The binding profiles of AAL, ECL, MAL I, and SNA were aligned well with recent microarray analyses (Figure S13) [68]. The binding analysis revealed that SNA exhibits a stronger preference for the α2,6-sialylated LacNAc epitope over the α2,6-sialylated LacdiNAc epitope, whereas ECL shows a marked preference for the LacdiNAc epitope. Furthermore, SNA exhibits a preference for the MGAT4 branch over the MGAT5 branch. Additionally, we found that core xylose modification enhances the glycan binding of MAL I compared to structures lacking core xylose. However, dual modifications, involving both core xylose and bisecting GlcNAc, significantly diminish its binding signal for core-xylosylated N-glycans (Figure S13).

We next used the constructed glycan microarray to investigate the binding specificities of antibodies against *Schistosoma japonicum*, one of the predominant *Schistosoma* species that have caused widespread infections in Asia. As it is difficult for us to obtain enough samples of *Schistosoma*-infected patients in China, we investigated the binding activities of polyclonal antibodies from *Schistosoma*-immunized rabbits, which is a widely used model for investigation of *Schistosoma* infections [69–72]. During the life cycle of *Schistosoma*, cercariae infect hosts and transform into schistosomula once penetrate the skin barrier. Only the schistosomula that successfully reach the mesenteric veins could develop to adult male and female worms [73, 74]. The soluble egg antigens (SEA) secreted by the eggs deposited in the liver are key components causing host pathology, leading to granuloma formation, tissue fibrosis, and subsequent severe complications such as cirrhosis, portal hypertension, and colonic lesions [75, 76]. Therefore, we set out to study the antibodies against *Schistosoma* and SEA in this work. Serum containing polyclonal antibodies from *Schistosoma japonicum*-infected rabbit (1500 cercariae per rabbit) [77], and SEA-immunized rabbit were prepared as reported previously [78].

The constructed glycan microarray slides were incubated with the serum diluted 1:100. Then, the slides were washed, and Alexa Fluor 647 goat anti-rabbit IgG antibody was added. The

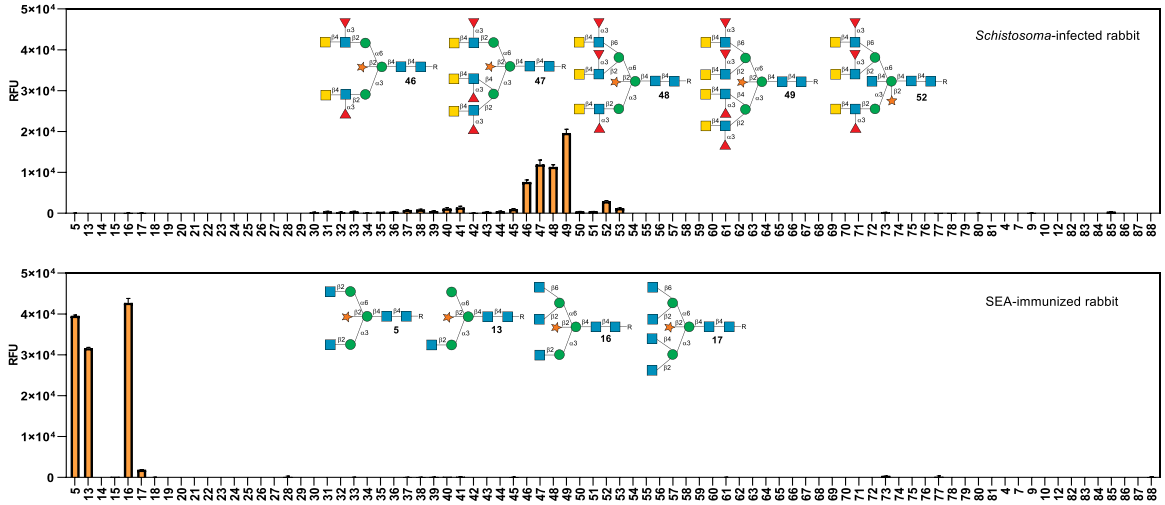


FIGURE 5 | The binding profiles of IgG polyclonal antibodies from *Schistosoma*-immunized rabbits and healthy rabbit with the constructed glycan array (82 structure-defined glycans). The binding profiles of IgA and IgM polyclonal antibodies are shown in supporting information.

fluorescence signals were recorded using a microarray scanner. The binding results showed that IgG polyclonal antibodies from *Schistosoma*-infected rabbit could recognize the structures processing fucosylated LacNAc, LacdiNAc epitope, and fucosylated LacdiNAc epitopes, while only a background level of binding signals was observed in healthy rabbit (Figure S14). These results accord well with the previously reports [45, 49]. The structures containing the LacdiNAc or fucosylated LacNAc epitope exhibit comparatively weaker binding signals (**30** to **41**) when compared to the corresponding structures featuring the fucosylated LacdiNAc epitope (**46** to **49**). Interestingly, the additional bisecting GlcNAc modification strongly inhibits the antibody recognition (**50** to **53**). Compounds **47** and **48**, which incorporate triantennary branches, exhibit stronger binding signals compared to the corresponding bi-antennary structure (**46**). Notably, the tetra-antennary structure (**49**) demonstrates the better binding relative to **47** and **48**. These results indicated that multi-branched core-xylosylated N-glycans processing fucosylated LacdiNAc epitopes are ideal candidates for development of vaccine against *Schistosoma*. Meanwhile, we also studied the binding activities of IgM and IgA polyclonal antibodies from *Schistosoma*-immunized rabbit. IgM shows a similar binding profile with IgG antibodies, while IgA also recognized structures containing fucosylated LacdiNAc epitopes, although the binding signals were relatively weak (Figures S15 and S16).

In contrast to the antibodies from *Schistosoma*-infected rabbit, IgG polyclonal antibodies from SEA-immunized rabbit could not bind the structures as discussed above (Figure 5). This is probably because that the cell surface N-glycans of *Schistosoma* varies with developmental stage and among species [79]. Unlike the polyclonal antibodies from *Schistosoma*-infected rabbit, where more branches have stronger binding signal, antibodies from SEA-immunized rabbit have a strict branch preference. The binding profile shows that IgG polyclonal antibodies strongly bind compounds **5**, **13**, and **16**, while no binding signals towards the corresponding structures that process no core xylose were observed (**4**, **7**, **9**, and **10**). This result demonstrates the importance of core xylose in the IgG antibody recognition. In the meantime, no signals towards **14** and **15**, and only weak binding signal towards compound **17** were observed, indicating that the terminal naked GlcNAc residue attached to MGAT1 branch is necessary for antibody recognition, while the attachment to the MGAT4 branch strongly inhibits the antibody binding. These results also indicated that compounds **5**, **13**, and **16** can be the ideal candidates for the development of therapeutic vaccine to alleviate the symptoms caused by SEA. In addition, we also investigate the binding activities of IgM and IgA antibodies from SEA-immunized rabbit. Both IgM and IgA show a similar binding profile with IgG antibodies (Figures S15 and S16). Moreover, all the tested antibodies from either *Schistosoma*-infected rabbit or SEA-immunized rabbit fail to bind any sialylated glycans, indicating that the sialic acid group shield the antibody recognition. This probably because sialic acid group is a generally native epitope in the host, which cannot elicit immune response. In addition, the binding results show that many compounds process the potential for application in clinical diagnosis as they display a unique binding profile toward the polyclonal antibodies against *Schistosoma japonicum*, which are widely used in clinical diagnosis of *Schistosoma* infections.

3 | Conclusion

Core-xylosylated N-glycans represent a ubiquitous class of N-glycans widely distributed across nature. However, they are extremely difficult to prepare due to the structural complexity. Although core-xylosylated N-glycans does not exist in humans, they express abundantly in plants and *Schistosoma* and have been implicated in allergy and other medical conditions. Recently, studies also highlighted the potential of core-xylosylated N-glycans in the development of vaccines against *Schistosoma* infections, as antibodies targeting these glycans were shown to effectively kill schistosomula in vitro. Schistosomiasis, a chronic disease caused by parasitic flukes of the genus *Schistosoma*, remains highly prevalent in many low and middle-income countries. Although schistosomiasis has been identified as one of the ten diseases for which the development of an effective vaccine is most urgently needed, progress in this area remains limited. In this work, we developed a powerful platform that enables

the efficient preparation of core-xylosylated N-glycans. By the described strategy, a comprehensive glycan library containing a variety of typical N-glycan structures was constructed successfully. With the glycan microarray made from this glycan library, we investigated the binding activities of the antibodies against *Schistosoma japonicum* and many structures that strongly bind antibodies were discovered. This work provides an insightful understanding for the rational design of core-xylosylated N-glycan-based vaccine candidates against *Schistosoma* infections. In addition, we also believe that this work will accelerate the application of core-xylosylated N-glycans in other research areas.

Author Contributions

Fangyu Wei: methodology, formal analysis. **Xiao Tian:** methodology, formal analysis. **Dingyi Mao:** methodology. **Yang Dai:** methodology. **Jiabin Zhang:** methodology. **Liuqing Wen:** conceptualization, project administration, supervision, investigation, funding acquisition, writing-review and editing, writing-original draft.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting information of this article

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: The supporting information is available free of charge at Figures S1–S15, NMR spectra for all compounds, additional experimental details, materials, and methods.

Supporting File 2: anie72584-sup-0002-SI-NMR Spectra.pdf.