RSC Advances

REVIEW

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Cite this: *RSC Adv.*, 2026, **16**, 6786

Recent advances in the synthesis of α-dystroglycan *O*-mannose glycans

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α-Dystroglycan (α-DG) is an important component of the extracellular domain of the dystrophin complex, with extensive and diverse *O*-mannosylation modifications, which can widely participate in various physiological and pathological processes. In particular, core M1, core M2, and core M3 *O*-mannose glycans, which are post-translational modifications of α-DG, are shown to play critical roles in muscle and brain development. However, elucidating their precise mechanisms has been hampered by inherent structural heterogeneity, creating an urgent demand for efficient methods to obtain homogeneous glycans. Despite their structural complexity, tremendous progress has been made in the synthesis of *O*-mannose glycans and glycopeptides in recent years. By systematically comparing synthetic strategies and methodologies, this review highlights recent progress in the chemical, enzymatic, and chemoenzymatic synthesis of the three major *O*-mannose glycan types of α-DG. In addition, key synthetic challenges, including stereoselective glycosylation, site-specific functionalization, and scalability, are discussed. Finally, current limitations and future perspectives in *O*-mannose glycans synthesis are outlined, aiming to inspire further methodological innovation and biological applications.

Received 31st December 2025
Accepted 27th January 2026

DOI: 10.1039/d5ra10119k

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1 Introduction

α-Dystroglycan (α-DG) is an extensively *O*-glycosylated transmembrane glycoprotein encoded by the DAG1 gene that serves as a critical component of the dystrophin-associated glycoprotein complex (DGC).[1–3] It plays a key role in maintaining muscle integrity, neuronal development, and cell adhesion by linking the extracellular matrix (ECM) to the intracellular cytoskeleton (Fig. 1A).[4] Unlike conventional glycoproteins, α-DG is uniquely modified by *O*-mannose glycans, a rare class of glycosylation that is essential for its functional interactions with ECM ligands such as laminin,[5] agrin,[6] perlecan,[7] neurexin,[8] pikachurin[9] and slit.[10] Defective *O*-mannosylation of α-DG leads to various forms of congenital muscular dystrophies (CMDs),[11–16] including Walker–Warburg syndrome (WWS),[17] muscle-eye-brain disease (MEB),[18] Fukuyama congenital muscular dystrophy (Fukuyama CMD),[19,20] and limb-girdle muscular dystrophy (LGMD).[21] These disorders are collectively defined as dystroglycanopathies (DGPs), a group of congenital muscular dystrophies caused by defects in the glycosylation of α-DG.[22] Clinically, these disorders manifest from the neonatal period and are characterized by progressive muscle degeneration, evolving from muscle weakness to loss of mobility and respiratory failure, intellectual disability due to abnormal brain development, and impaired neuronal migration. Some cases also present ocular abnormalities such as microphthalmia and retinal dysplasia.[23,24] These conditions not only significantly reduce patients' quality of life and life expectancy, but the glycosylation defects are also associated with cancer progression and metastasis in various malignancies.[25–27] However, despite the severity of these disorders, there are currently no effective treatments nor efficient diagnostic strategies available for DGPs. Thus, *O*-mannose glycans are essential for maintaining the normal structure and function of α-DG in muscles and the brain.[28] Beyond its structural role, α-DG also functions as a receptor for multiple pathogens, including *Mycobacterium leprae* and arenaviruses such as Lassa fever virus (LFV).[29–31]

In α-DG, *O*-mannose-based glycans constitute nearly 50% of the total *O*-glycans.[32] Recent advances in glycobiology have significantly accelerated the characterization of *O*-mannose glycans, leading to better understanding of laminin-binding glycan structures. To date, three distinct *O*-mannose glycan structures involved in α-DG post-translational modifications have been identified and classified based on the linkage of *N*-acetylglucosamine (GlcNAc) to mannose (Man) (Fig. 1B): core M1, GlcNAcβ1-2Manα1-*O*-Ser/Thr; core M2, GlcNAcβ1-

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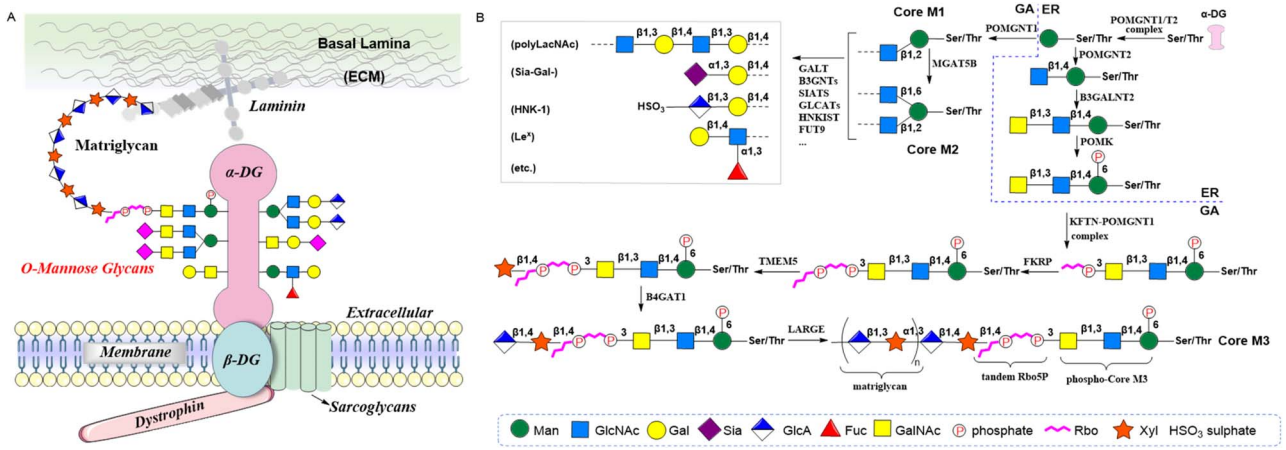
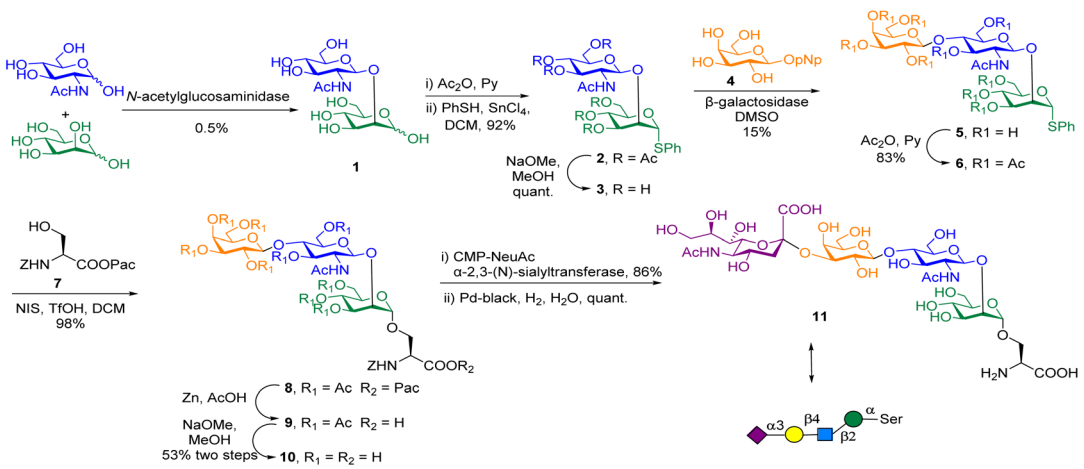


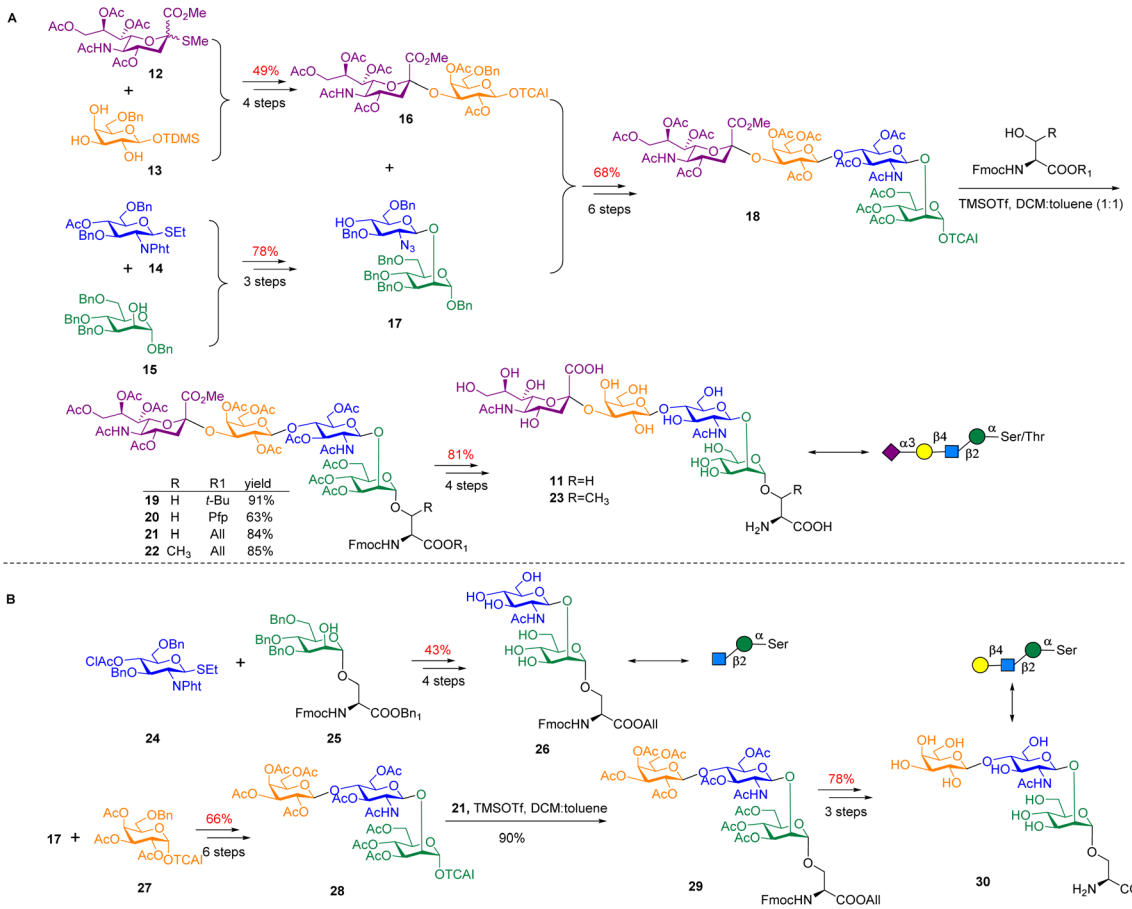
Fig. 1 The DGC and *O*-mannosylated glycans identified on α-DG. (A) The heavily glycosylated peripheral membrane protein α-DG participates in the DGC and mediates interactions with ECM proteins through matriglycan. α-DG also functions as a receptor for multiple pathogens, including *Mycobacterium leprae* and arenaviruses such as Lassa fever virus. (B) Biosynthesis pathway of core M1, core M2 and core M3 glycans on α-DG. Abbreviations: Man, mannose; GlcNAc, *N*-acetylglucosamine; Gal, galactose; Sia, sialic acid; GlcA, glucuronic acid; Fuc, fucose; GalNAc, *N*-acetylgalactosamine; Rbo, ribitol; Xyl, xylose; POMT1/T2, protein *O*-mannosyltransferases 1 and 2; POMGNT1, protein *O*-linked mannose *N*-acetyl-glucosaminyltransferase 1; MGAT5B, mannosyl α1,6-glycoprotein β1,6 *N*-acetyl-glucosaminyltransferase; GALTs, galactosyltransferases; B3GNTs, β1,3-*N*-acetylglucosaminyltransferases; SIATs, sialyltransferases; GLCATs, glucuronyltransferases; HNK1ST, HNK-1 sulfotransferase; FUT9, α1,3-fucosyltransferase-9; POMGNT2, protein *O*-linked mannose β-1,2-*N*-acetylglucosaminyltransferase 2; B3GALNT2, β1,3 *N*-acetylgalactosaminyltransferase 2; POMK, protein *O*-mannose kinase; FKTN, fukutin; FKRP, fukutin-related protein; TMEM5, transmembrane protein 5; B4GAT1, β1,4-glucuronyltransferase 1; LARGE, like-acetylglucosaminyltransferas.

6(GlcNAcβ1-2)-Manα1-*O*-Ser/Thr, and core M3, GalNAcβ1-3GlcNAcβ1-4(phospho-6)-Manα1-*O*-Ser/Thr.[33,34] On this basis, these proteins undergo further modifications including galactosylation, sialylation, and the addition of human natural killer factor-1 (HNK-1) epitope, Lewis X and polylactosamine on *O*-mannose glycans.[34,35] These elaborations generate highly heterogeneous and complex glycan structures that create unique carbohydrate epitopes, which are believed to play key roles in brain development. Moreover, compared with core M1 and core M2, the structure of core M3 was clarified later. Its complete configuration [(3GlcAβ1-3Xylα1)$_n$-3GlcAβ1-4Xylβ1-4Rbo5P-1Rbo5P-3GalNAcβ1-3GlcNAcβ1-4(phospho-6)-Manα1-*O*-Ser/Thr] was not fully characterized until 2016.[36,37] Notably, recent studies have confirmed that all gene defects impairing core M3-type glycan maturation have been identified as causative factors of DGPs.[38] This unequivocally demonstrates that defective core M3-type glycan constitutes the fundamental pathological mechanism underlying DGPs.

The biosynthesis of *O*-mannose glycans on α-DG takes place in the endoplasmic reticulum (ER) and the Golgi apparatus (GA) requiring the harmonic activity of multiple enzymes (Fig. 1B).[11] The process is initiated in the ER, where the protein *O*-mannosyltransferase complex (POMT1/POMT2) enzyme complex catalyzes the transfer of an *O*-mannose residue to the hydroxyl group of serine or threonine residue of α-DG, forming an α-glycosidic bond.[39,40] Within the GA, protein *O*-linked mannose



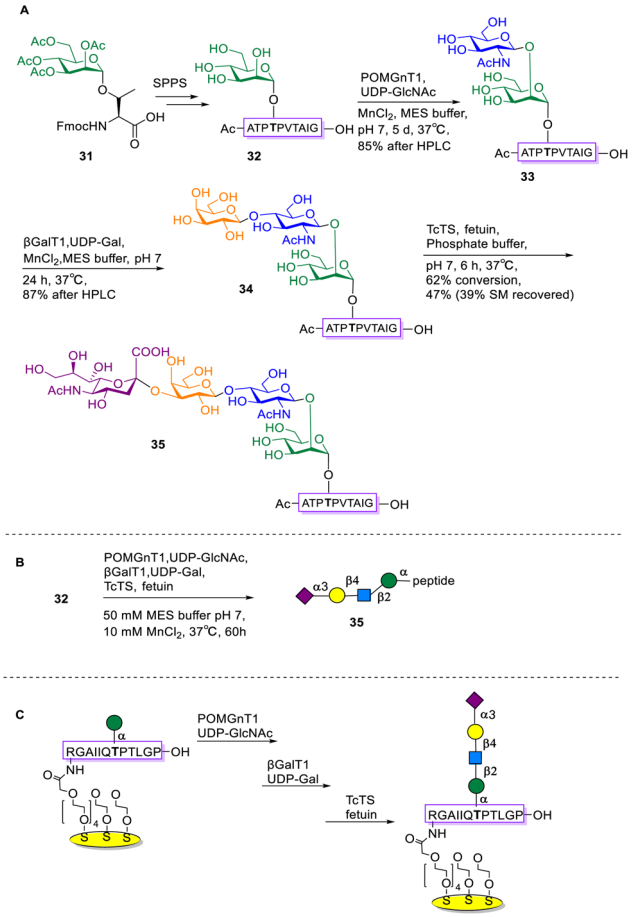
Scheme 1 First chemoenzymatic synthesis of core M1 tetrasaccharide by Ajisaka group. Abbreviation: Pac = phenacyl; Z = benzyloxycarbonyl (Cbz).



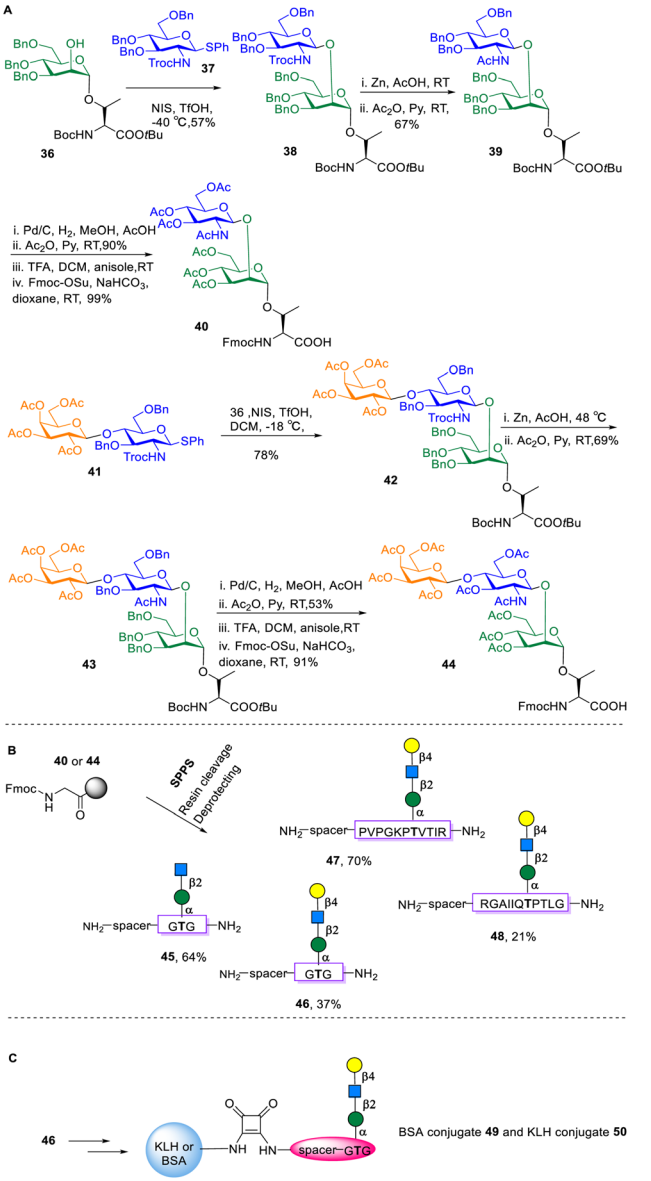
Scheme 2 Chemical synthesis of the core M1 glycoamino acids by Ito group. (A) Synthesis of the core M1 tetrasaccharide amino acid. (B) Synthesis of the core M1 di- and trisaccharide amino acid. Abbreviation: Pht = phthalyl; Pfp = pentafluorophenyl; ClAc = chloroacetyl.

β1,2-*N*-acetylglucosaminyltransferase 1 (POMGNT1) subsequently adds a β1,2-linked GlcNAc moiety to the *O*-mannose, yielding core M1.[12] Core M1 serves as the biosynthetic precursor for core M2 glycans. Under the catalysis of mannosyl α1,6-glycoprotein β1,6-*N*-acetylglucosaminyltransferase (MGAT5B), a β1,6-linked GlcNAc is added to core M1, resulting in core M2.[41] Additionally, both core M1 and core M2 can undergo glycan chain elongation through the action of various glycosyltransferases, such as galactosyltransferases (GALTs), β1,3-*N*-acetylglucosaminyltransferases (B3GNTs), sialyltransferases (SIATs), glucuronyltransferases (GLCATs), HNK-1 sulfotransferase (HNK1ST), and α1,3-fucosyltransferase-9 (FUT9), leading to the formation of multiple epitope structures depicted in Fig. 1B.[34,42] In comparison to other glycans, the biosynthesis of core M3 exhibits greater complexity. The process initiates in the ER, where protein *O*-linked mannose β1,4-*N*-acetylglucosaminyltransferase 2 (POMGNT2) catalyzes the transfer of a β1,4-linked GlcNAc to the initial Man residue.[43] This is followed by β1,3-*N*-acetylgalactosaminyltransferase 2 (B3GALNT2)-mediated attachment of a β1,3-linked GalNAc to the GlcNAc moiety. Subsequently, protein *O*-mannose kinase (POMK) phosphorylates the C6 hydroxyl group of the mannose residue.[44] The resulting phosphorylated trisaccharide undergoes further elongation in the GA through sequential enzymatic modifications: First, ribitol-5-phosphate transferase fukutin (FKTN) transfers ribitol-5-phosphate (Rbo5P) to the 3-hydroxyl group of GalNAc. Fukutin-related protein (FKRP) then catalyzes the addition of a second Rbo5P to the first, forming a tandem Rbo5P–Rbo5P structure.[37,45] Ribitol xylosyltransferase 1 (RXYLT1; previously designated transmembrane protein 5, TMEM5) subsequently transfers a β1,4-linked xylose (Xyl) to the second Rbo5P, followed by β1,4-glucuronyltransferase 1 (B4GAT1)-mediated addition of a β1,4-linked glucuronic acid (GlcA) to the xylose residue.[16,36,46] The final maturation step involves the alternating transfer of α1,3-linked xylose and β1,3-linked glucuronic acid residues by like-acetylglucosaminyltransferase (LARGE), generating the characteristic repeating -3GlcAβ1-3Xylα1- disaccharide units that constitute the functional matriglycan structure.[47,48]

Although the structures and biological significance of *O*-mannose glycans on α-DG have been extensively elucidated, their precise physiological roles and disease-related molecular mechanisms remain to be fully clarified.[49] Access to structurally defined, homogeneous, and scalable *O*-mannose glycans would not only provide ideal probes for functional studies but also open new avenues for the diagnosis and treatment of DGPs.[50,51] Nevertheless, the inherent heterogeneity of native *O*-mannose glycans produced by biological systems makes it challenging to



Scheme 3 Synthesis of glycopeptides, containing *O*-mannosyl glycan NeuNAcα2-3Galβ1-4GlcNAcβ1-2Manα. (A) Three-step enzymatic synthesis of glycopeptides. (B) "One-pot" enzymatic cascade synthesis glycopeptide. (C) Enzymatic oligosaccharide synthesis of a glycopetide on a surface.



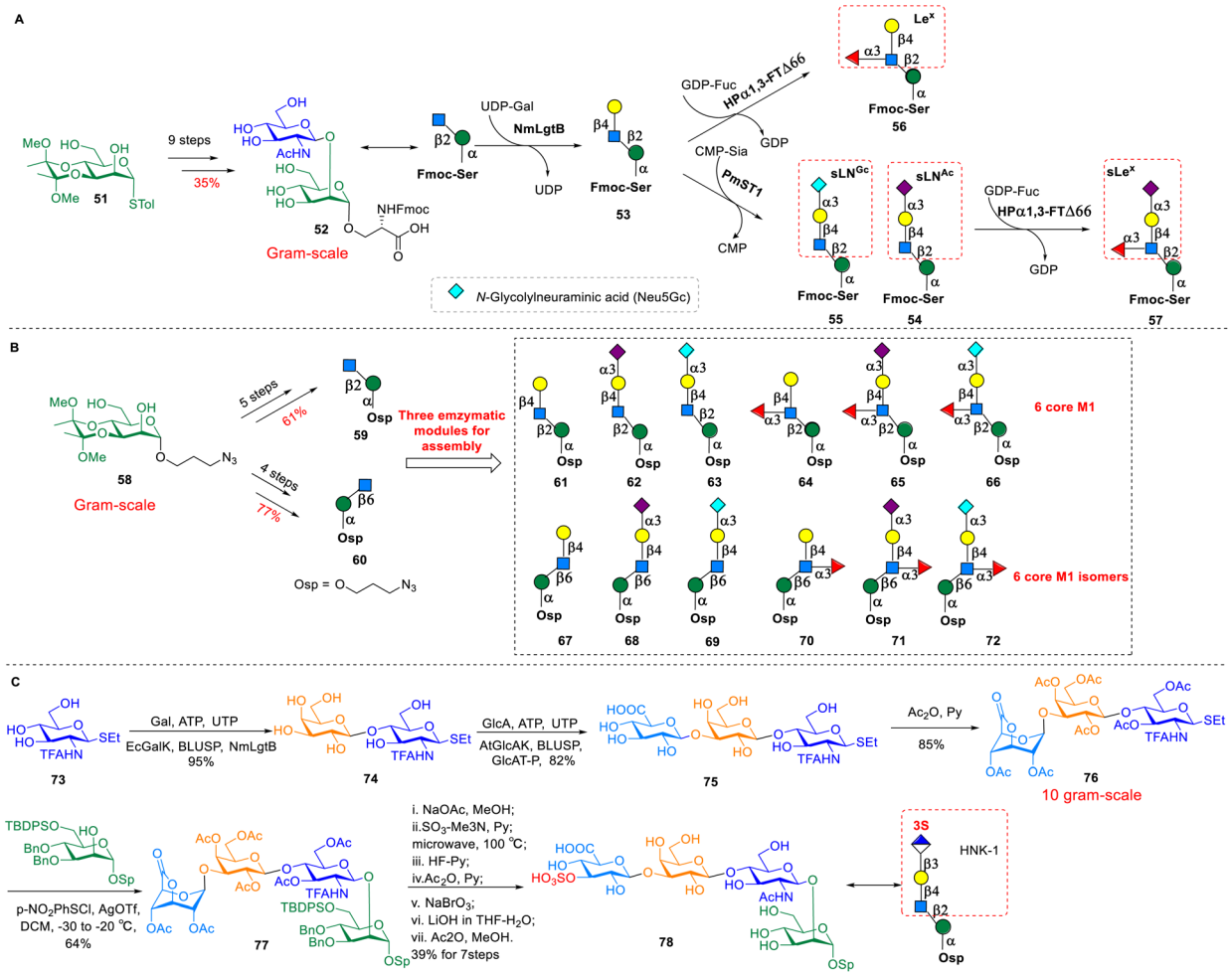
Scheme 4 Synthesis of glycopeptides and glycoconjugate vaccines by Westerlind group. (A) Synthesis of glycosylated threonine building blocks **40** and **44**. (B). Synthesis of glycopeptides **45**–**48**. (C) Synthesis of BSA conjugate **49**, and KLH conjugate **50**.

obtain homogeneous materials. In contrast, chemical, enzymatic, and chemoenzymatic approaches have collectively emerged as powerful alternative strategies for producing homogeneous *O*-mannose glycans.[52,53] Significant progress has been made in this field in recent years. This review systematically and comprehensively summarizes recent advances in the synthesis of *O*-mannose glycans on α-DG employing these methodologies.

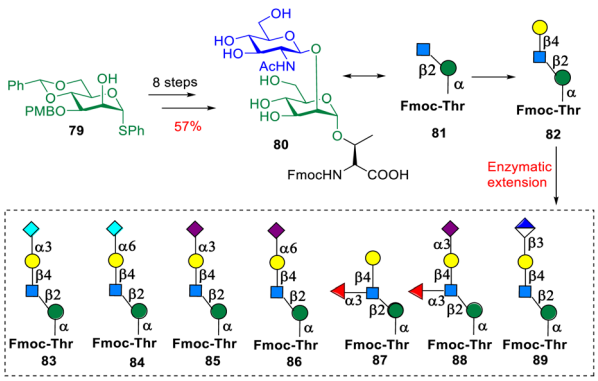
2 Synthesis of α-DG core M1 *O*-mannose glycans

Core M1 *O*-mannose glycans constitute one of the most fundamental structural motifs decorating α-DG. Over the past decades, considerable efforts have been devoted to their construction through both purely chemical and enzymatic approaches. These sustained efforts have resulted in significant achievement in the efficient and stereoselective synthesis of core M1 structures.

In the 1990s, following the initial characterization of the tetrasaccharide NeuNAcα2-3Galβ1-4GlcNAcβ1-2Manα-by Endo and co-workers, its total synthesis became a primary objective for the scientific community.[54] In a landmark study, Ajisaka and co-workers achieved the first chemoenzymatic synthesis of α-(2,3)-sialylated tetrasaccharidic glycosyl amino acid **11** (Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα-*O*-Ser), establishing a key methodology for glycopeptide research (Scheme 1).[55] Using enzymatically synthesized disaccharide **1** as the starting substrate,[56] a thiophenyl group was introduced at the anomeric position of its mannose residue through a series of protection and deprotection steps, yielding compound **3**.[55] Galactosylation of glucosamine at the 4-hydroxy group, catalyzed by a galactosidase from *B. bifidum*, provided trisaccharide **5** in 15% yield. The glycosylation between the acetylated compound **6** and the serine derivative **7** was performed with *N*-iodosuccinimide (NIS) and triflic acid (TfOH) in



Scheme 5 Chemoenzymatic synthesis of core M1 *O*-mannose glycans by Cao group. (A) Sequential OPME synthesis of the Neu5Gc, Lex, and sLex bearing core M1 glycans. (B) Enzymatic assembly of six core M1 *O*-mannose glycans along with their corresponding C-6-arm isomers. (C) First enzymatic assembly of core M1 *O*-mannose glycans with sulfated HNK-1 epitope. Abbreviation: TFA = trifluoroacetyl.



Scheme 6 SSEE strategy synthesis of core M1 *O*-mannose glycans by Li group. Abbreviation: PMB = *p*-methoxybenzyl.

dichloromethane (DCM) at −78 °C, affording the glycosyl amino acid **8** in 98% yield. Following the removal of all protecting groups except the Fmoc group, compound **10** was elaborated to the core M1 tetrasaccharide amino acid **11** through a sialyltransferase-catalyzed sialylation[57] and subsequent Fmoc deprotection. Unfortunately, this synthetic route proceeded in 11 steps with an overall yield of only 0.026%. Concurrently, Ito and co-workers developed a chemical convergent strategy for the synthesis of core M1 glycosyl amino acids (Scheme 2A).[58] Stereoselectivity was controlled through neighboring group participation and modulation of solvent effects. The total synthesis, which included the preparation of monosaccharide building blocks, comprised approximately 50 steps and proceeded in less than 1% overall yield.[58] Although this method afforded higher reaction yields compared to Ajisaka's chemoenzymatic approach, it required a considerably longer and more labor-intensive synthetic route. In 2000, the team further applied this synthetic strategy and optimized the reaction pathway to successfully obtain two core M1 glycosyl amino acids (Scheme 2B), the disaccharide **26** (GlcNAcβ1-2Manα-*O*-Ser) and the trisaccharide **30** (Galβ1-4GlcNAcβ1-2Manα-*O*-Ser).[59]

In addition to glycan synthesis, the preparation of pure, homogeneous glycopeptides with well-defined sequences is essential for further investigation of their biological functions.[60,61] To this end, Flitsch and co-workers accomplished the first

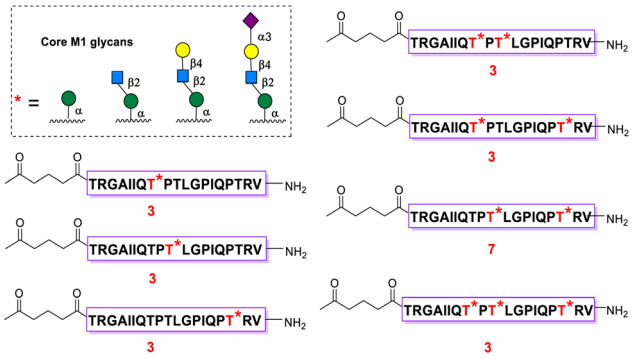
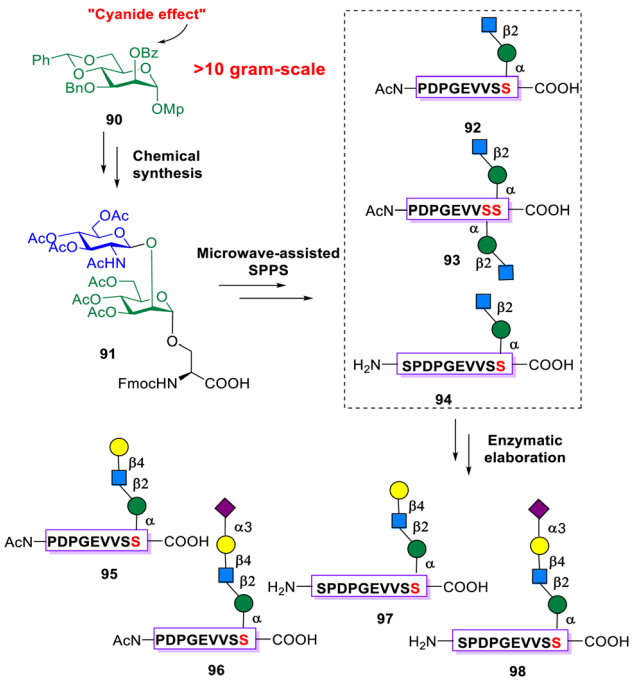


Fig. 2 Chemoenzymatic synthesis of core M1 *O*-mannosylated glycopeptides by Nishimura group.

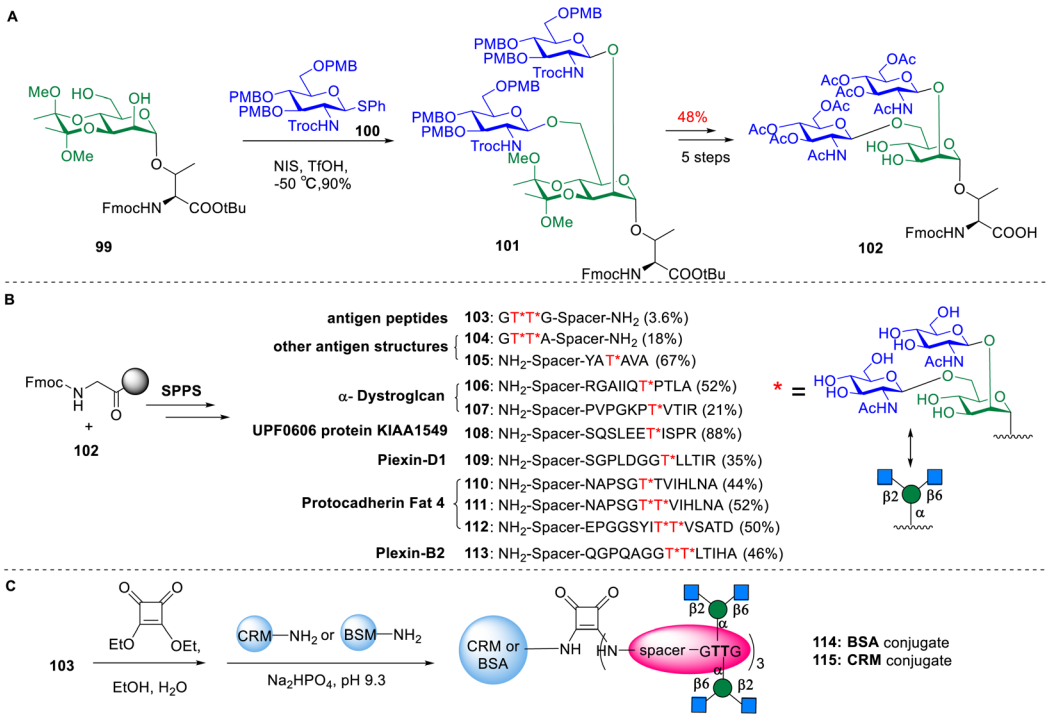


Scheme 7 Synthesis of core M1 *O*-mannosylated glycopeptides by the Peng group *via* microwave-assisted SPPS and enzymatic glycan elaboration.

synthesis of glycopeptides containing the core M1 structured *O*-mannose glycan NeuNAcα2-3Galβ1-4GlcNAcβ1-2Manα *via* a biomimetic stepwise assembly from the reducing end using three consecutive enzymatic glycosylation steps (Scheme 3).[62] Specifically, GlcNAc, Gal, and Neu5Ac were introduced using human POMGnT1, bovine β1,4-galactosyltransferase (β1,4-GalT), and a *trans*-sialidase from Trypanosoma cruzi (TcTS),[63] respectively (Scheme 3A). Furthermore, the authors developed a one-pot enzymatic cascade reaction for efficient synthesis of the target tetrasaccharide (Scheme 3B) and showcased solid-phase synthesis of the desired glycopeptides directly on a gold microarray platform (Scheme 3C). The resulting intermediates are currently being used to study the role of this unusual glycan in mediating the binding of α-DG to its various receptors.

Shortly after the enzymatic synthesis, Westerlind and co-workers subsequently achieved the chemical synthesis of a series of glycopeptide chains and glycoconjugate vaccines containing GlcNAcβ1-2Manα disaccharide and Galβ1-4GlcNAcβ1-2Manα trisaccharide motifs (Scheme 4).[64] The team prepared peracetylated Fmoc-protected glycosylated threonine building blocks **40** and **44** *via* a convergent glycosylation strategy (Scheme 4A), which were then incorporated into short glycopeptides using optimized Fmoc solid-phase peptide synthesis (SPPS) (Scheme 4B). The resulting glycopeptide antigen **46** was site-specifically conjugated to bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) through a diethyl squarate linker, yielding fully defined vaccine constructs **49** and **50** (Scheme 4C). Notably, while the induced antibodies demonstrated high specificity against the target antigenic structure, they retained residual cross-reactivity toward the peptide backbone. This limitation renders these antibodies unsuitable for broad detection of *O*-mannose glycans presented across diverse peptide contexts.

Research on the synthesis of core M1 glycans has evolved significantly, transitioning from initial low-yielding multistep chemical routes and restricted enzymatic methods to contemporary strategies dominated by efficient chemo-enzymatic approaches. Notably, Cao and co-workers reported several highly efficient chemical and chemoenzymatic strategies for the synthesis of core M1 glycans (Scheme 5).[65–67] In 2015, they chemically synthesized disaccharide serine **52**, obtained in gram scale *via* Ley's diketone protection and late-stage amino-acid introduction, which served as the universal acceptor for a sequential one-pot multienzyme (OPME) cascade.[65] This overcomes a major limitation of large-scale enzymatic glycan synthesis, namely the high costs caused by the extensive use of costly sugar nucleotides and enzymes. Three orthogonal glycosyltransferase modules were integrated to deliver, for the first time, the Neu5Gc, Lex, and sLex bearing core M1 glycans **55–57**, together with the known trisaccharide **53** and tetrasaccharide **54** (Scheme 5A). In 2018, the same chemoenzymatic strategy was employed to chemically synthesize two disaccharide intermediates **59** and **60**, which were subsequently subjected to enzymatic assembly using a three-enzyme module system to yield six core M1 *O*-mannose glycans **61–66** along with their corresponding C-6-arm isomers **67–72** (Scheme 5B).[66] In 2019, they further achieved the first total synthesis of a more complex core M1 tetrasaccharide **78** bearing the sulfated HNK-1 epitope through further chemical transformation of key intermediate **77** (Scheme 5C).[67] Moreover, Li and co-workers developed an efficient scaffold synthesis/enzymatic extension (SSEE) strategy to prepare 9 core M1 glycans **81–89** by combining gram-scale convergent chemical syntheses of scaffold and strictly controlled sequential glycosyltransferase-catalyzed enzymatic extension (Scheme 6).[68] Employing an analogous approach, Nishimura and co-workers synthesized a comprehensive library of 25 distinct core M1 *O*-mannosylated glycopeptides for high-throughput microarray analysis (Fig. 2).[69] This systematic screening uncovered specific ligand-recognition patterns and associated conformational change at the *O*-mannosylated position. In addition, building upon their previously established "cyanide effect" strategy, Peng and co-workers accomplished



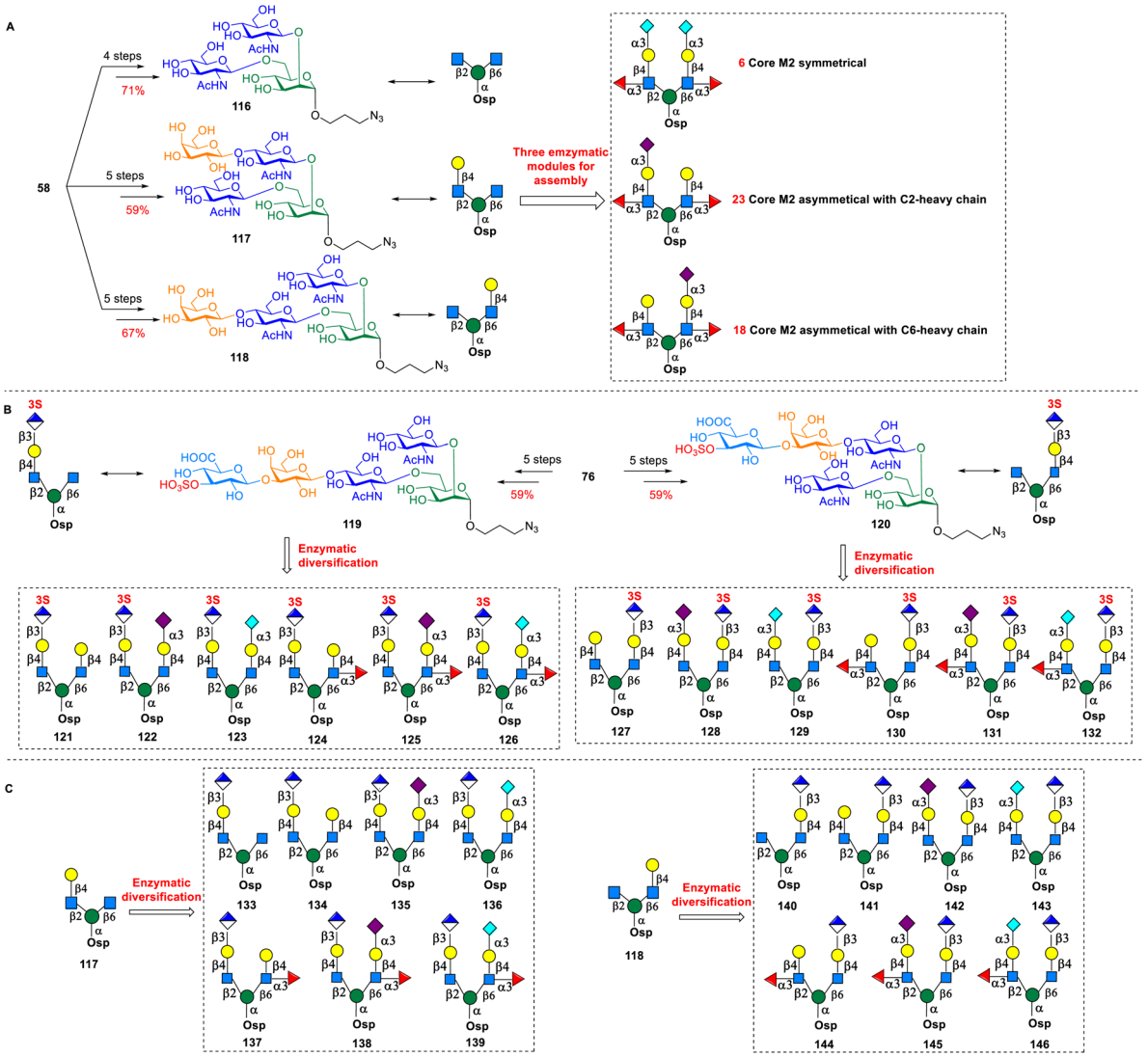
Scheme 8 Synthesis of core M2 *O*-mannose glycopeptides and glycoconjugate vaccines. (A) Synthesis of glycosylated threonine building blocks (B). Synthesis of glycopeptides. (C) Synthesis of BSA and CRM conjugates.

regioselective protection at the mannose 2-OH position, facilitating the high-yield synthesis of a pivotal intermediate **90** and gram-scale production of core M1 mannosylated amino acid **91** (Scheme 7).[35] Subsequently, using glycopeptides **92** and **93** prepared *via* microwave-assisted SPPS as acceptor substrates, they successfully generated four glycopeptides **95–98** featuring sialylated and non-sialylated core M1 mannose structures through enzymatic glycan elaboration.

3 Synthesis of α-DG core M2 O-mannose glycans

The synthesis of branched core M2 glycans lagged significantly behind that of linear core M1 *O*-mannosyl glycans. In 2017, Westerlind and co-workers accomplished the first chemical total synthesis of eleven glycopeptides **103–113** and two glycoconjugates **114** and **115** containing the branched core M2 structure [GlcNAcβ1-6(GlcNAcβ1-2)-Manα1] (Scheme 8).[70] Using a synthetic vaccine approach, they successfully elicited highly selective antibodies that specifically recognize the branched epitope without cross-reacting with linear core M1 glycans—demonstrating a markedly superior recognition profile compared to traditional concanavalin A (ConA) lectin. This work fills a crucial gap in the research tools for branched *O*-mannosylation and provides reliable molecular probes for investigating its functional roles in neurodegenerative diseases and cancer metastasis.

In 2018, employing a strategy analogous to that used for core M1 assembly, Cao and co-workers devised a concise synthetic route starting from mannose derivative **58**, enabling the efficient preparation of three pivotal core M2 intermediates **116–118** in approximately five steps (Scheme 9A).[66] Subsequently, the team employed an enzymatic extension strategy to achieve the diversity-oriented assembly of 57 distinct *O*-mannosylated core M2 structures. This collection encompassed 6 symmetric, 23 C2 "heavy chain"-branched asymmetric, and 18C6 "heavy chain"-branched asymmetric core M2 *O*-mannose glycans. This work presents the first systematic construction of the most comprehensive core M2 *O*-mannose glycan library. Furthermore, using glycan microarray technology, the study revealed specific recognition patterns between these glycans and brain proteins as well as immune molecules. In a pivotal expansion of the glycan library, Cao and co-workers also utilized enzymatic diversification to generate 28 structurally defined core M2 *O*-mannose glycans incorporating sulfated epitope **121–132** (Scheme 9B) or non-sulfated HNK-1 epitope **133–146** (Scheme 9C), which represent functionally critical motifs in neural systems, starting from chemically synthesized intermediates **117–120**.[67] During the same period, Li and co-workers employed a developed SSEE strategy to synthesize 36 core M2 glycosyl amino acids (Scheme 10).[68] Subsequent glycan microarray profiling further uncovered the fine specificities of these glycans towards various glycan-binding proteins and specific antisera. In 2022, Peng and coworkers reported the synthesis of a series of glycopeptides incorporating sialylated or non-sialylated core M2 *O*-mannose glycopeptides **170–173** using a microwave-assisted SPPS strategy (Scheme 11).[35] Among these, glycopeptide **173** was obtained for the first time. Remarkably, this novel synthetic approach demonstrated that glycosyltransferases



Scheme 9 Chemoenzymatic synthesis of core M2 *O*-mannose glycans. (A) Enzymatic assembly of symmetrical, C2 and C6 "heavy chain"-branched asymmetrical core M2 *O*-mannose glycans from **58**. (B) Synthesis of core M2 *O*-mannose glycans with sulfated HNK-1 epitope. (C) Synthesis of core M2 *O*-mannose glycans with nonsulfated HNK-1 epitope.

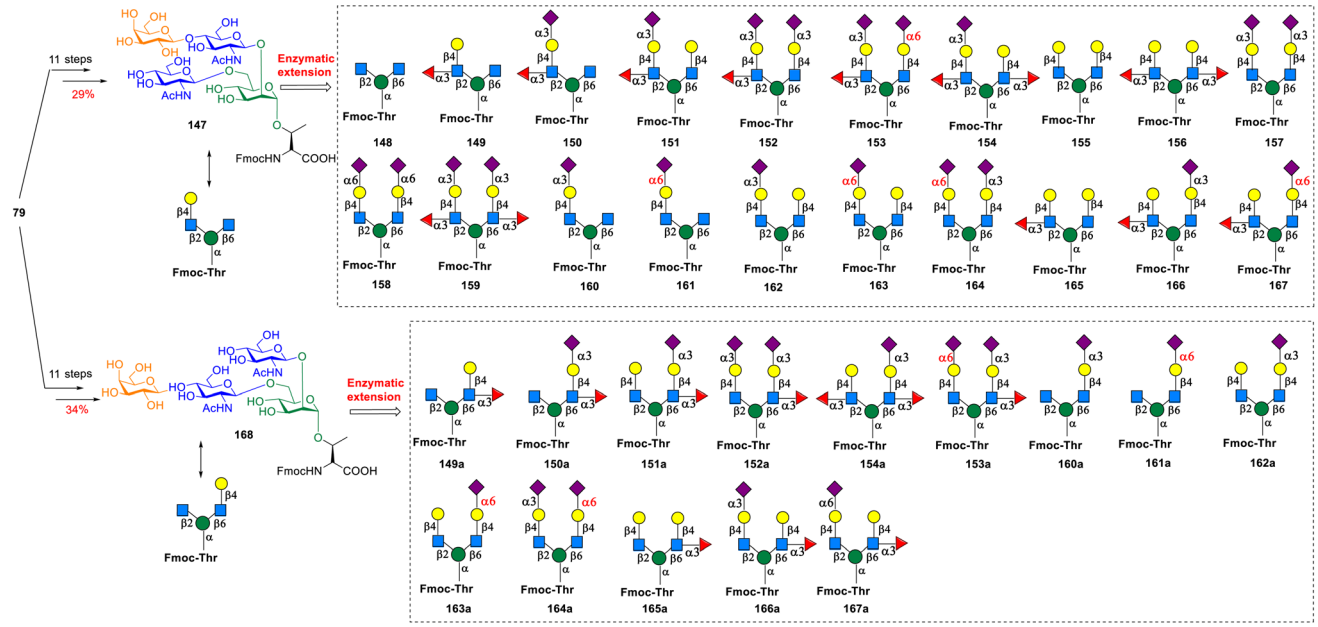
maintain considerable catalytic activity even toward *N*-terminally exposed glycopeptide substrates. This finding provides a promising strategy for the synthesis of extended, structurally defined α-DG glycopeptides.

4 Synthesis of α-DG core M3 *O*-mannose glycans

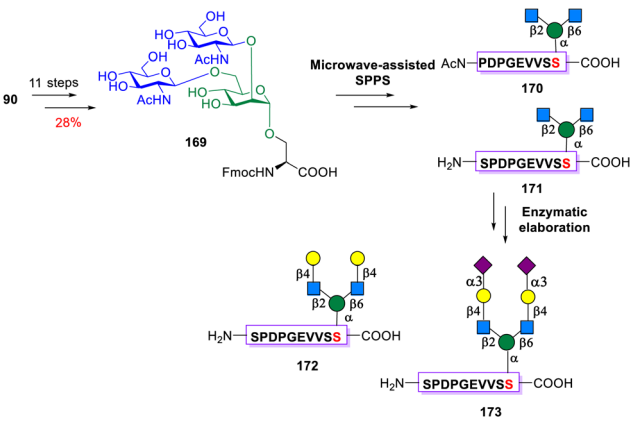
As depicted in Fig. 1, the structure of core M3 displays considerable structural complexity, consisting of three primary components: (1) the core trisaccharide with mannose phosphorylated at the 6-position; (2) the tandem Rbo5P structure; (3) matriglycan, which is a polysaccharide composed of the repeating disaccharide units, and is a critical component of the core M3 *O*-mannose glycan. Here, we provide a detailed account of the synthetic progress of these three parts.

4.1 Synthesis of the phosphorylated core M3 trisaccharide

Early efforts in the synthesis of core M3 glycan primarily focused on the construction of the phosphorylated core M3 trisaccharide. In 2011, Boons and co-workers reported the first linear synthetic strategy for the preparation of a phosphorylated trisaccharide glycopeptide derived from α-DG (Scheme 12A).[71] This synthetic route employed four key building blocks **174–177** to sequentially construct the phosphorylated trisaccharide-threonine **180**, which was subsequently incorporated into an automated SPPS system. Through iterative coupling cycles and deprotection steps, the glycopeptide **182** was efficiently assembled. In addition, they also provided the trisaccharide and confirmed that its NMR data matched the previously reported spectral features of the isolated compound, thereby verifying the structural assignment of this unusual phospho-glycan. In 2022, Peng and co-workers described a divergent synthetic



Scheme 10 Synthesis of core M2 *O*-mannose glycoamino acids *via* SSEE strategy from **79**.



Scheme 11 Chemoenzymatic synthesis of core M2 *O*-Mannosyl glycopeptides by Peng group.

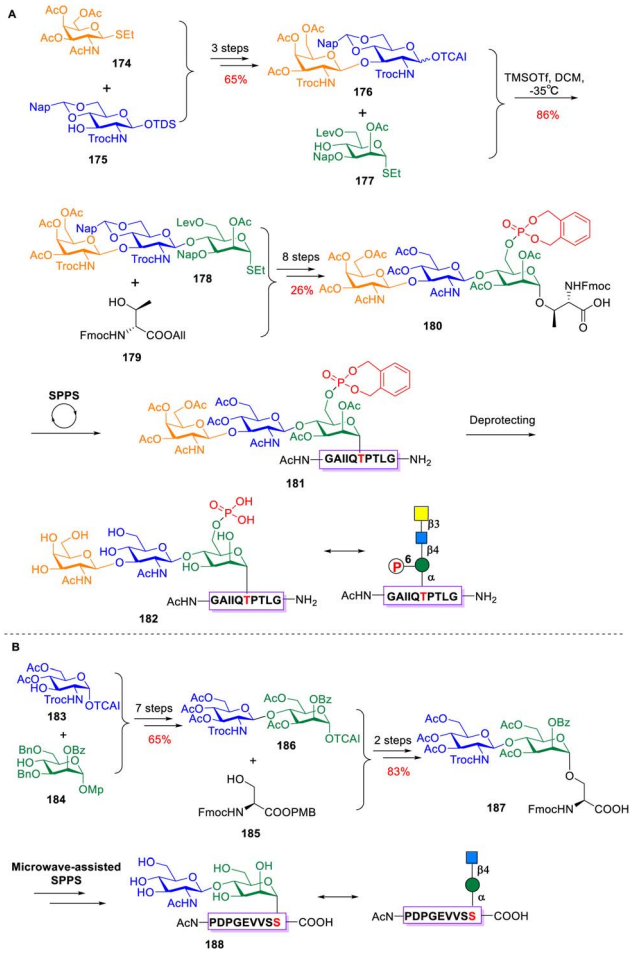
approach to access core M3 mannose glycan structures.[35] As highlighted in the previous section, this synthetic strategy integrated facile preparation of core M3 glycopeptide assembly *via* optimized microwave-assisted SPPS, culminating in the successful synthesis of glycopeptide **188** containing a partial core M3 glycan motif (Scheme 12B).

4.2 Synthesis of the tandem Rbo5P structure

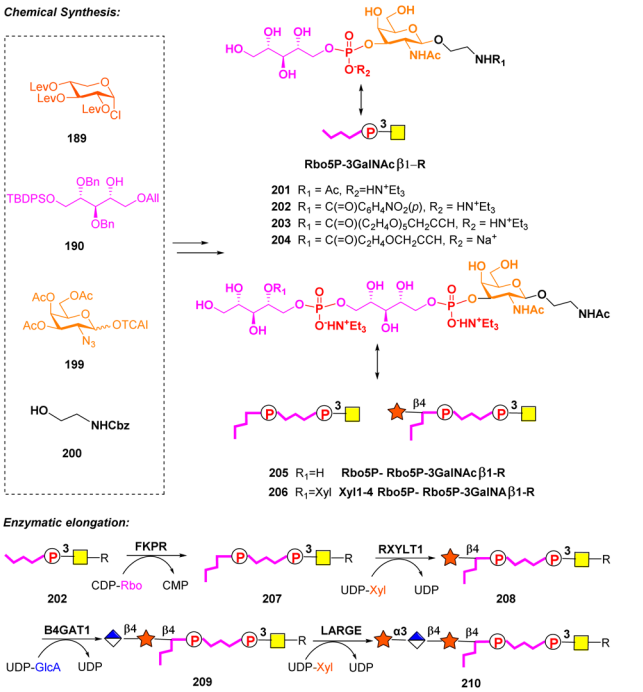
The tandem Rbo5P modification, a process mediated by FKTN and FKRP that transfers Rbo5P to the O-3 of GalNAc and O-1 of Rbo5P, respectively, is essential for the functional maturation of α-DG and was first identified in mammals. In 2019, Tamura and co-workers reported the first synthesis of partial structures of core M3 glycans incorporating the tandem Rbo5P linkers,[72] which included compounds **196** (Xylβ1-4Rbo), **197** (Xylβ1-4Rbo5P), and **198** (Xylβ1-4Rbo5P-1Rbo). The synthesis was achieved *via* a chemical approach to precisely control regio- and stereoselectivity (Scheme 13). Building on this work, the group further developed chemical and chemoenzymatic strategies to prepare three types of partial core M3 *O*-mannose glycans containing tandem Rbo5P motifs and their derivatives **201–206**. Moreover, they demonstrated enzymatic glycan elongation using these synthetic acceptors, revealing that Rbo5P-3GalNAcβ derivatives constitute the minimal glycan structure necessary for FKRP-mediated Rbo5P transfer and may function as precursors for the elongation of the core M3 *O*-mannose glycan (Scheme 14).[73] Recently, Willems and co-workers synthesized four alkyne-tagged Rbo5P derivatives and demonstrated that the *S*-acyl-2-thioethyl (SATE)-protected phosphorylated probes enabled specific labeling of overexpressed α-DG in mammalian cells.[74]

4.3 Synthesis of matriglycan

Matriglycan, a high-molecular-weight polysaccharide located at the terminus of the core M3 *O*-mannose glycan and composed of repeating [-3GlcAβ1-3Xylα1-]$_n$ units, mediates the critical linkage between α-DG and the basement membrane by serving as the direct ligand for laminin.[75] However, the highly stereoselective construction of 1,2-*cis*-xylopyranosides remains a formidable challenge in the synthesis of the -XylαGlcAβ- repeating unit. This difficulty arises from the distinctive structural and electronic properties of xylose. In contrast to its C6-analogue glucose, the xylose ring exhibits greater conformational flexibility and a stronger tendency to undergo S_N1-type glycosylation pathways, owing to its electronic features, which collectively compromise stereochemical control. As a result, many established glycosylation protocols that successfully form 1,2-*cis*-linkages in glucopyranosides prove inadequate for



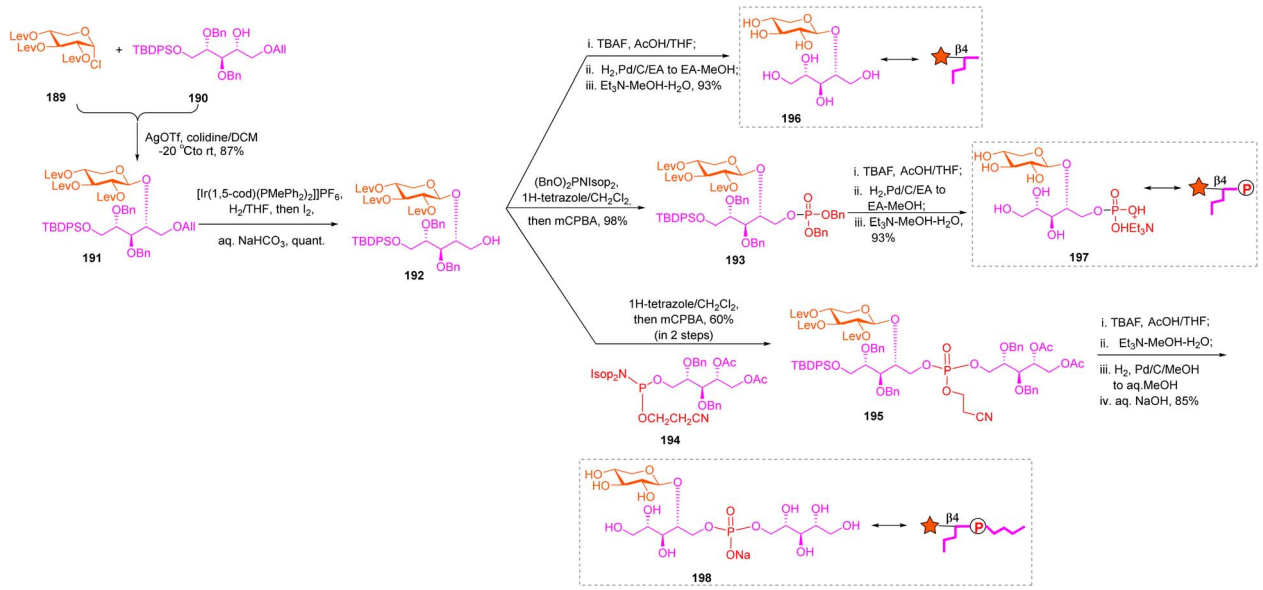
Scheme 12 Synthesis of core M3 glycopeptides *via* SPPS. (A) Chemical synthesis of phospho-trisaccharide glycopeptide **182** and (B) synthesis of disaccharide glycopeptide **188**. Abbreviation: Lev = levulinoyl; Nap = 2-naphthylmethyl.



Scheme 14 Chemical and chemoenzymatic synthesis of three types of partial core M3 *O*-mannose glycans containing tandem Rbo5P motifs.

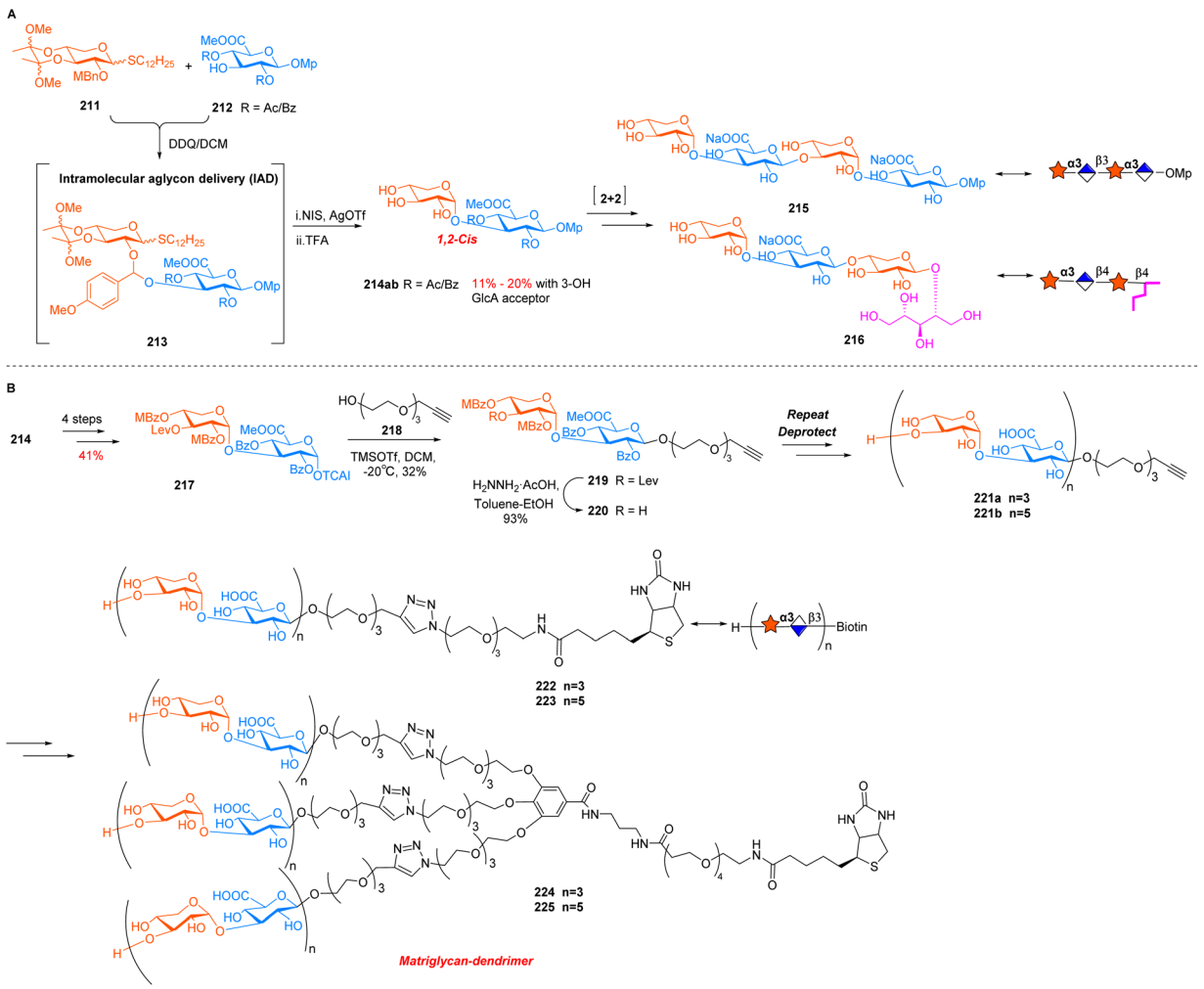
xylose, generally affording poor to moderate stereoselectivity that depends inefficiently on the anomeric effect.

Consequently, considerable efforts have been devoted to developing α-selective xylosidation methods to enable the successful synthesis of matriglycan. In 2020, Tamura and co-workers combined conformation fixation and intramolecular aglycon delivery (IAD) strategies, employing 4-methoxybenzyl

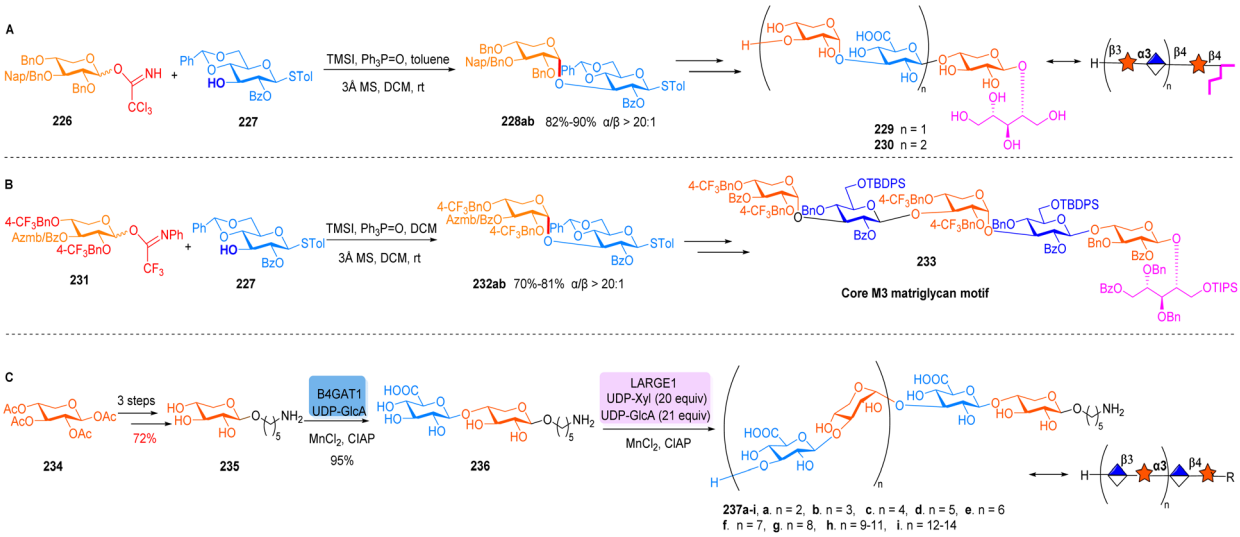


Scheme 13 Chemical synthesis of tandem Rbo5P-containing glycans **196** (Xylβ1-4Rbo), **197** (Xylβ1-4Rbo5P), and **198** (Xylβ1-4Rbo5P-1Rbo).

ether at the 2-position of xylose to construct 1,2-*cis*-xylopyranoside formation.[76] By exploiting this method, the matriglycan-repeating tetrasaccharides **215** and **216** were synthesized (Scheme 15A). Based on this, in 2025, they also reported the synthesis of an alkyne-linked matriglycan-repeating hexasaccharide **221** and its biotin conjugate **222** (Scheme 15B).[77] In a further report, by the stepwise addition of the corresponding disaccharide unit for regioselective and stereoselective synthesis, they first removed the levulinoyl (Lev) group with hydrazine acetate, followed by glycosylation with the donor, and repeated the process to obtain the matriglycan-repeating decasaccharide **223** and two dendrimers **224** and **225** comprising three branches of matriglycan-repeating hexa- and decasaccharides (Scheme 15B). However, the application of the IAD strategy to α-linked disaccharide construction is limited by low efficiency and the necessity for lengthy synthetic routes. To enhance the synthetic efficiency of matriglycan, in 2023, Peng and co-workers established a preactivation-based, additive-modulated 1,2-*cis*-xylopyranosylation protocol employing *O*-xylopyranosyl trichloroacetimidate **226** as glycosyl donors, with trimethylsilyl iodide (TMSI) as a promoter in the presence of triphenylphosphine oxide (Ph_3PO) as an additive (Scheme 16A).[78] The matriglycan constituent tetrasaccharide **229** and hexasaccharide **230**, which contain a tandem Rbo5P structure, were efficiently synthesized *via* this synthetic strategy. In a recent report, Xiao and co-workers also demonstrated that 1,2-*cis*-xylosylation could be accomplished through a synergistic combination of reagent modulation (TMSI and Ph_3PO), 3-*O*-acyl group remote participation, and electron-withdrawing effects of 4-trifluoromethylbenzyl group, which achieved the synthesis of core M3 matriglycan motif **233** (Scheme 16B).[79] In addition to chemical synthesis, enzymatic elongation has also been employed, as noted earlier, by Endo and coworkers to introduce the disaccharide repeating units into matriglycan (Scheme 14).[73] In 2022, Boons and co-workers reported a chemo-enzymatic strategy for the assembly of matriglycan oligosaccharides **237a–i** bearing a precisely defined number of tandem repeats (Scheme 16C).[80] Extension of the core chain was accomplished by iterative enzymatic glycosylation with large excesses of UDP-GlcA and UDP-Xyl, furnishing products that



Scheme 15 Synthesis of matriglycan constituent by Tamura group. (A) Synthesis of matriglycan-repeating tetrasaccharide **215** and **216** by intramolecular aglycon delivery (IAD) strategies (B) synthesis of glycodendrimers **224** and **225** containing matriglycan repeating hexa- and decasaccharides.



Scheme 16 Strategies for stereoselective construction of matriglycan-repeating α-xylopyranosides. (A) Synthesis of matriglycan constituent by apreactivation-based, additive-modulated trichloroacetimidate glycosidation strategy and (B) Reagent modulation, remote participation, electron-withdrawing synergistic effects and (C) enzyme-mediated specificity control.

encompass two to fourteen disaccharide repeats. A notable limitation, however, was that oligosaccharides with nine or more disaccharide repeats could not be isolated as a single homogeneous fraction by HPLC. Furthermore, utilizing glycan microarray technology and cell-surface glyco-engineering approaches, their work established the length-dependent binding of matriglycan on α-DG to laminin, Lassa virus (LASV) GP1, and the clinically important antibody IIH6. It was demonstrated that introducing synthetically defined matriglycans onto the surface of cells lacking either α-DG or *O*-mannosylation could restore infection by a Lassa-pseudovirus. Additionally, free matriglycans were shown to inhibit viral infection of wild-type cells in a dose and length dependent manner. Ultimately, their findings indicate that matriglycan alone is necessary and sufficient for IIH6 staining, laminin and LASV GP1 binding, and Lassa-pseudovirus infection, and further reveal that increasing its chain length enhances ligand-binding capacity.

5 Conclusions and perspectives

The field of *O*-mannose glycan synthesis has witnessed remarkable progress, evolving from pioneering low-yielding chemical routes to sophisticated and highly efficient chemoenzymatic strategies. This review has chronicled the successful synthesis of all three major α-DG *O*-mannose glycan types—cores M1, M2, and the highly complex core M3. For *O*-mannose glycan synthesis, chemical approaches offer flexible structure design but often involve long, low-efficiency routes, whereas enzymatic approaches are efficient and selective but limited by the narrow substrate specificity of glycosyltransferases. Therefore, productive glycosylation approaches and low-cost, broadly applicable glycosyltransferases are still needed. Yet, while the syntheses of core M1 and M2 glycans have been extensively established, the construction of core M3 remains nascent, primarily limited to the preparation of its fragmentary components. The lack of access to the fully assembled, precisely defined core M3 glycan has consequently restricted comprehensive studies of its physiological functions and disease-related molecular mechanisms. Therefore, obtaining the complete *O*-mannosylated structure is of paramount importance. It is evident that the development of highly efficient strategies, which seamlessly integrate the strengths of chemical and enzymatic approaches, represents the most promising direction for the future synthesis of homogeneous *O*-mannose glycans and glycopeptides. Simultaneously, embracing automation and enzymatic synthesis will be key to streamlining production, reducing costs, and providing scalable access to these biologically vital glycans.[81,82]

Author contributions

Yue Yang: conceptualization, writing – original draft, writing – review & editing. Huiran Hao: writing – original draft, writing – review & editing. Huarong Shao: investigation. Jinhua Zhang: conceptualization, supervision. Weilu Tian: formal analysis. Han Zhang: validation. Beibei Liu: visualization. Fei Liu: conceptualization, resources, supervision. Peixue Ling: supervision, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software, or code have been included, and no new data were generated or analysed as part of this review.

Acknowledgements

This work was financially supported by the Shandong Provincial Natural Science Foundation (ZR2025QC2270Z), the National Key Research and Development Program (2024YFF0618302), the Shandong Provincial Key R&D Program (Competitive Platform Project, 2025CXPT145, 2025CXPT035), the Shandong Province Key R&D Program (Innovation Capacity Enhancement Project for Technology-Based Small and Medium-Sized Enterprises, 2025TSGCCZZB0800),the National Natural Science Foundation of China (Grant No. 82404439), and the Shandong Provincial Natural Science Foundation (ZR2023QB236).

References

1 J. M. Ervasti, K. Ohlendieck, S. D. Kahl, M. G. Gaver and K. P. Campbell, *Nature*, 1990, **345**, 315–319.
2 O. Ibraghimov-Beskrovnaya, J. M. Ervasti, C. J. Leveille, C. A. Slaughter, S. W. Sernett and K. P. Campbell, *Nature*, 1992, **355**, 696–702.
3 S. Liu, T. Su, X. Xia and Z. H. Zhou, *Nature*, 2025, **637**, 1261–1271.
4 R. Barresi and K. P. Campbell, *J. Cell Sci.*, 2006, **119**, 199–207.
5 J. M. Ervasti and K. P. Campbell, *J. Cell Biol.*, 1993, **122**, 809–823.
6 M. A. Bowe, K. A. Deyst, J. D. Leszyk and J. R. Fallon, *Neuron*, 1994, **12**, 1173–1180.
7 J. F. Talts, Z. Andac, W. Göhring, A. Brancaccio and R. Timpl, *EMBO J.*, 1999, **18**, 863–870.
8 S. Sugita, F. Saito, J. Tang, J. Satz, K. Campbell and T. C. Südhof, *J. Cell Biol.*, 2001, **154**, 435–446.
9 S. Sato, Y. Omori, K. Katoh, M. Kondo, M. Kanagawa, K. Miyata, K. Funabiki, T. Koyasu, N. Kajimura, T. Miyoshi, H. Sawai, K. Kobayashi, A. Tani, T. Toda, J. Usukura, Y. Tano, T. Fujikado and T. Furukawa, *Nat. Neurosci.*, 2008, **11**, 923–931.
10 K. M. Wright, K. A. Lyon, H. Leung, D. J. Leahy, L. Ma and D. D. Ginty, *Neuron*, 2012, **76**, 931–944.
11 T. Endo, *J. Biochem.*, 2015, **157**, 1–12.
12 A. Yoshida, K. Kobayashi, H. Manya, K. Taniguchi, H. Kano, M. Mizuno, T. Inazu, H. Mitsuhashi, S. Takahashi, M. Takeuchi, R. Herrmann, V. Straub, B. Talim, T. Voit, H. Topaloglu, T. Toda and T. Endo, *Dev. Cell*, 2001, **1**, 717–724.
13 C. J. Moore and J. E. Hewitt, *Glycoconjugate J.*, 2009, **26**, 349–357.
14 F. Sciandra, K. I. Gawlik, A. Brancaccio and M. Durbeej, *Trends Biotechnol.*, 2007, **25**, 262–268.
15 A. A. Zambon and F. Muntoni, *Neuromuscular Disord.*, 2021, **31**, 931–942.
16 T. Willer, K.-i. Inamori, D. Venzke, C. Harvey, G. Morgensen, Y. Hara, D. Beltrán Valero de Bernabé, L. Yu, K. M. Wright and K. P. Campbell, *eLife*, 2014, **3**, e03941.
17 T. Roscioli, E.-J. Kamsteeg, K. Buysse, I. Maystadt, J. van Reeuwijk, C. van den Elzen, E. van Beusekom, M. Riemersma, R. Pfundt, L. E. L. M. Vissers, M. Schraders, U. Altunoglu, M. F. Buckley, H. G. Brunner, B. Grisart, H. Zhou, J. A. Veltman, C. Gilissen, G. M. S. Mancini, P. Delrée, M. A. Willemsen, D. P. Ramadža, D. Chitayat, C. Bennett, E. Sheridan, E. A. J. Peeters, G. M. B. Tan-Sindhunata, C. E. de Die-Smulders, K. Devriendt, H. Kayserili, O. A. E.-F. El-Hashash, D. L. Stemple, D. J. Lefeber, Y.-Y. Lin and H. van Bokhoven, *Nat. Genet.*, 2012, **44**, 581–585.
18 D. E. Michele, R. Barresi, M. Kanagawa, F. Saito, R. D. Cohn, J. S. Satz, J. Dollar, I. Nishino, R. I. Kelley, H. Somer, V. Straub, K. D. Mathews, S. A. Moore and K. P. Campbell, *Nature*, 2002, **418**, 417–421.
19 Y. K. Hayashi, M. Ogawa, K. Tagawa, S. Noguchi, T. Ishihara, I. Nonaka and K. Arahata, *Neurology*, 2001, **57**, 115–121.
20 T. Toda, K. Kobayashi, E. Kondo-Iida, J. Sasaki and Y. Nakamura, *Neuromuscular Disord.*, 2000, **10**, 153–159.
21 L. Xu, P. J. Lu, C.-H. Wang, E. Keramaris, C. Qiao, B. Xiao, D. J. Blake, X. Xiao and Q. L. Lu, *Mol. Ther.*, 2013, **21**, 1832–1840.
22 M. Kanagawa, *Int. J. Mol. Sci.*, 2021, **22**, 13162.
23 E. Martin-Rendon and D. J. Blake, *Trends Pharmacol. Sci.*, 2003, **24**, 178–183.
24 J. R. Mendell, D. R. Boué and P. T. Martin, *Pediatr. Dev. Pathol.*, 2006, **9**, 427–443.
25 C. M. Dobson, S. J. Hempel, S. H. Stalnaker, R. Stuart and L. Wells, *Cell. Mol. Life Sci.*, 2013, **70**, 2849–2857.
26 V. M. Panin and L. Wells, *Curr. Protoc. Protein Sci.*, 2014, **75**, 12.12.11–12.12.29.
27 A. S. Sanji, M. J, M. J. Gurav, S. K. Batra and V. B. Chachadi, *Int. J. Biol. Macromol.*, 2024, **260**, 129318.
28 J. L. Praissman and L. Wells, *Biochemistry*, 2014, **53**, 3066–3078.
29 M. Katz and R. Diskin, *Front. Mol. Biosci.*, 2024, **11**, 1371551.
30 S. Kunz, M. Rojek Jillian, M. Perez, F. Spiropoulou Christina and B. A. Oldstone Michael, *J. Virol.*, 2005, **79**, 5979–5987.
31 L. T. Jae, M. Raaben, M. Riemersma, E. van Beusekom, V. A. Blomen, A. Velds, R. M. Kerkhoven, J. E. Carette, H. Topaloglu, P. Meinecke, M. W. Wessels, D. J. Lefeber, S. P. Whelan, H. van Bokhoven and T. R. Brummelkamp, *Science*, 2013, **340**, 479–483.
32 M. Imperiali, C. Thoma, E. Pavoni, A. Brancaccio, N. Callewaert and A. Oxenius, *J. Virol.*, 2005, **79**, 14297–14308.
33 M. Koff, P. Monagas-Valentin, B. Novikov, I. Chandel and V. Panin, *Glycobiology*, 2023, **33**, 911–926.
34 H. Manya and T. Endo, *Biochim. Biophys. Acta, Gen. Subj.*, 2017, **1861**, 2462–2472.
35 T. Li, Y. Zhang, T. Li, H. Zhuang, F. Wang, N. Wang, R. R. Schmidt and P. Peng, *Chin. J. Chem.*, 2022, **40**, 1571–1577.
36 H. Manya, Y. Yamaguchi, M. Kanagawa, K. Kobayashi, M. Tajiri, K. Akasaka-Manya, H. Kawakami, M. Mizuno, Y. Wada, T. Toda and T. Endo, *J. Biol. Chem.*, 2016, **291**, 24618–24627.
37 M. Kanagawa, K. Kobayashi, M. Tajiri, H. Manya, A. Kuga, Y. Yamaguchi, K. Akasaka-Manya, J.-i. Furukawa, M. Mizuno, H. Kawakami, Y. Shinohara, Y. Wada, T. Endo and T. Toda, *Cell Rep.*, 2016, **14**, 2209–2223.

38 W. Sharaf-Eldin, *J. Mol. Neurosci.*, 2025, **75**, 28.
39 H. Manya, A. Chiba, A. Yoshida, X. Wang, Y. Chiba, Y. Jigami, R. U. Margolis and T. Endo, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 500–505.
40 L. Bai, A. Kovach, Q. You, A. Kenny and H. Li, *Nat. Struct. Mol. Biol.*, 2019, **26**, 704–711.
41 K.-i. Inamori, T. Endo, J. Gu, I. Matsuo, Y. Ito, S. Fujii, H. Iwasaki, H. Narimatsu, E. Miyoshi, K. Honke and N. Taniguchi, *J. Biol. Chem.*, 2004, **279**, 2337–2340.
42 T. Endo, *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.*, 2019, **95**, 39–51.
43 M. C. Manzini, D. E. Tambunan, R. S. Hill, T. W. Yu, T. M. Maynard, E. L. Heinzen, K. V. Shianna, C. R. Stevens, J. N. Partlow, B. J. Barry, J. Rodriguez, V. A. Gupta, A. K. Al-Qudah, W. M. Eyaid, J. M. Friedman, M. A. Salih, R. Clark, I. Moroni, M. Mora, A. H. Beggs, S. B. Gabriel and C. A. Walsh, *Am. J. Hum. Genet.*, 2012, **91**, 541–547.
44 T. Yoshida-Moriguchi, T. Willer, M. E. Anderson, D. Venzke, T. Whyte, F. Muntoni, H. Lee, S. F. Nelson, L. Yu and K. P. Campbell, *Science*, 2013, **341**, 896–899.
45 M. Kanagawa and T. Toda, *J. Biochem.*, 2018, **163**, 359–369.
46 J. L. Praissman, D. H. Live, S. Wang, A. Ramiah, Z. S. Chinoy, G.-J. Boons, K. W. Moremen and L. Wells, *eLife*, 2014, **3**, e03943.
47 M. M. Goddeeris, B. Wu, D. Venzke, T. Yoshida-Moriguchi, F. Saito, K. Matsumura, S. A. Moore and K. P. Campbell, *Nature*, 2013, **503**, 136–140.
48 K. Inamori, T. Yoshida-Moriguchi, Y. Hara, M. E. Anderson, L. Yu and K. P. Campbell, *Science*, 2012, **335**, 93–96.
49 M. O. Sheikh, S. M. Halmo and L. Wells, *Glycobiology*, 2017, **27**, 806–819.
50 E. Sasaki, Y. Hayashi, Y. Kimura, S. Sashida, N. Hamano, K. Nirasawa, K. Hamada, F. Katagiri, Y. Kikkawa, T. Sakai, A. Yoshida, M. Kawada, S.-I. Hirashima, T. Miura, Y. Endo-Takahashi, M. Nomizu and Y. Negishi, *J. Contr. Release*, 2021, **329**, 1037–1045.
51 M. Van Scherpenzeel, E. Willems and D. J. Lefeber, *Glycoconjugate J.*, 2016, **33**, 345–358.
52 Z.-F. Hu, K. Zhong and H. Cao, *Curr. Opin. Chem. Biol.*, 2024, **78**, 102417.
53 S. Ma, J. Gao, Y. Tian and L. Wen, *Org. Biomol. Chem.*, 2024, **22**, 7767–7785.
54 A. Chiba, K. Matsumura, H. Yamada, T. Inazu, T. Shimizu, S. Kusunoki, I. Kanazawa, A. Kobata and T. Endo, *J. Biol. Chem.*, 1997, **272**, 2156–2162.
55 I. Matsuo, M. Isomura and K. Ajisaka, *Tetrahedron Lett.*, 1999, **40**, 5047–5050.
56 H. Fujimoto, M. Isomura, T. Miyazaki, I. Matsuo, R. Walton, T. Sakakibara and K. Ajisaka, *Glycoconjugate J.*, 1997, **14**, 75–80.
57 M. A. Williams, H. Kitagawa, A. K. Datta, J. C. Paulson and J. C. Jamieson, *Glycoconjugate J.*, 1995, **12**, 755–761.
58 J. Seifert, T. Ogawa and Y. Ito, *Tetrahedron Lett.*, 1999, **40**, 6803–6807.
59 J. Seifert, T. Ogawa, S. Kurono and Y. Ito, *Glycoconjugate J.*, 2000, **17**, 407–423.
60 K. T. Schjoldager, Y. Narimatsu, H. J. Joshi and H. Clausen, *Nat. Rev. Mol. Cell Biol.*, 2020, **21**, 729–749.
61 C. Li and L.-X. Wang, *Chem. Rev.*, 2018, **118**, 8359–8413.
62 R. Šardzík, A. P. Green, N. Laurent, P. Both, C. Fontana, J. Voglmeir, M. J. Weissenborn, R. Haddoub, P. Grassi, S. M. Haslam, G. Widmalm and S. L. Flitsch, *J. Am. Chem. Soc.*, 2012, **134**, 4521–4524.
63 M. A. Ferrero-García, S. E. Trombetta, D. O. Sánchez, A. Reglero, A. C. Frasch and A. J. Parodi, *Eur. J. Biochem.*, 1993, **213**, 765–771.
64 J. Yu and U. Westerlind, *ChemBioChem*, 2014, **15**, 939–945.
65 Y. Zhang, C. Meng, L. Jin, X. Chen, F. Wang and H. Cao, *Chem. Commun.*, 2015, **51**, 11654–11657.
66 C. Meng, A. Sasmal, Y. Zhang, T. Gao, C.-C. Liu, N. Khan, A. Varki, F. Wang and H. Cao, *Angew Chem. Int. Ed. Engl.*, 2018, **57**, 9003–9007.
67 T. Gao, J. Yan, C.-C. Liu, A. S. Palma, Z. Guo, M. Xiao, X. Chen, X. Liang, W. Chai and H. Cao, *J. Am. Chem. Soc.*, 2019, **141**, 19351–19359.
68 S. Wang, Q. Zhang, C. Chen, Y. Guo, M. R. Gadi, J. Yu, U. Westerlind, Y. Liu, X. Cao, P. G. Wang and L. Li, *Angew Chem. Int. Ed. Engl.*, 2018, **57**, 9268–9273.
69 H. Hinou, S. Kikuchi, R. Ochi, K. Igarashi, W. Takada and S.-I. Nishimura, *Bioorg. Med. Chem.*, 2019, **27**, 2822–2831.
70 J. Yu, O. C. Grant, C. Pett, S. Strahl, S. Stahl, R. J. Woods and U. Westerlind, *Chemistry*, 2017, **23**, 3466–3473.
71 K.-F. Mo, T. Fang, S. H. Stalnaker, P. S. Kirby, M. Liu, L. Wells, M. Pierce, D. H. Live and G.-J. Boons, *J. Am. Chem. Soc.*, 2011, **133**, 14418–14430.
72 T. Tamura and J.-I. Tamura, *Tetrahedron Lett.*, 2019, **60**, 465–468.
73 J.-I. Tamura, T. Tamura, S. Hoshino, R. Imae, R. Kato, M. Yokono, M. Nagase, S. Ohno, N. Manabe, Y. Yamaguchi, H. Manya and T. Endo, *ACS Chem. Biol.*, 2022, **17**, 1513–1523.
74 L. D. Murphy, S. Akkad, A. Lopez, M. E. Batiste-Simms, G. L. McNeil, E. W. Wan, K. E. Huxley, L. Julyan, M. Shandell and L. I. Willems, *RSC Chem. Biol.*, 2025, **6**, 1832–1836.
75 T. Yoshida-Moriguchi and K. P. Campbell, *Glycobiology*, 2015, **25**, 702–713.
76 T. Tamura, Y. Omura and J.-I. Tamura, *J. Org. Chem.*, 2020, **85**, 12935–12946.
77 K. Kotera, R. Miyamoto, G. Mochizuki, T. Tamura, N. Manabe, Y. Yamaguchi and J.-I. Tamura, *Carbohydr. Res.*, 2025, **547**, 109328.
78 T. Li, M. Zhang, P. Lv, Y. Yang, R. R. Schmidt and P. Peng, *J. Org. Chem.*, 2024, **89**, 804–809.
79 J. Wan, Y. Ji, L. Wang, R. Yang, K. Li, Q. Xian, X. Wang, G. Lu and G. Xiao, *Angew Chem. Int. Ed. Engl.*, 2025, **64**, e202424048.
80 M. O. Sheikh, C. J. Capicciotti, L. Liu, J. Praissman, D. Ding, D. G. Mead, M. A. Brindley, T. Willer, K. P. Campbell, K. W. Moremen, L. Wells and G.-J. Boons, *Nat. Commun.*, 2022, **13**, 3617.
81 Y. Wu, Y. Sun, C. Pei, X. Peng, X. Liu, E. W. Qian, Y. Du and J.-J. Li, *RSC Adv.*, 2024, **14**, 17397–17405.
82 T. Li, L. Liu, N. Wei, J.-Y. Yang, D. G. Chapla, K. W. Moremen and G.-J. Boons, *Nat. Chem.*, 2019, **11**, 229–236.